

# medicina

BUENOS AIRES, VOL. 84 Supl. V - 2024



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La Tapa  
Todo, 2016  
Daniela Kantor

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# **REUNIÓN CONJUNTA SAIC SAFIS ALACF 2024**

**LXIX REUNIÓN ANUAL DE LA  
SOCIEDAD ARGENTINA DE INVESTIGACIÓN CLÍNICA  
(SAIC)**

**XXVI SOCIEDAD ARGENTINA DE FISIOLÓGÍA  
(SAFIS)**

**ASOCIACIÓN LATINOAMERICANA DE CIENCIAS FISIOLÓGICAS  
(ALACF)**

19-22 de noviembre de 2024  
Usina del Arte – Ciudad Autónoma de Buenos Aires

**EDITORES RESPONSABLES**

Dr. Rodolfo Rey  
Dra. Graciela Cremaschi  
Dr. Ernesto Alejandro Aiello

# **JOINT MEETING SAIC SAFIS 2024**

**LXVIII ANNUAL MEETING OF  
THE ARGENTINE SOCIETY OF CLINICAL RESEARCH  
(SAIC)**

**XXVI ARGENTINE SOCIETY OF PHYSIOLOGY  
(SAFIS)**

**LATIN AMERICAN ASSOCIATION OF PHYSIOLOGICAL SCIENCES  
(ALACF)**

November 19-22, 2024  
Usina del Arte – Ciudad Autónoma de Buenos Aires

**RESPONSIBLE EDITORS**  
Dr. Rodolfo Rey  
Dr. Graciela Cremaschi  
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## **LA TAPA**

**Edgardo Hartkopf. Esquina de Tango 1980**

**Técnica:** Óleo sobre lienzo.

Nacido en Gualeguaychú (provincia de Entre Ríos), se estableció en Rosario en 1940. Profesor Nacional de Dibujo y Pintura de la Escuela de Bellas Artes de la Universidad Nacional del Litoral. Ejerce como docente alternando con trabajos en su atelier. Realiza su primera exposición en Gualeguaychú en 1957. En 1965, expone en la Galería Stilnuovo de Venado Tuerto (provincia de Santa Fe). En 1975, obtiene mención honorífica en el Salón Pictórico Anual de la Escuela Municipal de Artes Plásticas M. Musto de Rosario. Expone sucesivamente en Rosario en 1975 en Galería Borgherese, en 1976 en Fontana Artis, en 1977 en Galería Fienze, y en 1978 expone en el XII Salón Anual del Museo Castagnino de Rosario. Sus trabajos reflejan paisajes rurales y urbanos del Litoral.

Gentileza de la familia Hartkopf.

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## PALABRAS DE BIENVENIDA DE LOS PRESIDENTES DE LAS SOCIEDADES

Colegas y amigos:

*Es un privilegio para mí, como Presidente de SAIC, darles la bienvenida a este Congreso Anual de Sociedades de Biociencias, organizado conjuntamente con SAFIS y ALACF. Agradezco a los Dres. Gracielita Cremaschi y Alejandro Aiello por acompañar a SAIC en esta ocasión de características tan particulares y por la confianza depositada durante la organización del Congreso. No es necesario que insista sobre lo difícil que ha sido este período de armado del congreso, porque es la misma situación la que afecta a toda nuestra comunidad científica. Han sido la firme convicción de realizar la Reunión Anual y el compromiso de los miembros del Consejo Directivo de SAIC elementos cruciales para lograr un programa de calidad, a la altura de la tradición de nuestra sociedad. Destaco particularmente el denodado trabajo de la Dra. Mariana Tellechea, Secretaria, y la Dra. María Fernanda Riera, Tesorera de SAIC, puntales esenciales en la concreción de esta reunión. El apoyo del Vicepresidente, Dr. Javier Cotignola, y la Prosecretaria, Dra. Geraldine Gueron, han sido también determinantes. La experiencia transmitida por la Dra. Caroline Lamb, Secretaria en 2023, y por los Presidentes que me precedieron, el Dr. Daniel Alonso y la Dra. Isabel Lüthy, fueron de enorme ayuda. La empatía y el ofrecimiento de colaboración de expresidentes representaron demostraciones de solidaridad sobre las que pudieron construirse la confianza y el grado de optimismo necesarios en momentos como pocas veces ha pasado la SAIC a lo largo de su historia. Quiero recordar a Fabiana, mi esposa, que fue, mientras estuvo, un puntal de apoyo en la intimidad diaria a lo largo de este desafío que comenzó hace más de dos años.*

*Como es notorio, debimos cambiar la sede de nuestro Congreso luego de una consulta a los socios. También es claro que la cantidad de actividades debió ser ajustada, todo ello por razones presupuestarias. Aprovecho para agradecer a las empresas auspiciantes por habernos apoyado en el contexto actual que también las afecta. Este año contamos con 5 conferencias plenarias de destacados colegas nacionales e internacionales, 11 simposios, 3 mesas redondas, 10 sesiones de presentaciones orales y 5 de posters, además de las clásicas sesiones de trabajos presentados a los premios Cherny, Bigand, Gador, mAbxien-ce, Faryna-Raveglia y del Consejo de Genética. Agradezco a los auspiciantes de estos premios y a los jurados, así como a los invitados a las conferencias y simposios, particularmente a quienes se desplazaron desde el exterior sin apoyo económico de SAIC. A los socios que facilitaron dichos contactos, les hago llegar un muy especial reconocimiento.*

*No hace muchos años, en 2019, la Dra. Mónica Costas, entonces Presidenta de la Sociedad, se refería a la necesidad que tiene nuestro país del sector de ciencia y tecnología para construir conocimiento y producir bienes y servicios que mejoren la calidad de vida de todos. Decía Mónica que “el modelo de país lo hacemos entre todos y sin ciencia, tecnología, salud y educación, no hay modelo de país posible”. Tristemente, hoy se aplica el proverbio popular “¡qué bien estábamos cuando estábamos mal!”. Cinco años después la situación es claramente peor. La reflexión se impone para poder comprender cómo pudimos llegar a esta situación. Y no exime de autocrítica. Sin duda, nuestro país no está aislado, y la denostación de la ciencia y del conocimiento basado en evidencia pululan globalmente. Pero la Argentina está viviendo una situación que va a contramano de su historia. Nuestro país se caracterizó por estar entre los líderes del continente en la generación de conocimientos gracias a un modelo basado en una educación y una ciencia sustentadas por el Estado. Mientras que los países más desarrollados siguen teniendo hoy ese modelo, el nuestro parece intentar copiar modelos de subdesarrollo, donde el foco está en la generación de renta como el valor más importante. Transferir todo a organizaciones privadas va contra el principio de solidaridad, caracterizado por que “cada uno participa según sus posibilidades y recibe según sus necesidades”.*

*Como el mundo en general, el sistema científico se construye en base al aporte colectivo. La visión individualista, cortoplacista, aporta mucho menos al desarrollo del sistema (y del mundo) que el trabajo*

colectivo. El avance de la ciencia en las últimas décadas se ha sustentado esencialmente en el trabajo colaborativo de grandes equipos multidisciplinarios. A diferencia de lo que ocurría cuando se fundó la SAIC, en que el mismo profesional atendía pacientes por la mañana y realizaba sus investigaciones en el laboratorio por la tarde, actualmente la investigación traslacional con mayor repercusión en el progreso del conocimiento y su aplicación a la salud se ha basado en el trabajo en red de grandes consorcios. Ello requiere una actitud despojada de ambiciones personales. Nuestro sistema es exigente al evaluar los aportes que realizamos. De ese modo reasegura su calidad. Lo crítico es definir cómo evaluar un aporte, si focalizando en los logros individuales de cada investigador (sus artículos, sus tesis doctorales dirigidas, sus subsidios de investigación obtenidos) o midiendo su legado en conocimientos, desarrollos y formación de capacidades para la sociedad.

En ese mismo sentido, resulta controvertido el concepto de formación de recursos humanos en ciencia y tecnología. Recurso es un medio de cualquier clase que, en caso de necesidad, sirve para conseguir lo que se pretende. Es fácil entender la necesidad de recurrir a medios materiales para llevar adelante un proyecto. Parecemos habernos acostumbrado a homologar al ser humano a los recursos materiales para conseguir lo que se pretende. Quizá así sea, tristemente, para emprendimientos cuyos objetivos apunten a un logro material. Una visión alternativa es ver a las personas que forman parte del emprendimiento como responsables del logro o a la sociedad como beneficiaria del logro y de la formación de individuos con capacitaciones diversas.

La autocrítica requiere también una reflexión sobre el concepto de mérito, esa acción o conducta que hace a una persona digna de premio o alabanza. Nuestro sistema evalúa resultados, más que acciones o conductas, pero genera un orden de mérito. Entre el esfuerzo estéril y el resultado obtenido más gracias al contexto que a cualidades propias, es necesario plantearse dónde se sitúa un concepto equilibrado de mérito. Momentos críticos como el que estamos viviendo por la extrema falta de recursos para llevar adelante nuestras tareas invitan a meditar sobre la excesiva dependencia de los fondos provenientes del Estado. Durante décadas, nuestro sistema ha demostrado insuficiente capacidad para involucrar al sector privado en el apoyo al desarrollo científico y tecnológico. No podemos endilgarle todo a los políticos o quienes tienen a cargo la gestión. Cada uno de nosotros tiene parte de responsabilidad. Todos nos llevamos cosas de nuestro paso por la vida, algunos dejan a la sociedad más de lo que reciben. Invito al esfuerzo comunitario para dejar a las generaciones futuras una sociedad mejor, más justa y equitativa. Para ello, es crítico revertir la creciente concepción individualista y la mirada equivocada sobre la existencia de una contribución escasa de la comunidad y del Estado al bienestar general.

**Rodolfo Rey**  
Presidente SAIC 2024

*Me siento muy feliz por tener la oportunidad de hablarles en nombre del Consejo Directivo de la Asociación Latinoamericana de Ciencias Fisiológicas (ALACF) para darles la bienvenida al Congreso ALACF 2024, que en esta ocasión se realiza en el marco del Congreso Conjunto de la Sociedad Argentina de Fisiología (SAFIS) y de la Sociedad Argentina de Investigación Clínica (SAIC). Antes que nada, quiero agradecer profundamente la generosidad de SAIC y SAFIS para albergar nuestra reunión científica en este magnífico Congreso. Particularmente agradezco a los presidentes, Dr. Rodolfo Rey y Dra. Graciela Cremaschi, y a las comisiones directivas ambas sociedades por su cordialidad y la excelente disposición para llevar a cabo este evento en estos tiempos difíciles que nos tocan vivir en nuestro país.*

*SAFIS y ALACF están emparentadas de modo muy estrecho ya que SAFIS, que fue fundada en 1950 por el Dr. Bernardo Houssay, ha sido el motor de la fundación de la Sociedad Latinoamericana de Ciencias Fisiológicas (ALACF), que tuvo lugar en Montevideo en abril de 1957. A partir de ese año se han celebrado reuniones en México, Venezuela, Brasil, Uruguay, Chile, Cuba y varias veces en Argentina. Esta primera secuencia de Congresos Científicos se cerró en el año 2009 con el XXIII Congreso en la ciudad de Pucón, Chile, cuando se discontinuaron las reuniones del consejo directivo. En el año 2019, la Dra. Alicia Mattiazzi, expresidenta de SAFIS y ese momento Editora en Jefe de la revista *Physiological Minireviews* (PMR), también en ese momento órgano oficial de SAFIS, me convocó para encarar una nueva aventura, refundar ALACF. Para ello redactamos un nuevo estatuto, se convocó a una reunión de Consejo Directivo y se designó Presidente al Dr. Luis Sobrevía, reconocido investigador de la Universidad Católica de Chile, y en ese momento Presidente de la Sociedad Chilena de Ciencias Fisiológicas.*

*El Consejo Directivo de ALACF está compuesto por los presidentes de las Sociedades de Fisiología de Latinoamérica y un comité ejecutivo formado por el presidente, el secretario general, el tesorero y el editor en jefe del PMR. Su objetivo primordial es promover la consolidación y difusión de los conocimientos producidos en Fisiología en Latinoamérica, contribuyendo a la interacción, colaboración y flujo de recursos humanos entre los grupos de investigación de las Sociedades miembro. ALACF organiza reuniones anuales o bianuales junto con una de las Sociedades de Fisiología incluidas en la ALACF y además participa de manera central en la organización de los Congresos Panamericanos de Fisiología (PANAM), que se realizan cada tres o cuatro años en nuestra región, y que involucra además de las sociedades latinoamericanas a las Sociedades de Fisiología de Canadá y Estados Unidos. La ALACF además forma parte de *Unión Internacional de Ciencias Fisiológicas (IUPS)*.*

*Desde su refundación a fines de 2019 y debido a la pandemia, ALACF organizó tres Congresos virtuales, Chile 2020, Argentina 2021 y México 2022. En el 2023 ALACF organizó junto con la Sociedad Chilena de Ciencias Fisiológicas el Congreso Panamericano de Ciencias Fisiológicas en la ciudad de Puerto Varas, que fue presencial y resultó un éxito de convocatoria. Desde ya quedan todos invitados al próximo Congreso Panamericano, que se realizará en la ciudad de Monterrey, México, en 2027.*

*ALACF también se enorgullece de difundir la investigación en el área de Fisiología mediante la edición de una revista electrónica, *Physiological Mini-Reviews* (PMR), anteriormente órgano oficial de SAFIS y actualmente de ALACF. El PMR ha sido editado exitosamente durante años por la Dra. Alicia Mattiazzi. A partir del presente año esa tarea la cumple el Dr. Luis Sobrevía, y como novedad de gran relevancia les cuento que a partir del año que viene será administrada por Bentham Science Publishers, luego de un convenio celebrado con ALACF. Desde ya los invito a enviar artículos para ser considerados para su publicación.*

*Para esta reunión, se ha confeccionado un interesante programa científico. En conjunto con SAFIS hemos organizado seis Conferencias y dos Simposios con distinguidos disertantes del país y del extranjero, entre estos últimos los Dres. Luis Sobrevía (Universidad Católica de Chile), Gerardo García Rivas (Tecnológico de Monterrey, México), Kim E. Barrett (University of California at Davies), Eugenio Cingolani (Cedars-Sinai Medical Center, California), Maria Elisa Calcagnotto (Universidade Federal do Rio Grande do Sul, Porto*

*Alegre, Brazil), Barbara Goodman (University of South Dakota), Norma Bobadilla (Universidad Nacional Autónoma de México), César Romero (Emory University School of Medicine, Atlanta), Alexis González (Pontificia Universidad Católica de Valparaíso, Chile), Marta Casado Pinna (Instituto de Biomedicina de Valencia) y Julie Massart (University Rennes, Francia). Además, hemos organizado tres simposios conjuntos con SAFIS y SAIC, dónde además de distinguidos disertantes nacionales hemos invitado a Paola Casanello (Universidad Católica de Chile), Genaro Ramirez-Correa (UT Health Rio Grande Valley, Texas) y Romana Netea-Maier (Radboud University Medical Center, Países Bajos). Por último, se presentarán comunicaciones científicas y cuatro resúmenes seleccionados competirán por el Premio ALACF.*

*Para ir terminando, además de decirles muchas gracias a los disertantes, que han realizado un gran esfuerzo para participar del programa científico, quiero también agradecer a los coordinadores de los Simposios, Conferencias y de las sesiones de comunicaciones orales, a los Jurados de los Premios y por supuesto, muy especialmente a los miembros del Consejo Directivo de ALACF, con los cuales seguiremos trabajando para organizar los Congresos de los años venideros. También agradezco el apoyo infinito que constantemente me brinda mi familia.*

*Me gustaría finalizar destacando que la calidad de nuestra ciencia se ubica por encima de las posibilidades económicas, edilicias y de equipamiento de nuestra región. En parte somos capaces de desafiar estas dificultades gracias al entusiasmo y obsesión por contestar las preguntas que nos plantea la naturaleza, que constituye el motor que nos mueve a pesar de las tempestades, y que queda reflejado año tras año, en el interés que los jóvenes demuestran en los congresos como el de SAIC y SAFIS, dónde la mayoría de nosotros hemos dado los primeros pasos de este camino, que al mismo tiempo nos desvela y nos enamora.*

*Les agradezco su atención y espero disfruten del Congreso.*

**Ernesto Alejandro Aiello**  
Presidente ALACF

*Estimados colegas, invitados especiales y queridos asistentes, en nombre de la Comisión Directiva de la Sociedad Argentina de Fisiología (SAFIS), es un honor y un gran privilegio darles la más cordial bienvenida a la Reunión Conjunta SAIC-SAFIS-ALACF, un evento que nos congrega en torno a los avances científicos en investigación básica, traslacional y clínica en distintos áreas de la biomedicina. La fisiología es un campo fascinante y es una disciplina transversal y central en la ciencia biomédica, ya que nos permite comprender los mecanismos y funciones del cuerpo humano y de otros organismos, incluyendo entre muchas otras la respiración, la circulación, hasta la regulación hormonal y las respuestas celulares. Cada nuevo descubrimiento no solo amplía nuestro conocimiento, sino que también abre puertas a aplicaciones clínicas, terapias innovadoras y mejoras en la calidad de vida de los seres humanos. Precisamente, en estos tiempos en los que la interdisciplinariedad y el avance tecnológico están impulsando nuevas formas de investigación este congreso es una plataforma ideal para explorar cómo estas nuevas herramientas están siendo aplicadas para resolver problemas fisiológicos que, hasta hace poco, parecían insuperables.*

*Este congreso tiene un significado especial, no solo por el gran nivel académico de los disertantes y las investigaciones que aquí se presentarán, sino también por el contexto en el que ha sido organizado. Todos sabemos que estamos atravesando una de las peores crisis económicas de la historia de ciencia en el país. El desfinanciamiento, los recortes presupuestarios y las limitaciones en los recursos han puesto enormes desafíos en el camino de quienes, día a día, trabajamos para avanzar en el conocimiento científico. Hoy estamos aquí, demostrando que la ciencia sigue viva, que nuestros esfuerzos por avanzar en la investigación no cesan, y que la comunidad científica es más resiliente que nunca.*

*Organizar este evento no ha sido una tarea sencilla. Nos hemos encontrado con numerosas dificultades, desde la falta de financiamiento hasta obstáculos logísticos. Sin embargo, quiero destacar el enorme esfuerzo y la dedicación de todas las personas involucradas en su organización, que no se dejaron vencer por las circunstancias adversas. Mi más profundo agradecimiento al Dr. Rodolfo Rey por su generosidad que ha facilitado la participación de nuestra sociedad y de la ALACF en la reunión conjunta. También a la comisión directiva de SAIC por su gran trabajo. Hago extensivo mi agradecimiento al Dr. Alejandro Aiello y al Council de ALACF que han posibilitado generar un programa científico con renombrados disertantes nacionales e internacionales, a los que ya se ha referido el Dr. Aiello. A lo largo de estas jornadas, escucharemos a expositores de renombre que han dedicado sus carreras al avance del conocimiento en fisiología. Especialmente quiero darles la bienvenida y expresar mi reconocimiento y gratitud por su desinteresada participación que prestigia esta reunión.*

*También agradezco a toda la comisión directiva de SAFIS, en especial a su secretario, Dr. Germán Gonzales y a su tesorera, Dra. Alicia Klecha por su dedicación y trabajo.*

*Además de las conferencias y simposios tendremos dos importantes workshops, uno de educación en fisiología y otro organizado por nuestra Comisión de Investigadores Jóvenes (CIJ) conjuntamente con la recientemente constituida CIJ de SAIC. Tenemos también sesiones de comunicaciones orales, de posters y nuestro premio María Cristina Camilión de Hurtado, cuyo financiamiento le agradecemos a su familia.*

*Quiero destacar la importancia de esta reunión como un espacio para el diálogo y la colaboración. Estoy segura de que surgirán nuevas ideas, nuevas preguntas y, por qué no, nuevas colaboraciones que llevarán nuestro campo aún más lejos.*

*Finalmente agradezco a cada uno de ustedes por su presencia, y espero que tengan una exitosa reunión que pueda ser marco para el aprendizaje, el intercambio de ideas innovadoras y la camaradería. Que este congreso sea un faro de esperanza y resiliencia para todos los que creemos en el poder transformador de la ciencia.*

*Muchísimas gracias y sean todos muy bienvenidos a disfrutar del congreso.*

**Graciela Cremaschi**  
Presidente SAFIS 2024

**CONFERENCE OF EDUCATION IN PHYSIOLOGY - SAFIS/ALACF***Tuesday 19th November 11:00 - 12:00 - Anexo (streaming)***Chair: Irene Ennis, Analia Tomat****HOW TO IMPROVE STUDENT LEARNING OF PHYSIOLOGY USING INNOVATIVE TECHNOLOGIES.****Barbara E. Goodman***Editor-in-Chief, Advances in Physiology Education. Division of Basic Biomedical Sciences, University of South Dakota, USA.*

There are many ways to enhance student learning in physiology courses including using active learning techniques during lecture-based classes, designing team-based and student-centered learning opportunities throughout the course, and using various online resources to develop learning activities. [Advances in Physiol-](#)

[ogy Education](#) features a number of different article types with evidence for improving student learning in physiology. Various ideas for encouraging student learning will be modeled and discussed during this session. In addition, information will be provided for how to publish peer-reviewed educational scholarship.

**CONFERENCE SAFIS/ALACF I.***Tuesday 19th November 15:00 - 15:50 - Anexo (streaming)***Chair: Alejandro Aiello, Graciela Cremaschi****FETOPLACENTAL DYSFUNCTION IN GESTATIONAL DIABETES MELLITUS AND MATERNAL OBESITY: A POTENTIAL THREAT FOR PROGRAMMING CARDIOVASCULAR DISEASE.****Luis Sobrevia***Cellular and Molecular Physiology Laboratory (CMPL), Department of Obstetrics, Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile.*

Gestational diabetes mellitus (GDM) is a condition defined by hyperglycemia or impaired glucose tolerance identified during pregnancy, with a global prevalence estimated at 14%. In Chile, the prevalence of GDM has surged to 13%, nearly tripling over the past decade. GDM is linked to an array of maternal and perinatal complications and elevates the risk of long-term metabolic disorders, including recurrent GDM in subsequent pregnancies, obesity, and type 2 diabetes mellitus in offspring and adulthood. The predisposition to GDM is notably higher in women with pre-pregnancy obesity compared to those with normal pre-pregnancy weight, with a five-fold increased risk in the former group. It is essential to distinguish between women with gestational diabetes (pre-pregnancy obesity followed by GDM onset) and women of normal pre-pregnancy weight who develop GDM, as these groups exhibit distinct metabolic profiles and divergent impacts on fetoplacental vascular function, which can adversely affect maternal, fetal, and neonatal outcomes. Our research has focused on evaluating

the impact of GDM and gestational diabetes on fetoplacental vascular endothelial function. We have observed an upregulation in nitric oxide (NO) production, a critical endothelium-derived vasodilator, within the fetoplacental endothelium in GDM pregnancies, potentially attributed to intracellular alkalization in human umbilical vein endothelial cells. Additionally, hydrogen sulfide (H<sub>2</sub>S), a gasotransmitter involved in vascular tone modulation and endothelial protection against hyperglycaemic damage, demonstrates dual regulatory effects on endothelial NO synthase activity. These effects are contingent on intracellular pH (pHi) and other modulatory factors, including insulin. The crosstalk between H<sub>2</sub>S and NO signaling pathways in GDM and gestational diabetes remains inadequately characterized. We explored the mechanistic regulation of NO and H<sub>2</sub>S interplay and how these interactions may differ in the context of gestational diabetes, potentially elucidating novel therapeutic targets for mitigating endothelial dysfunction in these conditions.

**CONFERENCE SAIC I - Alfredo Lanari.***Tuesday 19th November 15:00-15:50 - Auditorium***Chair: Rodolfo Rey**

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## EXPERIENCES OF TRANSLATIONAL CANCER RESEARCH FROM ACADEMIC AND HOSPITAL INSTITUTIONS

**Daniel Alonso**

*Center of Molecular and Translational Oncology, Department of Science and Technology, National University of Quilmes, and CONICET. Buenos Aires, Argentina.*

Translational medicine seeks to accelerate the process of bringing scientific discoveries from the laboratory to the patient. However, the so-called ‘valley of death’ in biomedical research represents a central obstacle in this process, where projects stagnate due to the disconnection between scientific approaches and clinical practice, the complexity of clinical trials, insufficient funding, difficulties in scaling production processes, or limitations in meeting necessary regulations, among the most significant factors. For over two decades, our team has been addressing various lines of work in experimental oncology, seeking new contributions for the treatment of cancer in adult and pediatric patients. Through the establishment of public-private consortia involving entities from the academic scientific field, hospitals, and pharmaceutical companies, we have been able to advance both repurposed drugs and novel antitumor agents to clinical trials. We have investigated the antidiuretic compound desmopressin from preclinical murine models and veterinary trials to clinical protocols in humans, for its repurposing as a coadjuvant in breast cancer surgery and other gynecological cancers, as well as a neoadjuvant hemostatic in colorectal cancer with bleeding. Based on these findings, the start-up company KVR Pharmaceuticals (Canada) is

developing these repurposed indications for desmopressin in highly regulated markets. The monoclonal antibody racotumomab, initially developed by the Center of Molecular Immunology (Cuba) and Elea Laboratories (Argentina), has advanced through various preclinical and clinical stages until its first indication as maintenance therapy in advanced non-small cell lung cancer, and is currently under clinical scrutiny in high-risk pediatric neuroblastoma. The proapoptotic peptide CIGB-300, developed at the Center for Genetic Engineering and Biotechnology (Cuba), has progressed to clinical trials as a therapeutic agent for managing genital warts and cervical lesions. Nowadays, it is undergoing preclinical studies in lung and breast cancer. The compound 1A-116, created by our group as a Rac1 inhibitor, has completed preclinical studies, including efficacy tests in models of glioblastoma and hormone-independent breast cancer, and more recently, in psoriasis-like lesions using a topical formulation. The various challenges that have arisen when tackling these journeys have left several lessons learned. In particular, the nonlinear innovation model emerges as an appropriate approach to address the complexity and interactivity required in biomedical research aimed at clinical application.

### CONFERENCE SAFIS/ALACF II.

*Wednesday 20th November 10:30 - 11:20 - Anexo (streaming)*

**Chairs: Enrique Sánchez Pozzi, Luis Sobrevía**

## THE WORLD WITHIN – MECHANISMS AND CONSEQUENCES OF INTERACTIONS OF THE GUT WITH RESIDENT AND INVADING MICROORGANISMS

**Kim E. Barrett**

*School of Medicine, University of California, Davis, Sacramento, California, United States.*

The gastrointestinal tract maintains a life-long association with a complex and dynamic microbial ecosystem known as the microbiota. The gut microbiota is comprised of bacteria, viruses, fungi, archaea and protists, and has co-evolved with the host over thousands of years. Health is associated with a more diverse microbiota, and diversity is reduced in several disease states, as well as during aging. The microbiota also varies between individuals, but there is a high degree of functional redundancy. Both positive and negative factors regulate the make-up of the microbiota, including microbial co-metabolism, microbe-microbe and host-microbe antagonism, nutrient availability, and GI motility. The microbiota supplies numerous beneficial functions to the host, and we can consider ourselves to be symbiotic “super-organisms”. At the same time, the gut epithelium fulfills a physiological imperative to absorb the products of digestion as well as fluid and electrolytes, while maintaining a selective barrier that excludes the microbiota, pathogens and toxins in health. We have studied the effects of various commen-

sal bacteria selected for beneficial properties, probiotics, on gut and cognitive function using both *in vitro* models as well as mouse models of colitis. Our data indicate that specific commensal bacteria can ameliorate transport and barrier dysfunction caused by both invasive pathogens and inflammatory cytokines. Furthermore, probiotics reduce diarrhea, epithelial dysfunction, and cognitive impacts of experimental colitis. We have also studied the pathophysiological correlates of gut infection with invasive pathogens such as non-typhoidal *Salmonella* species, which are common causative agents of foodborne diarrheal illnesses and associated mortality. Our findings suggest that these bacteria cause diarrhea by suppressing the expression of absorptive electrolyte transporters and by modifying epithelial dynamics such that secretory epithelial lineages predominate. Overall, our work, and that of many other laboratories, implies that intestinal homeostasis requires precise control of epithelial transport and barrier function, and that endogenous and exogenous factors, including commensals, pathogens, and

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probiotics, dynamically modulate the physiological properties of intestinal epithelial cells. Ultimately, we hope that further understanding of this area will reveal novel

therapeutic mechanisms and/or targets for both infectious and inflammatory diarrheal disease states.

### CONFERENCE SAIC II - Taquini Conference.

Wednesday 20th November 10:30 - 11:20 - Auditorium

Chair: **Geraldine Gueron**

#### UNUSUAL ANTI-OBESITY ACTIONS OF CARBON MONOXIDE IN MICE FED A HIGH-FAT DIET

**Roberto Motterlini<sup>1</sup>, Roberta Foresti<sup>1</sup>**

<sup>1</sup>INSERM U955, Faculty of Health. University Paris Est Créteil.

Obesity leads to accumulation of adipose tissue and is associated with the development of insulin resistance. Carbon monoxide (CO), a signaling gasotransmitter produced endogenously in mammals during the degradation of heme by the enzyme heme oxygenase, participates in a variety of important physiological processes and has recently emerged as a key modulator of energetic metabolism. The advent of CO-releasing molecules (CO-RMs), a class of compounds that deliver controlled amounts of CO in cells and tissues, confirmed the potential of using this gas as a therapeutic for the treatment of a diverse array of ischemic, inflammatory and metabolic disorders. Here we will show that oral administration of CORM-401, a manganese-based compound that delivers CO with high efficiency in vivo, significantly reduces body weight gain and markedly improves glucose homeostasis in mice fed a high fat diet (HFD). We further demonstrate that CO accumulates in adipose tissue and uncouples mitochondrial respiration in adipocytes, resulting in a switch toward glycolysis. This effect was accompanied

by enhanced sensitivity to insulin, with lowering of glycemia and increased AKT phosphorylation. We will also present more recent data supporting a role of CO in reprogramming the gut microbiota during obesity. In fact, a detailed study to determine CO distribution in different organs following CORM-401 administration revealed that CO is abundantly localized in liver but also in colon, cecum and faeces, suggesting that CO could also affect the microbiota to combat obesity. Indeed, we observed that mice under HFD develop microbiota dysbiosis compared to mice fed a standard diet and that treatment with CORM-401 promoted an enrichment of specific bacterial species that are associated with a healthy metabolic profile and are beneficial for the host. Our combined data indicate that, once released from CORM-401, CO reaches several body compartments where it exerts specific positive pharmacological effects against obesity, suggesting that the interaction of CO with multiple sensitive targets in the body could be exploited for therapeutic application in metabolic diseases.

### CONFERENCE SAIC III - Council of Genetics.

Wednesday 20th November 13:30 - 15:00 - Auditorium

Chair: **Ariel López, Santiago Andres Rodriguez Segui**

#### PRECISION MEDICINE IN DIABETES: FROM SPECIFIC DIAGNOSIS TO PERSONALIZED TREATMENT.

**Gustavo Frechtel**

Instituto de Inmunología, Genética y Metabolismo (INIGEM) - CONICET

Diabetes is a heterogeneous disease characterized by different clinical presentation or phenotypes that have hyperglycemia as the common denominator that identifies them from the diagnosis of the disease. These phenotypes differ from each other due to the alteration in the functioning of certain molecules due to modifications in the genetics and immunology of these patients, as well as the impact of the environment. The advances in the immunogenetics have allowed the detection and identification of some of these phenotypes such as neonatal diabetes, MODY (Maturity Onset Diabetes in the Young), adult-onset autoimmune diabetes (LADA), etc. Precision medicine oriented towards diabetes aims to achieve a precise diagnosis of each of the phenotypes to establish personalized pharmacological treatments that imply greater pharmacological efficacy and lower adverse ef-

fects, so, the correct pharmacotherapy for the right patient at the right time. Just as an example, some specific cases of neonatal diabetes that were treated with insulin with poor results in glycemic control are currently treated with sulfonylureas with excellent results, or LADA that is treated early with insulin, MODY that receives its appropriate therapeutic intervention, with pharmacological or with changes in lifestyle according to the specific subtype. Progress are being made in the phenotypic identification of patients with type 2 diabetes. This is the most frequent type of presentation, in which clinical phenotypes are being detected according to the presence of insulin resistance,  $\beta$ -cell failure and comorbidities such as body fat distribution, ectopic fat, hypertension or chronic complications such as kidney or cardiovascular disease. These phenotypes are beginning to be correlated with



genetic variants. This advance in the identification of phenotypes and their corresponding genotypes allows us, through precision medicine, to obtain treatments that

improve glycemic control, avoid complications and ultimately improve the quality of life of our patients.

### CONFERENCE SAFIS/ALACF III -

*Wednesday 20th November 15:10 - 16:00 - Anexo (streaming)*

**Chair: Cecilia Mundiña, Martin Vila Petrof**

#### CARDIAC BIOLOGICAL PACEMAKERS: PRESENT AND FUTURE

**Eugenio Cingolani**

*Department of Cardiology, Smidt Heart Institute, Cedars-Sinai Medical Center, Los Angeles, California.*

Heart electrogenesis begins in the sinoatrial node and progresses through the conduction system to generate the heartbeat. Disorders of the conduction system can lead to bradycardia, where heart rates are too slow to sustain circulation, necessitating electronic pacemaker implantation. A standard pacemaker includes a subcutaneous generator and battery module, connected to one or more endocardial leads. Newer leadless pacemakers, however, can be implanted directly into the right ventricular apex, offering single-chamber pacing without the need for a subcutaneous generator.

Modern pacemakers are generally reliable, with programmable modes that can be customized to address specific clinical requirements. Future advances in pacemaker technology are expected to include alternative energy sources and dual-chamber leadless pacing, offering enhanced versatility in treatment.

Despite their effectiveness, current electronic pacemakers have limitations. These include risks of lead or generator malfunctions, reduced autonomic responsiveness, potential interference with strong magnetic fields, and device-related infections. As an alternative, biological pacemakers—developed through somatic gene transfer, cell fusion, or cell transplantation—present promising options. Somatic reprogramming techniques, which involve introducing genes encoding transcription factors to convert working myocardium into a functional sinoatrial node, have progressed furthest along the translational pathway.

As electronic pacemakers evolve to become smaller and less invasive, biological pacemakers may broaden therapeutic possibilities for conduction system disorders, offering a more natural alternative that addresses some limitations of current devices.

### CONFERENCE SAIC IV - Christiane Dosne de Pasqualini.

*Thursday 21st November 15:00-15:50*

**Chair: Mauricio Menacho Márquez, Edith Kordon**

#### THE COMPLEXITY OF BREAST CANCER: EXPLORING THE ROLE OF THE ADAPTOR PROTEIN P140CAP IN INSTRUCTING A PROTECTIVE ANTI-TUMOR IMMUNE RESPONSE.

**Paola Defilippi<sup>1</sup>, Matteo Poncina<sup>1</sup>, Andrea Scavuzzo<sup>1</sup>, Lucrezia Rosgen<sup>1</sup>, Francesca Nigrelli<sup>1</sup>, Daniela Tosoni<sup>2</sup>, Salvatore Pece<sup>2</sup>, Emilia Turco<sup>1</sup>, Vincenzo Salemm<sup>1</sup>**

*<sup>1</sup>Department of Molecular Biotechnology and Health Sciences, University of Torino, 10126, Torino, Italy. <sup>2</sup>European Institute of Oncology IRCCS, 20141 Milan, Italy.*

Breast cancer (BC) is one of the most commonly cancers in USA with 290.560 estimated new cases and 43.780 estimated deaths in 2022. Due to the heterogeneous clinical behavior of BC in response to currently available multimodal treatments, the development of new targeted therapies and biomarkers for improved patient management remains an unmet clinical need. In addition to genetic diversity and plasticity of cancer cells, the activity of different types of immune cells in the microenvironment can change the effectiveness of cancer therapies. p140Cap is an adaptor protein encoded by the SRCIN1 gene whose expression is associated with a significantly reduced probability of developing distant recurrence and improved overall survival in HER2-positive BC patients. The tumor suppressor activity of p140Cap has been largely attributed to its intrinsic ability to interact with pro-

teins involved in different cancer-associated biological networks. Gain or loss of function experiments demonstrated that p140Cap may affect tumor growth and metastasis formation by controlling the signaling pathways involved in tumorigenesis and metastatic features. Notably, p140Cap has also been described as a direct binder of  $\beta$ -Catenin, although the functional significance of this interaction and its possible relevance to BC remain elusive. Here we show that p140Cap plays a critical role in coordinating local and systemic events that ultimately inhibit the function of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs), which create an immunosuppressive, tumor-promoting environment both in the primary tumor and in premetastatic niches. Through integrative transcriptomic and preclinical studies, it has been revealed that p140Cap regulates an epi-

static axis by inhibiting  $\beta$ -Catenin. This inhibition restricts tumorigenicity and the self-renewal of tumor-initiating cells, thereby limiting the release of the inflammatory cytokine G-CSF. G-CSF is necessary for PMN-MDSCs to perform their tumor-promoting functions both locally and systemically. Mechanistically, p140Cap's inhibition of  $\beta$ -Catenin relies on its ability to localize within and stabilize the  $\beta$ -Catenin destruction complex, enhancing  $\beta$ -Catenin inactivation. Clinical studies in women have shown that low p140Cap expression correlates with a reduced presence of tumor-infiltrating lymphocytes and more aggressive tumor types. This was observed in a large

cohort of real-life breast cancer patients, underscoring the potential of p140Cap as a biomarker for therapeutic interventions targeting the  $\beta$ -Catenin/tumor-initiating cells/G-CSF/PMN-MDSC axis to restore an efficient anti-tumor immune response. Additionally, the cancer stem cells are implicated in response to chemotherapy. Preliminary data indicate that the presence of p140Cap leads to increased chemosensitivity to chemotherapeutic agents and subsequently higher cell death related to chemotherapy, indicating p140Cap as a possible predictive biomarker in breast cancer.

#### CONFERENCE SAFIS/ALACF IV.

Thursday 21st November 15:00-15:50.

Chair: Celeste Villa-Abrille, Bruno Buchholz

#### HEART, METABOLIC SYNDROME AND MITOCHONDRIAL FUNCTION: NEW INSIGHTS INTO A COMPLEX RELATIONSHIP.

**Gerardo García Rivas.**

*Institute for Obesity Research, Tecnológico de Monterrey, Hospital Zambrano-Hellion, San Pedro Garza-García, Nuevo León, Mexico.*

The progression of cardiometabolic diseases is driven by a complex interplay of factors, including energy deprivation, alterations in calcium metabolism, inflammation, and apoptosis. These conditions may arise from mitochondrial dysfunction, highlighting mitochondria as potential therapeutic targets. Our research group has utilized preclinical models of ischemia-reperfusion injury (I/R), pulmonary arterial hypertension, lethal arrhythmia, and heart failure (HF) to propose that regulating mitochondrial post-translational modifications (PTMs) could reduce cardiovascular dysfunction. Specifically, the mitochondrial permeability transition pore (mPTP) plays a crucial role in the pathophysiology of various cardiac diseases. Growing evidence from proteomic studies has shown that hyperacetylation and oxidation of several key components of the mPTP occur in response to changes in the extracellular and intracellular environment, as well as energetic demand. These PTMs may create a fine and complex regulatory mechanism that can alter mitochondrial function and affect cellular fate under pathological conditions. While our understanding of the intricate relationships between these PTMs is still developing, it may reveal new, promising therapeutic targets and treatment approaches. One focus of research is Cyclophilin D (CypD), which mediates the mPTP and contributes to mitochondrial damage. CypD's activity is regulated by its acetylation/deacetylation state, which depends on Sirtuin-3 (SIRT3), a mitochondrial deacetylase. Given that metabolic syndrome decreases SIRT3 activity and expression, we tested the hypothesis that hyperacetylation of CypD promotes mitochondrial dysfunction in this pathological state. In our studies, obese rats exhibited

decreased SIRT3 mitochondrial expression, accompanied by a hyperacetylated mitochondrial profile, including CypD. Cardiac mitochondria from obese animals were found to be more prone to mPTP opening than those from controls, suggesting that activating SIRT3 could be a potential target for reducing ventricular dysfunction and slowing the progression of HF. Additionally, we have proposed targeting mitochondrial channels as a novel approach to understanding the general mechanism of excitation-contraction energy coupling. Intracellular  $\text{Ca}^{2+}$  mishandling is a fundamental mechanism of I/R that results in mitochondrial dysfunction and cardiomyocyte death. This is mediated by mitochondrial  $\text{Ca}^{2+}$  (mCa $^{2+}$ ) overload, which is facilitated by the mitochondrial calcium uniporter (MCU) channel. To investigate this further, we evaluated the effects of a specific inhibitor and siRNA targeting MCU in cardiomyocytes subjected to I/R injury. Silencing MCU in these myocytes led to reductions in necrosis and apoptosis levels, as well as decreased activity of caspases 3/7, 8, and 9. Recently, in angiotensin II (ANGII)-induced hypertrophic cardiac cells, we observed that these cells overexpress MCU and exhibit bioenergetic dysfunction. However, silencing MCU prevented cell hypertrophy and mitochondrial dysfunction by blocking mitochondrial calcium overload, increasing mitochondrial reactive oxygen species, and activating nuclear factor kappa B-dependent hypertrophic and pro-inflammatory signaling. Collectively, our data suggest that targeting MCU through genetic downregulation or pharmacologic inhibition may offer a potential strategy for preventing cardiac remodeling and mitochondrial dysfunction.

**CONFERENCE SAFIS/ALACF.***Thursday 21st November 16:00 - 16:50 - Anexo (Streaming)***Chair: Alejandro Sodero****ELECTROPHYSIOLOGY OF DENDRITIC SPINES: INFORMATION PROCESSING, DYNAMIC COMPARTMENTALIZATION, AND SYNAPTIC PLASTICITY.****Maria Elisa Calcagnotto.***Department of Biochemistry, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.*

Dendritic spines, with their heterogeneous morphological, biochemical, and functional features, play a pivotal role as postsynaptic components in central nervous system synapses. These structures undergo dynamic morphological reorganization throughout development, aging, and in response to behavioral cues. Dendritic spines not only integrate and filter signals to the soma but also facilitate diverse connections with axons from different sources. They undergo geometric changes that provide rapid adjustments to postsynaptic ionic balance, protein trafficking, and signaling events, ultimately regulating synaptic efficacy and long-term plasticity. The cellular components of the synapse—including the neuronal pre- and postsynaptic compartments, glial cells such as astrocytes, NG2 cells, and microglia, along with the extracellular matrix, collectively referred to as the tetrapartite synapse—are morphologically and functionally

plastic and crucial for synapse development, physiology, and pathology. For a long time, the limitations of available methods prevented direct measurement of electrical signals from individual spine membranes. However, recent advancements have unveiled important biophysical properties of dendritic spines. These breakthroughs are due to the development of novel, integrated imaging and electrophysiological techniques that enable the study of functional dendritic characteristics. This includes research on backpropagating action potentials, synaptic potentials mediated in dendritic spines in different biochemical and electrical compartments, and the spatiotemporal dynamics of dendritic spines involved in synaptic plasticity. These discoveries significantly contribute to our understanding of dendritic spine dynamics and neuronal behavior.

**CONFERENCE SAIC V- Closing Conference.***Friday 22nd November 11:00 - 11:50 - Auditorium***Chair: Rodolfo Rey****ALTERATIONS IN THE ACTIN CYTOSKELETON IN SMN-DEPRIVED NEURONS AS REVEALED WITH SUPER-RESOLUTION MICROSCOPY. IMPLICATION FOR THE PATHOGENESIS OF SPINAL MUSCLE ATROPHY.****Alfredo Caceres and Mónica Remedi.***Centro Investigación Medicina Traslacional Severo R. Amuchástegui (CIMETSA) - Instituto Universitario Ciencias Biomédicas Córdoba (IUCBC), Av. Naciones Unidas 420, 5009 Córdoba, Argentina.*

Spinal Muscle Atrophy (SMA) is the leading neurological genetic disease causing infant mortality. This axonopathy is caused by reduced levels of the Survival of Motor Neuron (SMN) protein due to mutations in the *SMN* locus gene. The actin cytoskeleton, the driving force for growth cone motility, guidance, target recognition and axon growth, has been implicated in the pathogenesis of SMA. However, up to date almost no study has examined in detail the organization/dynamics of actin structures in axons of SMA spinal motor neurons (MN). We have now studied the consequences of SMN deprivation on the organization, abundance and dynamics of axonal and growth cone actin-based cytoskeletal structures using super-resolution microscopy and multidimensional high-resolution microscopy. We were particularly interested in analyzing the recently discovered membrane periodic actin-spectrin skeleton (MPS), a key component of the neuronal cytoskeleton, with a functional role in target-in-

duced axonal degeneration. As model systems we have used cultures of rat spinal motor neurons (MN) deprived of SMN by RNA interference. To visualize actin cytoskeletal organization we used STED nanoscopy. The results obtained indicate that SMN deprivation produces rapid and massive disassembly of the MPS without altering other actin-based structures, such as growth cone actin arcs, radial striations, lamellipodial veils, axonal actin trails, or axonal dynamic and stable microtubules. MPS dismantling is associated with increased axonal diameter including areas of localized swellings and is followed by axonal fragmentation and degeneration; interestingly, agents that stabilize actin filaments (e.g. Jaspaklinolide) prevent MPS loss and axonal shattering. Previous findings highlight a robust interplay between profilin and ROCK, its upstream kinase, in SMN-deprived neurons. This interaction potentially leads to diminished ROCK-mediated cofilin phosphorylation, thereby increasing actin sever-

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ing activity and promoting actin depolymerization. Supporting this notion, our experiments demonstrate that treatment with Y27632, a specific ROCK inhibitor, or expression of S3A-cofilin, a constitutively active mutant, induces disassembly of the MPS in spinal MN. Conversely, expression of S3E cofilin, an inactive mutant lacking actin severing activity, prevents MPS disassembly triggered

by the ROCK inhibitor or SMN suppression. Collectively, these results underscore the MPS as a pivotal target of SMN deprivation-induced axonal degeneration, primarily through reduced cofilin phosphorylation and subsequent actin depolymerization. Thus, safeguarding the MPS emerges as a promising therapeutic strategy for SMA and potentially other neurodegenerative diseases.

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**SYMPOSIUM SAIC I. Tuesday 19th November 13:00 - 14:30 - Auditorium**  
**PANCREATIC CANCER: A ROUND TRIP FROM THE BENCH TO THE BED**  
**Chair: Juan Iovanna, Carlos Davio**

**ADVANCES IN UNDERSTANDING METASTATIC PANCREATIC CANCER: HOW CAN WE APPLY THESE INSIGHTS IN PATIENT CARE**

**Marcela Carballido**

*Hospital de Gastroenterología Dr Carlos Bonorino Udaondo. Hospital Universitario Austral.*

Pancreatic cancer ranks 12th in incidence and 7th in mortality worldwide. Despite substantial resources being allocated to research this disease, progress in improving overall survival has been limited, with a survival rate of just 10% across all stages.

As a result, pancreatic cancer is expected to become the second leading cause of cancer-related death by 2030, following lung cancer.

In clinical practice, healthcare professionals have the responsibility to offer the best possible treatment, which is only feasible if it is approached from a multidisciplinary perspective. Due to the nonspecific nature of the initial symptoms and the aggressive progression of the disease, 50% of patients are diagnosed with metastatic pancreatic cancer that is no longer amenable to curative treatment, and their median survival is typically less than one year.

From a genetic point of view, pancreatic cancer can be driven by both germline and somatic mutations. Among germline mutations, those in the HR-DDR genes are the most common, particularly mutations in BRCA1, BRCA2, and PALB2. This information is crucial for defining the first-line treatment and guiding future therapeutic strategies. For this reason, international guidelines recom-

mend performing germline genetic testing in all patients with advanced pancreatic cancer. In terms of somatic mutations, KRAS mutations are the most prevalent, occurring in 90% of patients. Among the remaining 10% of patients with wild-type KRAS, mutations in MMR genes, kinase fusion genes, or MAPK pathway activation may be present.

Patients with druggable mutations who are eligible for targeted therapies can have a median survival of up to 3 years, which is three times the survival rate of the broader pancreatic cancer population.

Unfortunately, there are currently few therapeutic options for point mutations: KRAS G12C inhibitors (e.g., adagrasib, sotorasib), PARP inhibitors for maintenance therapy in BRCA mutation carriers, and drugs approved for tumors with RET, NTRK, or other fusion mutations, regardless of the cancer type.

Nevertheless, numerous promising investigations are underway, and these may potentially transform the prognosis and management of pancreatic cancer in the future. Ultimately, only collaborative, multidisciplinary, and translational efforts will lead to improved outcomes in this complex and challenging disease.

**PERSONALIZING PANCREATIC CANCER TREATMENTS: A JOURNEY FROM BENCH TO BEDSIDE AND BACK AGAIN**

**Nelson Dusetti**

*Cancer Research Center of Marseille, CRCM, Inserm, CNRS, Paoli-Calmettes Institut, Aix-Marseille University, Marseille, France.*

Pancreatic adenocarcinoma is a highly heterogeneous disease, with each tumor exhibiting unique molecular characteristics that significantly influence its progression and response to treatment. As a team of translational researchers, our journey began with pressing clinical questions, leading us to model the disease using preclinical models such as primary cell cultures, patient-derived xenografts (PDX), and organoids, all directly derived from patient tumors.

From these preclinical models, we developed molecular signatures that predict sensitivity to standard pancreatic cancer treatments: Gemcitabine, Abraxane, and FOLFIRINOX. Remarkably, these signatures require only

a few micrograms of total RNA extracted from formalin-fixed paraffin-embedded biopsies, which are routinely collected in clinical practice. The signatures are applicable across all stages of the disease whether localized, locally advanced, or metastatic, including liver metastasis biopsies.

By stratifying patients based on these signatures, we can personalize treatment by selecting the most appropriate and least toxic therapy that offers the greatest benefit. This approach aims to improve patient management by avoiding treatments to which the tumor is likely to be resistant, thereby reducing unnecessary toxicity and paving the way for therapeutic de-escalation. These signatures

have now allowed us, in an unprecedented way, to identify patients who will be multi-resistant. These patients are of particular interest for developing new treatments, as their molecular characteristics reveal previously unobserved mechanisms of resistance and potential ther-

apeutic targets.

This continuous loop between clinical challenges and laboratory research encapsulates our journey from bench to bedside and back again, driving forward the personalization of pancreatic cancer treatment.

## NEW INSIGHTS INTO THE EPIGENETICS OF PANCREATIC CANCER

**Martin E., Fernandez-Zapico**

*Schulze Center for Novel Therapeutics, Division of Oncology Research, Department of Oncology, Mayo Clinic, Rochester, MN, USA.*

Pancreatic cancer (PC), a malignancy predicted to be the 2<sup>nd</sup> cause of cancer death in the US by 2040, has well-established highly recurrent mutations in four driver genes including *KRAS*, *TP53*, *CDKN2A* and *SMAD4*. Unfortunately, these genetic drivers are not currently therapeutically actionable. Despite extensive sequencing efforts, few additional druggable drivers have been identified. In the absence of targetable mutations, chemotherapy remains the mainstay of treatment. Further, the role of the above driver mutations on PC initiation/early development is well-established. However, these mutations alone cannot account for PC heterogeneity, discern early from advanced disease as well as predict treatment response. For example, two consensus PC

subtypes with significantly different prognosis, classical and basal, are defined by differential gene expression as opposed to mutational profile. In addition, somatic variants can rarely distinguish between PCC primary tumors and metastases. In fact, PC metastasis is driven by enhancer reprogramming rather than secondary somatic drivers. Thus, together these findings demonstrated that key pathogenic features are largely conferred by the epigenetic make-up of PC. In recent years, emphasis has been given to the re-organization of enhancers and large topological domains, these are essential regulatory elements controlling oncogenic gene expression. Here, we discuss the mechanism driving these nuclear changes and their role in PC development and therapeutics.

**SYMPOSIUM SAIC/SAFIS/ALACF I. Tuesday 19th November 13:00 - 14:30 - Anexo**

**BIOCHEMICAL MECHANISMS IN THE DEVELOPMENT AND PREVENTION OF METABOLIC DISORDERS**

**Chair: Valeria Zago, Marcela Vazquez Prieto**

## NUTRIOMIC MECHANISMS OF FUNCTIONAL LIPIDS-RICH MILK FAT IN THE PREVENTION OF METABOLIC DYSFUNCTION-ASSOCIATED FATTY LIVER DISEASE IN A MURINE MODEL

**Claudio Bernal**

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Metabolic dysfunction-associated fatty liver disease (MAFLD) is a more comprehensive and accurate term for a multisystemic disease characterized by liver steatosis, accompanied by the presence of overweight/obesity, diabetes mellitus, or metabolic dysfunction. This disease is primarily characterized by excessive lipid accumulation in the liver, and the progression is accompanied by inflammation, i.e., steatohepatitis and potentially fibrosis, liver cirrhosis and liver cancer. MAFLD affects approximately 25% of the global population, and currently, no approved therapeutics are available. Functional lipids are known to regulate epigenetic processes, which, in turn, can modulate gene expression and metabolic responses, potentially contributing to the prevention of liver steatosis, glucose intolerance, and inflammation. Among functional lipids, ruminic acid (RA) and its precursor, trans-vaccenic acid (VA), have garnered increasing attention due to their ability to regulate the balance between hepatic triglyceride input/output, as well as triglyceride content in adipose tissue and muscle, glucose uptake and oxidation, and reduction of oxidative

stress and inflammatory markers. Both RA and VA are present in low amounts in milk fat, but their levels can be increased by supplementing the cows' diet with n-3 polyunsaturated fatty acids-rich oils, leading to functional milk fat (FMF). Thus, the aim of this presentation is to offer some insights into the potential role, as well as the molecular and biochemical pathways involved, in the prevention of MAFLD by a FMF enriched with VA and RA, using a murine model. Male Wistar rats, fed high-fat diets, which develop liver steatosis associated with obesity and glucose intolerance -key characteristics commonly observed in MAFLD- served as the experimental animal model. While the outcomes from these animal studies cannot be directly extrapolated to humans, the advances in understanding the biological mechanisms underlying the beneficial effects of FMF enriched in RA and VA may offer valuable insights into mitigating or attenuating the development and progression of MAFLD and certain metabolic alterations seen in non-communicable chronic diseases.

## CARDIOMETABOLIC EFFECTS OF ZINC DEFICIENCY AND ZINC SUPPLEMENTATION DURING DIFFERENT STAGES OF LIFE

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Experimental animal models and clinical studies highlights the physiological role of zinc in the cardiovascular and metabolic systems. This micronutrient has antioxidant, anti-inflammatory and anti-apoptotic properties. It also modulates gene expression, the activity of multiple enzymes, organ growth, and the synthesis, storage, secretion and signalling pathways of insulin. Intracellular zinc homeostasis is finely regulated by transporting proteins and metallothionein.

Zinc deficiency during different periods of life has become an important health concern in developing and developed countries, particularly among pregnant women and children having an imbalanced diet, overweight, obesity or diet-related diseases. Moderate zinc deficiency during critical periods of prenatal and/or postnatal growth can be considered a risk factor for the development of hypertension, obesity, diabetes, renal insufficiency and metabolic syndrome in adult life. Different mechanisms can be involved in the developmental programming of these alterations, including activation of oxidative, apoptotic and inflammatory processes, dysfunction of the nitric oxide and renin angiotensin systems, changes in glucose-insulin homeostasis, modifications in cytokines profile secreted by adipose tissue, alterations in organogenesis, among others.

On the other hand, zinc supplementation could be a

valuable strategy in mitigating the development of the cardiometabolic alterations associated with metabolic syndrome. In this regard, we have recently demonstrated that dietary zinc supplementation decreases the metabolic alterations induced in rats exposed to a high fat and fructose diet during postnatal growth, by improving glucose tolerance and lipid profiles, and by reducing oxidative stress and adipocyte hypertrophy in the visceral adipose tissue. Moreover, zinc supplementation can have a potential protective role against cardiovascular complications associated with metabolic syndrome, by attenuating the increase in blood pressure levels, left ventricle hypertrophy and vascular structural alterations induced by a high fat and fructose diet.

Considering these results, a greater knowledge about zinc homeostasis and the physiological function of zinc transporters could be important to develop new therapies for the prevention and treatment of cardiovascular and metabolic disorders. Further clinical research is needed to elucidate the efficacy and doses of zinc supplementation, in pregnant women, neonates, infants, children, and adults exhibiting adequate or deficient zinc intake. Finally, learning about healthy eating habits and food fortification strategies should be explored and implemented to ensure adequate dietary zinc intake in individuals at different stages of life.

## EARLY PROGRAMMING OF ADIPOSE TISSUE IN THE NEXT GENERATION BY MATERNAL OBESITY

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Maternal obesity is the most relevant risk factor for developing obesity and metabolic syndrome in the offspring. The mother's inflammatory and metabolic status-related to obesity- can be transferred to the developing embryo and fetus. At this point, cell metabolism can be programmed. In this presentation, an overview of the mechanisms by which maternal obesity can alter fetal immune function, fetal adiposity and placental transporters will be described. We set the study at birth and follow-up (4 months) of the offspring of women with pregestational obesity who participated in a randomized controlled trial with docosahexaenoic acid (DHA) supplementation

during pregnancy. The results of this follow-up on neonatal body composition, metabolic markers (lipid profile, HOMA-IR, leptin, adiponectin) and placental fatty acid transporters will be presented and discussed. The results presented will shed light on our current research interest, where the mechanism by which the chronic low-grade inflammation in the mother can affect the offspring's progenitor mesenchymal stem cells, compromising their early cell commitment. These molecular changes are critical to the infant's programming of the risk of obesity and chronic diseases and are fundamental to understanding how and when prevention needs to start.

**SYMPOSIUM SAIC II. Wednesday 20h November 8:30 - 10:00 - Auditorium**

**INNOVATIVE THERAPEUTIC APPROACHES TARGETING INFLAMMATION AUTOIMMUNITY AND NEUROPROTECTION**

**Chair: Javier Cotignola, Geraldine Gueron**

INVESTIGATING THE ANTI-INFLAMMATORY AND CYTOPROTECTIVE ACTIONS OF HYCOS,

## NRF2 ACTIVATORS THAT SIMULTANEOUSLY RELEASE CARBON MONOXIDE

**Roberta Foresti, Roberto Motterlini**

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Heme oxygenase-1 (HO-1) is one of several targets proteins that are dependent on the activation of NRF2, the transcription factor recognized as the master regulator of the stress response in mammalian systems. HO-1 degrades heme to carbon monoxide (CO) and biliverdin/bilirubin, which possess anti-inflammatory and antioxidant properties, respectively.

There exist several NRF2 activators, including naturally-derived compounds, such as curcumin and sulforaphane, but also endogenous products of metabolism, such as itaconate and fumarate, which induce NRF2 to promote cytoprotective activities. To exploit the positive pharmacological effects of NRF2 activation and CO, we recently developed new hybrid compounds (HYCOs) consisting of CO-releasing molecules (CORMs) conjugated to fumaric esters known to activate Nrf2/HO-1. Fourteen newly-synthesized compounds were tested in human monocytes and keratinocytes in vitro as well as in vivo models of inflammation. The effects of HYCOs were compared to dimethyl fumarate (DMF), a known fumaric ester used in the clinic, and a CORM alone.

Selected HYCOs efficiently increased intracellular CO, up-regulated Nrf2/HO-1 and elicited a strong reduction

in anti-inflammatory markers in monocytes stimulated by lipopolysaccharide (LPS). This effect was stronger than that observed with DMF or CORM alone, indicating the enhanced potency of HYCOs compared to the separate entities. In vivo, HYCOs given orally to mice exerted beneficial effects by accelerating skin wound closure and reduced psoriasis-mediated inflammation. In a LPS murine model of systemic inflammation, HYCO-3 markedly reduced inflammation in liver, heart and brain in an NRF2- and CO-dependent manner.

In new unpublished data we also found that HYCOs protect a human cardiac cell line from hypoxia-reoxygenation damage, as demonstrated by maintenance of cell viability, decrease in lactate dehydrogenase release and preservation of cellular myoglobin content. Importantly, HYCOs were given at the onset of reoxygenation, recapitulating more closely a potential clinical setting and indicating their potential usefulness also in this additional stressful condition.

These results support the idea that HYCOs possessing a dual mode of action could be applied to combat inflammation and oxidative stress.

## THERAPEUTIC CARBON MONOXIDE EXPOSURE TARGETS T CELL ACTIVATION DURING SYSTEMIC AUTOIMMUNITY

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Systemic Lupus Erythematosus (SLE) is an autoimmune disease with impaired regulation of immune cells. Although survival of SLE patients has considerably improved in the past 50 years, they still have higher mortality and morbidity. Furthermore, it is currently acknowledged that adverse effects associated with immunosuppressive therapy and chronic inflammation are responsible for increased morbidity. Thus, new therapies are needed to enhance efficacy and reduce the adverse effects of the current lupus treatments. In the last decade, the anti-inflammatory properties of heme oxygenase-1 (HO-1) and one of its enzymatic products, carbon monoxide (CO), have shown to be potential targets for immune-mediated disease treatment. Heme oxygenase-1 (HO-1) is an inducible enzyme that catabolizes heme degradation into Fe<sup>2+</sup>, biliverdin, and carbon monoxide (CO). HO-1 can modulate the function of some hemeproteins, including nitric oxide synthase, soluble guanylate cyclase, peroxidase, and catalase through the degradation of the heme group and the release of CO, which may bind to surrounding heme groups, inhibiting its activity. HO-1

deficiency is associated with an increased susceptibility to uncontrolled inflammation with subsequent leukocytosis, splenomegaly, and lymph node swelling in affected individuals. This work aimed to evaluate HO-1 levels in immune cells from SLE patients and whether HO-1 product (carbon monoxide-CO) ameliorates disease in lupus-prone mice. Blood was obtained from 43 SLE patients and 30 healthy controls. We sorted CD14<sup>+</sup> monocytes and CD4<sup>+</sup> T cells by FACS, and HO-1 expression was measured using RT-PCR and FACS. Lupus murine models FcγRIIb KO, NZBW F1, and MRL/Fas mice were exposed to CO (250ppm/1h/day). We measured immune cells by FACS and autoantibodies by ELISA. Renal damage was evaluated by the amount of proteinuria with urine reagent strips and kidney histological analysis. HO-1 levels (mRNA and protein) were decreased in monocytes from SLE patients compared to healthy controls. In addition, HO-1 levels were also decreased in lupus-prone FcγRIIb KO mice. Furthermore, CO exposure to lupus-prone mice ameliorated kidney function, such as proteinuria and glomerulonephritis in



FcgRIIb KO and MRL/Fas mice. CO therapy decreased anti-nuclear antibodies in all lupus mice tested and improved anemia in MRL/Fas mice. In addition, CO modulated different immune cell populations, such as monocytes/granulocytes and T cells. In conclusion, we found a significant decrease in HO-1 expression in monocytes

from SLE patients, suggesting that an imbalance in HO-1 may promote disease progression. The success of HO-1 product-CO therapy in lupus-prone mice highly suggests that HO-1 modulation could be used as a therapeutic approach to SLE treatment.

#### DESIGN, SYNTHESIS, AND PRECLINICAL EVALUATION OF DAD9: A NOVEL DOPAMINERGIC AGONIST WITH NEUROPROTECTIVE EFFECTS FOR PARKINSON'S DISEASE

Rosana Chehín<sup>1</sup>, María del Milagro Terán<sup>1</sup>, Valentina Budeguer Isa<sup>1</sup>, Hernán Cruz<sup>2</sup>, Rodrigo H. Tomas-Grau<sup>1</sup>, María Laura Guayán<sup>1</sup>, Agustín Pernicone<sup>3</sup>, Verónica Manzano<sup>3</sup>, Carla Beatriz Delprato<sup>4</sup>, Diego de Mendoza<sup>4</sup>, Oscar Varela<sup>3</sup>, Diego Ploper<sup>1</sup>

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The escalating prevalence of Parkinson's disease (PD) underscores the need for innovative therapeutic interventions since current palliative measures, including the standard L-Dopa formulations, face challenges of tolerance and side effects while failing to address the underlying neurodegenerative processes. Here, we introduce DAD9, a novel conjugate molecule that aims to combine symptomatic relief with disease-modifying strategies for PD. Crafted through knowledge-guided chemistry, the molecule combines a non-antibiotic doxycycline derivative with dopamine, preserving neuroprotective attributes while maintaining dopaminergic agonism. This com-

pound exhibited no off-target effects on PD-relevant cell functions, and sustained antioxidant and anti-inflammatory properties of the tetracycline precursor. Furthermore, it effectively interfered with the formation and seeding of toxic  $\alpha$ -Synuclein aggregates without producing detrimental oxidative species. In addition, DAD9 was able to activate dopamine receptors, and docking simulations shed light onto the molecular details of this interaction. These findings position DAD9 as a potential neuroprotective dopaminergic agonist, promising advancements in PD therapeutics.

**SYMPOSIUM SAIC III. Wednesday 20th November 8:30 - 10:00 - Sala de Camara**

#### THE IMPACT OF INFLAMMATION ON REPRODUCTIVE HEALTH: FROM FERTILITY TO PREGNANCY AND INTRAUTERINE PROGRAMMING

Chair: Romina Higa, Marina Peluffo

#### THE PRO-INFLAMMATORY AXIS OF THE RENIN ANGIOTENSIN SYSTEM (RAS) AT THE MATERNAL-FOETAL INTERFACE. A MECHANISM IN THE FINE-TUNING OF EMBRYO SELECTIVITY AND QUALITY CONTROL OF IMPLANTATION?

Rosario Macchi<sup>1,2</sup>, Marisa Castro<sup>1,2</sup>, Guillermo Blanco<sup>2</sup>, Andrea Canellada<sup>1,2</sup>

1. Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Inmunología, Buenos Aires, Argentina.

2. Universidad de Buenos Aires -CONICET, Instituto de Estudios de la Inmunidad Humoral Dr. Ricardo Margni (IDEHU), Buenos Aires, Argentina.

Implantation of low-quality embryos is prevented by an endometrial quality control program (QC) that is hampered in women with altered endometrial decidualization. During decidualization, endometrial stromal cells (ESC) undergo mesenchymal-to-epithelial reprogramming, with production of inflammatory factors and recruitment of immune cells favoring trophoblast invasion. As gestation progresses, ESC acquire mobility to encapsulate the embryo and anti-inflammatory features. Mitochondrial functions, potentially affected by endogenous and exogenous factors, have recently been associated with ESC decidualization and its QC.

Activation of the angiotensin II (Ang II)/Ang II type-1 receptor (AT1R) axis exerts vasoconstrictive, proinflammatory and profibrotic effects. This classical axis is physiologically regulated through the binding of Ang II and

other RAS peptides to specific receptors, constituting a complex network (extended RAS). An uteroplacental RAS has been described. Its role in endometrial decidualization and a link to pregnancy-associated diseases has been proposed.

We reported that Ang II, through AT1R, increased decidualization markers in human ESC decidualized *in vitro* for 4 days (dHESC) and influenced their interaction with trophoblasts. Conditioned media of Ang II-treated dHESC increased the migration of trophoblast cells, in a CXCL8- and CCL2-dependent manner and favored the expansion of blastocyst-like spheroids on a monolayer of these cells. Mechanisms involved included JNK activation and the increase of ROS in trophoblasts. Those results could be associated with a dose-dependent fine-tuning of implantation. We made functional genomics analyses of

publicly available scRNAseq decidual samples and bulk RNAseq endometrial biopsies from patients with history of recurrent miscarriage (RM), infertility due to unknown causes and normal controls (NC) that revealed deregulation of RAS genes in RM samples that showed predictive potential. Differentially expressed genes were associated to metabolism, vesicle trafficking and cell migration functions in ESC and macrophages (Mac). We explored functions related to mitochondrial metabolism and vesicular trafficking in Mac differentiated in the presence of CM of dHESC-treated (dCM+) or not (dCM-) with Ang II and in Ang II-treated or untreated dHESC, by image cy-

tometry and single cell approach. Ang II induced changes consistent with increased metabolism in dHESC. Presence of large vesicles with extracellular localization were observed, characterized by a strong mitochondrial mark, but without membrane potential (MMP). Mac(dCM-) showed intercellular projections with cell-cell contacts and formed large size multinucleated cells with a shared mitochondrial network and high MMP. Mac(dCM+) located in clusters separated from (dCM-) and (LPS+) Mac. We postulate that Ang II may indirectly modulate Mac reprogramming through an action on ESC that could involve mechanisms mediated by extracellular vesicles.

#### BUTYRATE, A POST-BIOTIC WITH ANTIINFLAMMATORY PROPERTIES, AMELLIORATES THE PROGRAMMING OF METABOLIC ALTERATIONS IN THE OFFSPRING FROM RATS WITH OBESITY

**Verónica White, Florencia Heinecke, Jeremías Flores-Quiroga, Marina Labiano y Victoria Deschamps.**

*Centre for Pharmacological and Botanical Studies School of Medicine, Buenos Aires University, CONICET.*

A substantial body of research on obesity has indicated that overweight is merely the most visible aspect of a much larger problem: the prevalence of metabolic impairments. Obesity can give rise to a range of metabolic impairments, including fatty liver disease, stroke and inflammatory bowel disease. Furthermore, obesity has been demonstrated to alter the equilibrium of the intestinal microbiota. The intestinal microbiota metabolises dietary fibre, producing postbiotics such as butyrate, a short-chain fatty acid that reduces inflammation and modulates metabolic and epigenetic processes.

The prevalence of obesity has increased for a number of environmental reasons, as well as a result of non-genetic parental transmission. Maternal obesity has been shown to impact the metabolic program of the offspring, leading to an increased vulnerability to develop metabolic issues such as obesity, fatty liver and intestinal inflammatory diseases.

We developed an experimental model in rats in which obesity is induced by a diet rich in saturated fats, both prior to and during the periods of pregnancy and lactation. At term gestation, these rats displayed increased weight, insulinemia, triglyceridemia and liver lipid accumulation. Their fetuses were also overweighted and showed liver lipid accumulation. Additionally, fetal livers exhibited increased lipid peroxidation, nitration, and nitric oxide production, indicating pro-oxidant-related tissue damage. The intestines of fetuses from rats with obesity displayed

pro-inflammatory and oxidative markers, including elevated lipoperoxidation, nitrotyrosine content, and nitric oxide production, along with a reduction in Claudin 3, a tight-junction protein crucial for epithelial barrier integrity. Also, the adult offspring showed elevation in pro-inflammatory and oxidative markers, along with augmented intestinal permeability. Despite the absence of overweight or adipose tissue accumulation, an elevation in circulating hepatic enzymes was observed. Additionally, the livers exhibited lipid overload, oxidative damage, and augmented expression of the pro-inflammatory cytokines IL1 $\beta$  and IL6.

Other groups have demonstrated that pregnant and non-pregnant patients with obesity exhibit decreased faecal levels of butyrate. Consequently, we elected to supplement the diet of the progenitor rats with butyrate during gestation and lactation.

Interestingly, butyrate prevented the increase in circulating triglycerides and decreased the levels of IL1 $\beta$ . Additionally, maternal treatment with butyrate prevented fetal overweight and liver lipid overaccumulation. The beneficial effects of butyrate persisted in the offspring, preventing the development of markers of fatty liver.

Our findings demonstrate that maternal obesity programs significant liver and intestinal metabolic impairments, and that maternal oral treatment with butyrate can ameliorate this malprogramming. Future studies will be conducted to determine the mechanisms involved.

#### CHRONIC INFLAMMATION OF THE MALE GENITAL TRACT IMPAIRS FERTILITY

**Rubén Darío Motrich**

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Male infertility accounts for up to 50% of all infertility cases, with urogenital inflammation, particularly chronic prostatitis, contributing to 15% of these cases. Chronic prostatitis patients show semen inflammation and reduced sperm quality. Given that the seminal fluid is

crucial not only for fertilization but also for inducing immunoregulation on the female genital tract (FGT) to support embryo implantation and pregnancy development, chronic prostatitis could have further effects beyond semen inflammation.

To investigate the impact of Experimental Autoimmune Prostatitis (EAP) on male fertility, immunomodulation in the FGT, and offspring development.

C57BL/6 male mice were immunized with prostate antigens (PA) or saline (C) to induce EAP. The PA-specific immune response, prostate histopathology, and sperm quality were evaluated. Mating experiments with female BALB/c mice were performed to assess fertility parameters, uterine immune cell infiltration and cytokine/chemokine expression, and offspring development. Statistical analysis was performed using the Mann-Whitney test or ANOVA ( $p < 0.05$ ).

EAP males exhibited chronic prostate inflammation, semen inflammation and oxidative stress, and reduced sperm quality. Females mated with EAP males showed significantly increased uterine leukocyte infiltration, up-

regulated expression of IL-1 $\beta$  and IL-17A, along with reduced IL-10, and downregulated expression of embryonic factors crucial for implantation (LIF, IL-6 and IGFBP-1) whereas increased expression of the pro-apoptotic factor TRAIL. Additionally, females mated with EAP males showed significantly lower fertility indexes, and higher rates of pre- and post-implantation embryo loss. Strikingly, offspring sired by EAP males exhibited reduced growth and decreased sperm quality when adults. Chronic prostatitis causes semen inflammation and disrupts immune regulation in the FGT, compromising fertility and negatively impacting offspring development. These findings highlight the need to properly diagnose and treat chronic prostatitis to safeguard fertility and prevent long lasting complications on the offspring.

**SYMPOSIUM CARDIOLOGY SAIC/SAC/SAFIS/ALACF I. Wednesday 20th November 8:30 - 10:00 - Anexo**  
**ADVANCES IN MECHANISMS OF CARDIAC DYSFUNCTION AND ITS POTENTIAL TREATMENTS**  
**Chairs: Gustavo Pérez, Martin Aladio**

**VAGAL NEUROMODULATION FOR MYOCARDIAL INFARCTION: EMERGING MECHANISTIC INSIGHTS AND FUTURE DIRECTIONS**

**Bruno Bucholz**

Dysautonomia, characterized by sympathetic hyperactivity and diminished parasympathetic activity has been implicated in the pathogenesis of many cardiovascular diseases including hypertension, myocardial ischemia, arrhythmia, and heart failure. Strong basic evidence suggested that restoring parasympathetic activity by vagal nerve stimulation (VNS) improves ventricular function, adverse myocardial remodeling, and survival in different ischemic heart failure models. However, clinical studies of VNS for heart failure with reduced ejection fraction have had mixed and inconclusive results to date. The infarct size is one of the most important determinants of the evolution of ischemic heart disease. In recent years, several experimental studies have shown that VNS reduces acute myocardial infarct size by different mech-

anisms. Recently we studied the effects and mechanisms of pre-ischemic VNS as a technique to improve parasympathetic activity in acute myocardial ischemia or ischemia/reperfusion injury, and its long-term effects on left ventricular remodeling and function. We also discuss the potential limitations to translate these preclinical myocardial protection results to patients. Disruptions of neural and intrinsic heart cellular pathways in patients who suffer cardiovascular diseases and comorbidities could interfere in clinical vagal protection on myocardial infarction. In the complex interplay of the brain-heart axis, both electrical and mechanical cardiac injuries can occur due to dysautonomia in the context of cerebral ischemia, which may benefit from an increase in parasympathetic tone.

**DECIPHERING CARDIAC SIGNALING NETWORKS IN DIABETIC CARDIOMYOPATHY: UNCOVERING THERAPEUTIC TARGETS USING QUANTITATIVE PROTEOMICS OF O-GLCNAcylation AND PHOSPHORYLATION Crosstalk**

**Marzieh Ayati<sup>1</sup>, Amit Das<sup>1</sup>, Miguel A. Garza<sup>2</sup>, Junfeng Ma<sup>3</sup>, Nazareno Paolucci<sup>4,5</sup> and Genaro A. Ramirez-Correa<sup>2</sup>**

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Type 2 diabetes mellitus (T2D) affects approximately 11.6% of US residents. Diabetic cardiomyopathy is a common complication of diabetes, leading to cardiac muscle dysfunction characterized initially by relaxation abnormalities, followed by contraction defects, cardiac hypertrophy, and ultimately, heart failure. Historically, cytosolic calcium levels and the dynamic phosphorylation

of sarcomere proteins have been recognized as major regulators of cardiac relaxation and contraction. In recent decades, O-GlcNAcylation has also emerged as a significant post-translational modification (PTM) that regulates cardiac contraction. O-GlcNAcylation, which regulates glucose metabolism, increases due to glucose overload of the hexosamine biosynthesis pathway.

We have demonstrated that excessive O-GlcNAcylation impairs cardiac myofilament and mitochondrial function, and that removing excessive myofilament O-GlcNAcylation improves contractile function. O-GlcNAcylation and phosphorylation can also activate or inhibit kinases involved in cardiac contractility, such as the Ras-dependent p38/ERK1/2-MAPK pathways. Both phosphorylation and O-GlcNAcylation modify proteins at serine and threonine residues, and crosstalk between these two PTMs has been observed in various cellular processes. We generated global myofilament site-specific O-GlcNAcylation and phosphorylation data in normal and type 2 diabetic hearts of mouse models. We compared the proteome of diabetic and control mice under stress (high-frequency heart rate pacing in isolated perfused hearts - Langendorff). In this study, we present the results of our analysis of the site-specific interplay between O-GlcNAcylation and phosphorylation in diabetic cardiomyopathy. We simultaneously determined the full proteome with TMT labeling and, in two other fractions, the

site-specific modifications of O-GlcNAc and phosphorylation at Ser/Thr residues. We quantified these PTMs by normalizing their signal to the TMT signal of the full proteome.

Our phospho-proteomics data show that 22 kinases from the Insulin/IGF/MAPK, Ras, and EGFR pathways are significantly activated in diabetic hearts. Additionally, there is a significant correlation between the fold change of phosphorylation and O-GlcNAcylation at the same residue in the protein. We also investigated whether the proximity on the protein sequence affects the correlation between phosphorylation and O-GlcNAcylation of two intra-protein residues. Our results suggest that closely positioned intra-protein residues have a higher correlation between phosphorylation and O-GlcNAcylation, and as the sites become more distant, their correlation decreases. However, whether the Insulin/IGF/MAPK, Ras, and EGFR pathways are affected in the human heart and whether their alteration plays a role in the mechanisms of diabetic cardiomyopathy remains unknown.

#### UNDERSTANDING PROARRHYTHMOGENIC ALTERNANS IN THE HYPERTENSIVE MYOCARDIUM: IMPACTS OF CALCIUM REGULATION

**Matilde Said**

*Centro de Investigaciones Cardiovasculares "Dr. Horacio Cingolani", UNLP-CONICET, La Plata, Argentina.*

Left ventricular hypertrophy (LVH), an early cardiac adaptation resulting from hypertension, is acknowledged as an independent risk factor for cardiovascular disease. The hypertrophied myocardium of spontaneously hypertensive rats (SHR), a well-established model of human essential hypertension, exhibits increased susceptibility to developing alternans. This phenomenon refers to the beat-to-beat variability in action potential duration, contraction strength, or amplitude of  $Ca^{2+}$  transients at a constant stimulation frequency. Clinically, it is observed as T-wave alternans, which has emerged as a significant predictor of malignant arrhythmias and sudden cardiac death. Various experimental conditions and interventions can induce alternans, revealing their multifactorial nature. Empirical studies and mathematical models predominantly indicate that defects in intracellular  $Ca^{2+}$  handling -particularly those involving the sarcoplasmic reticulum (SR)- are central to the development of alternans. Both SR  $Ca^{2+}$  release via ryanodine receptors (RyR2) and SR  $Ca^{2+}$  reuptake by the  $Ca^{2+}$  pump (SERCA2a) have been identified as key contributors to this process. These  $Ca^{2+}$  movements must be efficient and

temporally synchronized; however, as stimulation rates increase, the myocyte's ability to cycle  $Ca^{2+}$  becomes overwhelmed. This limitation has been linked to the onset of cardiac alternans, which typically occurs at elevated heart rates. Impaired  $Ca^{2+}$  dynamics in various cardiac diseases can lead to alternans manifesting at lower frequencies, thus increasing the risk of lethal arrhythmias. Our results demonstrate that from 6-months of age, SHR hearts exhibit increased vulnerability to mechanical, global, and subcellular  $Ca^{2+}$  alternans compared to age-matched normotensive rats. This earlier onset of alternans correlates with structural alterations in the T-tubule system -membrane invaginations essential for efficient excitation-contraction coupling- as well as increased refractoriness of SR  $Ca^{2+}$  release, while the rate of SR  $Ca^{2+}$  uptake remains unchanged. In summary, although substantial progress has been made in elucidating LVH and its implications for cardiac health, further exploration of the mechanisms governing  $Ca^{2+}$  handling and their relationship with cardiac alternans is essential for the advancement of effective antiarrhythmic therapies.

**SYMPOSIUM ENDOCRINOLOGY SAIC-SAFIS-ALACF II.** *Wednesday 20th November 13:30 -15:00 - Anexo (streaming)*  
**Chair: Florencia Cayrol, Karina Formoso**

#### IGF1/IGF1R SYSTEM OF LIGANDS AND RECEPTORS: FRIEND AND FOE

**Patricia Pennisi**

*Centro de Investigaciones Endocrinológicas Dr. César Bergadá, CEDIE. CONICET – FEI, Buenos Aires, Argentina.*

The insulin and insulin-like growth factor (IGF) system, which includes ligands (IGF1, IGF2 and insulin) and re-

ceptors (IGF1R and IR), plays important physiological roles in ensuring cellular survival and proliferation across various tissues. IGF1 is well known for its critical roles in promoting growth during both pre- and post-natal life, as well as regulating neurological development in mammals. Direct evidence of IGF1's involvement in human fetal development comes from the positive correlation between birth length and IGF1 expression in term and pre-term human fetuses, as well as in neonates. During fetal development, IGF1 expression is independent of growth hormone (GH); however, after birth, circulating IGF1 levels depend on liver production, which is tightly regulated by pituitary-derived GH. In addition to its classical endocrine role, many extrahepatic tissues produce measurable quantities of IGF1, which exhibits tissue-specific paracrine and autocrine activities.

Both IGF1 and IGF2 activate the IGF1 receptor (IGF1R), leading to mitogenic, anti-apoptotic, and pro-survival signaling activities. Several components of the IGF system are known to be present in tumors from both adult and pediatric patients. Specifically, IGF1R is a membrane receptor belonging to the tyrosine kinase family, functioning primarily through the phosphorylation of intracellular molecules, including those in the Ras-Raf-MAPK and PI3K-AKT signaling cascades. Recently, it has become clear that receptor endocytosis is crucial for forming signaling complexes, fine-tuning hormonal signals, and

maintaining proper receptor homeostasis and regulation. The signaling that occurs following the interactions between IGFs and their receptors in tumor cells has received extensive attention when investigating the role of IGF1R in cancer. Increased IGF1R expression has been observed during progression to metastatic phenotypes and is associated with worse prognosis in several types of cancer. Despite being a transmembrane receptor, intact IGF1R has been detected in the nuclei of both malignant and non-malignant human cells. The phosphorylation of IGF1R and subsequent SUMOylation are necessary for the nuclear translocation of the receptor. However, knowledge about the actions of nuclear IGF1R remains limited. Recent reports have demonstrated that once in the nucleus, IGF1R can interact with specific regulatory regions and upregulate the expression of genes related to various cellular processes. Although the function of IGF1R in the nucleus is still unclear, it has already been associated with poor prognosis in patients with breast cancer, anaplastic lymphoma, synovial sarcomas, and gliomas.

The novelty and complexity of the interplay between classical gene regulation resulting from membrane-based IGF1R signaling, along with internalization, trafficking, and the recently described gene regulation by nuclear IGF1R, merit further investigation.

## MECHANISMS OF FUNCTIONAL PROGRAMMING OF MYELOID CELLS IN ENDOCRINE TUMORS

**Romana Netea-Maier**

*Department of Internal Medicine, Division of Endocrinology, Radboud University Medical Center, Nijmegen, The Netherlands*

In the past decades, immunotherapy has established itself as a new hallmark in cancer treatment. Immune-checkpoint inhibitors (ICIs) and chimeric antigen receptor (CAR) T-cell therapy, both of which are focusing on immune cells of lymphoid lineage, are illustrative examples of therapies that have impacted the outcome of different patient categories. Increasingly however, more evidence indicates the important role of myeloid cells in onco-immunology. For example, tumor-associated macrophages (TAMs) are myeloid cells present in the tumor micro-environment (TME), showing an anti-inflammatory and pro-tumoral phenotype. Recent research starts to unravel mechanisms by which macrophages, and other myeloid cells, can be reprogrammed by tumoral as well as hormonal factors. In the presentation, the mechanisms of functional programming of myeloid cells will

be addressed, specifically in the context of endocrine tumors. Like in other tumors, myeloid cells are important players in the TME of endocrine tumors.

In endocrine tumors, particular those which are also producing hormones, the chronic exposure to these hormones can impact as well the functional programming of myeloid cells both within the TME and systemically. In the last years, several studies, both by our group and others, have elucidated the effects of different hormones on the phenotype of myeloid cells in physiological and pathological conditions. These novel insights are not only of interest for understanding the pathogenesis of these tumors and their short- and long-term complications, but may have implications for current oncological treatments and open avenues for new therapeutic modalities.

## DRUG RESISTANCE IN PITUITARY ADENOMAS. A GREAT CHALLENGE

**Alejandra Abeledo-Machado<sup>1</sup>, Josep Argerich<sup>2,3</sup>, Agustín Yanéff<sup>4</sup>, Dana Bornancini<sup>1</sup>, Milagros Peña-Zanoni<sup>1</sup>, Mariela M. Gironacci<sup>5</sup>, Carina Shayo<sup>6</sup>, Francisco Ciruela<sup>2,3</sup> and Graciela Díaz-Torga<sup>1</sup>.**

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Among the pituitary tumors PROLACTINOMAS, are the most frequently observed in the clinic. The dopamine receptor 2 (D2R) agonists represent a highly effective first-line therapy. However, there is a subset of prolactinomas (between 15 and 20%) that do not respond to the treatment or become resistant. These RESISTANT prolactinomas represent a major challenge for clinical management as there are no alternative treatments. The molecular mechanisms underlying their escape from dopaminergic regulation are not understood and may include alterations in D2R signaling. For several years our question was: WHAT to do when dopamine agonists don't work, looking for alternative inhibitory systems. Then we focused on 3 intra-pituitary inhibitory systems: TGF $\beta$ 1, activins and the membrane progesterone receptors. But during the last years, our focus has been on the WHY: why D2R doesn't work. Then we start studying whether D2R dimerization disrupts its signaling in lactotrophs promoting resistance to D2R agonists. It was reported that the bradykinin receptor type 2 (B2R) is overexpressed in prolactinomas. Then we postulated that the increased

B2R expression in prolactinomas could facilitate D2R-B2R dimerization disturbing D2R signaling, promoting resistance to D2R agonists. We first characterized the bradykinin receptors in the pituitary, and we found that B2R is the most expressed, mainly in lactotrophs cells. Then, the formation of B2R-D2R complexes in cultured cells transiently expressing both receptors was validated using the NanoBiT technology. Interestingly, while the stimulation of D2R did not alter B2R-induced intracellular calcium mobilization, B2R stimulation abolished D2R signaling (modulation of cAMP levels). The existence of B2R-D2R complexes in human pituitary adenomas biopsies was evaluated using the ALPHALisa approach. B2R-D2R complexes were detected in human prolactinomas and nonfunctioning pituitary adenomas (NFPA), but not in mixed (prolactin + growth hormone) secreting adenomas. These results suggest that overexpression of B2R in resistant prolactinomas may promote the formation of B2R-D2R complexes, with B2R disrupting D2R signaling, thus generating resistance to D2R agonists.

**SYMPOSIUM SAIC IV. Wednesday 20th November 13:30 - 15:00 - Auditorium**  
**APPROACHES AND OPPORTUNITIES FOR TRANSFER IN TRANSLATIONAL MEDICINE**  
**Chair: Juan Garona, Paula Heller, Hugo Ortega, Daniel Alonso**

**MANUFACTURING OF PHARMACEUTICAL DOSAGE FORMS CONTAINING  
 NANOCRYSTALS USING 3D PRINTING STRATEGY**  
**Santiago Daniel Palma**

UNITEFA- CONICET. Facultad de Ciencias Químicas – Universidad Nacional de Córdoba

When a drug or biologically active molecule is administered in a conventional pharmaceutical dosage form (tablet, injectable, etc.), the drug is released rapidly and unrestrictedly into the body. The physicochemical properties of the drug (solubility, pKa, log P, molecular weight, etc.) influence its eventual dissolution and absorption through biological membranes, and therefore its bioavailability. One of the main challenges that researchers in the pharmaceutical industry currently face is that although new drug candidates are very active in terms of binding to their target receptors, their bioavailability is affected due to their low solubility in physiological fluids. Consequently, the effectiveness of pharmacotherapy with these drugs may be reduced. There are important groups of drugs that present these unfavorable properties, compromising their efficacy or safety and thus requiring new formulation strategies to overcome such deficiencies. The conference will focus on the convergence of two technologies developed by our research group that offer synergistic alternatives to improve the biopharmaceutical performance of drugs: nanocrystals and 3D printing. These technologies also allow for rapid translation to the

clinic due to their simplicity and scalability. Nanocrystals (NCs) are described as a highly useful tool for increasing drug efficacy, as it is possible to obtain particles with sizes below 1  $\mu$ m that can easily redisperse in aqueous environments. The key characteristic of NCs is the large surface area of the particles, which leads to faster saturation of the dissolution layer, consequently increasing the dissolution rate. NCs are composed entirely of the drug, providing the additional advantage of achieving a high "system load capacity" compared to other nanoparticles that can carry a maximum of approximately 25-30% of the active ingredient. These advantages, along with the simplicity of the formulation and ease of scaling, have notably attracted the attention of the pharmaceutical industry. On the other hand, additive manufacturing (AM), more commonly known as 3D printing (3DP), has become a promising tool in many fields of production, including pharmaceuticals. 3DP allows for the creation of solid objects from pre-designed digital models by adding material layer by layer until the three-dimensional shape stored in a digital file is achieved. By simply modifying the file, it is possible to create a wide variety of geomet-

ric structures with different sizes and shapes, using the same printer. In the field of pharmaceuticals, conventional manufacturing methods for oral solid dosage forms (OSDF) (tablets or capsules) are adapted to produce large batches of identical shape (same geometry, size, and dose). 3DP technology represents a versatile, simple, and precise design tool for obtaining OSDFs with unique and differential potentialities such as:

- The ability to create solid structures of different shapes and sizes without loss of precision using the same equipment and without changes in setup.
- The ability to combine materials with different physico-chemical properties (hydrophobicity/hydrophilicity), even placing them in different layers or surfaces of the structure without mixing them.
- The ability to produce multiple different OSDFs with the same equipment: tablets, liquid capsules, films, vaginal ovules, etc.
- The ability to create innovative geometries that are difficult to achieve with traditional manufacturing, such as hollow or porous structures that allow solids to float.
- The ability to design personalized dosage forms, adapting the dose to the patient's body mass and specific metabolic needs.
- The ability to adjust the release kinetics, enabling the creation of immediate or controlled-release OSDFs as required. Changes in shape, material composition, or even the way materials are arranged within the OSDF are some techniques that have been explored to modify release.
- The ability to group numerous active pharmaceutical

ingredients (APIs) (even incompatible ones) into a single OSDF.

Various production methods have been explored for 3DP of medicines: a) fused deposition modeling (FDM), b) powder deposition method, c) semi-solid micro-extrusion or pressure-assisted micro-syringe (PAM), d) selective laser sintering (SLS), and e) photopolymerization or stereolithography (SLA). Although the basic principle of 3D printing is the same, each of these methods uses a completely different 3D printer and presents different drawbacks that hinder their use in pharmaceutical systems. For this reason, our research group decided to modify a PAM printer by adding a thermostatic jacket to control the temperature of the syringes in a range from 25 to 80 °C, which enabled the development of a patented process – Melting Solidification Printing Process (MESO-PP) that can be schematically visualized in the image below. This technique uses materials with a melting temperature in the range of 40 to 60 °C as “ink” and differs from the aforementioned techniques by not using extreme temperatures, not requiring prior processes (such as the manufacture of filaments or inks, or powder flow control), not using organic solvents or water, not requiring drying times, and, most importantly, by allowing the use of materials already tested in pharmaceutical technology under similar conditions. Based on the above and given the difficulty in obtaining solid dosage forms with NCs, we advanced in loading NCs into 3D printed forms. The results of this combination of technologies will be presented during the conference.

## TRANSLATIONAL RESEARCH: DIALOGUE OF KNOWLEDGE AND HYPERCOMPLEX PROBLEMS

**Mario Rovere<sup>1</sup>, Marcela Belardo<sup>1</sup>, Verónica González<sup>1</sup>**

<sup>1</sup> *Escuela de Gobierno en Salud “Floreal Ferrara”, Ministerio de Salud de la Provincia de Buenos Aires.*

Health is a reflection of social justice and an essential factor for the development of societies, influenced by the social and economic environment in which people live. However, the traditional biomedical approach has dominated the analysis of health problems, reducing their causes to infectious agents, genetics, or biological factors, while neglecting broader social influences. This approach limits the understanding of health inequalities, which are often explained solely by access to medical services, without considering how social conditions significantly affect the health of the population.

It is crucial to recognize that social factors play a determining role in public health. The context in which individuals live, their working conditions, socioeconomic status, and environment deeply influence their well-being. This approach allows for a more comprehensive understanding of how policies and social conditions affect population health, broadening the traditional view that often focuses on conventional indicators like employment or education. Through this perspective, it becomes clear that individual risks explain only a limited part of the variability in the occurrence of diseases, and that modifying individual

behavior is insufficient when such behavior is shaped by broader social factors.

One of the most important challenges is the difficulty of integrating scientific knowledge about social determinants into political decision-making. Over time, there has been a disconnect between the world of science and politics, partly caused by how research is validated and funded, with an emphasis on academic publications and citation indexes, which often distance scientific knowledge from the real problems of societies. In politics, the media often plays a predominant role in decision-making, frequently replacing scientific rigor with the immediacy of news, making it difficult to properly incorporate public health research into policies.

Despite these challenges, it is proposed that the key to addressing complex health problems is to develop an approach that allows for the “translation” of scientific knowledge about social determinants into concrete political actions. This translation process is not mechanical, but involves a deep understanding of different perspectives and contexts. Social phenomena, such as poverty, working conditions, and support networks, must be addressed

comprehensively to design effective policies that improve population health. Translational research is essential in this regard, as it connects science with policy and helps

to break down the barriers that exist between the two worlds.

### EARLY PHASE STUDIES IN ARGENTINA

**Ventura A. Simonovich MD**

*Clinical Pharmacology, Hospital Italiano de Buenos Aires*

Early phase studies represent an area of high complexity and risk within clinical pharmacology, requiring highly trained personnel and sophisticated infrastructure. Currently, Argentina has seven accredited centers to carry out these studies, which positions the country as a relevant player in the international clinical research landscape. This platform is strategic for several reasons. Argentina stands out for having one of the largest numbers of biotechnology startups in Latin America and for having researchers who are making significant advances in various health fields, from oncological treatments to the development of vaccines. Therefore, it is essential to have an environment that allows clinical validation according to international standards.

Over the years, ANMAT has established itself as a reference in the evaluation of clinical studies. Through the Circular 1 instrument, it has facilitated an open and constructive dialogue with those submitting trials, which has improved approval times and clarity in reviews. Today,

Argentina is beginning to conduct first-in-human trials to the global pharmaceutical industry, a possibility unimaginable five years ago. In particular, trials in advanced therapies and oncology are leading this advancement, building capacity in research centers and giving patients access to new treatment options for complex and rare diseases. However, there are significant challenges. The lack of state funding puts at risk the ability of many research groups to advance to clinical phases. Therefore, it is essential to review the financing mechanisms for research lines.

Scientific societies such as the Argentine Association of Experimental Pharmacology must play an active role in this context, for example acting as intermediaries between researchers and biotechnology investment funds. By promoting new and different forms of financing, combined with an increase in early phase studies, Argentine science will be able to be projected beyond our borders.

**SYMPOSIUM SAIC V. Wednesday 20th November 13:30 - 15:00 - Sala de Camara**

**NEURONAL REPAIR IN NEUROLOGICAL DISORDERS**

**Chair: Ramiro Quinta, Mercedes Lasaga**

### NEURODEGENERATION IN TRANSGENIC MOUSE MODELS OF THE TDP-43 PROTEINOPATHIES ALS/FTD

**Lionel Muller Igaz<sup>3</sup>**

*IFIBIO Houssay (CONICET-UBA), Facultad de Medicina, UBA, Buenos Aires.*

TAR DNA-binding protein 43 (TDP-43) is a ubiquitously expressed RNA-binding protein with critical roles in RNA metabolism. Aberrant aggregation of TDP-43 is a hallmark of several neurodegenerative diseases, collectively termed TDP-43 proteinopathies. Notably, TDP-43 pathology is observed in nearly 97% of amyotrophic lateral sclerosis (ALS) cases and in 50% of frontotemporal dementia (FTD) cases, underscoring its central role in these disorders. In ALS/FTD, TDP-43 mislocalization from the nucleus to the cytoplasm, accompanied by hyperphosphorylation, ubiquitination, and cleavage, contributes to motor neuron degeneration. Similarly, in FTD, TDP-43 aggregates drive neurodegeneration, leading to cognitive and behavioral impairments. Animal models, including mice, zebrafish, and *Drosophila*, have been critical in unraveling the molecular mechanisms of TDP-43 toxicity and exploring therapeutic interventions. These models have different degrees of success in reflecting important aspects of TDP-43 dysfunction, provid-

ing valuable insights into its role in neurodegeneration and the shared pathogenic mechanisms between ALS and FTD. We have previously generated and characterized transgenic mice conditionally overexpressing either nuclear (WT) or cytoplasmic ( $\Delta$ NLS) forms of human TDP-43 in forebrain neurons. I will present our efforts to understand the pathophysiological roles of TDP-43, using these inducible murine models that recapitulate key features of the ALS/FTD spectrum. We have evidence of region-specific neuronal loss, mild at early stages (1 month) after transgene induction and more pronounced at late (6 months) time points. Regions involved include CA1 and Dentate Gyrus of the hippocampus, and motor, somatosensory and prefrontal cortices. These changes are preceded by multiple behavioral abnormalities, including cognitive, motor and social deficits, all present in ALS/FTD patients. To assess early changes (previous to overt neurodegeneration), we additionally studied a) alterations in plasticity-related gene expression; b) cor-



ticospinal tract degeneration, associated with the loss of axons from the upper motor neurons from the motor cortex, and c) ultrastructural changes using Transmission Electron Microscopy (TEM) analysis, revealing synaptic

changes in hippocampal and cortical regions. Our findings contribute to our understanding of the pathological mechanisms underlying TDP-43 proteinopathies like ALS and FTD.

### NEURONAL CHOLESTEROL REGULATION DURING EXCITOTOXICITY AND SIRT1 INHIBITION

**Alejandro Sodero**

*Instituto de Investigaciones Biomédicas (BIOMED), Pontificia Universidad Católica Argentina (UCA) y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.*

The primary origin of brain cholesterol is de novo synthesis. The entry of circulating cholesterol to the brain is prevented by the shielding properties of the blood-brain-barrier. In the adult brain, ~80% of the cholesterol is in the lipid-rich myelin membranes, while the rest of the cholesterol constitutes an active pool implicated in a vast number of cellular functions.

I will show that neuronal cholesterol levels get reduced due to higher catabolism in response to excitotoxicity, which has been demonstrated to occur in acute neuronal injury (brain trauma, stroke, epileptic seizures) as well as in chronic neurodegenerative diseases. The loss of neuronal cholesterol due to excessive activation of glu-

tamatergic receptors leads to significant changes in the depolarization-evoked calcium responses.

In addition, I will present data supporting the participation of the protein deacetylase SIRT-1 in the maintenance of normal levels of cholesterol synthesis in the adult brain. SIRT-1 has been demonstrated to play an important role in the organism aging, adapting the transcription of genes implicated in metabolic processes via deacetylation of transcription factors.

All these findings open new questions on the consequences that modifications in neuronal/synaptic cholesterol might have in the context of neurological diseases and aging.

### UNVEILING MOLECULAR MECHANISMS ASSOCIATED WITH ANTI-GANGLIOSIDE ANTIBODY-MEDIATED INHIBITION OF PERIPHERAL NERVE REPAIR

**Pablo H.H. Lopez**

*Investigador Independiente CONICET, Dpto. Química Biológica Ranwell Caputto-CIQUIBIC, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.*

Anti-ganglioside antibodies (anti-Gg Abs) are known to inhibit nerve repair in Guillain Barré Syndrome, contributing to delayed recovery in about 30% of patients. These antibodies primarily halt axon regeneration by targeting gangliosides at the growth cones of regenerating nerves, disrupting the cytoskeleton and preventing further extension. Our previous research showed that the inhibition involves the activation of the small GTPase RhoA and its downstream kinase, ROCK, partially mirroring mechanisms seen with other axon regeneration inhibitors. We also found that ROCK inactivates collapsing response mediator protein-2 (CRMP-2), negatively impacting the tubulin cytoskeleton. However, the specific membrane transducers involved in these signaling events remained unclear. Using a proteomic approach along with in vivo and in vitro models of nerve repair, we recently identified Tumor necrosis factor receptor 1A (TNFR1A) as the membrane transducer that mediates the inhibitory

effects of anti-Gg Abs targeting GD1a ganglioside (but not Abs targeting a structurally-related gangliosides). We hypothesize that anti-Gg Abs may also disrupt the cytoskeleton of non-neuronal cells, despite their low ganglioside expression. We observed that regenerating nerves exposed to anti-Gg Abs showed impaired myelin debris clearance. Further investigation revealed that these antibodies affected macrophage function—reducing myelin phagocytosis through ROCK-dependent inactivation of cofilin—without impacting Schwann cells. Additionally, impaired myelin clearance was absent in mice treated with Y-27632, a RhoA/ROCK inhibitor, or in mice lacking gangliosides or TNFR1A. Overall, our findings reveal a novel transducer for the inhibition of nerve repair by anti-Gg Abs and highlight a new cellular target for therapeutic intervention, potentially reducing neurological issues associated with high levels of these antibodies in Guillain-Barré Syndrome.

**SYMPOSIUM SAFIS/ALACF I. Thursday 21th November 10:30 - 12:00 - Anexo (Streaming)**

**ADVANCES IN MECHANISMS OF RENAL DYSFUNCTION AND ITS POTENTIAL TREATMENTS**

**Chair: Sara Molinas, Carolina Caniffi**

### PREVENTION OF MALADAPTIVE REPAIR MECHANISMS INDUCED BY ACUTE KIDNEY INJURY AND ITS TRANSITION TO CHRONIC KIDNEY DISEASE

**Norma Bobadilla**

*Unidad de Fisiología Molecular, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México,*

Mexico City, Mexico.

A mild or severe episode of acute kidney injury (AKI) leads to chronic kidney disease (CKD) due to an abnormal reparative process, known as a maladaptive response. The sodium-glucose cotransporter 2 (SGLT2) inhibitor, dapagliflozin, has shown nephroprotective effects in patients with CKD; however, the molecular mechanisms responsible for this protection are unknown. This study evaluated whether early and transient administration of dapagliflozin after AKI induced by ischemia/reperfusion is sufficient to prevent subsequent CKD.

**Materials and Methods:** Forty-one male Wistar rats (300-350 g) were randomized into three groups: Sham surgery, renal bilateral ischemia for 30 min (IR), and IR + dapagliflozin, 1 mg/kg/day (IR+Dapa). Daily treatment with Dapa started 24 hours after AKI and continued for only 10 days. After this period, half of each group was euthanized, and the other half was followed for 5 months before euthanasia. Renal hemodynamics, histology, ultrastructure, and expression of relevant mRNA or proteins were analyzed. Differences between groups were analyzed using ANOVA/Kruskal-Wallis with a significance level of  $p < 0.05$ .

Ten days after AKI, the rats that received Dapa showed early restoration of renal blood flow (RBF) and recovery of creatinine clearance (CrCl) compared to the vehicle-treated IR group. Fewer ultrastructural alterations were found in proximal tubule cells, with notable preservation of mitochondrial morphology. Additionally, IR rats showed an accumulation of proteins related to mitochondrial damage, deficient mitophagy, and loss of NAD<sup>+</sup> homeostasis, all of which were reversed with Dapa.

Furthermore, a reduction in myeloid cell infiltration in the renal cortex was observed in the IR+Dapa group compared to the IR group. Finally, we demonstrated that this brief treatment was sufficient to prevent CKD after five months.

A brief treatment with dapagliflozin after AKI is sufficient to prevent abnormal repair and CKD in rats. This effect involves improved renal circulation and restoration of mitochondrial homeostasis. These results indicate that maladaptive response can be modulated early after severe AKI, thus preventing the development of long-term consequences.

## DIABETES, OXIDATIVE STRESS AND KIDNEY DAMAGE, ROLE OF THE INTRATUBULAR RENIN ANGIOTENSIN SYSTEM

Alexis González

*Institute of Chemistry, Pontificia Universidad Católica de Valparaíso, Valparaíso 2950, Chile.*

A hallmark of diabetes mellitus (DM) is the hyperglycemia but also hyperglycosuria, along with the presence of proteinuria and signs of renal damage that includes deposition of extracellular matrix, glomerular damage and tubulointerstitial fibrosis. All these aspects are usually observed during advanced stages of the disease. Among the injury markers observed in chronic DM in experimental animal models we have described particularly in the collecting duct cells the upregulation of profibrotic factors such as transforming growth factor-beta 1 (TGF- $\beta$ 1), connecting tissue growth factor (CTGF), plasminogen activator inhibitor (PAI-I), fibronectin and renal NADPH oxidase (NOX)-4-dependent production of reactive oxygen species (ROS). On the other hand, it has been shown that intratubular renin angiotensin system is augmented in animal models of diabetic disease, in particular the expression of angiotensinogen is upregulated in the proximal tubule and secreted to the luminal fluid. Also, renin and prorenin is highly produced by the renal collecting ducts during diabetic conditions. We have shown that cultured collecting duct cells treated with nanomolar concentrations of recombinant prorenin undergo to epithelial-mesenchymal transition and have increased levels of intracellular ROS, activation of MAPK pathway, and upregulation of profibrotic factors. Prorenin binds to a new described protein able to bind of renin or prorenin. This protein called the (pro)renin receptor (PRR)

is expressed in the apical plasma membrane of kidney intercalated collecting duct cells and triggers intracellular pathways related to the upregulation of profibrotic genes. However, the underlying mechanisms contributing to the stimulation of these pathways remain unclear. Here we discuss some of the mechanisms proposed related to the induction of kidney damage mediated by the activation of the intratubular renin angiotensin system, mainly through the prorenin-dependent activation of PRR and its intracellular cascade leading to upregulation of profibrotic factors. Our studies showed that high glucose conditions lead to upregulation and translocation of PRR. We have also showed that high glucose increases prorenin secretion in collecting duct cells and causes the accumulation of Krebs' cycle intermediary  $\alpha$  ketoglutarate, which is able to upregulate PRR expression through the  $\alpha$  keto-glutarate receptor 1 (OXGR1), which in turn causes prorenin-dependent PRR activation and the expression of profibrotic factors TGF- $\beta$ 1, CTGF and ROS. Importantly, pharmacological blockade of PRR using PRO20, impairs the increases of profibrotic expression. In recent studies we were able to demonstrate that this pathway also depends on glucose transport via GLUT1 since GLUT1 blockade prevented the high glucose dependent increases in profibrotic protein expression. Furthermore, ROS scavenging prevented these effects. Our cumulated data indicate that glucose transportation via GLUT1 is impli-

cated in the PRR-dependent upregulation of profibrotic factors and is mediated mainly via a mechanism depend-

ing on ROS formation in renal collecting duct cells exposed to high glucose.

## ADVANCES IN RENAL AUTOREGULATION: ROLE OF NMDA RECEPTORS AND THEIR IMPACT IN HYPERTENSION

**César Romero**

*Renal Division, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia, USA.*

N-methyl-D-aspartate receptors (NMDAR) are amino acid gated channels that are well studied in brain physiology; however, their functions in the kidney are poorly understood. We explored the expression of NMDAR along the nephron, using in-situ hybridization and other molecular biology techniques. We also evaluate their role in renal autoregulation using tubule microperfusion. We further explored their interaction with ENaC channel and the impact of this receptor on blood pressure regulation,

using several knockout mice models.

We found that NMDARs are expressed along the rodent nephron, including ENaC-positive cells, with higher expression in females. Epithelial NMDAR mediates renal vasodilation through the connecting-tubule-glomerular feedback, by increasing ENaC activity. NMDAR inhibition, specifically the subunit GluN2C is linked to increased blood pressure.

### SYMPOSIUM SAIC. Thursday 21th November 13:00 - 14:30 - Auditorium CANCER GENETICS: FROM BASIC SCIENCE TO CLINICAL PRACTICE

**Chair: Caroline Lamb, Catalina Lodillinsky, Marcela Bolontrade**

## DECODING DISEASES: BIOINFORMATICS APPLICATIONS IN CANCER AND AMYOTROPHIC LATERAL SCLEROSIS RESEARCH

**Cristina Marino-Buslje<sup>1</sup>, Elizabeth Martínez-Pérez<sup>1</sup>, Miguel A. Molina-Vila, Macarena Alonso<sup>1</sup>, Franco L. Simonetti<sup>1</sup>.**

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Malignant tumors arise from somatic mutations and genomic or epigenomic alterations, leading to a loss of cellular control. Our study explores two treatment strategies derived from mutation patterns. First, we analyze co-occurrence and mutual exclusivity of mutations, which can influence prognosis and drug responses, emphasizing the need for multitargeted therapies. While past studies focused on specific malignancies and considered whole genes, we performed a comprehensive analysis of co-dependencies of individual somatic mutations across various tumors. By applying multitesting with conditional and expected mutational probabilities, we discovered rules governing driver and nondriver mutation co-dependencies. We identified co-mutation and exclusion networks, some specific to certain cancers and others widespread, offering insights into multitargeted antitumor therapies. Recurrent co-mutations suggest effective drug combinations, while exclusions indicate combinations less likely to succeed. Secondly, we addressed Tumor Mutation Burden (TMB) as a biomarker for immune checkpoint blockade (ICB) therapy response. While TMB is increasingly being used in clinical trials, patient selection remains challenging due to inadequately designed sequencing panels. We developed a bioinformatics method to select gene panels and mathematical mod-

els for accurate TMB prediction. Our approach used tumor-specific, forward-step gene selection, linear regression-based panel generation, and rigorous internal and external validation. We proposed cancer-specific panels for 14 malignancies, offering reliable TMB estimates to enhance patient selection for ICB therapy. Lastly, we studied Amyotrophic Lateral Sclerosis (ALS), a neurodegenerative disease lacking biomarkers and effective treatments. Using graph convolutional neural networks (GCNNs), we analyzed protein-protein interaction (PPI) networks and gene expression data from ALS patients and controls. We tested the model on a ALS microarray datasets and validated it with a different dataset. We integrated gene expression data with PPI networks, comparing GCNN performance against logistic regression, random forest, and XGBoost classifiers trained on gene expression data alone. The GCNN, using Graph Layer-wise Relevance Propagation (GLRP), identified stable gene sets, with 46 genes consistently ranked in the top 50 across runs, outperforming the other models. We demonstrated that integrating PPI network information with gene expression profiles using the GCNN-GLRP method is effective for classifying ALS cases. More importantly, the stability of the results regarding the relevant genes enables robust future biomedical analyses.

## MULTIGENE PANEL TESTING IN YOUNG PEOPLE WITH DIGESTIVE CANCER: SEARCHING

FOR UNDERLYING HEREDITARY SYNDROMES. FIRST EXPERIENCE  
IN A PUBLIC HOSPITAL IN ARGENTINA

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**Background and Aim:** Hereditary cancer syndromes infer high risks of developing cancer throughout life. Their identification has enormous implications for those affected, since it provides valuable prognostic and predictive information; and for their families, since it allows the detection of relatives at risk in whom surveillance from early ages and prophylactic surgeries reduce cancer mortality in up to 60%. **Method:** Between January 2021 and May 2022, 500 patients were attended at the weekly hereditary cancer genetic counseling clinic of the Hospital of Gastroenterology Bonorino Udaondo Oncology Section, in Buenos Aires, Argentina. From these, 140 had gastrointestinal cancer diagnosed at younger than 50yo, and 72 were eligible for our study (mean age 37,2yo): 57 (79%) had CRC, 10 (14%) had gastric cancer (GC), and 5 (7%) had pancreatic cancer (PC). All colorectal tumors were screened for MMR deficiency by MSI testing and/or IHC analysis. Blood underwent DNA extraction using standard methods and all patients underwent germline testing for 30 cancer susceptibility genes in our Laboratory or in commercial laboratories. **Results:** Among 72 patients with early-onset digestive cancer, pathogenic (P) or likely pathogenic (LP) cancer susceptibility gene mutations were found in 19 patients (26%): 11 (15%) in Lynch syndrome (LS) genes, 4 (5,5%) in colorectal polyposis

genes, and 4 (5,5%) in genes more traditionally associated with breast cancer. When analyzing results by organ, 18/57 (31,5%) CRC's had MMR-deficient tumors, of which 11 (61%) had P/LP variants in Lynch syndrome genes. 33/57 (58%) patients had MMR-proficient CRC's, and 3 (9%) had a positive test: 1 in BRCA1, 1 in BRCA2, and 1 in ATM. Lastly, 6/57 (10,5%) of patients with CRC had a Polyposis syndrome clinical diagnosis, and four of them (67%) had APC P/LP variants. One of 5 (20%) patients with PC had a positive test (BRCA1), and none of 10 patients with GC had a positive study, despite the fact that 3/10 had clinical suspicion of Hereditary Diffuse Gastric Cancer. Only MMR-deficient CRC and first-degree cancer family history significantly predicted a positive genetic test (<0.001). **Conclusions:** Because 1 of every 4 patients with early-onset digestive cancer has at least 1 pathogenic or likely pathogenic cancer susceptibility gene mutation, genetic counseling and testing with a broad multigene panel should be considered for all of them, regardless of age at diagnosis, family history of cancer, and tumor phenotype. We have shown that it is feasible to carry out such studies in a public institution in our country, and we believe it is essential to pool resources to democratize new forms of molecular diagnosis in these patients.

INHERITANCE AND BREAST CANCER: CURRENTS CHALLENGES AND FUTURE DIRECTIONS

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**Introduction:** The incorporation of genetic counseling and testing into breast cancer care is essential for prevention, early detection and personalized treatment.

In Argentina, access to comprehensive genetic services remains limited, particularly in the public healthcare sector.

**Objective:** This study aims to explore the challenges encountered in implementing genetic testing for breast cancer in a public hospital in Argentina. Through a comparison of two phases: Phase 1 (January 2015 to June 2023) and Phase 2 (July 2023 to November 2024), we aimed to identify where improvements had occurred and where ongoing barriers remained, to improve the accessibility and effectiveness of genetic testing.

**Methods:** A retrospective analysis was conducted on all patients who were referred for genetic counseling in our center during both phases. We investigated phase-spe-

cific data, including patients meeting National Comprehensive Cancer Network (NCCN) criteria, the percentage of those for whom testing was requested and completed, and the types of tests conducted. Barriers to testing were also examined. Phase 2 genetic test results were analyzed to identify the types of genes with pathogenic variants and the prevalence of variants of unknown significance (VUS).

**Results:** In Phase 1, 1032 patients were evaluated, compared to 337 patients in Phase 2. The proportion of patients meeting NCCN criteria for genetic testing was 81.01% in Phase 1 and 83.38% in Phase 2. Among these, the percentage of patients for whom genetic testing was requested rose from 68.78% in Phase 1 to 88.61% in Phase 2. Test completion rates decreased from 44% in Phase 1 to 36.9% in Phase 2.

In Phase 2, of the 92 patients who completed genetic

testing, 69.6% underwent only BRCA1/2 testing, while 28.3% underwent multigene panel testing. The multigene panels identified pathogenic variants in multiple genes, including *TP53*, *PTEN*, *FANCA*, and others. Additionally, 30.8% of the multigene panel results included variants of unknown significance (VUS). Among patients who did not complete genetic testing, loss to follow-up increased significantly from 81% in Phase 1 to 94.9% in Phase 2.

**Discussion:** The results demonstrate improvements in some areas and persistent challenges in others. Among the advances is the annual number of patients evaluated, which grew from approximately 121 per year in Phase 1 to 224 per year in Phase 2. This enhancement reflects successful efforts to optimize workflows and improve referrals, likely supported by increased education for breast cancer specialists and the inclusion of genetic counselors in core teams.

The proportion of patients meeting NCCN criteria for whom genetic testing was requested also improved, increasing from 68.78% in Phase 1 to 88.61% in Phase 2. This suggests improved awareness and adherence to testing guidelines among referring clinicians. However, the test completion rate declined, with only 36.9% of requested tests being completed in Phase 2 compared to 44% in Phase 1.

Among patients who did not complete genetic testing, the loss to follow-up is a critical obstacle to achieving optimal outcomes. Many patients who were lost to follow-up likely experienced denied insurance coverage for genet-

ic testing, an issue that goes undocumented when they do not return for further counseling. This indicates that while direct reports of financial barriers decreased, the underlying problem remains and is likely underreported in Phase 2.

Financial limitations continue to pose structural challenges, particularly for patients relying on public healthcare. Although industry-sponsored BRCA1/2 testing has expanded access for some, it restricts the scope of testing. Multigene panels, which can identify pathogenic variants in a broader range of cancer-associated genes, were used in only 28.3% of cases in Phase 2. This limited access underscores the pressing need for systemic funding solutions to ensure equitable access for all patients.

The genetic testing results highlight the critical role of multigene panels, which identified pathogenic variants in genes such as *TP53*, *PTEN*, and *FANCA*—mutations that would have been missed with BRCA1/2-only testing. Moreover, the high prevalence of VUS (30.8% in multigene panels) illustrates the complexities involved in interpreting these findings and providing effective counseling for patients.

**Conclusion:** To address these challenges, efforts should focus on improving genetic counseling workflows to minimize patient attrition, advocating for policy changes to expand insurance coverage for comprehensive testing, and enhancing education for patients and healthcare providers about the benefits of genetic testing

**SYMPOSIUM SAIC VI. Thursday 21th November 13:00-14:30-Sala de Cámara**

#### **TOXICOLOGY**

**Protecting the environment to take care of our health: an integrative approach**

**Chair: Claudia Cocca, Andrea Randi**

#### **EFFECTS OF ENVIRONMENTAL CHEMICALS ON MAMMARY GLAND ORGANOIDS**

**Laura Kass<sup>1,2</sup>**

<sup>1</sup>Instituto de Salud y Ambiente del Litoral (ISAL, UNL-CONICET), <sup>2</sup>Cátedra de Patología Humana, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe.

Previous studies have indicated that exposure to environmental chemicals can influence the development of the mammary glands. It has been shown that exposure to these compounds during the perinatal period alter mammary alveolar development during gestation and lactation and modify milk composition in lactating dams. Because milk is nutritionally important for the offspring and because mammary tissue can develop and differentiate *in vitro*, we wanted to investigate whether exposure to various endocrine-disrupting chemicals (EDCs) found in common products, such as bisphenol A (BPA, found in plastics and dental sealants) or benzophenone-3 (BP3, found in sunscreens), affects ductal morphogenesis and functional differentiation of the mammary gland using three-dimensional *in vitro* models. Cell culture is a valuable method for toxicological assessment, as it saves time and reduces the number of animals required compared to traditional testing, which is an important goal in

any research involving living organisms. Different methodological strategies can be used to study the potential mechanisms of action of EDCs. To accomplish our objective, mammary glands were aseptically removed from untreated 8-week-old female mice, mechanically and enzymatically digested to isolate mammary organoids, and then embedded in a 50:50 mixture of Geltrex/Collagen I or in Matrigel to assess branching progression and functional differentiation, respectively. The organoids cultured under conditions that promote branching were exposed to BP3 for nine days. The progression of ductal branching in organoids exposed to BP3 was similar to that observed in organoids exposed to the vehicle. However, BP3 altered the mRNA expression of the progesterone receptor and its downstream mediators, suggesting that exposure to BP3 may later affect alveolar growth. Functional differentiation of the mammary gland occurs during lactation and has previously been shown to be sensitive

to BPA exposure *in vivo*. Therefore, organoids cultured under differentiation conditions (with lactogenic hormone supplementation) were exposed to BP3, BPA or vehicle for three days and then milk protein expression was evaluated. BPA and the lowest concentration of BP3 tested were found to increase beta-casein mRNA levels, which was linked to reduced methylation of its promoter and increased protein expression. In contrast, exposure to BPA

decreased alpha-lactalbumin mRNA and protein expression and increased DNA methylation levels. In summary, these findings demonstrate that exposure to low levels of BP3 and BPA impairs the development and differentiation of the mammary gland *in vitro*. Furthermore, mammary organoids serve as a valuable experimental model for evaluating the effects of EDCs at different stages of mammary development.

## RATS AS INDICATORS OF ENVIRONMENTAL QUALITY: THE MATANZA-RIACHUELO BASIN AS A CASE STUDY

**Olga Suárez, Mariel Trípodí, Emiliano Muschetto y Diego Hancke**

*Laboratorio de Roedores Urbanos. Departamento de Ecología, Genética y Evolución. FCEN, UBA, IEGEBA, CONICET.*

The Matanza-Riachuelo Basin (MRB) is one of the most urbanized areas in the country and one of the 10 most polluted basins in the world, mainly due to the discharge of sewage and industrial waste. The presence of toxic pollutants from petrochemical industries, tanneries and meat processing plants has caused a very complex contamination situation in the MRB. The sharp increase in the presence of arsenic and heavy metals such as cadmium, copper, chromium, mercury, nickel and lead is not only an ecological problem but also a serious risk to people's health because there are no natural mechanisms for their degradation.

According to the WHO, 25% of deaths and illnesses worldwide are linked to environmental risks. According to the same organization, the human losses registered due to exposure to chemical substances in the world during 2019 were 2 million, half of them as a consequence of exposure to lead. Exposure to toxic metals can affect various organs and organ systems such as the hematopoietic system, liver, lungs and kidneys, cause damage to genetic material and the central nervous system. Metals classified as carcinogenic, including lead, are of particular public health concern because they induce damage even at low levels of environmental exposure. The population residing in the vulnerable neighborhoods of the MRB are generally poor, low-income people living in unsuitable structures near open-air dumps with high levels of rodent infestation, without basic services and

with little access to health systems, leaving them even more exposed to environment-related diseases. The brown rat (*Rattus norvegicus*) is, among the rodents commonly referred to as commensals, the species that has best adapted to urban environments by relying on people for food and shelter. Because of its close interaction with humans, in addition to other features of its biology and behavior, it is considered an appropriate species to monitor the quality of the urban environment. We quantified the bioaccumulation of lead in different soft organs in rats captured between 2014 and 2015 in urban settlements located in the lower and middle basin of the Matanza Riachuelo river and also evaluated the presence of damage in the genetic material of bone marrow in these specimens. Our results showed the existence of a positive relationship of both lead accumulation in tissues (liver and kidney) of *R. norvegicus* and the frequency of bone marrow micronuclei with environmental concentrations of toxic metals. These findings indicate that *Rattus norvegicus* can be used as a sentinel species for toxic metal contamination and provide information on the potential risk to which people living in the MRB are exposed, mainly in the lower basin, an area with a higher concentration of urban settlements and social vulnerability. This method could be useful for monitoring urban areas where there is no data available on the concentration or sources of contaminants present.

**SYMPOSIUM SAFIS/ALACF IV. Thursday 21th November 13:00 - 14:30 - Anexo (Streaming)**

**GASTROENTEROLOGY**

**Unraveling Mechanisms of Liver Metabolic Disease**

**Chair: Silvina Villanueva, Daniel Francés**

**BENEFICIAL EFFECTS OF HEPATIC CYCLOOXYGENASE-2 EXPRESSION AGAINST NAFLD/NASH: A POTENTIAL THERAPEUTIC TARGET**

**Marta Casado Pinna**

*Metabolic Experimental Pathology Department of Metabolism, Inflammation and Aging. Instituto de Biomedicina de Valencia. Spain.*

Prostaglandin H synthase, or cyclooxygenase (COX), is essential for synthesizing prostaglandins (PGs) and thromboxanes from arachidonic acid (AA). COX-2 is gen-

erally absent in most tissues but can be induced by various stimuli, particularly in specific cell types like Kupffer cells. However, adult hepatocytes do not induce COX-2

expression in response to pro-inflammatory factors. Although COX-2's role in the liver has been studied using inhibitors and knockout models, the effects of its constitutive expression have not been extensively explored. We developed a transgenic mouse model (hCOX-2-Tg) with human COX-2 driven by the human Apo E promoter. Our studies show that constitutive COX-2 expression in hepatocytes protects against insulin resistance, obesity, and inflammation in high-fat diets. COX-2 also offers protection against non-alcoholic steatohepatitis (NASH), liver fibrosis, and ischemia-reperfusion injury. These findings suggest that COX-2 may be a key player in developing therapeutic options for liver diseases. This study aims to evaluate COX-2 as a therapeutic target for NAFLD and NASH, focusing on its protective effects even when pathology develops before enzyme overexpression. Conditional COX-2 transgenic mice (B6-Tg(ROSA)26Sor<sup>tm1(CAG-hCOX-2-PP2A-GFP)</sup>/Upme, also known as ihCOX-2-TG) were used in this study. Steatosis, steatohepatitis, and fibrosis were induced using a diet consisting of 60% kcal from fat, devoid of choline, and containing 0.1% methionine for six weeks (Research diet reference A06071302). To achieve COX-2 overexpression, floxed

mice were administered intravenously with  $10^{11}$  copies of AAV.TBG.PI.Cre.rBG (Addgene, #107787-AAV8) or pAAV.TBG.PI.eGFP.WPRE.bGH (#105535-AAV8) after either one or three weeks on the diet. COX-2 activity, liver damage, and liver leukocyte infiltration were subsequently analyzed.

ihCOX-2-TG mice exhibited reduced liver lipid accumulation and lower plasma cholesterol levels. Additionally, these mice displayed less hepatic fibrosis, as evidenced by a reduction in protein levels associated with fibrosis (such as ERK, JNK, and STAT-1) and an increase in STAT-3 levels. Flow cytometry analysis revealed that ihCOX-2-TG mice had a lower percentage of CD4<sup>+</sup> T cells and myeloid-derived dendritic cells, as well as reduced numbers of pro-inflammatory cells.

Our findings highlight the potential of COX-2 as a therapeutic target in treating NAFLD/NASH by decreasing hepatic steatosis and fibrosis, even after the disease has been established. Moreover, immune cell profiling suggests a reduction in cells associated with the initiation and progression of NAFLD/NASH, including pro-inflammatory and pro-fibrotic cells.

## XENOBIOTIC-INDUCED HEPATIC METABOLIC DYSFUNCTION

**Julie Massart**

*Institut NUMECAN (Nutrition Metabolisms and Cancer), UMR\_A 1341, UMR\_S 1241, INSERM, University Rennes, INRAE, F-35000 Rennes, France.*

Metabolic-associated steatotic liver disease (MASLD), often associated with obesity, encompasses a broad spectrum of hepatic lesions, including simple steatosis, steatohepatitis, cirrhosis, and hepatocellular carcinoma. The contribution of xenobiotic exposure—whether from pharmaceuticals or environmental toxicants—to the progression of MASLD is a growing concern in hepatology. In individuals with obesity, these exposures can worsen pre-existing steatosis or accelerate the progression of fatty liver to steatohepatitis. Our research has elucidated key interactions between xenobiotic exposure and metabolic dysregulation in the liver.

In MASLD, the altered activity of cytochrome P450 (CYP) enzymes, including reduced CYP3A4 and elevated CYP2E1 activity, significantly impacts drug metabolism. Using advanced in vitro liver models, we demonstrated

that drugs metabolized by CYPs—such as troglitazone by CYP3A4 and acetaminophen by CYP2E1—exhibit modified hepatotoxic profiles under MASLD-like conditions. Furthermore, we investigated mechanisms underlying drug-induced steatosis, a common hepatic lesion reported with various pharmaceuticals. While severe mitochondrial dysfunction can directly lead to lipid accumulation, our findings suggest that in cases without overt mitochondrial impairment, mechanisms like impaired very low-density lipoprotein (VLDL) secretion, likely due to endoplasmic reticulum (ER) stress, are more frequently implicated.

Our work underscores the importance of considering xenobiotic interactions in MASLD, particularly as these interactions may increase drug toxicity risks and alter drug safety profiles in at-risk populations.

## THE IMPACT OF INFLAMMATION ON BILE FORMATION

**Enrique Sánchez Pozzi**

*Enrique Sánchez Pozzi. Instituto de Fisiología Experimental (CONICET-UNR).  
Fac. Cs. Bioquímicas y Farmacéuticas – UNR, Rosario, Argentina.*

Sepsis is one of the first pathological conditions where inflammation's influence on bile formation was described. In animal models, it was demonstrated that inflammatory cytokines decrease bile flow and lower the expression of canalicular transporters such as the bile salt export pump (BSEP) and the multidrug resistance-associated

protein 2 (MRP2). One probable mechanism of action for cytokines is the internalization and endocytosis of canalicular transporters to a subapical space, a phenomenon already noted with other cholestatic agents like estradiol-17 $\beta$ -D-glucuronide or tauroolithocholate.

In experiments using polarized hepatocyte cultures,

we demonstrated that the inflammatory cytokines TNF and IL-1 $\beta$  decrease MRP2-mediated transport and confirmed, using confocal microscopy, that they internalize the transporter. This mechanism is mediated by intracellular signaling, involving mediators such as ERK1/2, PI3K, AKT, and ROS formation. These same mechanisms have been implicated in other models of cholestasis.

The cholestatic action of cytokines is not limited to sepsis. Conditions with elevated and sustained cytokine levels could decrease bile formation or create an intracellular environment with activated signaling proteins, sensitizing hepatocytes to other cholestatic agents. For instance, in *in vitro* studies, low concentrations of TNF that slightly affect MRP2-mediated transport were found to increase the cholestatic action of estradiol-17 $\beta$ -D-glucuronide. Estrogen-induced cholestasis is of particular interest, as sustained estrogen levels increase TNF and IL-1 $\beta$ . The

importance of cytokine increases was demonstrated by administering dexamethasone, a corticosteroid that reduces cytokine synthesis. This steroid prevented the cholestatic action of a 5-day ethinylestradiol administration, an accepted model of estrogen cholestasis.

A prevalent pathological condition of special relevance today is metabolic syndrome. One of its characteristics is sustained low-grade inflammation. Using a fructose administration model of the syndrome, we demonstrated decreased bile flow and biliary excretion of bile salts and bilirubin. While this may not be clinically significant for patients with metabolic syndrome, it could contribute to the increased susceptibility these patients have to drug-induced damage.

These findings demonstrate that elevated cytokine levels, even without reaching sepsis levels, can affect bile formation and potentiate the effects of drugs and hormones, leading to pathologies.

**SAIC I ROUNDTABLE I** *Tuesday 19th November 9:00-10:30 - Auditorium*

**TRANSFER & ARTICULATION WITH THE PRODUCTIVE SECTOR**

**Chair: Gabriela Berg**

**FROM RNA DISCOVERIES TO REAL-WORLD APPLICATIONS: BRIDGING SCIENCE AND SOCIETY**

**Carla Borini Etichetti, Uciel Chorostecki and Silvana Spinelli.**

*Kresko RNAtech Corporation. Rosario.*

The translation of basic research into consumer-focused innovations is a challenging aspect of biotechnology. Kresko RNAtech exemplifies this journey through its work in identifying bioactive dietary-RNAs and developing ingredients focused on health and wellness. These molecules are present in all fresh food and botanicals; however, they are often missing from the food that reaches our tables due to their high instability and loss during production and commercial processing. By leveraging a multidisciplinary approach that integrates bioinformatics, cellular biology, and AI-driven data analytics, Kresko has pioneered the identification, stabilization, and functional

validation of dietary-RNAs, overcoming key challenges related to their degradation. We have created the only library of dietary RNAs, enabling us to reconnect with nature and address modern lifestyle-related health issues like no other company. The transition from scientific discovery to practical application involves navigating complex landscapes, including scaling up production, addressing regulatory considerations, and achieving product-market fit. Through our innovative approach, we are not just advancing biotechnology but also setting new standards for how science can meaningfully impact everyday health.

**RESEARCH AND INNOVATION IN PEDIATRIC HEALTHCARE:**

**A VISION FROM A PEDIATRIC HOSPITAL IN ARGENTINA**

**Paula Schaiquevich**

*CONICET, Unidad de Tratamientos Innovadores, Coordinación de Investigación, Hospital de Pediatría JP Garrahan*

Technological innovation in healthcare refers to the application of scientific and technological knowledge to improve diagnosis and treatment for real-life patients considering a cost-effective approach. In pediatric healthcare, research and innovation is essential to improve cures and find new treatment. Importantly, innovation allows reducing health disparities, providing equal opportunities of advanced and cost-effective treatments to all patients with the consequent increase in overall survival, better health and reduction of morbidities. The industry has made remarkable progress in technologies in favor of healthcare delivery. However, most of these

advancements have focused on adult populations commonly excluding children for various logistic, methodological, and ethical reasons. Moreover, the market for children's healthcare is assumed to be too small to warrant sufficient costs and to attract industry investment.

An ideal framework for boosting advancements in pediatric healthcare innovation should bring together healthcare professionals including clinicians and specialists, who visualize the unmet clinical needs, and researchers that are experts on the fields of developments. Despite on its infancy in our country, there should be a close relationship between hospitals and academics, engaging



representatives of industry as well as national health authorities to make advancements clinically and commercially viable. Human resources as well as capital, usually scarce in the pediatric context, are needed.

During the last decade, innovations in medical technologies have included cutting-edge treatments involving novel pharmaceutical and biotechnology products, drug deliveries technologies, and molecular diagnostics. Personalized treatments using targeted drugs, pharmacogenomics, and/or model informed precision dosing provide huge steps for optimizing patient treatment according to

individual requirements. Telemedicine, the use of digital tools to enable remote delivery of healthcare services, telesurgery, patient monitoring, and discussions among clinical teams, has become central in our hospital and in the country, reducing geographical barriers for access to high-level care. Also, virtual reality helps training professionals.

In this round table we will discuss about the use of innovative technologies in pediatric healthcare within the context of a public pediatric hospital in our country, delving into the challenges in the field.

### TRANSFORMING SCIENTIFIC KNOWLEDGE INTO INNOVATION: FROM PHOSPHATIDYLCHOLINE TO EXOSOMES

**Claudia Banchio**

*EXO+ startup, IBR-CONICET, Rosario.*

After years of researching the role of phosphatidylcholine in neuronal differentiation, we began to question what the physiological carrier of this molecule in the brain might be. This led us to exosomes, small particles surrounded by a phospholipid membrane that carry a variety of molecules, promoting neural stem cell proliferation and their differentiation into functional neurons, even in damaged environments.

Given that neurodegenerative diseases are characterized by neuronal death, and no treatments currently exist to replace lost neurons, we realized these particles could hold great potential as a treatment for such conditions. Additionally, with the anticipated rise in life expectancy in the coming years, neurodegeneration is set to become the next global health crisis. This was the problem we set out to solve, and exosomes appeared as a promising solution.

Determined to bring this idea to life, we sought a way to build a startup. In 2022, we joined the SF500 Build program, spending nearly six months in a completely different world; learning new terms, strategies, and the complexities of entrepreneurship. For scientists grounded in basic research, this transition was particularly challenging, as basic science often isn't accustomed to the fast-paced demands of product development. Balancing the

roles of scientist and professor made the journey even more difficult, but ultimately, we were selected for the program's pre-seed investment, and Exomas was born. Our strong foundation in science has been crucial, and now we are navigating the intricate process of translating that science into a product, bridging the gap between research and real-world application.

Our future plans for Exomas focus on advancing the pre-clinical and clinical development of our exosome-based therapies to treat neurodegenerative diseases. In the short term, we aim to complete the necessary preclinical studies to file an Investigational New Drug (IND) application with regulatory authorities. Once we reach this stage, our strategy is to partner with larger pharmaceutical or biotech companies to conduct clinical trials and eventually bring the product to market.

For other researchers looking to bring their discoveries from the lab to the market through a startup, my first piece of advice is to be patient and understand that the path from science to market is long and challenging. Basic science doesn't always align with the pace and demands of the business world, so it's essential to have the right support, whether through incubators, accelerators, or mentors.

**Committee on Education - SAIC I ROUNDTABLE I Tuesday 19th November 9:00-10:30 - Sala de Cámara**  
**HOW TO ENGAGE STUDENTS IN BIOMEDICAL SCIENCE CAREERS "AND NOT DIE TRYING"**  
**Chair: Graciela Calabrese, Sandra Zárate**

#### EXPERIENTIAL DEVICES FOR CAREER OUTREACH, THE EXPERIENCE OF "VIVÍ LA UNER"

**Andrea María Teresita Carraza and Andrea Mercedes Suarez**

*Secretaría de Extensión Universitaria y Cultura, Facultad de Bromatología, Universidad Nacional de Entre Ríos.*

The dissemination of careers in Argentina is conducted through a variety of initiatives and programs implemented by universities and faculties. These endeavors aim to inform high school students about the available academic options and to facilitate their access to higher education.

In recent years, the National University of Entre Ríos (UNER) has undertaken an innovative project for the promotion of careers, encompassing the academic offerings of its nine faculties: Bromatology (Guaqueguaychú); Engineering and Agricultural Sciences (Oro Verde); Ed-

ucation Sciences, Social Work, and Economic Sciences (Paraná); Health Sciences (Concepción del Uruguay - Villaguay); Administration Sciences and Food Sciences (Concordia). This project, known as the Itinerant Fair "Viví la UNER," aims to introduce high school students and the general public to the diverse disciplines, careers, services and activities offered by the public and tuition-free university throughout the province, fostering curiosity and exploration among prospective students while providing information and guidance on the available academic pathways.

The Faculty of Bromatology actively participates in this initiative by offering various experiential and participatory activities to fair visitors. The event offers informative talks detailing professional practices by institution alumni, as well as a dedicated space led by faculty pedagogical advisors named "Contemplating My Life Project." Attendees are guided through the facilities, exploring approximately thirty discipline-specific stands featuring engaging ac-

tivities and practical experiences in classrooms, central courtyards and laboratories, showing the professional practices and academic content covered throughout the educational journey. Additionally, spaces are provided for student well-being, offering available services and support.

The fair aims to engage the local community, transforming public spaces into educational hubs for interaction. "Viví la UNER" serves not solely as an informative event but also fosters a meaningful connection between the university and educational communities, providing a platform for dialogue and academic exploration.

Over the course of three editions of this itinerant fair at the Faculty of Bromatology, the inaugural event in 2022 welcomed 29 high schools from the city and surrounding areas, with 700 attendees. This number increased in 2023 to 31 schools and 1000 visitors, and in 2024, 36 institutions participated with 1300 attendees.

### STRATEGIES IN SPREADING OF CAREERS OF THE FACULTY OF PHARMACY AND BIOCHEMISTRY OF UBA: OPENING DOORS TO MIDDLE SCHOOL

**Tamara Zaobornyj and Christian Höcht**

*Secretaría de Extensión Universitaria y Bienestar Estudiantil, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.*

Currently, the 3 undergraduate courses and the 3 technical courses taught at the Faculty of Pharmacy and Biochemistry-UBA (FFyB) have vacant positions in the working areas where they are offered. The Secretariat of University Extension and Student Welfare (SEU-BE-FFyB) aims to bring the careers of the Faculty closer to middle school students, generating creative spaces close to the new generations, to make these professions visible and increase the student enrollment of the faculty. We worked together with the Office of Student Guidance (DOE) of the Secretariat of Institutional Relations, Culture and Communication of the Rectorate of the University. First, a course on guiding dissemination on the careers and activities of this academic unit was designed to train undergraduate students in skills for the guiding dissemination, that is, the development of proposals aimed at enabling people to expand the information they have and participate in meaningful experiences about the different occupations, their tasks, and objectives. The planning and implementation of the proposal required the creation of an interdisciplinary teaching body that links the specific contents of dissemination, orientation, and communication; with those of the relevant careers. At the end of the training, the students become disseminators of the FFyB and participate in various activities that include: visits of secondary schools that comprise talks on careers as well as visits to different spaces of FFyB

with dissemination activities; similar events for students who spontaneously come to DOE and FFyB interested in the academic offerings of the Faculty; special days of FFyB such as the anniversary; meetings of new students of FFyB; profession exhibitions outside FFyB. Another relevant initiative was the establishment of a 2-week approach for middle school students at "Activities to approach the world of work, higher education and citizenship building" (ACAP) in alliance with the government of the City of Buenos Aires. The faculty created 10 different experiences that allowed students to go through different real-life situations in which graduates from FFyB may be involved. These activities engaged students to pursue higher education, approach the world of work and enrich their options for a life project. Although the impact of these actions on enrollment has not yet been evaluated 1 year after their implementation, it can be concluded that this experience resulted in multiple learnings. The FFyB students who participated in the proposal developed new skills for communication and planning. Moreover, the faculty achieved a collaborative construction of tools and strategies for guiding dissemination and the planning of proposals focused on group and experimental learning. Finally, the devices designed and put into practice open the possibility of multiple spaces and activities to actively publicize the academic offer and the scope of the careers of FFyB-UBA.

### STRATEGIES FOR THE PROMOTION OF SCIENTIFIC AND TECHNOLOGICAL CAREERS AT THE UNIVERSIDAD NACIONAL DE QUILMES

**María Belén Sabaini.**

*Observatorio en Enseñanza de la Ciencias Exactas y Naturales (OEACEN), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes.*

The promotion of scientific and technological careers at the Universidad Nacional de Quilmes (UNQ) is based on a combination of educational, communicative, and coordination strategies with the secondary level. Below, a series of experiences and projects will be presented that align with the institution's interest in strengthening the connection between different educational levels and reflect an institutional policy committed to ensuring the right to education and the continuity of higher studies.

One of the main initiatives is the organization of tours of the university. The objective of this activity focuses on allowing students to explore the facilities and imagine and experience the possibility of becoming part of the university community. These guided visits include stops at various research laboratories and teaching centers, serving as a key tool for students to observe the advancements and projects being carried out, thereby increasing their interest in these disciplines.

Furthermore, since 2018, the EXPO-UNQ has been held: an annual event showcasing the different degree programs offered by the university, with a particular emphasis on those related to science and technology. During this exhibition, prospective students can participate in talks, learn about the curricula, interact with faculty and advanced students, and explore the innovative projects being developed in various academic units. Additionally, in continuity with these initiatives, participation in other similar events, workshops, and exhibitions organized at

our institution takes place. These participations represent a valuable opportunity to interact with students and educators from other institutions in the province of Buenos Aires, enabling the sharing of proposals and educational experiences while promoting the creation of inter-institutional collaboration networks.

Another key aspect is the organization of practical workshops in the UNQ laboratories. In these workshops, high school students have the opportunity to experience firsthand how work is conducted in areas such as biotechnology, bioinformatics, and engineering. These workshops not only allow young people to become familiar with the tools and methodologies used in scientific research but also provide a space for solving problems and developing practical skills, emphasizing how these areas contribute to addressing social, environmental, and economic issues, among others.

Finally, the Departamento de Ciencia y Tecnología has the Programa de extensión Vinculando a la Biotecnología con la Sociedad (ViBioS). This program consists of three projects: 1) Ciencia en De(C)construcción; 2) Compostando y Cultivando ConCiencia; and 3) Habitar con Salud. Through these initiatives, students can apply their knowledge to solving local issues, reinforcing the idea that science and technology careers are not only fundamental for academic development but also for generating social impact in the region and democratizing access to knowledge.

**SAIC/SAFIS I ROUNDTABLE I Thursday 21 November 17:00-18:30 h – Anexo (streaming)**  
**RESOURCES TO OVERCOME THE FUNDING CRISIS IN THE LOCAL SCIENTIFIC SYSTEM**  
**Chair: Bruno Berardino**

**COLLABORATIONS IN SCIENCE: HOW TO GET THE MOST OUT OF A HALF-FULL GLASS**

**Graciela Díaz-Torga**

*Laboratorio de Fisiopatología Hormonal. Instituto de Biología y Medicina Experimental (IBYME-CONICET)*

For many years our governments have been allocating a percentage of GDP (Gross Domestic Product) to Argentine science, much lower than that of other countries where we compete in our research. Our national grants have been completely devalued for many years. But, this year, we are experiencing the worst crisis in science and

technology registered in our country. We are not even receiving the quotas of the grants already granted. How can we survive such a tragedy? How much longer will we be helpless? In this round table, I will present various strategies I have used to survive in science, in Argentina, with little money in a competitive world.

**HERRAMIENTAS DE VINCULACIÓN TECNOLÓGICA**

**Dr. Luis Acosta**

*Gerencia de Vinculación Tecnológica - Coordinación de Salud Alimentos y Biotecnología, CONICET.*

Technology transfer is a fundamental process to boost innovation, development and the economic growth in a country. Understanding the various tools and mechanisms that facilitate the transformation of scientific and technological knowledge into new products and commercial services is necessary to ensure the success of the transfer. For this purpose, we will focus on the High Level Technology Services (STANs), which involves standardised activities such as testing,, analysis, advis-

ing and institutional consultancy. Additionally, we will discuss other tools such as Technical Assistance (TA) and Research and Development (R&D) agreements, and the advantages of establishing a contract with CONICET. Finally, we will present examples of licensing and commercialisation agreements while discussing key aspects of them and its applications.

Through concrete examples, we will show the scope of these tools by discussing how they can help the transfer

processes, regulate important aspects of R&D projects, and reduce the risks associated with sharing confidential data that can lead to intellectual property rights. Suc-

cessful cases will be presented that illustrate how these collaborations can generate mutual benefits and contribute to the development of new economic sectors.

## SCIENCE AND INDUSTRY IN ARGENTINA: TURNED THEIR BACKS ON EACH OTHER

**Dr. Rodrigo Ramele**

*Instituto Tecnológico de Buenos Aires (ITBA). Buenos Aires, Argentina. Cofundador y director de Ingeniería de NeuroAcoustics Inc.*

Argentina has a solid foothold in science which was grounded on a rich university ecosystem. Many reforms during the twentieth century shaped the academic structure of the country towards a very distinctive and singular organization. Success of those movements were crowned by three Nobel prizes in physiology, medicine and chemistry, something quite unique in the Latin American region.

However, this very particular science production system didn't manage to establish itself as the substrate of corporate and industrial innovation. Argentina never managed to perform a successful industrialization.

How was it possible that a very successful scientific system was unable to be used to drive innovation and create wealth? We can highlight two reasons and speculate that they have a cultural origin.

First, the twentieth century reforms that precisely shaped the system, had in its origin influences from socialists and anarchists' immigrants that fled from Europe. Hence, it always had a seed of anti-commercialization ideas that lasted to this day.

Second, industrialization attempts were driven mostly as import substitution and the lack of stable state policies in this regard, swinging policies like a pendulum, triggered a risk-averse cultural behaviour that always prioritized short term gains and basic strategies to survive. Importing technology already developed by the Global North was safer.

In concrete, the proposal stated here aims to consider the problem from this vantage point and aims to attack it bottom-up.

Argentine scientists need to break the cultural tie that forbids to push scientific discoveries towards business opportunities. First, they need to connect with businessmen, with CEOs, with investment funds in all their forms, like VC (Venture Capitalists), Angel Investors, FRO (Focused Research Organizations), SME (Small-Medium Enterprise) and startup entrepreneurs. Why not sharing their fancy meetings or inviting them to formal scientific conferences? They need to go out, to knock doors and purposely try to establish those links. In the end, scientists aim for impact and creating a product that can change people's life has a lot of it.

On the other side of the equation, CEOs need to understand that in today's heavily competitive environment in the international arena the only way in which they can have an edge that drives market share capture is to develop new ideas. This can only come from the development of scientific discoveries, that are transformed into technological feats.

Perhaps current science crisis can be considered as an opportunity as well and push us to break these cultural ties that exists on both sides of the equation and ask the science community and the corporate ecosystem, to turn around, face each other, and establish a more productive win-win relationship.

**ROUNDTABLE on Science Policy Thursday 21 November 18:30 - 19:30 h - Auditorium**  
**Chair: Graciela Cremaschi, Rodolfo Rey**

**Valeria Levi**

*Coordinadora General de RAICYT, Vicedecana de la Facultad de Ciencias Exactas y Naturales de la UBA, Investigadora Principal de CONICET en IQUIBICEN, CABA.*

**Nicolás Rendtorff**

*Secretario de Ciencia y Técnica de la UNLP, Investigador Principal CONICET en CETMIC, La Plata.*

**Jorge Aliaga**

*Miembro del Directorio de CONICET por el Consejo de Universidades, Investigador Principal de CONICET, Secretario de Planeamiento y Evaluación Institucional de la Universidad Nacional de Hurlingham.*

**WORKSHOP SAFIS I - Roundtable on Education in Physiology.**

*Tuesday 19 th November 9:00 - 11:00 - Anexo (streaming)*

**Chair: Sebastian Caffera**

TEACHING AND LEARNING PHYSIOLOGY IN DIFFERENT CONTEXTS  
PRESENTATION OF EXPERIENCES

**Irene Ennis**

*Profesora Titular de Fisiología y Física Biológica de la Facultad de Medicina de la Universidad de La Plata.*

**Flavio Francini**

*Profesor Titular de Biología de la Facultad de Medicina de la Universidad de La Plata.*

**Emiliano Diez**

*Profesor Adjunto Efectivo del Instituto de Fisiología de la Facultad de Ciencias Médicas de la Universidad Nacional de Cuyo.*

**ABSTRACT N/A**

**WORKSHOP SAFIS II.**

*Wednesday 20th November 11:30 - 11:50 - Anexo (streaming)*

**Chair: Alicia Mattiazzi**

PUBLISHING FOR BEGINNERS: A WORKSHOP FOR EARLY-CAREER RESEARCHERS.

**Kim Barret**

*Editor in Chief, The Journal of Physiology, and Luis Sobrevía; Regional Editor - Central and South America,  
The Journal of Physiology.*

**ABSTRACT N/A**

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**SAIC AWARD - Fundación BIGAND - Multidisciplinary call for young investigators.***Thursday 21th November 8:30-10:00-Auditorium***Jury: Adali Pecci, Claudia Gonzalez Deniselle, Alejandro Curino****GENETIC MYOPATHIES MODELING USING HUMAN INDUCED PLURIPOTENT STEM CELLS AND THEIR DIFFERENTIATION INTO THE MUSCLE LINEAGE****Lucia Moro, Guadalupe Amin, Sheila Castañeda, Federico Zabalegui, Joaquin Smucler, Denisse Saulnier, Agustina Scarafía, Julia Halek, Mateo Lacava, Sol Renes, Gustavo Sevlever, Ariel Waisman, Santiago Miriuka***Laboratorio de Investigación Aplicada a Neurociencias (LIAN), Instituto de Neurociencias (INEU-CONICET), Fundación para la Lucha contra las Enfermedades Neurológicas de la Infancia (FLENI), Belén de Escobar, Buenos Aires, Argentina.*

Each year, around 8 million people are born with genetic diseases that currently have no cure. The aim of this study is to investigate genetic myopathies by differentiating human induced pluripotent stem cells (iPSCs) that carry different pathological mutations into cardiomyocytes (CMs) and skeletal myocytes. These studies will allow the identification of therapeutic targets and drug discovery. We have modeled the arrhythmogenic dysplasia (AD, characterized by the replacement of CMs with fibroadipose tissue in the heart) by generating edited iPSCs with specific mutations in the generally involved genes PKP2 and PKG. We are also studying two muscular dystrophies (MD) associated with mutations in the FHL1 or DES genes after generating patient-derived iPSCs. In AD, CMs derived from these edited iPSCs showed alterations in desmosome junctions and increased lipid accumulation compared to controls. In the DES associated MD, the DES variant studied (c.1059\_1061dupGGA) was first described by our group. We investigated

the clinical presentation of patients, consistent with myofibrillar myopathy, we generated iPSC lines from three siblings, and determined that the mutation likely affects DES filament polymerization based on bioinformatics modeling. Functional studies in HEK cells and C2C12 myocytes revealed the formation of protein aggregates in the presence of the mutation. Moreover, CMs differentiated from the patient iPSCs showed lower proportion of DES+ CMs and disorganized DES distribution compared to control. To study the pathogenic effects of FHL1 and DES in skeletal muscle, we developed a differentiation protocol using wild-type iPSCs, where we obtained DES and myosin heavy chain positive cells (muscle specific markers), after 45 days of differentiation. We are currently differentiating patient iPSCs into this lineage. In summary, we have developed efficient strategies for genetic disease modelling in addition to obtaining relevant information for their comprehension.

**DEVELOPMENT OF IVERMECTIN AS AN IMMUNOPOTENTIATING AND COADJUVANT THERAPEUTIC STRATEGY IN COMBINATION WITH  $\alpha$ -PD-1 IMMUNOTHERAPY FOR THE MANAGEMENT OF MISMATCH REPAIR-PROFICIENT COLORECTAL TUMORS****Candela Llavana<sup>1,2</sup>, Luisina Solernó<sup>1,2</sup>, Valeria I. Segatori<sup>1,2,3</sup>, Martín Ledesma<sup>1,4</sup>, Melina Cohen<sup>5,6</sup>, Maximiliano R. Ferrero<sup>5,6</sup>, Florencia Gottardo<sup>1,2,3</sup>, Carla Capobianco<sup>1,2</sup>, Giselle A. Martínez<sup>1,2</sup>, Daniela L. Papademetrio<sup>7,8</sup>, Karina Yonamine<sup>1,9</sup>, Cecilia Curvale<sup>1,9</sup>, Julieta Saenz<sup>1,10</sup>, Esteban Vogel<sup>1,10</sup>, María Laura Luján<sup>1,11</sup>, Lisandro Vimo<sup>11</sup>, Viviana Tassi<sup>1,10</sup>, Juan Iovanna<sup>12,13</sup>, Raúl Matanó<sup>1,9</sup>, Daniel F. Alonso<sup>1,2,3</sup>, Juan Garona<sup>1,2,3</sup>.**<sup>1</sup>*Biomedical Cancer Research Unit (IBioCAN), Center of Translational Medicine (CEMET, Unit N°6), El Cruce "Néstor Kirchner" High Complexity Hospital (HEC), Buenos Aires, Argentina.*<sup>2</sup>*Center for Molecular and Translational Oncology (COMTra), Science and Technology Department, National University of Quilmes, Buenos Aires, Argentina.*<sup>3</sup>*National Council of Scientific and Technical Research (CONICET), Buenos Aires, Argentina.*<sup>4</sup>*Genomic Unit, Center of Translational Medicine (CEMET, Unit N°6), El Cruce "Néstor Kirchner" High Complexity Hospital (HEC), Buenos Aires, Argentina*<sup>5</sup>*Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany*<sup>6</sup>*Biomedicine Research Institute of Buenos Aires (IBioBA), Buenos Aires, Argentina*<sup>7</sup>*Institute for the Study of Humoral Immunity (UBA-CONICET), Pharmacy and Biochemistry School, University of Buenos Aires.*<sup>8</sup>*Translational Research Unit, High Complexity Bicentenario Hospital, Esteban Echeverría, Buenos Aires, Argentina.*<sup>9</sup>*Gastroenterology Unit, El Cruce "Néstor Kirchner" High Complexity Hospital (HEC), Buenos Aires, Argentina*<sup>10</sup>*Pathology Unit, El Cruce "Néstor Kirchner" High Complexity Hospital (HEC), Buenos Aires, Argentina*<sup>11</sup>*Oncology Unit, El Cruce "Néstor Kirchner" High Complexity Hospital (HEC), Buenos Aires, Argentina*<sup>12</sup>*Cancer Research Center at Marseille (CRCM), INSERM, Marseille University and Institut Paoli-Calmettes Institute,*

Marseille, France.

<sup>13</sup>El Cruce "Néstor Kirchner" High Complexity Hospital (HEC), Buenos Aires, Argentina

Colorectal cancer (CRC) represents a public health challenge considering its prevalence and mortality. The use of immune checkpoint inhibitors (ICi) has been a paradigm shift in its management. However only a small fraction of patients bearing immunologically "hot" and DNA mismatch repair (MMR) deficient tumors respond favorably to such therapies.

In this context, and considering its antitumor effects, reversal of drug resistance and impact on immune function, the aim of this project was to assess the repurposing of the antiparasitic agent ivermectin (IVM) as an immunopotentiating strategy to improve the therapeutic response to ICi in CRC.

A large panel of *in vitro*, molecular and cellular, *in silico* and bioinformatic, and *in vivo* methodologies were used on cold MMR proficient CRC models with limited response to chemo- and immunotherapy.

Using pharmacologically relevant concentrations, IVM impaired aggressive traits in tumor cells, enhanced response to chemo and induced immunogenic cell death

(ICD), characterized by the release of inflammatory damage-associated molecular patterns. IVM treatment was also associated with modulation of PD-L1 expression and extracellular levels of lactate, IL2 and IL17, all key immune regulators of the tumor microenvironment (TME). Immunization using IVM heavily-treated CRC cells led to a robust protection against tumor and metastatic outgrowth, confirming ICD-induction *in vivo*. Moreover, treatment with IVM significantly enhanced CD4<sup>+</sup> and CD8<sup>+</sup> T cell infiltration, helping convert immunologically cold tumors hot. Finally, addition of IVM (5 mg/kg) to  $\alpha$ -PD-1 (10 mg/kg) resulted in a cooperative and robust inhibition of CRC primary growth and metastatic disease ( $p < 0.05$ ).

Through a dual antineoplastic and immune-enhancing mechanism, IVM is postulated as a safe and cost-effective adjuvant agent to be administered concomitantly with  $\alpha$ -PD-1 immunotherapy, in order to improve its therapeutic action in cold and low-immunoreactive CRC tumors.

#### UNRAVELING THE MECHANISMS OF ALCOHOL HANGOVER: THE ROLE OF ACETALDEHYDE AND THE POSSIBLE PROTECTIVE ACTION OF N-ACETYL CYSTEINE.

**Analia Karadayian<sup>1</sup>, Lautaro Carrere<sup>1</sup>, Czerniczyniec Analia<sup>2</sup>, Lores-Arnaiz S<sup>1</sup>**

<sup>1</sup>Instituto de Bioquímica y Medicina Molecular Profesor Alberto Boveris (UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina. <sup>2</sup>Laboratorio de Investigación Aplicada a Neurociencias, Instituto de Neurociencias, Fundación Para La Lucha Contra Las Enfermedades Neurológicas de La Infancia (LIAN-INEU, Fleni-CONICET), Provincia de Buenos Aires, Argentina.

Ethanol induces mitochondrial dysfunction and oxidative stress in brain cortex. Alcohol hangover (AH) involves physical and mental symptoms when blood alcohol concentration (BAC) approaches zero. Acetaldehyde, the byproduct of ethanol metabolism, was postulated as a primary contributor to AH-induced mitochondrial dysfunction, particularly at brain cortex synapses. We aimed to evaluate the possible role of acetaldehyde in AH synaptic damage and the potential beneficial effect of N-acetylcysteine (NAC). Male Swiss mice were used in two different experimental approaches depending on the use of 4-methylpyrazole (4-MP, an ADH

inhibitor) or NAC. Animals were divided into four groups: saline (control), 4-MP (10 mg/kg) or NAC (500 mg/kg), ethanol (3.8 g/kg, AH group), and 4-MP/ethanol or NAC/ethanol. Animals were sacrificed after 6 hours (BAC=0), and brain cortex synaptosomes were isolated. Results showed that reducing acetaldehyde levels significantly restored mitochondrial respiration, ATP synthesis, and

coupling efficiency compared to the AH group ( $p < 0.05$ ). Treatment with 4-MP fully prevented the reduction in enzymatic activity of mitochondrial complex I-III caused by AH, while the activity of complex IV only partially recovered, remaining 50% lower than in the control group ( $p < 0.05$ ). 4-MP fully restored ATP production and mitochondrial membrane potential, which were reduced by 41% and 48% in the AH group, respectively ( $p < 0.05$ ). However, 4-MP intervention did not affect nitric oxide metabolism, suggesting that residual ethanol plays a role after ADH inhibition. NAC treatment significantly prevented impairments in oxygen uptake, mitochondrial membrane potential, ATP production, and enzymatic activity of the mitochondrial respiratory complexes due to AH ( $p < 0.05$ ). These findings underscore acetaldehyde as a key factor in the development of AH and demonstrate NAC's potential to counteract AH pathology representing a promising strategy for mitigating its harmful effects.

**SAFIS AWARD - "Camillon de Hurtado" Award (SAFIS).***Thursday 21th November 8:30 - 10:00 - Anexo (Streaming)***Jury: Gerardo Garcia Rivas; Carolina Caniffi, Germán E. González****TRPV4 CHANNELS ENHANCE HYPOOSMOTIC STRESS-INDUCED INOTROPIC RESPONSE IN SPONTANEOUSLY HYPERTENSIVE RATS AND CONTRIBUTE TO THE SLOW FORCE RESPONSE****Racioppi MF<sup>1</sup>, Pons L<sup>1</sup>, Díaz RG<sup>1</sup>, Perez NG<sup>1</sup>, Gonano LA<sup>1</sup> y Vila Petroff M<sup>1</sup>.**<sup>1</sup>*Centro de investigaciones cardiovasculares Horacio Cingolani, CONICET-UNLP.*

**Introduction:** Hypoosmotic stimulation produces a transient positive inotropic effect (PIE) associated with an increase in intracellular Ca<sup>2+</sup>. However, the underlying mechanisms remain elusive. The Transient Receptor Vanilloid 4 channel (TRPV4) responds to osmotic and mechanical stimuli promoting Ca<sup>2+</sup> entry and could contribute to the hypotonic swelling-induced PIE. The role of TRPV4 has not been studied in spontaneously hypertensive rat (SHR) hearts.

**Objectives:** To determine if TRPV4 contribute to swelling-induced PIE in Wistar rats and if this response is enhanced in SHR. To examine the potential mechanisms involved in TRPV4 activation and if they are also activated by cardiac stretch.

**Methods:** Cardiomyocytes were isolated from 6-10 month old Wistar and SHR rats. Contractility was assessed in HEPES isotonic solution (IS; 300 mOsm) in hypotonic solution (HS; 217 mOsm). Cell shortening was measured by video edge-detection (Ionoptix). Ca<sup>2+</sup>-dependent contractile response to stretch, the slow force

response (SFR), was examined in isolated papillary muscles from SHR hearts stretched from 92 to 98% of their maximal length.

**Results:** While TRPV4 inhibition with GSK2193874 (300 nM) didn't affect the HS-induced PIE in Wistar myocytes, it was significantly reduced in SHR ( $p \leq 0.05$  Anova). GSK2193874 also blunted the SFR ( $p \leq 0.05$  Anova). To examine the role of caveolae and microtubules in transmitting mechanical cues that activate TRPV4 in HS, myocytes were treated with 5 mM methyl- $\beta$ -cyclodextrin to disrupt caveolae, or with 10  $\mu$ M Colchicine, to inhibit microtubule polymerization. Both these strategies prevented the HS-induced PIE in SHR myocytes ( $p \leq 0.05$  t-Test).

**Conclusion:** TRPV4 do not contribute to the HS-induced PIE in Wistar rats but provide additional Ca<sup>2+</sup> entry that amplifies this response in SHR. In SHR TRPV4 can also be activated by cardiac stretch contributing to the SFR. Intact caveolae and microtubule integrity are required for TRPV4 activation in SHR myocytes.

**MOLECULAR BASIS OF LONG-TERM CARDIAC FUNCTION IMPROVEMENT FOLLOWING AT-MUSE ALLOTRANSPLANTATION IN AN OVINE MODEL OF ACUTE MYOCARDIAL INFARCTION****Cristian Nuñez Pedrozo<sup>1</sup>, Agustina Varela<sup>1</sup>, Francisco Borzone<sup>1</sup>, Pablo Cutine<sup>2</sup>, Gustavo Giunta<sup>2</sup>, Tomas Peralta<sup>1</sup>, Eduardo Guevara<sup>2</sup>, Miguel Cerdá<sup>2</sup>, Mario Embón<sup>2</sup>, Alberto Crottogini<sup>1</sup>, Luis Cuniberti<sup>1</sup>**<sup>1</sup>*Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMETTyB-Universidad Favaloro-CONICET)*<sup>2</sup>*Hospital Universitario Universidad Favaloro***Background and Aims**

Acute myocardial infarction (AMI) remains a leading cause of mortality worldwide. In a recent long-term study, we reported that intramyocardial administration of adipose tissue (AT)-derived Muse cells after AMI improved left ventricular function, regardless of non-effect on infarct size. The aim of this study was to elucidate the long-term beneficial effects of AT-Muse allotransplantation.

**Methods**

AT-Muse cells were isolated from donor sheep. AMI was induced by permanent ligation of the left anterior descending coronary artery. Sheep were randomized in two groups: 9 animals received 10 intramyocardial injections of 2x10<sup>7</sup> AT-Muse cells PKH26+ (AT-Muse group) and 8 received PBS (Vehicle group). Sheep were euthanized 35 days post-AMI and histological samples of the heart and remote organs were analyzed via histological staining, immunohistochemistry and immunofluorescence.

**Results**

PKH26+ cells were detected in the heart (n=3), periph-

eral blood (n=4), and remote organs (n=2) at 35 days post-treatment, although no evidence of tumorigenesis was observed in any of these tissues. Based on the improvement in left ventricular function, reduced thinning of the infarcted anterior wall, and thickening of the septal wall, we observed a reduction in cardiomyocyte area within the septal wall and a significant increase in arteriolar density in the infarct border zone ( $p < 0.05$ , Student's t-test). Additionally, no change was observed in the degree of interstitial fibrosis between treatments ( $p > 0.05$ , Mann-Whitney U test). Finally, less than 20% of AT-Muse cells exhibited partial differentiation into cardiac and vascular lineages during long-term follow-up.

**Conclusion**

AT-Muse cell transplantation had a high survival rate and proved to be safe in the chronic phase of AMI. The improvement in cardiac function could be due to a positive paracrine modulation limiting septal cardiomyocyte hypertrophy and inducing angio- and arteriogenesis of the area surrounding the infarct.



**ALACF AWARD.***Thursday 21th November 8:30 - 10:00 - Anexo (Streaming)***Jury: Daniel Vigo, Graciela Cremaschi, Germán E. González****MITOCHONDRIAL CA<sup>2+</sup> OVERLOAD IS AN EARLY RISK FACTOR FOR LETHAL VENTRICULAR ARRHYTHMIAS BY IMPAIRING BIONEGETICS AND SUPERCOMPLEX ASSEMBLY****Felipe de Jesús Salazar-Ramírez<sup>1</sup>, Judith Bernal-Ramírez<sup>1</sup>, Luis Luévano-Martínez<sup>1</sup>, Christian Silva-Platas<sup>1</sup>, Abraham Méndez-Fernández<sup>1</sup>, José Velasco-Bolom<sup>2</sup>, Flavio Contreras-Torres<sup>2</sup>, Gerardo García-Rivas<sup>1\*</sup>**<sup>1</sup>*Cátedra de Cardiología y Medicina Vasculard, Escuela Nacional de Medicina, Tecnológico de Monterrey. Hospital Zambrano-Helion, San Pedro Garza-García, N.L., México 66278,*<sup>2</sup>*Tecnológico de Monterrey, Escuela de Ingeniería y Ciencias, Monterrey, NL, 64849, Mexico**E-mail: gdejesus@tec.mx*

A leading cause of death within the cardiovascular patient population is ventricular arrhythmias, which are associated with elevated catecholamine levels. Since mitochondrial calcium (Ca<sup>2+</sup>) transport is necessary to elicit an adrenergic response and constant stimulation leads to mitochondrial Ca<sup>2+</sup> overload and dysfunction in cardiac tissue, we evaluated its role and the effect of its modulation in arrhythmogenesis.

The objective was to evaluate the effects of mitochondrial Ca<sup>2+</sup> transport modulation in ventricular arrhythmogenesis induced by catecholamine overload.

12-15 week-old C57bl/6 male mice were administered intravenously with either Ru360, a mitochondrial Ca<sup>2+</sup> transport inhibitor, or normal saline. A baseline ECG was recorded, after which Isoproterenol (ISO, 400mg/kg) was given subcutaneously, and ECG recording was kept for another 20 minutes. Afterward, cardiomyocytes and mitochondria were isolated for characterization studies.

ISO administration caused ventricular tachycardia and

fibrillation, while pretreatment with Ru360 prevented ventricular arrhythmias. ISO-treated heart mitochondria had a higher Ca<sup>2+</sup> content, indicating overload, and compromised function and membrane integrity; as well as increased peroxide production, electron leak and ROS-driven post-translational modifications and erratic cellular Ca<sup>2+</sup> dynamics. This impaired mitochondrial function correlates with decreased supercomplex activity and assembly, which Ru360 pretreatment prevented as well as all other previously mentioned changes found.

Mitochondrial Ca<sup>2+</sup> overload promotes arrhythmias by disrupting supercomplex activity and assembly, which in turn induces mitochondrial dysfunction and increases oxidative stress. This impairs cellular Ca<sup>2+</sup> dynamics and enables the appearance of anomalous activity. Mitochondrial Ca<sup>2+</sup> transport modulation proved to be an effective tactic to prevent arrhythmias, which points towards a potential new target for the development of new anti-arrhythmic therapies.

**SILDENAFIL REDUCES T-TUBULE DISORGANIZATION AND ALTERS ALTERNANS SUSCEPTIBILITY IN HYPERTROPHIC HEARTS OF SPONTANEOUSLY HYPERTENSIVE RATS****Margarita Rodríguez, Javier Torres, Cecilia Mundiña-Weilenmann, Matilde Said***Centro de Investigaciones Cardiovasculares, CCT-La Plata, CONICET, Facultad de Ciencias Médicas, UNLP*

In a recent study we demonstrated that hypertrophic hearts from spontaneously hypertensive rats (SHR) exhibit increased susceptibility to alternans. Alternans is a beat-to-beat oscillation in the duration of action potential, the amplitude of Ca transient and the strength of contraction, and often precedes severe ventricular arrhythmias. We found that the early onset of Ca alternans of SHR hearts was associated to a delay in the recovery of Ca release from the sarcoplasmic reticulum and a reduction in the T-tubule periodicity. Sildenafil (SIL), a phosphodiesterase 5 inhibitor, has been reported to preclude T-Tubule disorganization and correct Ca mishandling in different animal models of heart failure. The purpose of this study was to test whether SIL mitigated the structural change in the T-tubule system and reversed the increase vulnerability to alternans of SHR. SHR rats were treated daily with SIL (100mg/kg/day) in drinking acidic water or placebo for 3 months. Arterial blood pressure and echocardiographic parameters were measured before and af-

ter treatment. Ca dynamics and alternans were studied in isolated myocytes loaded with a Ca fluorescent indicator. SIL was able to significantly decrease systolic blood pressure (185±7 to 162±6mmHg, n=5-7) and hypertrophy (left ventricular mass:SHR:1161±28, SIL:1021±46 mg, n=5-7). Myocytes from SIL-treated animals began to alternate at a higher stimulation frequency than myocytes from non-treated rats (frequency threshold:5.8±0.2 vs 4.4±0.2Hz, n=20 cells from 5 hearts in each group, p<0.05). This effect was associated to an acceleration in the recovery of SR Ca release and an increase in T-Tubule integrity, measured by TT-power (SHR:55.1±0.7, SIL:58.6±0.7ua, p<0.05). The overall results demonstrated beneficial effects of SIL treatment in preventing T-tubule remodeling and delaying the early onset of alternans in SHR hearts, highlighting a novel mechanism underlying the therapeutic benefits of SIL in hypertensive hypertrophy.

**CROSSTALK BETWEEN  $\beta$ 1 ADRENERGIC SIGNALING, COAGULATION AND ORGAN FUNCTION IN ANIMAL MODELS OF SEPSIS: ADVANCES FROM A CLINICAL-BASED APPROACH**  
**Yolanda Prado<sup>1,2</sup>, FM Llancahuen<sup>1,2</sup>, Cristóbal Aravena<sup>1,2</sup>, Cristian Reyes<sup>3</sup>, Carmen-Gloria Feijoo<sup>3</sup>, Christian Hidalgo<sup>4</sup>, Felipe Simon<sup>1,2</sup>**

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**Introduction:** Sepsis is the organ dysfunction triggered by the dysregulated host response to an infection. The presence of coagulation abnormalities and its subsequent progression to disseminated intravascular coagulation (DIC) constitutes a significant risk for organ failure and mortality in septic patients. Although elevated plasma catecholamine levels during sepsis correlated with unfavorable outcomes, there is a lack of studies addressing the involvement of  $\beta$ -Adrenergic receptors ( $\beta$ -AR) subtypes in coagulation alterations during sepsis.

**Objective:** The aim is evaluating the contribution of  $\beta$ -AR in sepsis-induced coagulopathy. **Methods:** Male SD rats aged 8 weeks were injected with bisoprolol ( $\beta$ 1-AR antagonist) or butaxamine ( $\beta$ 2-AR antagonist) at 5 mg/kg and 10 mg/kg respectively, or saline (n=3). After 1 h, animals were infused with endotoxin (LPS O55:B8, 30 mg/kg, 300  $\mu$ L/h) or saline. After 3 h, blood and organs were collected for coagulation, biochemical and histological

analysis. Additionally, wildtype or transgenic (Tg(fli1:eGFP)y1 (fli1:eGFP)) zebrafish larvae knockout for  $\beta$ 1-AR were treated with endotoxin (20 nL) or saline to evaluate thrombus formation. Results are presented as mean  $\pm$ SD. Differences were assessed by two-ways ANOVA and Tukey's post-hoc test. Experimental protocols were approved by the Bioethics and Biosafety Committee of the Universidad Andrés Bello. **Results:** Endotoxin-treated rats exhibited alteration in DIC-related parameters and organ dysfunction.  $\beta$ 1-AR inhibition prevented these alterations. In zebrafish larvae,  $\beta$ 1-AR knockout prevented thrombus formation and disturbances in platelet flow. **Conclusions:** The  $\beta$ 1-AR stimulation modulate DIC-related parameters in endotoxemic rats and thrombus formation in zebrafish larvae.  $\beta$ 1-AR stimulation could be considered as target to reduce coagulation alterations and organ dysfunction septic patients.

**LEAP2 ANOREXIGENIC EFFECTS PERSIST IN A DIET-INDUCED OBESITY MOUSE MODEL**

**Lucia Giovanini<sup>1</sup>, María Paula Cornejo<sup>1</sup>, Florencia Heredia<sup>1</sup>, Daniel Castrogiovanni<sup>1</sup>, Mario Perello<sup>1</sup>**

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Liver-expressed antimicrobial peptide 2 (LEAP2) is a newly discovered endogenous ligand of the growth hormone secretagogue receptor (GHSR), which is mainly expressed in the brain and is strongly implicated in the regulation of energy balance. In humans and rodents, LEAP2 is mainly produced in the jejunum and liver, and its plasma levels increase in mice and in patients with obesity, presumably as a part of an adaptive response to counteract energy surplus. Here, we aim to test the hypothesis that mice with diet-induced obesity (DIO) develop resistance to LEAP2 like other known hormones such as insulin or leptin. We used male and female wild type mice that were fed with either regular chow (RC mice) or high fat diet (HFD mice) during 20 weeks. HFD mice developed overweight (Unpaired t-test, n=8-10 per group, p<0,005), elevated plasma levels of glucose (2-way ANOVA, Sidak's test n=8-10 per group, p<0,005) and LEAP2 and did not respond to the orexigenic effects

of ghrelin. We performed RT-qPCR to quantify LEAP2 mRNA levels in the jejunum, liver and hypothalamus, and found that LEAP2 mRNA levels increased in jejunum (Unpaired t-test, n=5 per group, p<0,005), but not the liver, of mice. LEAP2 mRNA was not found in the hypothalamus of RC or HFD animals. We also measured GHSR expression in the hypothalamus and found no differences between RC or HFD mice. Moreover, we performed an anti-LEAP2 immunostaining in the jejunum and observed a higher number of LEAP2 positive cells in HFD mice. Finally, we found that HFD mice responded to the anorexigenic and body-weight reducing effects of centrally-administered LEAP2. Altogether, our results suggest that LEAP2 levels are elevated in our DIO model and that these animals continue to respond to exogenous LEAP2, highlighting the potential of this peptide to treat individuals with obesity.

**SAIC AWARD - Fundación Gador - Metabolic syndrome and related disorders.**

Thursday 21th November 8:30 - 10:30 - Sala de Cámara

**Jury: Victoria Lux-Lantos, Guillermo Juvenal, Basilio Kotsias**

**THE HEPATOPROTECTIVE EFFECT OF HEMIN IS ASSOCIATED WITH AN IMPROVEMENT IN MITOCHONDRIAL DYSFUNCTION AND LIPID OXIDATION IN A STEATOHEPATITIS MODEL**

**Morena Wiszniewski<sup>1,2</sup>, Malena Gromez<sup>1</sup>, Diego Mori<sup>1</sup>, Cora Beatriz Cymeryng<sup>1,3</sup>, Esteban Martin Repetto<sup>1,4</sup>.**

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<sup>3</sup>Universidad de Buenos Aires. Facultad de Medicina. Departamento de Bioquímica Humana. Cátedra de Bioquímica Humana I. Buenos Aires, Argentina

<sup>4</sup>Universidad Nacional de Entre Ríos, Facultad de Bromatología. Entre Ríos, Argentina Km. 7, B8000FWB Bahía Blanca, Argentina. \* both authors contributed equally to this work.

We have previously characterized steatohepatitis in rats fed sucrose 30% in the drinking water, and shown that hemin, an heme oxygenase-1 (HO-1) inducer, mitigates hepatic damage by reducing inflammatory injury, oxidative and endoplasmic reticulum stress. Considering the role of mitochondria dysfunction in the metabolic dysfunction associated steatotic liver disease, this study aimed to assess the effect of hemin on mitochondrial well-being and its association with liver lipid metabolism. Male Wistar rats were fed a control diet with tap water (C, n=12) or 30% sucrose water (SRD, n=12) for 10 weeks. SRD rats were then split into two groups: one received hemin (SRD+H, 15mg/kg/48h i.p., n=12) and the other vehicle. Treatments continued for two more weeks.

Liver from SRD fed rats showed a greater number of mitochondria (by transmission electron microscopy) ( $p < 0.05$  vs. C) and a reduction in the percentage of small mitochondria at expense of intermediate size (by confocal microscopy). This effect was not observed after HO-1

induction ( $p < 0.05$  vs. SRD). Studies on mitochondrial dynamics show that SRD induces fusion protein OPA1, which expression was attenuated in the SRD+H group ( $p < 0.05$  vs. C;  $p < 0.01$  vs. SRD), with no changes in the fission marker DRP1 between groups. Although the mitophagy marker parkin was elevated in both SRD groups ( $p < 0.05$  vs. C); p62, LC3-II and LAMP1 levels suggest an impaired autophagy in SRD-treated rats that was recovered by hemin treatment ( $p < 0.05$  and  $p < 0.001$ ). Hemin also induced the expression of mitochondrial beta oxidation markers, phosphorylation of AMPK and nuclear translocation of PGC1a ( $p < 0.01$  vs. C or SRD). An increased mitochondria-lipid vacuole association was also detected in the SRD+H group ( $p < 0.01$  vs. C and SRD). Our findings indicate that hemin restores the autophagic flux, facilitating mitochondrial clearance via mitophagy and is associated with an improvement in fatty acids oxidation.

#### IDENTIFICATION OF BIOMARKERS BASED ON METAGENOMIC STUDIES OF THE GUT MICROBIOTA IN CHRONIC METABOLIC DISEASES

**Andrea L. Millán<sup>1</sup>, María C. Pautasso<sup>1</sup>, María del R. Taussig<sup>2</sup>, Mailén Rojo<sup>1</sup>, Hernán Pérez<sup>1</sup>, Alejandra Duarte<sup>1</sup>, Ignacio Cassol<sup>2</sup>, Ariel P. López<sup>1</sup>, Juan P. Bustamante<sup>2,3</sup>, Gustavo D. Frechtel<sup>1</sup>.**

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A study at Hospital de Clínicas “José de San Martín” aimed to evaluate the effects of physical activity and diet interventions on lifestyle and gut microbiota (GM) in individuals with obesity (OB), prediabetes (preDT), and type 2 diabetes (T2D), compared to a reference group (CNT). Materials and Methods: The study included 47 OB, 29 preDT, 13 T2D, and 23 CNT individuals. Fecal samples were analyzed for GM composition using metagenomic sequencing and bioinformatics tools. Clinical, biochemical, and anthropometric parameters and statistical analyses included correlations and comparisons were assessed with SPSS.

Results: At baseline (T0), Firmicutes (Fir) and Bacteroidetes (Bac) were the predominant phyla. The T2D group had a lower abundance of Verrucomicrobia compared to CNT ( $p = 0.072$ ). The OB, preD, and T2D groups had a higher Fir/Bac ratio, which was negatively correlated with weight ( $p = 0.025$ ,  $r = -0.220$ ), BMI ( $p = 0.05$ ,  $r = -0.190$ ), and inflammation (hsCRP) ( $p = 0.036$ ,  $r = -0.211$ ). *Faecalibac-*

*terium* was less abundant in OB and preDT compared to CNT, with *Faecalibacterium prausnitzii* decreasing as metabolic conditions worsened ( $p = 0.036$  and  $p < 0.001$ , respectively). Lower species diversity was associated with higher hsCRP.

After 6 months of lifestyle interventions (T6), OB showed significant reductions in waist circumference ( $p = 0.001$ ) and HbA1c ( $p < 0.001$ ). The preDT group had significant decreases in HbA1c ( $p < 0.001$ ), weight ( $p < 0.001$ ), waist circumference ( $p = 0.001$ ), and BMI ( $p = 0.003$ ). The T2D group saw declines in HbA1c ( $p = 0.001$ ), triglycerides ( $p = 0.032$ ), total cholesterol ( $p = 0.021$ ), and hsCRP ( $p = 0.012$ ). GM composition and the Fir/Bac ratio remained unchanged.

Conclusions: The study demonstrated that 6 months of lifestyle changes led to a GM composition in OB, preDT, and T2D groups that progressively resembled the CNT group, correlating with improvements in clinical, biochemical, and anthropometric parameters.

## MITOCHONDRIA AS A TARGET OF CARDIOPROTECTION CARRIED OUT BY ORAL ADMINISTRATION OF STEVIOSIDE: INSIGHTS INTO AKT INVOLVEMENT

**Victoria Evangelina Mestre Cordero<sup>1,2</sup>, María de las Mercedes Fernández Pazos<sup>1,2</sup>, Federico Joaquín Reznik<sup>1</sup>, Romina Hermann<sup>1,2</sup>, María Gabriela Marina Prendes<sup>1,2</sup>, Tamara Zaobornyj<sup>1,3</sup>**

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*3 CONICET- Universidad de Buenos Aires. Instituto de Bioquímica y Medicina Molecular Profesor Alberto Boveris (IBIMOL) Buenos Aires, Argentina.*

Mitochondria are crucial in cardiac energy metabolism during ischemia-reperfusion (Is-Rs), where stress alters their function and may cause cellular damage. We aimed to evaluate the impact of oral stevioside (S, 168 mg/kg for 15 days) on mitochondrial function in hearts subjected to Is-Rs using a Langendorff perfusion model, and to assess the involvement of Akt using the inhibitor wortmannin (W, 100 nM) 15 min before Is.

Female Wistar rat hearts were used, with mitochondria isolated at pre-ischemic and post-Is-Rs stages. Mitochondria were energized with pyruvate/glutamate and malate or succinate, and their function was evaluated through ATP synthesis, O<sub>2</sub> consumption, membrane potential ( $\Delta\psi$ ), respiratory complex activities, and calcium retention capacity (CRC). Respiratory control ratio (RCR) of mitochondria and ATP/O ratio were evaluated to assess mitochondrial efficiency. Western blot was used to measure PGC1 $\alpha$  levels. Mitochondrial quantification and morphometrics were performed via electron microscopy. ANOVA, n=8/group.

S preserved mitochondrial state 3-oxygen consumption, RCR, ATP/O ratio and  $\Delta\psi$  after Is-Rs compared to controls ( $p < 0.05$  vs C and C+W), effects reversed by W. CRC increased with S ( $p < 0.01$  vs C and C+W), and was unaffected by W. S also enhanced 50% complex I-III activity both pre-ischemia and post-Is-Rs compared to controls ( $p < 0.01$ ), independent of W. While S increased pre-ischemic complexes II-III and IV activity ( $p < 0.05$  vs C and C+W), this was not sustained post-Is-Rs. Electron microscopy revealed that S increased pre-ischemic mitochondrial number/ $\mu\text{m}^2$ , preserved post-Is-Rs, an effect reversed by W only post-Is-Rs. S also conserved mitochondrial size post-Is-Rs ( $p < 0.05$  vs C; C+W and S+W), possibly linked to reduced edema. PGC1 $\alpha$  expression was elevated in S pre-ischemic conditions, with no changes induced by W.

These findings suggest that mitochondria are key targets in the cardioprotective action of S, which is only partially linked to Akt activation.

## THE IMBALANCE BETWEEN RENAL DOPAMINERGIC AND RENIN ANGIOTENSIN SYSTEMS INDUCED BY A HIGH-FAT DIET IS PREVENTED BY LOSARTAN AND METFORMIN

**Silvana María Cantú<sup>1,2</sup>, Hyun Jin Lee<sup>1,2</sup>, Christian Höcht<sup>3</sup>, Adriana Donoso<sup>1,2</sup>, Ana María Puyó<sup>1,2</sup>, Marcelo Roberto Choi<sup>1,4</sup>.**

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Renal sodium management and inflammation and their alterations associated to hypertension and cardio-renal-metabolic damage are regulated by the renal dopaminergic system (RDS) and the renin angiotensin system (RAS). This study evaluated if a high-fat diet is related to an imbalance in the RDS-RAS interaction as a renal mechanism for arterial hypertension development and whether this effect can be prevented by losartan (L) or metformin (M). Male Sprague-Dawley rats were studied for 8 weeks and randomly divided into six groups (n=4-6): C (Control): standard diet (SD) and tap water; HFD (High-Fat Diet): 50% w/w bovine fat added to the SD and tap water; CL (C+Losartan): C+30mg/kg/day of losartan in the water; HFDL (HFD+Losartan): CL+HFD; CM (C+Metformin): C+500mg/kg/day of metformin in the water; HFDM (HFD+Metformin): CM+HFD. Statistical

analysis: ANOVA with Tukey post-hoc tests. Both L and M, prevented HFD effects on FENa<sup>+</sup> and UNa<sup>+</sup>.V (HFDL or HFDM vs HFD  $p < 0.05$ , and HDF vs C,  $p < 0.01$ ); systolic blood pressure (HFDL or HFDM vs HFD, and HDF vs C,  $p < 0.01$ ); L-dopa/dopamine index (HFDL or HFDM vs HFD, and HDF vs C,  $p < 0.05$ ); and RDS and RAS proteins expression (measured by Western blot): OCTN 1/2/3 (HFDL or HFDM vs HFD, and HDF vs C,  $p < 0.05$ ); DRD1 (HFDL or HFDM vs HFD, and HDF vs C,  $p < 0.01$ ); AT1R (HFDL or HFDM vs HFD  $p < 0.01$ , and HDF vs C,  $p < 0.05$ ); Na<sup>+</sup>,K<sup>+</sup>,ATPase (HFDL or HFDM vs HFD, and HDF vs C,  $p < 0.05$ ). NF $\kappa$ B, TGF- $\beta$ 1 and fibrosis % increased were prevented by L and M (HFDL or HFDM vs HFD,  $p < 0.01$ ). Also, L and M reduced intracytoplasmic inclusions in the tubular cortex cells vs HFD (H&E stain), and improved glomerular ultrastructure evidenced

by TEM. To conclude, HFD promotes an imbalance between RDS and RAS in the kidney, favoring AngII and AT1R effects on sodium reabsorption and inflammation. M pleiotropic effects downregulate AT1R expression and

enhances RDS actions on natriuresis. L and M appear to be effective in preventing inflammatory and renal damage by HFD.

### SAIC AWARD - Fundación CHERNY - Multidisciplinary call.

Thursday 21th November 16:00 - 18:00

Jury: Claudia Lanari, Daniel Alonso, Héctor Targovnik, Alberto Crottogini and Alejandro De Nicola

#### UNCONVENTIONAL SECRETION OF THE AUTOPHAGY-RELATED PROTEIN VMP1 VIA EXTRACELLULAR VESICLES AS A POTENTIAL BIOMARKER FOR PANCREATIC CELL INJURY

Mariana S. Tadic<sup>1</sup>, Felipe J. Renna<sup>1</sup>, Malena Herrera López<sup>1</sup>, Tamara Orquera<sup>1</sup>, Fabiana López Mingorance<sup>1</sup>, Alejandro Ropolo<sup>1</sup>, Maria I. Vaccaro<sup>1\*</sup>

<sup>1</sup>Universidad de Buenos Aires, CONICET, Instituto de Bioquímica y Medicina Molecular Prof Alberto Boveris (IBIMOL), Buenos Aires, Argentina; mvaccaro@ffyb.uba.ar

Cellular stress activates various mechanisms, including autophagy and vesicular trafficking, to maintain homeostasis and cope with pathological conditions. One of these mechanisms involves the unconventional secretion of extracellular vesicles (EVs). Our study focuses on Vacuole Membrane Protein 1 (VMP1), an autophagy-related protein implicated in pancreatitis and cellular stress management. Here, we demonstrate that VMP1 is secreted into the extracellular medium as a component of EVs. We successfully purified VMP1-containing EVs (VMP1-EVs) from the extracellular medium of cells overexpressing VMP1, using ultracentrifugation and immune isolation techniques. Our findings confirm that VMP1 is integrated into the EV membranes. These VMP1-EVs have a diameter of approximately 150 nm, as determined by TEM and DLS analysis. Moreover, the secretion of VMP1-EVs decreases when mTOR is inhibited by starvation and PP242, as well as in response to

deficiencies in essential proteins involved in autophagosome formation, such as ATG5. Conversely, VMP1 secretion is increased under conditions of cellular stress, such as lysosomal blockade facilitated by BafA1 or CQ, as well as in experimental models of acute pancreatitis induced by supramaximal doses of cerulein. Notably, the secretion of VMP1-EVs under cellular stress/injury was accompanied by other autophagy-related proteins, such as LC3II and p62. Furthermore, VMP1-EVs are taken up by different host cells, suggesting that VMP1-EVs may be able to mediate remote communication between cells. In conclusion, we have demonstrated for the first time that VMP1 is unconventionally secreted as a component of EV membranes. The significant increase in VMP1-EV secretion under conditions of cell injury, such as in pancreatic acinar cells suffering pancreatitis, suggests that they could serve as a novel biomarker of cell damage in pancreatic disease.

#### PROTUMORAL INTRINSIC EFFECTS OF FOXP3 IN GLIOBLASTOMA

Matías García Fallit<sup>1,2</sup>, Jorge A. Peña Agudelo<sup>1</sup>, Nazareno Gonzalez<sup>1</sup>, Alejandro J. Nicola Candia<sup>1</sup>, Melanie Pérez Kuper<sup>1</sup>, Maicol Suarez Velandía<sup>1</sup>, Laura V. Gomez<sup>1</sup>, Yamila Zampini<sup>1</sup>, Ana Clara Romero<sup>1</sup>, Cristian Sobarzo<sup>1</sup>, Ivana Sánchez Rojas<sup>1</sup>, Hebe Durán<sup>3</sup>, Marina Perona<sup>3</sup>, Guillermo Videla Richardson<sup>4</sup>, Noelia Casares<sup>5,6</sup>, Juan José Lasarte<sup>5,6</sup>, Flavia Zanetti<sup>7</sup>, Marianela Candolfi<sup>1\*</sup>

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Glioblastoma (GBM), for which there have been no significant clinical advances in the last 20 years, is the most common malignant primary brain tumor. This disease carries a dismal prognosis due to its highly invasive nature and resistance to therapy. The transcription factor Forkhead box protein P3 (Foxp3) is known for its role in the immunosuppressive activity of regulatory T cells (Tregs), and has also been detected in tumor cells. However, the tumor-intrinsic role of Foxp3 is poorly understood. We have previously shown that administration of a

cell-penetrating Foxp3 inhibitory peptide (P60) improves the efficacy of antitumor vaccines and exerts direct antitumor effects in experimental breast cancer. Here, we aimed to assess the intrinsic effects of Foxp3 in GBM. Meta-analysis of transcriptomic data indicated that Foxp3 expression in GBM biopsies is associated with worse prognosis and correlates with the expression of markers of immune-suppression and epithelial-mesenchymal transition ( $p < 0.05$ ).

We detected expression of Foxp3 in GBM cell lines and

patient-derived cultures, which was upregulated by chemo- and radiotherapy ( $p < 0.05$ ). Foxp3 blockade using P60 reduced cell survival in GBM cells and enhanced their radio- and chemo-sensitivity. RNA-seq indicated that Foxp3 blockade reprograms the genetic profile of GBM cells, affecting the expression of over 4,000 genes, primarily related to cell cycle control. To improve the local availability of P60, we developed an adenoviral vector (Ad.P60) encoding P60 that effi-

ciently transduced GBM cells, enhancing their apoptotic response and reducing cell viability, proliferation, migration and chemoresistance ( $p < 0.05$ ). Local treatment with Ad.P60 in mice bearing intracranial GBM significantly reduced Treg infiltration, inhibited tumor growth and improved chemosensitivity compared with mice treated with control vector ( $p < 0.05$ ). Our results suggest that Foxp3 could be a valuable target to improve the treatment of these tumors.

#### INHIBITION OF INCREASED HMGB1 IN HUNTINGTON'S DISEASE BY GLYCYRRHICIN AMELIORATES MOTOR AND COGNITIVE FUNCTIONS

**Maria Friser Frederiksen<sup>1</sup>, Diego Rivas Castillo<sup>1</sup>, Julieta Saba<sup>1</sup>, Federico López Couselo<sup>1</sup>, Renata Capelli<sup>2</sup>, María Eugenia Gomez-Casati<sup>3</sup>, Juan Cruz Casabona<sup>2</sup>, Lila Carniglia<sup>1</sup>, Daniela Durand<sup>1</sup>, Mercedes Lasaga<sup>1</sup>, Carla Caruso<sup>1</sup>.**

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<sup>2</sup> Fundación Instituto Leloir (FIL), IIBBA-CONICET, Argentina.

<sup>3</sup> Instituto de Farmacología, Facultad de Medicina, Universidad de Buenos Aires, Argentina.

Huntington's Disease (HD) is a progressive neurodegenerative disorder caused by CAG expansion in the huntingtin (Htt) gene. This leads to a loss of striatal neurons, cognitive decline and motor dysfunction. HD patients show an unexplained sex-dependent difference in disease manifestations and course. We have previously shown that female HD mice manifest earlier motor dysfunction compared to male. High mobility group box 1 (HMGB1) is a nuclear protein involved in chromatin stabilization, transcription regulation, and DNA repair and has been proven to be involved in neurodegeneration. We aim to investigate HMGB1 expression in the cerebral cortex and striatum of zQ175 knock-in (HD) and wild-type (WT) mice at 4, 8, and 12 months (M) of age, as well as in HD patient-derived neural stem cells (NSC). Female HD mice show no differences in HMGB1 protein levels in the cortex while striatal levels show a significant increase at all age groups ( $p < 0.05$ ). On the other hand, male HD

mice showed an increase in striatal HMGB1 expression only at 8 months and no changes in the cortex. HMGB1 expression also increases in striatal immortalized murine STHdh-Q111 neurons expressing human mutant HTT (mHTT) compared to STHdh-Q7 cells ( $p < 0.05$ ) and in HD-patient derived neural stem cells ( $p < 0.05$ ). HMGB1 inhibition by glycyrrhizin (GLY, ip 100 mg/kg, once daily for 15 days) decreased HMGB1 expression in the striatum of 4-month-old female HD mice and improved motor function. Memory recognition was improved by GLY in HD mice of both sexes ( $p < 0.05$ ). GLY also decreased the number of mutant Htt aggregates in the striatum of HD mice ( $p < 0.05$ ). HMGB1 is selectively increased in the striatum of HD mice and NSC from HD patients and HMGB1 inhibition improves HD mice phenotype. Our results suggest that HMGB1 may play a role in HD pathogenesis.

#### DYNAMICS OF RESPIRATORY SYNCYTIAL VIRUS INFECTION IN THE CONTEXT OF COVID-19 IN THE METROPOLITAN AREA OF BUENOS AIRES

**Julia Dvorkin<sup>1,2</sup>, Gonzalo Guinazu<sup>1</sup>, Emiliano Sosa<sup>1</sup>, Sofía Jares<sup>1</sup>, María Agustina Wirth<sup>1</sup>, Pilar Goñi<sup>1</sup>, Yamila Alen<sup>3</sup>, Daniela Parada<sup>3</sup>, Damián Alvarez-Paggi<sup>1,2</sup>, Mauricio Caballero<sup>1,2</sup>**

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Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

Instituto de Cálculo, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

**Objectives** 1) Assess the excess of Respiratory Syncytial Virus (RSV) related lower respiratory tract infections (LRTI) after the release of non-pharmaceutical interventions (NPI) in Buenos Aires in 2022-2023 compared to pre-pandemic years in children under five; 2) Describe clinical and epidemiological differences in hospitalized patients between pre and post-pandemic periods; 3) Investigate changes in RSV antibody titers in children under 5 due to NPI.

**Methods** We conducted a multicenter, retrospective cohort study for objectives 1 and 2, using LRTI hospitaliza-

tion data from 2018-2023 across 4 hospitals. For objective 3, we enrolled children under 5 during 2020-2023 and collected serum samples and included pre-pandemic samples from serum biobanks.

**Results** 12907 LRTI hospitalizations were recorded, with 4284 RSV-positive cases. RSV positive patients were healthier than RSV negative ones. Pediatric intensive care unit (PICU) admissions increased significantly in the post-pandemic period: 26.68% (CI95% 24.48-28.97) in 2022-2023 vs 12.50% (CI95% 11.07-14.04) in 2018-2019 ( $p < 0.001$ ), both groups with similar comorbidities.

The mean age in months of patients admitted to PICU was also higher in the post-pandemic period (9.13 vs 7.38 months,  $p=0.034$ ). Using the Acosta-Irizarry method, we found a significant excess of PICU admission post-pandemic compared to 2018-2019. Differences in geometric mean titers (GMT) between different age groups and years were examined using Kruskal-Wallis test: we observed a significant reduction in the GMT between 7 and 36 months during 2020-2022, with recovery

to pre-pandemic values in 2023.

**Conclusions** After the pandemic receded, the burden of critical RSV-LRTI increased among infants in accordance with reduced antibody titers. Older patients were more affected compared to the pre-pandemic era, despite being equally healthy. This unusual situation generated an ideal context to understand and preview the potential impacts of RSV immunization.

#### SAIC AWARD - mAbxience Award: Endocrinology-Reproduction (SAIC).

Thursday 21th November 16:00-18:00

Jury: Silvina Meroni, Adriana Seilicovich, Carlos de Brasi

#### MOLECULAR STUDY OF *CDKN1B* GENE IN POSSIBLE MULTIPLE ENDOCRINE NEOPLASIA TYPE 4 CARRIERS. FIRST CASUISTIC IN PATIENTS BORN IN ARGENTINA

María Lorena Viale, María Pía Serra, Andrea Kozak, Eliana Miler, Cristabel Rubino, Analia Stigliano, Patricia Fainstein Day.  
*Laboratorio de Endocrinología Hormonal y Genético. Hospital Italiano de Buenos Aires.*

MEN4 is an autosomal dominantly inherited syndrome characterized by the appearance of parathyroid tumors (HPT) and pituitary tumors (PT) in association with tumors of the adrenal glands, kidneys, and reproductive organs. The gene related to this syndrome is *CDKN1B*, and encodes p27, a tumor suppressor protein, critical for controlling cell cycle regulation. The phenotypic expression of MEN 4 is not fully defined due to the limited number of patients described around the world. Genetic testing is recommended for patients exhibiting MEN1 symptoms in the absence of pathogenic genetic variants in *MEN1* gene. **AIM:** To develop the genetic test to study germline variants in *CDKN1B* gene in patients with clinical features of MEN1 born in Argentina. **METHODS:** We studied 33 potential carriers (21 women and 12 men; average age: 42.7 years ( $\pm 15.5$ )), who did not present any pathogenic variants in the *MEN1* gene. The coding and non coding exons, the promoter, and the flanking intronic regions were amplified by PCR. Sanger

Sequencing was performed and MLPA to detect large deletions, duplications, and/or insertions. **RESULTS:** We found a PV in the promoter region of the gene (5'UTR): c.-29\_-26delAGAG, a VUS: c.476-77C>T and the following benign variants: c.326T>G (exon1), c.-79T>C (5'UTR), c.\*9-222T>C (intron2) and c.\*9-317C>T (intron2). Additionally, we found 2 variants in non-coding exon 3: c.\*501G>A and c.\*956C>A. **CONCLUSION:** We found a PV in the promoter in a female patient of 64 years which presented a typical MEN1 phenotype characterized by HPT. We also found a VUS in intron 1 that has not been reported in the literature to date and the patient has a pituitary tumor. The variants found in non-coding exon 3 were classified as benign according to ACMG but there is no functional evidence. Our molecular study of the *CDKN1B* gene is the first in Argentina. Our observations will help to improve genetic counseling and management of this rare multiple endocrine neoplasia.

#### CONTRIBUTION OF THE EPIDIDYMIS BEYOND FERTILIZATION: RELEVANCE OF CRISP1 AND CRISP3 FOR SPERM DNA INTEGRITY AND EARLY EMBRYO DEVELOPMENT

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Numerous reports show that the epididymis plays a key role in the acquisition of sperm fertilizing ability but little is known on its contribution to embryo development. Our observations showed that mammalian CRISP (Cysteine-Rich Secretory Proteins), known to regulate calcium ( $Ca^{2+}$ ) channels, are relevant for embryonic development as judged by the finding that males with simultaneous mutations in epididymal *Crisp1* and *Crisp3* genes exhibited normal *in vivo* fertilization but impaired development to blastocyst after mating. In the present work, aimed to elucidate the mechanisms underlying this phenotype, we observed that embryo development failure was not due to a delayed fertilization known to lead to embryo develop-

ment defects, as no differences in sperm transport within the female tract after mating were observed. The finding that impaired embryo development was also detected in eggs fertilized by epididymal sperm inseminated in the uterus or used in *in vitro* fertilization revealed that the defects were already present at epididymal level. Of note, eggs fertilized *in vitro* by mutant epididymal sperm exhibited impaired meiotic resumption not due to defects in the  $Ca^{2+}$  oscillations triggered by egg activation, prompting us to examine potential sperm DNA defects known to require time to be repaired by the egg. Interestingly, higher levels of both DNA fragmentation and intracellular  $Ca^{2+}$  were observed for mutant than for control epididymal

sperm, supporting sperm DNA damage, linked to Ca<sup>2+</sup> dysregulation, as the main responsible for early development failure. Together, our results identified CRISP1/3 as novel male factors relevant for sperm DNA integrity and embryo development. Given the existence of human

CRISP homologues and the incidence of DNA fragmentation in infertile men, we believe these findings not only support the contribution of the epididymis beyond fertilization but will also contribute to a better understanding, diagnosis and treatment of human infertility.

#### EXPANDING PHENOTYPE FOR WT1 AND THE CRITICAL NEED FOR EARLY DETECTION

**María Celeste Mattone<sup>1</sup>, Carmen Turizo<sup>1</sup>, Natalia Pérez Garrido<sup>1</sup>, Silvia Gil<sup>1</sup>, Pablo Ramírez<sup>1</sup>, Roxana Marino<sup>1</sup>, María Laura Galluzzo Mutti<sup>1</sup>, Alicia Belgorosky<sup>1,2</sup>, María Gabriela Obregón<sup>1</sup>, Soledad Arbio<sup>1</sup>, Eugenia Di Meola<sup>1</sup>, Micaela Sole<sup>1</sup>, Marta Ciaccio<sup>1</sup>, Esperanza Berensztein<sup>1</sup>, Gabriela Guercio<sup>1,2,1</sup>, Mariana Costanzo<sup>1,1</sup>**

<sup>1</sup>Hospital de Pediatría "Prof. Dr. Juan P Garrahan", <sup>2</sup>CONICET

\*Comparten ultima autoría

**Introduction:** *Wilms' tumor suppressor gene 1 (WT1)* plays an essential role in urogenital development. Germline heterozygous variants in *WT1* are associated with complex, life-threatening, and heterogeneous phenotypes, highlighting the critical importance of early detection.

**Objective:** To analyze the phenotype, associated malignancies, complications, and follow-up of 15 pediatric patients (p) with differences in sexual development (DSD) associated with *WT1* variants followed in a single institution.

**Materials and Methods:** Data of DSD p with confirmed variants in *WT1* gene were analyzed. Phenotypes were evaluated according to karyotype and molecular findings (Sanger, whole exome sequencing, and NGS DSD-gene panel)

**Results:** *WT1* variants were identified in 15/83 p with disorders in gonadal development: 3/30 (10%) 46,XX ovotesticular/testicular (OT/T) DSD and 12/53 (22.6%) 46,XY gonadal dysgenesis.

All OT/T 46,XX p exhibited variants in exon 10, one had

gonadal germ cell malignancy at an early age. No renal involvement was observed.

Among 46,XY DSD, intron 9 splice-site (IVS9) variants were identified in 3 p with early-onset progressive nephropathy; 2/3 had complete gonadal dysgenesis (CGD), one with gonadoblastoma. Six p with variants in exons 8-9 presented with partial gonadal dysgenesis (PGD) and early-onset nephropathy. Novel variants were identified in 2 p with PGD and bilateral Wilm's tumor without nephropathy: p.(Tyr266Ter) in exon 3 and c.1264+1G>T in intron 7, both predicted as likely pathogenic (ACMG). A rare, not previously described association of congenital diaphragmatic hernia and CGD with proteinuria was detected in a newborn with a variant in exon 8 (p.(Arg-439Cys)).

**Conclusions:** *WT1* gene variants were frequent in our DSD cohort. We report two novel variants, and a remarkable and severe new phenotype expanding our knowledge of *WT1*-associated disorders. Awareness of early identification is crucial for effective clinical management and counseling.

#### SAIC AWARD - Irene Faryna y Roberto Raveglia - Oncology.

Friday 22th November 8:30 - 10:30 - Auditorium

Jury: Marianela Candolfi, Mónica Costas and Isabel Lüthy

#### SYNUCLEINS AS ONCOGENIC PLAYERS: EXPLORING THE IMPACT OF NEURODEGENERATION-LINKED PROTEINS ALPHA AND GAMMA SYNUCLEIN IN MELANOMA

**Lucia C. Zanotti<sup>1,2,3</sup>, Florencia Malizia<sup>1,2,3</sup>, Macarena Mamberto<sup>1,2,3</sup>, Ayleen Avila<sup>3</sup>, Nahuel Cesatti Lalue<sup>1,2,3</sup>, Luciano Anselmino<sup>1,2,3</sup>, Mauricio A. Menacho Márquez<sup>1,2,3</sup>**

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Synucleins are small, highly conserved proteins that play significant roles in both neurodegenerative disorders and cancer. These unstructured proteins are prone to aggregation, contributing to severe human diseases. Gene enrichment analyses associated with aS and gS reveal that all synuclein family members are involved in cell signaling pathways linked to disease, particularly in cancer. While the association of gS with cancer is well-established, recent studies have suggested a potential role for aS in this pathology. Our goal was to explore the role aS and gS in melanoma.

Through an initial bioinformatic analysis, we confirmed the expression of aS and gS in melanoma patients. Subsequently, we conducted experiments using human and mouse melanoma cell lines (SK-MEL28, A375, B16-F0, and B16-F10), where we verified the expression of these proteins through the use of specific antibodies.

To investigate the functional role of these proteins, we modulated endogenous expression levels using shRNA and expression vectors. The downregulation of aS and gS resulted in a significant decrease in proliferation, clonogenic capacity and migration. These findings were fur-



ther validated in an *in vivo* model where mice inoculated with these modified cells exhibited reduced tumor growth and diminished metastasis. Moreover, cells overexpressing synucleins were found to be associated with cytoskeletal reorganization, increased migration and enhanced focal adhesion formation. Nonetheless, they also exhibit a higher apoptotic rate.

In a complementary approach, we exogenously administered aS and gS. Rather than exerting cytotoxic effects,

these exogenous proteins were observed to promote proliferation, clonogenic potential, migration, and tumor growth.

In conclusion, our study confirms the involvement of synucleins in melanoma, affecting key cancer-related processes. The observed effects of these proteins on tumor growth and cell behavior underscore their potential significance in cancer research. Further investigations are needed to better understand their roles.

#### ATR, $\gamma$ H2AX, AND HSP27 AS PREDICTIVE/PROGNOSTIC BIOMARKERS IN PEDIATRIC BRAIN TUMORS: A RETROSPECTIVE PILOT STUDY

**Emanuel Martínez<sup>2</sup>, Darío Cuello Carrión<sup>1</sup>, Mariel Fanelli<sup>1</sup>, Silvina Gómez<sup>1</sup>, Marisa Francisco<sup>3</sup>, Gisela Drago<sup>2</sup>, Silvina Beatriz Nadin<sup>1</sup>.**

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Central nervous system (CNS) tumors are the most common solid neoplasms and the main cause of mortality in children with cancer. Treatment consists of surgery and chemotherapy. Many antineoplastic drugs target the DNA molecule, activating ATR, which leads to DNA repair or cell death. ATR phosphorylates the histone H2AX,  $\gamma$ H2AX, a biomarker of DNA damage. Overexpression of Hsp27 has been associated with chemotherapy resistance and poor prognosis. Despite advances in pediatric oncology, CNS tumors have an aggressive clinical course and high mortality, prompting the search for new monitoring and treatment options to optimize therapy and reduce side effects. We aimed to investigate the predictive and prognostic implications of ATR,  $\gamma$ H2AX, and Hsp27 in pediatric brain tumors. Thirty-two paraffin-embedded pre-chemotherapy tissues from pediatric CNS tumors were included. The protein expressions were assessed for intensity and proportion using immunohistochemistry. The patients were treated with chemotherapy

containing platinum analogs (cisplatin, carboplatin) and/or Vinca alkaloids (vincristine, vinblastine). The clinical response was classified as complete response (CR), partial response (PR), progressive disease (PD), or stable disease (SD). The mean follow-up was 62,44 months (range 7-124). Medulloblastomas showed lower ATR and  $\gamma$ H2AX proportions than other tumor types ( $P < 0.01$  and  $P < 0.05$ , respectively). Tumors from responder's patients (CR, PR) displayed lower expression levels of ATR ( $P < 0.05$ ), and nuclear and cytoplasmic Hsp27 ( $P < 0.01$  and  $P < 0.05$ , respectively) than non-responder's patients (SD, PD). Moreover, ATR levels  $\leq 50\%$  were associated with better progression-free survival (PFS). No associations between PFS and overall survival were found for other markers studied. Our data indicate that ATR and Hsp27 may be useful markers for predicting responses to chemotherapy. ATR could also be a promising indicator for PFS in pediatric patients with CNS tumors.

#### VAV2 EXPRESSION DRIVES KEY PROCESSES FOR MELANOMA PROGRESSION

**Macarena Mamberto<sup>1,2,3</sup>, Aylén Ávila<sup>2</sup>, Nahuel Cesatti Laluece<sup>1,2,3</sup>, Lucía C. Zanotti<sup>1,2,3</sup>, Florencia Malizia<sup>1,2,3</sup>, Luciano Anselmino<sup>1,2,3</sup> and Mauricio Menacho Márquez<sup>1,2,3</sup>.**

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Melanoma, the most dangerous form of skin cancer, continues to rise in incidence. Vav2, a guanosine nucleotide exchange factor (GEF) of the Rho GTPase family, plays a crucial role in melanoma progression. The role of Vav2 was studied in different processes associated to melanoma development.

Kaplan-Meier survival curves, generated using SKCM patient data, reveal that patients with elevated Vav2 expression have significantly reduced survival ( $p < 0.05$ ).

To further investigate Vav2's role, we modulated its expression in the murine melanoma cell line B16 F0. Mi-

croarray analyses revealed that reduced Vav2 levels led to the upregulation of pathways associated with cell adhesion, such as cell surface-integrin interactions and extracellular matrix organization. Conversely, expression of an active form of Vav2 downregulated these pathways. *In vitro* studies demonstrated that decreased Vav2 levels significantly reduced cell migration in both murine and human melanoma cells ( $p < 0.01$ ) (B16 and SK-MEL-28, respectively), while the oncogenic form of Vav2 markedly enhanced migration ( $p < 0.001$ ). Immunocytochemistry assays revealed that human cells with decreased

Vav2 expression exhibited fewer focal adhesions per cell ( $p < 0.001$ ), whereas cells expressing the active form of Vav2 showed larger focal adhesions compared to controls ( $p < 0.001$ ). Spheroid assays further indicated that cells with reduced Vav2 levels were less invasive than control cells ( $p < 0.05$  and  $p < 0.01$ , respectively). For *in vivo* validation, B16 F0 cells with modulated levels of Vav2 were subcutaneously injected into C57BL/6

mice. Tumors with reduced expression of Vav2 grew significantly less ( $p < 0.05$ ), while those expressing the wild type or oncogenic form of Vav2 grew significantly more compared to controls ( $p < 0.01$ ).

Vav2's ability to modulate these key processes highlights its potential as a critical therapeutic target in melanoma, offering new avenues for intervention aimed at improving patient survival and reducing metastatic risk.

#### EVALUATION OF THE IMMUNE RESPONSE, ABSCOPAL AND LOCAL EFFECT AND RADIOTOXICITY OF SEQUENTIAL BORON NEUTRON CAPTURE THERAPY (BNCT) IN A MODEL OF DIFFUSE LUNG METASTASES DERIVED FROM COLON ADENOCARCINOMA IN BDIX RATS.

**Debora N. Frydryk B. 1,2,3, Mónica A. Palmieri 3, Yanina V. Langle 4, Carolina B. Iglesias 4, Paula Curotto 1, Silvia I. Thorp 1, Marcela A. Garabalino 1, Emiliano C.C. Pozzi 1, Paula S. Ramos 1, Lucas Polti 5, Agustina M. Portu 1,6, Juana A. Solloguren 7, Martín V. Viale 8, Andrea Monti Hughes 1,6, Verónica A. Trivillin 1,6.**

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BNCT is based on the selective incorporation of  $^{10}\text{B}$  carriers in tumors followed by neutron irradiation. In this study, we evaluated tumor control, radiotoxicity, abscopal effect and immune response of a sequential BNCT protocol with a 7 day interval between irradiations (BNCT-Seq-7days) in a diffuse lung metastases model in BDIX rats. We also studied if Oligo-Fucoidan (O-Fuco, a seaweed extract with anti-inflammatory and anti-cancer effects) was capable of reducing BNCT induced radiotoxicity (dermatitis).

DHD/K12 colon cancer cells were intravenously (iv) injected in BDIX rats to induce lung metastases. BNCT-Seq-7days consisted of two BNCT applications: BPA-BNCT (Boronophenylalanine, 46.5 mg  $^{10}\text{B}/\text{kg}$ , iv) and, after 7 days, GB-10-BNCT (Decahydrodecaborate, 50 mg  $^{10}\text{B}/\text{kg}$ , iv). To study the abscopal effect, 24 h after GB-10-BNCT, we inoculated colon cancer cells subcutaneously in the right hind flank of the rats. O-Fuco (200mg/kg) was applied orally and topically in the skin area exposed to the neutron beam. Tumoral and system immune

cell populations were analyzed by cytometry. SHAM and  $T_0$  were control groups.

We observed that  $T_0$  group exhibited a lower lung mass relative to body weight vs SHAM ( $p \leq 0.01$ ). The percentage of metastases in the left lung lobe in SHAM was significantly higher ( $p \leq 0.05$ ) than in BNCT treated animals. Moreover, the percentage of microscopic lung metastases tended to be lower in BNCT groups vs SHAM. BNCT induced moderate to severe dermatitis. O-Fuco tended to reduce the percentage of animals with dermatitis. BNCT treated rats exhibited a significant volume reduction in their secondary tumor vs to SHAM ( $p < 0.01$ ), evidencing abscopal effect. However, cytotoxic NK and NKT immune cells of the SHAM group were slightly lower in the lung and secondary tumor compared to BNCT.

BNCT-Seq-7days would be potentially useful to treat diffuse lung metastasis and activate a local and systemic immune response, possibly controlling distant disease by an abscopal effect.

#### SAIC AWARD - "Council on Genetics and ASOCIACIÓN CIVIL, CULTURAL Y EDUCATIVA EDUARDO WILDE"

Friday 22th November 8:30 - 10:30 - Sala de Camara

Jury: Liliana Dain, Belen Almejum, Ezequiel Surace

#### RARE DISEASES PROJECT WITHIN THE FRAMEWORK OF THE FEDERAL GENOMICS AND BIOINFORMATICS NETWORK

Julián Sánchez Loria<sup>1</sup>, Florencia Medina<sup>1</sup>, Marisol Delea<sup>2</sup>, Silene Silvera Ruiz<sup>3</sup>, Carolina Ramirez<sup>4</sup>, Belén Brizuela Sánchez<sup>5</sup>, Agustín Izquierdo<sup>2</sup>, Gonzalo Castro<sup>3</sup>, Viviana Fanessi<sup>4</sup>, Mónica Ludojoski<sup>5</sup>, Sandra Rozental<sup>6,7</sup>, Romina Armandó<sup>8</sup>, Carlos David Bruque<sup>2</sup>, Tomás Poklepovich Caride<sup>1</sup>, and the EPF-RFGB taskforce.

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Programa de Enfermedades Poco Frecuentes - Ministerio de Salud de la Nación Argentina.*

**Objective:** To test a collaborative, multidisciplinary, and federal networking model that allows patients with rare diseases (RD) from public hospitals across the country to access exome studies.

**Materials and Methods:** The network comprises four regional nodes (Santa Cruz, Córdoba, Buenos Aires, and Misiones) and a central node at ANLIS-Malbrán. The regional nodes consist of a lead specialist operating in a molecular biology laboratory and a team of geneticists assigned based on the province where they practice.

The workflow begins with the inclusion of a patient with suspected RD by the attending geneticist. Blood samples are taken at the patient's care location and sent to the regional lead laboratory for processing. DNA is extracted and sent to the central node, where exome preparation, sequencing, and bioinformatic analysis are performed. The sequence analysis and report preparation are conducted by the regional lead, with the collaboration of the geneticist, the other nodes, and experts in the relevant pathologies if necessary. The final step involves genetic

counseling by the enrolling geneticist.

Alongside the workflow, specialists provide ongoing training sessions related to collaborative networking and the analysis of the genomic studies for all the lead specialists.

**Results:** To date, 76 exomes from 13 different provinces have been sequenced, covering a wide spectrum of RD such as Marfan syndrome, Wilson disease, and Ehlers-Danlos syndrome, among others. So far, 41 of the 76 exomes have been analyzed, of which 75% showed a Variant of Uncertain Significance (VUS), a Likely Pathogenic (LP) or Pathogenic (P) variant related to the patient's phenotype.

**Conclusions:** The networking model has been successfully implemented, improving access to genetic diagnosis for RD patients nationwide. This approach enhances diagnostic capabilities, professional training, and collaboration. Expanding this model could further improve assistance, training, and research in RD.

#### GENETIC VARIANTS IN *MYH7* AND *MYH6* GENES SUGGEST A POTENTIAL SHARED HAPLOTYPE

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**Introduction:** Hypertrophic cardiomyopathy (HCM) is characterized by unexplained left ventricular hypertrophy, which can impair the heart's ability to function properly. This condition is often associated with sarcomeric genetic variants that affect proteins crucial for the heart's structure and function. Common mutations linked to HCM typically involve genes encoding sarcomeric proteins, such as *MYH7* (beta-myosin), *MYBPC3* (cardiac myosin-binding protein C), and *MYH6* (alpha-myosin). *MYH7* encodes the beta heavy chain of cardiac myosin, which is predominantly expressed in the human ventricles, while *MYH6* encodes the alpha heavy chain, essen-

tial for heart contraction. Both genes are located in the same chromosomal region and may co-segregate either completely or partially.

**Material and Methods:** In this study, we examined four families with hypertrophic cardiomyopathy (HCM) who share the same genotype. Genomic DNA was extracted from blood samples, and whole exome sequencing (WES) was performed using the Illumina. The sequencing data were aligned to the human reference genome (GRCh38), and variants were annotated and analyzed using the Franklin - Genoox webserver. Linkage disequilibrium analysis was used to determine the co-inheri-

tance of these variants as a haplotype. Sanger sequencing was performed to confirm the specific variants and their co-segregation analysis.

**Results:** The analysis revealed the co-inheritance of the variants *MYH7* c.788T>C and *MYH6* c.5072G>A. The findings suggest that these variants co-segregate in a haplotype. These variants were consistently inherited together in affected individuals, supporting their potential

role in the development of HCM.

**Conclusion:** The identification of co-inherited variants in *MYH6* and *MYH7* reveals a haplotype that increases the risk of HCM. This approach can be effectively used in the search for new variants associated with HCM and in the potential screening of variants of uncertain significance in both genes.

## COMPREHENSIVE MOLECULAR PROFILING OF AN ARGENTINIAN PEDIATRIC COHORT OF PATIENTS WITH RASOPATHIES

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Rasopathies are heterogeneous genetic disorders with phenotypic overlapping features caused mainly by germline variants in RAS/MAPK pathway. Noonan syndrome (NS) is the most prevalent. These syndromes are transmitted as dominant traits, but recessive inheritance has also been recognized. Somatic variants have also been identified in this pathway. Molecular diagnosis is essential for the identification of these syndromes.

**Aim:** to perform a comprehensive molecular profiling of a pediatric cohort of patients with RASopathies and RAS/MAPK related disorders, and to assess the diagnostic yield of the molecular algorithm.

**Methods:** Our cohort consists of 320 unrelated individuals with Rasopathies and related disorders (Capillary Malformation-Arteriovenous Malformation syndrome, CM-AVM and Cutaneous Skeletal Hypophosphatemia syndrome, CSHS) evaluated at the Genetic Department of the Hospital Garrahan from 2014 to 2024. The main clinical characteristics were recorded. DNA samples were studied by Sanger sequencing and NGS.

**Results:** In 301/316 patients with Rasopathies relevant variants were detected in 16 different genes of the RAS/MAPK pathway, PTPN11 was implicated in 59% of NS cases. All variants were missense except for one deletion and two nonsense variants in LZTR1. Novel variants were identified in RAF1, SOS1 and LZTR1, three likely pathogenic and one of uncertain significance. Biallelic variants in LZTR1 were detected in three patients. Molecular studies confirmed the clinical diagnosis in 294 patients with RASopathies. Pathogenic variants in RASA1 were detected in two children with CM-AVM, and somatic variants in HRAS in two patients with CSHS. NGS increased the detection rate by 24%.

**Conclusions:** The molecular algorithm showed a high diagnostic yield (93%). The novel variants detected broadened the reported variants spectrum in RASopathies. The molecular diagnosis allow to confirm the clinical diagnosis, enable a targeted follow-up and an accurate genetic counseling.

## MOLECULAR ANALYSIS OF DYSTROPHINOPATHIES: THE PATHWAY TO PRECISION MEDICINE

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Muscular dystrophies (MD) are hereditary diseases caused by alterations in genes essential for muscle repair and regeneration. Dystrophinopathies, the most common form in children, are caused by alterations in the *DMD* gene. Diagnosing these diseases is difficult due to overlapping symptoms. Early detection is crucial to prevent patient deterioration. Thanks to NGS, diagnosing these diseases has become easier. This study generates a large amount of data that must be accurately classified following international guidelines. But variants of uncertain significance (VUS) remain challenging to categorize, being crucial their reclassification. Also, bioinformatics

tools can introduce errors that must be manually corrected for accurate variant calling. The aim of the study was to identify and characterize molecular alterations in the *DMD* gene in patients with a clinical suspicion of dystrophinopathy and in women at risk of being carriers.

A molecular algorithm was implemented: MLPA for CNVs and NGS for SNVs. In cases where no alteration in *DMD* was found, the search was extended to genes associated to other MDs. In cases of genotype-phenotype discordance, cDNA sequencing was performed from mRNA obtained from muscle biopsy to identify aberrant transcripts.

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The molecular algorithm confirmed the diagnosis of dystrophinopathy in 85% of the patients, increasing to 91.2% when including other associated genes. Women at risk of carrying *DMD* variants were evaluated, confirming carrier status in 32.8% of cases. Two VUS were reclassified and three errors in variant calling were found. An aberrant isoform was identified, helping explain the genotype-pheno-

type discordance observed in a patient. Variant analysis allowed the identification of specific therapies applicable to patients. Additionally, 80.3% of the alterations found in our cohort could be corrected by skipping an exon. In conclusion, an exhaustive analysis of the *DMD* gene was performed, achieving a differential diagnosis.

#### THE ENZYMES TDP1 AND MRE11 PARTICIPATE IN THE REGULATION OF DNA END RESECTION AND IN NASCENT STRAND DEGRADATION UPON FORK REVERSAL BY TWO DIFFERENT MECHANISMS

**Néstor Aznar, Camila Gosso, Irene Larripa, Marcela González-Cid, Marcelo de Campos Nebel**

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The processing of DNA double strand breaks (DSB) and the reversal of stalled forks following replication stress are two essential mechanisms for the maintenance of genomic integrity. Both processes include a nucleotide degradation step, called DNA end resection (DER) and nascent strand degradation (NSD), respectively. Etoposide (ETO) is a topoisomerase II (TOP2) poison that stabilizes TOP2-DNA complexes (TOP2cc). When the replication machinery encounters these complexes, it can lead to replication fork collapse associated to DSB, or to the reversal of the stalled forks. We have previously demonstrated that the nuclease Meiotic Recombination 11 (MRE11) works together with Tyrosyl-DNA Phosphodiesterase 1 (TDP1) in the removal of ETO-induced TOP2cc and DER. Here we analyzed the participation of TDP1 and MRE11 in NSD in HeLa cells to clarify the role of both enzymes in DNA degradation processes. The analyses of ETO-induced ssDNA formation resulting from DER showed that chemical inhibition of MRE11,

the genetic ablation of TDP1, or a combination of both caused a decrease in DER ( $P < 10^{-4}$ ). The decreased level of DER in the absence of TDP1 was not related to differences in the MRE11-chromatin loading. Besides, the DNA fiber analysis demonstrated that lack of TDP1 or MRE11 inhibition diminished significantly the NSD ( $P < 10^{-4}$ ) induced by ETO or hydroxyurea, both known inducers of stalled replication forks. However, MRE11 inhibition in cells lacking TDP1 showed an increased NSD. The analysis of cell survival by the MTT and the colony formation assays showed hypersensitivity to ETO of cells silenced in TDP1, with MRE11 inhibited or the combined defect ( $P < 0.05$ ). However, there was no additive or synergic effect when both conditions were joint together. Overall, despite the epistatic effect of MRE11 and TDP1 in replication stress-induced DER, our results demonstrate an involvement of TDP1 in NSD and suggest that different regulatory events are operating in both processes.

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## BIOINFORMÁTICA Y BLANCOS TERAPÉUTICOS

## P1 POSTERS

FECHA Y HORA: 19/11/2024 16:00-17:00 H

COORDINADORES: CARLOS DAVID BRUQUE, MARÍA ANDREA CAMILLETI, KARINA FORMOSO

## 1. 034 FLUOXETINE AS A GLOBAL SUMOYLATION INHIBITOR: UNRAVELING THE MOLECULAR MECHANISM THROUGH IN VITRO AND COMPUTATIONAL APPROACHES

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SUMOylation is a post-translational modification in which a small ubiquitin-like modifier (SUMO) is conjugated to a target protein. This conjugation not only alters the function and fate of a wide range of proteins, but also regulates multiple biological processes and has been implicated in many pathological conditions. Using in vitro SUMOylation assays followed by western blot, we showed that the widely used Selective serotonin reuptake inhibitor, fluoxetine decreases basal levels of FKBP51 SUMOylation (Relative SUMOylated Protein Levels at [FLX] = 1  $\mu$ M: 5.28 $\pm$ 0.33,  $p < 0.001$ ). We also showed, by using thermal shift assays, that unlike tricyclic antidepressants, fluoxetine acts as a global SUMOylation inhibitor that directly binds to UBC9 ( $\Delta T_m$  at [FLX] = 250  $\mu$ M: -0.70 $\pm$ 0.17  $^{\circ}$ C,  $p < 0.05$ ), the only E2 conjugating enzyme of the SUMOylation pathway, and by in vitro SUMO assays that fluoxetine selectively blocks the formation of the E2-S-SUMO intermediate (Relative SUMOylated Protein Levels at [FLX] = 10  $\mu$ M: 0.50 $\pm$ 0.05,  $p < 0.001$ ). Furthermore, we used computational methods to explore possible binding modes between UBC9 and FLX. First, we obtained a pose for the FLX binding mode from a biased molecular docking simulation. Then, we evaluated it by molecular dynamics simulations analysis; and finally, we found a system with an identifiable representative binding mode characterized by the specific interaction with four residues: Lys65, Lys74, Glu98 and Glu99.

## 2. 074 IDENTIFICATION OF POTENTIAL NOVEL EPIGENETIC BIOMARKERS FOR THE DIAGNOSIS OF PRECURSOR LESIONS OF COLORECTAL CANCER

Agustina R. Angeloni<sup>1</sup>, Victoria Bocanegra<sup>2</sup>, Rodrigo Luis Ongay<sup>3</sup>, Martin Toro<sup>3</sup>, Erika Gudino<sup>3</sup> and Emanuel M. Campoy<sup>1,2</sup>.

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**Introduction:** Colorectal cancer (CRC) is the second most common and fatal neoplasm in Argentina. Despite its preventable nature, CRC develops gradually, starting with epigenetic changes and mutations that lead to precursor lesions (PLs), some of which have a higher risk of progressing to aggressive neoplasms. DNA methylation

presents distinct profiles across different tissues, including PLs. This study aims to identify and characterize methylation profiles in PLs compared to normal tissues and cancerous tissues. **Methods:** We established a comprehensive, normalized database of PL methylation profiles using Illumina Methylation array data, covering approximately 450,000 potentially methylated CpG sites. This database integrates data from samples obtained through collaboration with regional gastroenterologists, alongside profiles from other research projects, including TCGA tumor samples, with detailed phenotype annotations such as histology and recurrence. **Results:** We developed an R application using the Shiny package for dynamic exploration and visualization of methylation data, enabling statistical calculations and comparison of methylation profiles across PLs, cancer, and normal tissues. Our analysis revealed distinct methylation profiles between PLs and normal tissues. High-risk PLs exhibited higher methylation levels, similar to those in cancer tissues, and these elevated levels persisted through different stages of CRC. By applying filters such as gene selection and median values, we identified approximately 30 genes with PL methylation levels significantly higher than in normal tissues at more than two sites. **Conclusion:** Our database and analytical tools effectively distinguish precursor lesions from normal tissues based on methylation profiles. High-risk PLs exhibit methylation patterns similar to cancerous tissues, enhancing the identification of key genes associated with increased risk, potentially improving early detection and risk assessment in colorectal cancer.

## 3. 112 LNCRNA PROFILING FOR COLORECTAL CANCER MOLECULAR SUBTYPES: VALIDATION AND APPLICATION IN BIOPSY AND BLOOD SAMPLES

Marcela Astiz<sup>1</sup>, Mariano Di Tommaso<sup>2</sup>, Melissa Hidalgo<sup>2</sup>, Natividad Perlo<sup>2</sup>, Rocio Laurini<sup>2</sup>, Leandro Michelena<sup>2</sup>, Leandro Spaletti<sup>2</sup>, Malena Solis<sup>2</sup>, Federico Campomenosi<sup>2</sup>, Carla Salmon<sup>2</sup>, Eugenia Salas<sup>1</sup>, Martín Rabassa<sup>1</sup>, Eduardo Alach<sup>2</sup>, Martín Abba<sup>1</sup>, Ezequiel Lacunza<sup>1</sup>.

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Colorectal cancer (CRC) screening with video-colonoscopy is advised for individuals aged 45 and older to detect polyps and prevent CRC. Although colonoscopy has greatly enhanced the detection of adenomas and cancers, the complex molecular diversity of these lesions underscores the need for precision medicine. Long non-coding RNAs (LncRNAs) play a role in colorectal tumorigenesis and may serve as biomarkers and therapeutic targets. In prior analyses using RNA-seq and microarray data, we identified distinct LncRNA signatures for CRC subtypes CMS1-4, showing high classification accuracy (AUC > 0.8), prognostic significance ( $p < 0.05$ ), and predictive value ( $p < 0.01$ ). This study aimed to validate several of these LncRNAs representative of each subtype by analyzing 36 pre-surgical colorectal biopsy samples (18 healthy, 18 CRC) and peripheral blood plasma samples. We assessed LncRNAs COLCA1 (CMS3), EGFRAS1 (CMS2), AFAP1AS1 (CMS1), and HAND2AS1 (CMS4) using RT-qPCR, revealing expression variability among samples. We identified at least two subgroups with high and low expression levels for each gene ( $p < 0.01$ ), highlighting tumor heterogeneity. Expression patterns varied by tumor location: AFAP1AS1 and EGFRAS1 were more expressed in the right and left colon, respectively, aligning with CMS1 and CMS2 locations. High COLCA1 expression was mainly in the right colon and rectum. HAND2AS1 was less detectable, likely due to its association with the rarer CMS4 subtype.

Detection of these LncRNAs in peripheral blood was successful in both healthy individuals and CRC patients, indicating their potential as liquid biopsy markers. Bioinformatics analysis of 1000+ colorectal tumor and adenoma samples offered more insights into these LncRNAs. Currently in its validation phase, this study offers a quantitative approach for screening LncRNAs in polyps and colorectal tumors using minimally invasive blood tests or standard colonoscopy, supported by accessible RT-qPCR technology.

**4. 174 STUDY OF FACTORS ASSOCIATED WITH OVERCROWDING: SITUATION DIAGNOSIS AND IDENTIFICATION OF PROGNOSTIC MARKERS**

Guillermo Keller<sup>1,2</sup>, Patricia Delnero<sup>1</sup>, Pablo Manjarin<sup>1</sup>, Silvana Oviedo<sup>1</sup>, Carlos Hurtado<sup>1</sup>, Lorena Romano<sup>1</sup>, Alejandro Aymar<sup>1</sup>.

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**Introduction:** Overcrowding in emergency departments (EOC) is a common and serious phenomenon in many countries, and lacks a standardized definition and measurement methods. In our country it is known but has not been studied. **Aim:** to propose an appropriate method for diagnosis and alerting the system in order to avoid complications of EOC. **Methodology:** For 1 year, the recognized parameters (% occupancy, length of stay, time to hospitalization, number of hospitalizations, hospital deviation) and additional proposed parameters (need for referral, use of supernumerary resources) were evaluated in an emergency department. **Results:** During the analysis period, 101,177 on-call consultations were recorded, of which 94% were completed (6% were withdrawn). The population had an average age of 34+/-23 (0-104) years, with 53% women. The initial consultation was performed in the general adult ward (54%), emergency room (0.6%), pediatrics (20%), obstetrics and gynecology (4.4%), traumatology (17%), and febrile clinic (4%). 2060 adult patients were admitted, who were significantly older, 56+/- 22 (22-104), 55.5% men, with a boarding time of 3+/-8 (1-15) days, hospital stay of 13+/- 21 (1-300) days, resulting in a boarding time percentage of 45+/-20%. The hospital deviation showed extreme variability ranges 15+/-40 (5-50)%. No significant association was found between boarding time and type of discharge (discharge, referral, death), or between hospital deviation, boarding time and other markers. **Conclusion:** The high variability of results and the lack of association between recognized markers and outcomes suggest the existence of functional rather than structural differences that can be corrected through process standardization strategies.

**5. 198 THE EXPRESSION OF ADRENERGIC RECEPTORS CORRELATE WITH RELEVANT PROTEINS AND PATHWAYS IN PUBLIC DATABASES FROM BREAST CANCER**

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Breast cancer is the most diagnosed female malignancy globally (GLOBOCAN 2022). We have previously investigated GEO data for the expression of adrenergic receptors and their relationship with disease-free survival. The present investigation aimed to analyze TCGA and METABRIC databases to assess their relation with relevant proteins and pathways for breast cancer. Negatively and positively correlated genes to adrenergic receptors were retrieved from METABRIC dataset (Spearman,  $p < 0.05$ ) and enrichment analysis performed with STRING (GOBP,  $FDR < 0.05$ ). Genes positively correlated to ADRA2B were associated with Golgi, probably linked to secretion, ADRA2C with angiogenesis, cell migration and protein phosphorylation and ADRAB2 positive, with enhanced immune system pathways. ADRA2A positively correlated were associated with cell adhesion, while negatively correlated with cell cycle pathway. ADRA2A positively correlates with ESR1, PGR, TP53 and GATA3 in the Metabric and TCGA databases ( $p < 0.0001$ ). ADRB2 also positive with ESR1 and PGR in TCGA but negative in Metabric for ESR1 and no correlation with PGR. ADRA2B negatively correlates with ESR1,

PGR, TP53 and GATA3 ( $p < 0.0001$ ). ADRA2C showed a positive correlation with ESR1 and GATA3 in TCGA but only positive correlation with PGR in Metabric highlighting the importance on interrogating different databases in order to conclude more accurately. All four adrenergic receptors negatively correlated with MKI67, PCNA, although with different slopes ( $p < 0.0001$ ), suggesting that their differential effect on disease-free survival is not due to different effects on proliferation, but probably on their correlation with steroid receptors or the pathways found. These adrenergic receptors related genes impact on relevant pathways for breast cancer which may explain the increased disease-free survival of patients with high expression of ADRA2A and ADRB2 in these databases.

**6. 303 MODELING OF THE ELECTROSTATIC POTENTIAL OF THE NA<sup>+</sup>/H<sup>+</sup> EXCHANGER ISOFORM 1 (NHE1) AND ANALYSIS OF ITS INTERACTION WITH CHP PROTEINS**

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The Na<sup>+</sup>/H<sup>+</sup> exchanger NHE1 is an electroneutral transporter present in the plasma membrane. It plays a role in regulating pH and cellular homeostasis. Signals from the extracellular environment and binding to calcineurin proteins (CHP1 and CHP2) activate NHE1. In healthy cells, NHE1 regulates the pHi in ranges from 6.99 to 7.15, while in cancer cells, the pHi range is from 7.12 to 7.7, generating an acidic pHe due to the excreted protons. This gradient reversal could coincide with aberrant regulation of NHE1. This work aimed to evaluate at a structural level the behavior of NHE1 charges at different pH ranges and whether the interaction with CHP1 and CHP2 proteins is involved in an increase in NHE1 activity in cancer cells. We modeled the electrostatic potentials (EP) of the NHE1 atoms between pH 6.5 and 7.5. We use the adaptive Poisson-Boltzmann Solver packages and the PDB database. We also used the Chimera software to study the interaction between NHE1 CHP1 and CHP2 molecules. We observed an asymmetric surface PE between the inner and outer face of the protein. The pH range of 7-7.5 in the modeling showed an increase in the negativity of the intracellular face, while the extracellular face remained mostly positive. In the interaction analysis, we found 20 and 21 amino acids of NHE1 potentially involved in the binding of CHP1 and CHP2, respectively (interaction distance: 3.8 Å). Most of them are hydrophobic, as already described. At the same time, we observed more hydrogen bond interactions between CHP2 and NHE1 than between CHP1 and NHE1, which could be associated with a greater proton efflux. We conclude that at pH 7, the positive charges on the external face could inhibit the Na<sup>+</sup>/H<sup>+</sup> influx/efflux mechanism of NHE1. Therefore, this mechanism could be associated with factors such as the interaction between CHP2 and NHE1. More studies are needed to elucidate this hypothesis.

**7. 410 INSIGHTS FROM A BIOINFORMATIC ANALYSIS: THERAPEUTIC POTENTIAL OF MUSE CELLS IN AN OVINE MODEL OF ACUTE MYOCARDIAL INFARCTION**

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**Background and Aims:** Acute myocardial infarction (AMI) remains a leading cause of mortality worldwide. Although cellular therapies aim to stimulate angiogenesis and promote cell survival, offering the potential for improved cardiac repair and recovery, there is currently no effective cellular therapy for the treatment of AMI. This

study aims to conduct a bioinformatic analysis of gene expression to identify key biological processes involved in the therapeutic potential of the novel adipose tissue-Multilineage-differentiating Stress Enduring (AT-MUSE) cells as an alternative to the currently most researched MSCs. **Methods:** Raw normalized microarray data files were retrieved from GEO (Gene Expression Omnibus). The differential expression genes between human AT-MSCs vs. AT-MUSE cells (GSE46353) and between sheep infarct border zone (bz-AMI) vs. Sham (GSE144509) were processed with GEO2R employing Limma pipeline (Benjamini & Hochberg FDR < 0.05, log<sub>2</sub> FC > 1, Significance level cut-off p < 0.05). Gene ontology (GO) analyses were performed to enrich GO terms using the Metascape database. **Results:** Tube morphogenesis (GO:0035239, LogP=-14.08; Log(q)=-11.60) and extracellular matrix organization (GO:0030198, LogP=-20.34; Log(q)=-17.48) were significantly enriched in bz-AMI and AT-MUSE cells. Compared to AT-MSCs, AT-MUSE cells exhibited overexpression of genes *PXDN*, *EFEMP2* and *LOXL2* (Log FC > 2 and p < 0.005). Additionally, when compared to Sham, bz-AMI exhibited overexpression of *TGFB2*, *COL1A1*, *COL1A2* and *COL3A1* (Log FC > 1 and p < 0.01). **Conclusions:** The upregulation of these genes in both the infarcted tissue and AT-MUSE cells indicates a potential relationship between these processes, suggesting that AT-MUSE cells may enhance or complement the reparative mechanisms within the infarction zone. AT-MUSE cells exhibit enhanced tube morphogenesis and matrix organization compared to MSCs, which may contribute to their superior regenerative potential.

## P2 POSTERS

FECHA Y HORA: 20/11/2024 11:30-12:30 H

COORDINADORES: ARIEL PABLO LÓPEZ, NATALIA CRISTINA FERNÁNDEZ

### 8. 285 THE ROLE OF THE VAV PROTEIN FAMILY IN CUTANEOUS MELANOMA: COMPARATIVE STUDY OF TRANSCRIPTOMIAL PROFILES

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Vav proteins are a family of RHO guanine nucleotide exchange factors (GEFs), which typically exhibit functional redundancy and are associated with proactive functions in cancer. However, their role of this three member family in melanoma remains largely unexplored. Our aim was to investigate the role of each member of the Vav family in melanoma, by bioinformatic techniques. Gene expression from cutaneous melanoma patients were obtained from the 'Cancer Genome Atlas' database. The patient cohort (n=460) was stratified based on high/low expression of Vav1, Vav2, and Vav3. Using the Kaplan-Meier estimator, survival plots were generated, and the log-rank test revealed an association between high Vav2 and poorer prognosis, whereas elevated Vav1 and Vav3 were correlated with increased patient survival probability (p<0.05). Gene set enrichment analysis was conducted using the GSEA. Immune and stromal cell infiltration in tumor tissues, Immune Score and Microenvironment Score were calculated using ESTIMATE and xCell algorithms. Both Scores showed a strong positive association with Vav1 and Vav3 (p<0.001). A robust positive correlation was identified between Vav1 and some types of immune cell signatures (p<0.001), but no significant correlation was observed for Vav2 or Vav3. Protein-protein interaction analysis, using STRING and Cytoscape, revealed that Vav1 and Vav3 share interactions with the tyrosine kinases BTK and ITK, but not with Vav2, suggesting a specific GEF function for each Vav. Our findings suggest that a favorable prognosis is linked to high Vav1 and Vav3 expressions, coupled with reduced Vav2 levels. This prognosis may arise from Vav1's impact on intercellular communication within the tumor microenvironment, while Vav3's role could regulate the activation of tumor cell signaling pathways, thereby

enhancing immunogenicity. Our study offers a comprehensive pipeline that could explore the implications of other proteins in various disease contexts.

### 9. 345 MACHINE LEARNING STRATEGIES TO ENHANCE PROGNOSTIC ACCURACY IN PROSTATE CANCER

Juan Bizzotto<sup>1,2,3</sup>, Agustina Sabater<sup>1,2,3</sup>, Pablo Sanchis<sup>1,2,3</sup>, Elba Vazquez<sup>1,2</sup>, Javier Cotignola<sup>1,2</sup>, Geraldine Gueron<sup>1,2</sup>

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Molecular signatures derived from gene expression profiling can play a critical role in guiding prostate cancer (PCa) treatment decisions. Transcriptomic data from high-throughput technologies generate thousands of measurements per patient, and complex dependencies among genes necessitate machine learning (ML) algorithms identify potential biomarkers. In this study, we evaluated ML strategies to filter, prioritize, and combine genes into a gene expression signature associated with disease progression. By leveraging 12 large-scale gene expression datasets, comprising 1,870 PCa patient samples from public repositories, we aimed to identify key stemness-associated genes and develop a risk score for predicting PCa patient outcomes. When available, we integrated clinical predictors with gene expression data. Our analysis demonstrated that using a multivariable Cox model followed by random forest is a robust strategy for selecting a core group of genes. Subsequently, we evaluated regression models (Cox, Ridge, and Lasso) to create a 7-gene risk score capable of risk stratification of PCa. This stemness-related signature was validated in five independent datasets. Survival analyses showed a significant association between higher risk scores and disease progression, across validation datasets, independent of clinicopathological features (Cox p < 0.001). In summary, we established a workflow to benchmark the performance of ML strategies in creating a gene expression signature that enhances prognostic accuracy and identifies key stemness-related genes associated with disease progression. These findings highlight the importance of integrating advanced computational methods with genomics and transcriptomics to drive personalized treatment strategies for PCa and support the development of robust prognostic models to guide clinical decision-making.

### 10. 368 CHARACTERIZATION OF *FMR1* ISOFORMS IN A HUMAN GRANULOSA CELL LINE

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The *FMR1* gene, located in the X chromosome, encodes for the FMRP protein and is involved in 4 genetic disorders. Through alternative splicing of its mRNA, the primary transcript of *FMR1* can generate numerous isoforms, suggesting that each of them may have a specific cellular role and a different biological function. In particular, we are interested in the Fragile X-associated primary ovarian insufficiency (FXPOI) and in the expression pattern of the isoforms in ovarian tissue. In previous studies, we identified several isoforms during folliculogenesis in the rat ovary, but due to experimental design, we could not detect all the putative-expressed isoforms. Similarly, there are no studies in human tissues that describe all the expressed isoforms and their complete sequence. Therefore, as a first objective of our study, we aimed to describe *FMR1* isoforms in a human granulosa cell line (KGN) using long-read sequencing. We isolated total RNA from KGN cells to synthesize cDNA. *FMR1* transcripts were obtained by PCR amplification using primers specific



to the first and last exon and the 3' UTR of the gene. Next, Oxford Nanopore Technologies (ONT) sequencing libraries were generated and the samples were sequenced using our sequencing platform. The results presented here correspond to two independent experiments. We described a total of 13 isoforms in human KGN cells, 7 of them have never been described before and present newly characterized splicing combinations. In conclusion, the new primer design combined with long-read sequencing allowed us to characterize previously described isoforms with known splicing sites and describe new isoforms that result from additional spliced exons.

**11. 411 A COMPREHENSIVE APPROACH TO IDENTIFYING THERAPEUTIC OPPORTUNITIES IN 5-FU-RESISTANT COLORECTAL CANCER: RAC1 PATHWAY AS A POTENTIAL TARGET**

Luciano Anselmino<sup>1-4</sup>, Florencia Malizia<sup>1-4</sup>, Aylén Avila<sup>3-4</sup>, Nahuel Cesatti Laluece<sup>1-4</sup>, Macarena Mamberto<sup>1-4</sup>, Lucía Zanotti<sup>1-4</sup>, Cecilia Farré<sup>1-4</sup>, Mauricio Menacho Márquez<sup>1-4</sup>

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Colorectal cancer (CRC) is the third most common cancer globally and a leading cause of death. 5-fluorouracil (5-FU) is a widely used chemotherapy for CRC, either alone or in combination, but resistance occurs in about 50% of cases. To understand the mechanisms behind CRC resistance or recurrence after 5-FU treatment, we conducted a comprehensive study using *in silico*, *in vitro*, and *in vivo* approaches. We identified differentially expressed genes between recurrent and non-recurrent phenotypes after 5-FU monotherapy across public gene expression datasets (FDR≤0.05, |logFC|>1). These DEGs were used for pathway enrichment (p-values≤0.05), gene set analysis (FDR-P ≤ 0.05, |NES|>2), and transcription factor motif detection (FDR-P<0.001, |NES|>3). Additionally, we created a merged gene expression matrix from patients treated with FOLFLOX and FOLFIRI regimens, applying feature selection and machine learning techniques to identify recurrence predictor genes. Our findings suggested Rac1 pathway as a potential target to overcome 5-FU resistance. Pull-down experiments confirmed significantly increased Rac1 activity in 5-FU-resistant CRC cells compared to sensitive ones. Guided by our bioinformatics analysis, we used drug repositioning databases to identify drugs that could restore 5-FU sensitivity (p<0.05). Notably, the novel Rac1 inhibitor 1A-116 significantly reduced the viability of 5-FU-resistant cells (p<0.05) and resensitized them to the treatment. It also reversed epithelial marker expression and morphological changes associated with resistance, restoring a control-like phenotype. *In vivo* studies further demonstrated that 1A-116 reduced tumor growth and metastasis. These results suggest that targeting Rac1 holds promise for developing new therapies for CRC patients resistant to 5-FU-based treatments.

**12. 468 SPATIAL EXPRESSION PATTERNS OF S100A4 AND S100A9 IN CERVICAL CANCER AND THEIR IMPACT ON IMMUNE EVASION**

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Cervical cancer is the second most common cancer affecting women worldwide, with squamous cell carcinoma (CESC) being the most prevalent subtype. Understanding the molecular mechanisms underlying CESC progression is crucial for developing more effective therapeutic strategies. The S100 family of proteins plays a pivotal role in both cancer development and immune response modulation. Previously, we demonstrated that patients with CESC have a higher number of S100A9<sup>+</sup> circulating cells compared to healthy individuals. However, the precise role of S100 proteins in CESC remains un-

clear. This study aimed to determine the gene expression patterns of S100A4 and S100A9, two immunomodulatory proteins, in CESC through bioinformatic analyses using TCGA and GTEx databases, alongside an analysis of high-throughput sequencing experiments. Our detailed analysis of gene expression data revealed that CESC is the second highest tumor type in terms of S100A9 expression, with a distinct pattern observed in tumor cells compared to normal tissue. Unlike other tumor types where S100A9 and S100A4 expressions are correlated, no such association was identified in CESC, prompting further investigation using high-throughput single-cell RNA sequencing datasets. Our findings indicate that S100A9 is markedly overexpressed in CESC tumor cells, while S100A4 expression is significantly reduced. In contrast, adjacent non-tumor cells exhibit elevated levels of S100A4. Further investigation into infiltrating immune cells revealed that CD8<sup>+</sup> cells exhibit increased S100A4 expression, coupled with signs of immune exhaustion, such as high levels of NFAT5, NR4A1, and NR4A2 expression. These results underscore a distinct spatial differentiation in the expression of S100A4 and S100A9 within the tumor microenvironment, potentially contributing to negative immune modulation in CESC.

**13. 474 EXPLORING CONSERVED DENV REGIONS AS TARGETS FOR VIRAL INHIBITION USING RFXCAS13D**

María Eugenia Oneto<sup>1\*</sup>, Fernando Gabriel Mazur<sup>2\*</sup>, Analía Vanesa Kachuk<sup>2</sup>, Marcos Mateo Miretti<sup>1,2</sup>, Sonia Lorena Espíndola<sup>1,2</sup>.

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\*The authors have contributed equally.

**Objective:** Develop an antiviral strategy against dengue virus serotypes by identifying conserved genomic regions as targets for the RfxCas13d system. **Methodology:** Subregions of 3'UTR, 5'UTR, and NS5 regions of the DENV genome were selected as targets due to their conservation and role in viral replication. All available DENV genomes from the BV-BRC platform were downloaded, aligned and manually curated to create specific datasets for each target region. These datasets were used to design RNA guides (gRNA) with Cas13Design, yielding over 500 results. The generated guides were evaluated for homology and thermodynamic properties using Blastn and RNAup, selecting the most promising ones. Representative sequences from different lineages (countries and years) were then analyzed to examine the structural variability of the target regions using LocARNA and RNAclust. This allowed the identification of conserved regions and the assessment of the gRNA robustness against the virus's genetic diversity. **Results:** We obtained a specific gRNA for each region: 3'UTR, 5'UTR, and NS5. Hybridization and MFE analyses showed that 5'UTR and NS5 targets are more favorable for RfxCas13d activity than 3'UTR. Thermodynamic and structural evaluations revealed lower variability sites in 5'UTR and NS5, while 3'UTR had less conserved sites. Despite greater structural heterogeneity in 3'UTR, the selected target included all serotypes. Clustering analysis of the three regions grouped model sequences with local lineages (AR-PY) and ancestral Southeast Asian lineages. **Conclusion:** The combined use of designed gRNAs is expected to enhance the antiviral strategy. The selected regions showed conserved structures across diverse DENV lineages, underscoring their potential as RfxCas13d targets. In summary, this study emphasizes the importance of investigating the structural variability of RNA in order to design effective universal antiviral strategies.

**14. 487 COMPREHENSIVE PROTEIN DOMAIN ANALYSIS TO DISTINGUISH HIERARCHICALLY AND FUNCTIONALLY RELATED CELL TYPES**

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Osteosarcoma (OS), is the most common malignant bone tumor, affecting 2% of the world's pediatric cancer population. Lung metastases pose a significant clinical challenge, with five-year survival rates unchanged since the 1970s. The tumor microenvironment (TME), including stromal cells such as fibroblasts and mesenchymal stem cells (MSC), is crucial in OS progression. Adopting another perspective to assess the relation among TME cells, we examined intracellular and extracellular compartments in MSC and OS cells, focusing on their protein domains. Samples were collected and subjected to mass spectrometry in triplicate. Data were scaled using Rstudio, and euclidean distances were calculated. Clustering yielded a cophenetic correlation of 0.991, clearly distinguishing MSC from OS cells. Despite their divergent biological behaviors, metastatic (LM7) and non-metastatic (SAOS2) OS cells exhibited homology in both intracellular and extracellular compartments. We identified three distinct protein groups: MSC intracellular proteins, extracellular proteins across all samples and OS intracellular proteins, underscoring the molecular homogeneity within these compartments. Principal Components Analysis (PCA) showed that the first component effectively distinguished samples based on intracellular and extracellular contributions, while the second differentiated these compartments within each cellular group. Key proteins in cellular stress responses like the apoptosis regulator BAX and the differentiation factor AHNK, and the tubulin family protein TUBA1C were upregulated in the intracellular domain across all samples, while extracellular matrix structural proteins like COL1A1 and FN1 were predominantly associated with the intracellular domain of MSC. These results show the potential of this approach to differentiate samples by cellular progeny, potentially reducing the need for repetitive biopsies and aiding in diagnosing or assessing a patient's risk of metastasis.

## BIOLOGÍA CELULAR Y MOLECULAR DE PROCESOS FISIOLÓGICOS Y PATOLÓGICOS

### O1 COMUNICACIONES ORALES

FECHA Y HORA: 20/11/2024 16:10-17:10 H

COORDINADORES: CECILIA PODEROSO,

ANA FERNANDA CASTILLO

LUGAR: SALA DE CÁMARA

#### 15. 050 HEME OXYGENASE-1 COUNTERACTS BONE-INDUCED STEMNESS IN PROSTATE CANCER CELLS

Agustina Sabater<sup>1,2,3</sup>, Inés Achinelli<sup>1,2</sup>, Pablo Sanchis<sup>1,2,3</sup>, Juan Bizzotto<sup>1,2,3</sup>, Gastón Pascual<sup>1,2</sup>, Rocío Seniuk<sup>1,2</sup>, Javier Cotignola<sup>1,2</sup>, Elba Vazquez<sup>1,2</sup>, Geraldine Gueron<sup>1,2</sup>, Ayelén Toro<sup>1,2</sup>.  
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In Argentina, Prostate Cancer (PCa) is the third leading cause of cancer-related death in males. PCa severity and mortality is often caused by metastatic disease, which is driven by PCa stem cells (PCSCs). Our previous research has highlighted the antitumoral effects of Heme Oxygenase-1 (HO-1) in PCa. Thus, we hypothesize that HO-1 induction is associated with a less stem-like phenotype in PCa. First, we evaluated the effect of hemin (HO-1 inducer and activator) on the stemness properties of PCa cells (PC3). We observed a reduction on colony formation of hemin treated PCa cells ( $p < 0.01$ ). Further, using the hanging drop assay we showed that hemin pre-treatment is associated with a reduction in the stem-like properties ( $p < 0.05$ ). Next, we employed an indirect co-culture system of hemin pre-treated or not PC3 cells and bone progenitors (MC3T3) to emulate PCa-bone crosstalk during metastasis. We assessed the expression of metastasis/stemness-related genes in PC3 co-cultured cells by RNA-seq. PCSC and pluripotency markers were upregulated in PC3 cells co-cultured with MC3T3. However, pretreatment of PC3 cells with hemin prevented this upregulation,

underscoring the protective effect of HO-1 induction against the pro-stemness effect triggered by bone cells. Furthermore, we performed bioinformatics analysis using publicly available PCa datasets ( $n=1185$ ) to assess the clinical relevance of HO-1 regulated genes. PCa bone metastases presented higher *ADAM15*, *BCL2L1*, *LTBR* and *SPINT1* expression than primary tumors ( $p < 0.001$ ), while this difference was exclusively observed in bone metastases compared to other metastatic sites ( $p < 0.01$ ). Overall, HO-1 induction regulates the expression of metastasis/stemness-related genes in PCa cells, promoting a shift towards a more differentiated phenotype and preventing the pro-stemness effect derived from the soluble interaction with bone progenitors, reinforcing the antitumoral role of HO-1 in PCa.

#### 16. 079 GαS- DEPENDENT SIGNALING IS REQUIRED FOR THE CORRECT ESTABLISHMENT OF A FUNCTIONAL β-CELL MASS AND PROPER PANCREATIC EXOCRINE TISSUE ARCHITECTURE AND FUNCTION IN THE ADULT MOUSE

Martina Rossotti<sup>1,2</sup>, Juan I. Burgos<sup>1,2</sup>, Dana Steffen<sup>3</sup>, Agustín Romero<sup>1,2</sup>, Silvio A. Traba<sup>1,2</sup>, Silvio Gutkind<sup>3</sup>, Santiago A. Rodríguez-Seguí<sup>1,2</sup>

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Heterotrimeric G-protein coupled receptor (GPCR) signaling pathway mediated by G protein  $\alpha$ -subunit ( $G\alpha_s$ ) plays a key role in the control of pancreatic  $\beta$ -cells function and proliferation. When deleted in  $\beta$ -cells, it has been shown to be required for its growth and maturation. However, the effects of deleting  $G\alpha_s$  during pancreas development still remain largely unexplored; therefore, we sought to investigate the role of  $G\alpha_s$ -dependent signaling during  $\beta$ -cell development. Conditional knockout mice in which the  $\alpha$ -subunit of the Gs protein was ablated only and specifically from the pancreatic compartment since the specification of this organ were generated by crossing  $G\alpha_s^{fl/fl}$  mice (control) with Pdx1-Cre transgenic mice, obtaining PGsKO mice. Mice phenotype was characterized in detail, including body weight, *in vivo* glucose tolerance tests and the pancreas of PGsKO and control were further analyzed by immunofluorescence using different combinations of pancreatic cell type markers, as well as other markers revealing tissue architecture, organization and exocrine function. PGsKO were found to be hyperglycemic from 4 weeks postnatal as a result of having fewer  $\beta$ -cells due to an increased number of  $\alpha$ -cells with unusual distribution in islets. In addition, PGsKO exhibit an acinar cell size mosaic pattern, which results in functional alterations and disorganization of the exocrine tissue; leading to their malabsorption phenotype. Furthermore, a greater number of insulin-positive cells outside PGsKO islets is observed, possibly as an adaptive response to the hyperglycemia produced by the diabetic condition. We conclude that pancreas-specific  $G\alpha_s$  deficiency has different effects on the endocrine and exocrine tissue:  $G\alpha_s$  is necessary for the correct establishment of pancreatic islets composition and for appropriate tissue architecture and function. Understanding the mechanisms involved holds great interest, and claims to be explored in detail in future work.

#### 17. 309 ALS-LINKED MUTANT SOD1 PROMOTES MITOCHONDRIAL DYSFUNCTION AND ALTERS CX3CR1 EXPRESSION IN BV2 MICROGLIAL CELLS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the progressive loss of motor neurons. Superoxide dismutase 1 (SOD1) mutations—nearly 20% of familial ALS cases—promote protein misfolding in motor neurons, leading to oxidative stress, mitochondrial dysfunction, and cell death. However, the damage accumulated in other nervous cells is also relevant to neurodegeneration. Microglia play a crucial role in ALS, transitioning from neuroprotective to neurotoxic as the disease progresses. Evidence found in other neurodegenerative diseases indicates that mitochondrial dysfunction regulates microglial activation and neuroinflammation. In this study, we aimed to investigate the impact of the ALS-linked mutant SOD1<sup>G93A</sup> on the mitochondrial network in BV2 microglial cells. Also, we studied the effect on CX3CR1 expression, a receptor involved in neuronal communication that regulates microglial activation. G93A cells showed a reduction in MitoTracker Red CMXRos retention ( $p < 0.001$ , vs control and WT), indicating higher amounts of cells with  $\Delta\phi_m$  loss. Overall, SOD1-expressing cells exhibited mitochondrial fragmentation compared to control. Under oxidative conditions ( $H_2O_2$  250  $\mu$ M, 90 min), we detected mitochondrial swelling in control and WT cells. Under this condition, G93A cells displayed higher  $\Delta\phi_m$  loss ( $p < 0.001$ , vs control and WT), along with mitochondrial fragmentation and swelling. Our immunocytochemical analysis revealed that expression of the G93A variant significantly increased CX3CR1 levels ( $p < 0.05$ ) compared to control and WT. Further research is required to determine the effects of  $H_2O_2$  on this parameter. In conclusion, our results suggest that ALS-linked mutant SOD1 impairs mitochondrial function and dynamics in BV2 microglial cells, increasing their susceptibility to oxidative stress-induced damage. Understanding the regulation of microglial activation in ALS is crucial to developing new strategies to prevent motor neuron degeneration.

**18. 383 PHYSIOLOGICAL IMPAIRMENT OF IN VITRO DIFFERENTIATED CARDIOMYOCYTES FROM PATIENT-DERIVED PLURIPOTENT STEM CELLS WITH A P.GLU353DUP DESMIN MUTATION**

Sheila Lucía Castañeda, Sol Renes, Joaquín Smucler, Julia María Halek, Federico Zabalegui, Guadalupe Amin, María Agustina Scarafia, Ariel Waisman, Santiago Gabriel Miriuka, Lucía Natalia Moro

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Desmin (DES) is a type III intermediate filament involved in cytoskeletal arrangement and organelle disposition in association with other myofibrils, intercellular junctions and nuclear membrane proteins in cardiomyocytes (CMs). So far, more than 50 DES mutations have reported cytoplasmic protein aggregate formation, mitochondrial disorders, conduction system disruption and cytoskeletal disorganization in CMs. We derived pluripotent stem cell (PSC) lines from a patient with muscular dystrophy (DESY-HE, XY) that has a heterozygous mutation in exon 6 of DES (c.1059\_1061dup-GGA; p.Glu353dup) and his healthy sibling (DESS, XX). We also generated a DESY-HE homozygous PSC line by CRISPR/Cas9 (DESY-HO). The aim of our work is to determine the pathological effect of p.Glu353dup mutation in CMs differentiated from DESS and DESY-HE/HO PSC lines in order to model the DES-associated cardiomyopathy *in vitro*. Firstly, we confirmed that the three cell lines differentiated to CMs by qPCR, flow cytometry (FC) and immunofluorescence (IF) of pluripotency (NANOG), mesoderm (TBXT), cardiac mesoderm (NKX2.5) and cardiac (TNNT1) biomarkers. We assessed that DES expression was lower in DESY-HE/HO with respect to DESS CMs by qPCR and IF. We also noticed changes in expression of cytoskeletal (CRYAB, KRT14, VIM), conduction system (HCN4, ISL1, SHOX2), inflammasome (NLRP3), mitochondrial (CYCS, CYB, NDUFAF1) and ubiquitination (RPS27A) genes by qPCR and WB and disposition of mitochondria by mitotracker staining. Lastly, we observed that DES arrangement was altered in DESY-HE CMs respect to DESS CMs after 100 days of culture. In

contrast to DESS CMs, vacuoles were observed in DESY-HE/HO CMs by bright field visualization. Altogether, a pathological effect of p.Glu353dup in DES can be observed in *in vitro* differentiated patient-derived CMs, which would allow the identification of therapeutic targets and drug screening in the future.

**19. 412 GENOMIC DYNAMICS OF ESTROGEN RECEPTOR BINDING SITES IN TAMOXIFEN USERS: IMPLICATIONS FOR ENDOMETRIAL CANCER**

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The DNA binding sites of estrogen receptor  $\alpha$  (ERbs) are key flexible genomic regions under hormone regulation. Tamoxifen is a widely applied therapy in breast cancer, affecting ER interactions and shifting their DNA-binding signature after prolonged exposure. Although tamoxifen inhibits the progression of breast cancer, it increases the risk of endometrial cancer. It has been shown that ER positioning in endometrial cells of patients previously treated with tamoxifen resembles the signature of ER in mammary cancer cells. Our previous results showed that nearly 50% of ERbs of endometrial adenocarcinoma cells are shared by PR. This could explain part of the progestin regulation of estrogen effects on ER and PR positive endometrial cancer cells. Here, we explore specific endometrial transcription factor cisomes, transcription and genomic structure of tamoxifen users, non-users and of Ishikawa cells, a model of endometrial adenocarcinoma cells. We found that the ER cisome of tamoxifen users is similar to Ishikawa ER cisome, while ER cisome of non-users is closer to transcriptionally active Ishikawa PR cisome. ER binding signal in the subset of regions bounded by ER and not co-occupied by PR in Ishikawa cells was higher in tamoxifen users than in non-users. This correlated with their H3K27ac signal, indicating that tamoxifen could activate enhancer regions bounded by ER that could not be co-regulated by PR. These results indicate that tamoxifen treatment could change the ER chromatin landscape of endometrial tumor cells. The subset of ERbs of tamoxifen users that are shared by Ishikawa ERbs is enriched in early estrogen response pathway genes. On the other hand, the subset of ERbs of non-users shared by Ishikawa PRbs is enriched in NOTCH signaling pathway genes. These results propose a possible mechanism to explain a context-dependent response of genomic regions to be taken into account to prevent a deregulation of endometrial cells under tamoxifen treatment.

**20. 464 ANGIOGENIC AND VASCULAR ANALYSIS: A COMPARATIVE STUDY BETWEEN HEALTHY AND TUMORAL BREAST ADIPOSE STROMA**

Priscila Pagnotta<sup>1,2</sup>, Sebastián Fandiño<sup>3</sup>, María Lujan Crosbie<sup>4</sup>, Natalia Santiso<sup>5</sup>, Anabela Ursino<sup>5</sup>, Celeste Frascarolli<sup>5</sup>, Alicia Amato<sup>5</sup>, Rubén Dreszman<sup>5</sup>, Juan Carlos Calvo<sup>1</sup> and Judith Toneatto<sup>1</sup>

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Angiogenesis is essential for growth, development, wound healing, and plays a pivotal role in diseases, including cancer. In breast cancer, the microenvironment, mainly adipose tissue, contributes to the progression, may promote angiogenesis. The aim was to evaluate the vasculature in human breast adipose stromal explants derived from tumors and a healthy context, and to assess the angiogenic potential of their released soluble factors. We performed a histological analysis of breast adipose explants stained with hematoxylin and eosin, obtained from breast cancer patients, including those from areas: adjacent to the tumor (ADJ) and >2 cm away (DIST), as well as alongside normal controls (Normal). We also treated the endothelial EA.hy926 cell line with conditioned media from ADJ (ADJ-CM) and Normal (Normal-CM). We used Student's t test with Welch's correction, with  $\alpha = 0.05$ . We observed that ADJ and DIST explants showed an increased capillary-to-larger vessel ratio (C/LV) compared to Normal, particularly in samples with predominant adipose tissue. All patients with body mass index > 30 had C/LV ratio  $\geq 2$ , and 22.2% of patients with C/LV ratio  $\geq 2$  were premenopausal, compared to 12.5% in those with a C/LV ratio < 2. Vascular alterations were found in 2/29 ADJ and 1/27 DIST explants. We also observed that EA.hy926 cells incubated with ADJ-CM increased proliferation, migration and polygon formation, with only 6% of cases lacking tubular structures. Normal-CM induced an increase in the number and average area of polygons, with 17% of cases lacking tubular structure formation. We conclude that vasculature and angiogenic potential are different between healthy and tumoral breast adipose stroma. In the cancer context, there are vascular alterations that may be influenced by patient-specific characteristics and indicative of active angiogenesis. In turn, soluble factors released by the tumor microenvironment enhance the angiogenic capacity of endothelial cells

### P1 POSTERS

FECHA Y HORA: 19/11/2024 11:00-12:00 H

COORDINADORES: YANINA BENZO,  
MARÍA EUGENIA FERMENTO,  
EMILIANA ECHEVERRÍA

#### 21. 075 SIMULTANEOUS REGULATION OF FKBP5 GENE EXPRESSION MEDIATED BY PROGESTERONE AND GLUCOCORTICOID RECEPTORS

Maximiliano Gutierrez 1, Norma Roxana Carina Alves 2, Adali Pecci 1,2, María Florencia Ogara 1

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The glucocorticoid and progesterone receptors (GR and PR respectively) are closely related members of the steroid receptor family of transcription factors (SR). Despite they share similar structural and functional properties, the cognate hormones display very distinct physiological responses and, even in tissues expressing both receptors they exert opposite biological actions in proliferation, differentiation and cell death. Results from our group demonstrated the existence of crosstalk between GR and PR in human breast cancer cells. On the other hand, FKBP5 is a member of the immunophilin protein family, which plays a role in protein folding and trafficking. The FKBP5 gene codifies a co-chaperone of the Hsp90 complex which interacts with SR including GR and PR. Genome-wide studies in tumor mammary epithelial cells (T47DA1/2) treated with Dexamethasone (DEX) [10nM] or/and R5020 [10nM], revealed the presence of several regions co-bound by both receptors along FKBP5 gene. ChIP assays confirmed the association of both GR and PR to a single HRE site in the FKBP5 gene. Moreover, RT-qPCR of cells treated with DEX or R5020 showed FKBP5 gene expression upregulation. This induction increased even more in the presence of both hormones ( $p < 0.01$ ), suggesting an enhancement in the activity of both receptors. Due to its characteristics, the FKBP5 gene was chosen as a molecular target to evaluate the interaction between PR and GR actions. Thus, we performed a luciferase gene expression assay under the control of the FKBP5 single HRE in which different amounts of the expression vectors hGR $\alpha$  and hPR $\beta$  were co-trans-

ected along with the reporter vector pFkbp5-HRE-Luc and treated with DEX [10nM] or/and R5020 [10nM] in HEK293T cells. The results showed that upon co-stimulation with both hormones LUC expression decreases concomitant PR/GR ratio increases ( $p < 0.05$ ). This would suggest possible interference of PR on GR activity in this cellular context.

#### 22. 091 STUDY OF THE ANTITUMOR MECHANISM OF IMIQUIMOD IN HEMANGIOMA CELLS

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The topical use of Imiquimod (IQ) is approved for treating some skin pathologies due to its antiviral and antitumor activity. IQ has shown benefits in treating infantile hemangiomas, being a potential alternative to beta blockers, which are associated with concerning side effects in infants. It works by stimulating the immune system but also directly inducing tumor cell death, independently of the immune action. We demonstrated that IQ selectively induces apoptosis in transformed cells compared to normal cells and, at sublethal concentrations, impairs some stages of the angiogenic process in hemangioma cells (HC). Additionally, IQ rapidly disrupts the redox balance in transformed cells, increasing reactive oxygen species (ROS) and significantly impacting enzymatic antioxidant defenses. To further understand the direct IQ-induced cytotoxicity in HC, we evaluated nitrosative stress resulting from intracellular drug accumulation in H5V cells treated with IQ (0-10  $\mu\text{g}/\text{mL}$ ) for 24 h. Reactive nitrogen species (RNS) were detected using the Griess method. A significant increase was observed in RNS/cell ratio at 10  $\mu\text{g}/\text{mL}$  IQ ( $p < 0.05$ ). Considering that RNS and ROS can lead to mitochondrial dysfunction, we evaluated the fluorescent MitoTracker CMXROS tracer in H5V cells treated with IQ (10  $\mu\text{g}/\text{mL}$ ) for 24 h. A decreased fluorescence and perinuclear mitochondrial concentration were noted. The ultrastructural analyses of mitochondria in H5V cells treated with IQ (10  $\mu\text{g}/\text{mL}$ ) for 4 h using transmission electron microscopy revealed changes in cristae morphology, mitochondrial size, clustering, and perinuclear localization. In summary, these findings support the direct cytotoxic effect of IQ on transformed HC and its potential use as an adjuvant for therapies for transformed/tumor cell pathologies of the skin.

#### 23. 106 ANTI-INFLAMMATORY ROLE OF EXTRACTS OF LIGARIA CUNEIFOLIA DURING TUMORAL CHEMOTHERAPY IN A COLORECTAL CANCER MODEL

Quiroz Lucía<sup>1</sup>, Birocco Ariadna<sup>1</sup>, Sol Cargneli<sup>1</sup>, Ricco Rafael<sup>2</sup>, Dobrecky Cecilia<sup>2</sup>, Calabrese Graciela C<sup>1</sup>.

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The use of the gold standard drug, 5-fluoruracil (5-FU) in long-term management of colorectal cancer (CRC) is associated with drug resistance in chemotherapy. Transcriptional factor NF-kappa-B plays a key role in drug resistance. Sociocultural shifts support the use of natural compounds and their derivatives as alternatives for the treatment of chronic diseases. The aim of this work is to evaluate *Ligaria cuneifolia* (Ruiz & Pav.) Tiegh. (Loranthaceae) (LC), the 'Argentine mistletoe', as a natural alternative to sensitize colorectal cancer cells to 5-FU. We study the ability of an infusion (CE) and flavonoid-enriched fractions (FEF), both of LC, to inhibit NF-kappa-B dependent inflammatory response. Samples of LC growing on *Prosopis chilensis* (Molena) Stuntz (Fabaceae) were collected in San Juan, Argentina. The plant material was subjected to an aqueous extraction to obtain a CE fraction (an infusion, the traditional way in which this species is consumed) as well as exhaustive extractions with methanol and combinations of methanol/water followed by partition with ethyl acetate to obtain a FEF. Their cytotoxicity was evaluated on

human colorectal carcinoma cell line HCT116 by MTT and NF- $\kappa$ B subcellular distribution by immunofluorescence studies. A decrease of 50% of viability was registered after adding CE (150  $\mu$ g/ml,  $p < 0.001$ ) or FEF (70  $\mu$ g/ml;  $p < 0.001$ ). FEF (50  $\mu$ g/ml) decreased cell viability in the presence of 5-FU (5  $\mu$ M) after 24 hs ( $p < 0.001$ ; control vs FEF and 5-FU vs FEF); exerting cell sensibilization to the drug. Moreover, the improvement of sensitivity to 5-FU was associated with the inhibition of NF- $\kappa$ B nuclear translocation. These results support the potential use of LC extracts, particularly FEF, as a strategy to modulate pharmacological stress tolerance to 5-FU related to NF $\kappa$ B signaling pathway involved in drug resistance.

**24. 208 HISTOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF A SPONTANEOUS LIVER TUMOR IN MICE**

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The ES2 tumor is a spontaneously occurring neoplasm in C3H/S strain mice, characterized both biologically and morphologically in our laboratory. This tumor is atypical, firm, well-defined, and exhibits rapid growth. While liver tumor diagnosis typically relies on classic histological findings,  $\alpha$ -fetoprotein (AFP) and Hepatocyte-specific Antigen (HepPar1) serve as useful hepatocyte markers for normal and tumor populations. This study aims to: (a) assess the utility of AFP and HepPar1 markers in characterizing the spontaneous ES2 mice tumor, and (b) evaluate tumor growth through mitotic and apoptotic activity using routine techniques with Hematoxylin and Eosin (H&E) staining and immunohistochemistry for cycling cells (Ki-67). Adult male C3H/S mice with subcutaneously grafted tumors were utilized for histological and immunohistochemical analysis. Proliferation (Ki-67), mitotic and apoptotic activity (H&E), and characterization markers (AFP, HepPar1) were examined in tumors of different sizes (0.5 cm and 1.5 cm in diameter). The results were quantified as positive cells/total cells for Ki-67, mitotic, and apoptotic activity, while AFP and HepPar1 immunostaining were categorized as either positive or negative. Our findings indicate positive immunostaining for AFP and negative for HepPar1, suggesting that the tumor may be a poorly differentiated hepatocellular carcinoma. Additionally, a decrease in proliferative activity and an increase in apoptotic phenomena were observed in the 1.5 cm tumor compared to the 0.5 cm tumor, which may indicate greater cell death relative to growth. This may be due to the rapid growth of the tumor, which did not allow for the development of proper vascularization to supply oxygen and nutrients to meet the high demands of the expanding tumor cells. This preliminary work presents a promising model for studying secondary tumor behavior and evaluating factors influencing tumor growth and progression.

**25. 370 EVALUATION OF C19MC MICRORNAS CLUSTER'S ROLE IN A HUMAN PLURIPOTENT STEM CELL MODEL OF HUMAN GASTRULATION**

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LIAN-CONICET, Fleni, Sede Escobar, Ruta 9 Km 52.5. Belén de Escobar. Buenos Aires. Argentina.

Human induced pluripotent stem cells (hiPSCs) and embryonic stem cells (hESC) possess the remarkable ability to self-renew and differentiate into various specialized cell types in vitro, making them an excellent model for studying the early stages of human development. Gastrulation is an early developmental process characterized by the epithelial-to-mesenchymal transition (EMT), which is crucial for mesodermal and endodermal development, and can be modeled during the differentiation of hESC/hiPSC-derived cardiomyocytes (CMs). The primate-specific C19MC cluster is the largest microRNA cluster in the human genome, comprising more than 100 kbp, and its expression decreases upon hiPSCs differentiation. We aim to

find the role of C19MC in human gastrulation, particularly during the EMT, using hiPSC and CMs as a model. To achieve this, we generated a knockout (KO) hPSC-C19MC(-/-) line with CRISPR/Cas9. Using a monolayer differentiation protocol that resembles mesodermal and cardiac development using small molecules (CHIR and IWR1), we evaluated the generation of immature CMs of cTNT+ cells by flow cytometry, for both WT and KO C19MC cell lines. Additionally, mesodermic and EMT markers were assessed by RT-qPCR, Western Blot (WB) and immunofluorescence assays. In this work, we demonstrate that hiPSCs with a complete C19MC KO fail to differentiate into CMs. Wound healing assays indicate a disruption of EMT in C19MC KO cells. RT-qPCR analysis revealed alterations in early differentiation markers, such as EOMES, TBX6, and MESP1, as well as EMT markers, including NCadherin, Snai1, and Twist. Mesodermal markers evaluated by RT-qPCR and immunofluorescence, such as PDGFR $\alpha$ , were also affected. Additionally, NCadherin alterations were confirmed by WB and IFA. Preliminary assays with an independent cell line, hESC-C19MC(-/-), support these results. In conclusion, this work suggests that the primate-specific cluster C19MC has a role regulating the EMT during mesoderm formation in our in vitro model.

**26. 414 OVEREXPRESSION OF THE P5-ATPase 13A2 ALTERS THE ACID GLUCOCEREBROSIDASE ACTIVITY IN SH-SY5Y CELLS**

Alejandra L. Marcos<sup>1,2</sup>, Débora E. Rinaldi<sup>1,2</sup>, Mariela S. Ferreira-Gomes<sup>1,2</sup> y Felicitas de Tezanos Pinto<sup>1,2</sup>

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The P-type ion pumps are membrane transporters energized by ATP-hydrolysis which are classified into five subfamilies termed P<sub>1</sub>-P<sub>5</sub>. ATP13A1-ATP13A5 genes that belong to this group have been identified in humans. Mutations of the ATP13A2 gene were associated with neurodegenerative diseases like Parkinson's Disease, Neuronal Ceroid Lipofuscinosis (CNL12), Hereditary Spastic Paraplegia (SPG78), Amyotrophic Lateral Sclerosis and most recently with colorectal cancer. ATP13A2 is localized in lysosomes and late endosomes. Dysfunction of this protein diminishes the lysosomal protein degradation, the autophagic flux and the exosome externalization. We have previously shown that ATP13A2 expression diminishes the bis (monoacylglycerol) phosphate (BMP) content and increased the expression of  $\alpha/\beta$  hydrolase domain-containing 6 (ABHD6), which is the enzyme responsible of BMP degradation. Moreover, treatment of cells with spermine -the recently found substrate of ATP13A2- increased the relocalization of ABHD6 towards the cytoplasm. As BMP is an anionic phospholipid that is essential for lipid degradation inside acidic compartments, these results suggest that ATP13A2 may be altering this process. By using SH-SY5Y cells overexpressing the human P5-ATP13A2 (ATP13A2) or an inactive mutant (ATP13A2-D508N), we found that ATP13A2 cells show higher activity of acid glucocerebrosidase (GCase) - which is involved in the degradation of glucosylceramide- and preliminary results show that the activity of this enzyme is reduced by spermine treatment. Altogether these results support the idea that spermine transport mediated by ATP13A2 is modulating the lipid digestion process inside acidic organelles.

**27. 466 MODULATION OF MICRORNA EXPRESSION IN BREAST CANCER CELLS BY ACYL-COA SYNTHETASE 4**

Luciano Martín Quevedo<sup>1</sup>, Jessica Prada<sup>1</sup>, María Mercedes Bigi<sup>1</sup>, Ulises Orlando<sup>1</sup>, Cecilia Poderoso<sup>1,2</sup>, Seong Wook Yang<sup>3</sup>, Ana Fernanda Castillo<sup>1,2</sup>

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Acyl-CoA synthetase 4 (ACSL4) expression is increased in some cancers and is associated with aggressiveness. This enzyme esterifies long chain fatty acids, particularly arachidonic acid. Increased ACSL4 expression promotes aggressive phenotype in breast cancer cells and enhances breast tumor growth in vivo. We sought to understand the molecular events driven by this enzyme that led to a highly aggressive cellular phenotype, focusing on microRNAs (miRNAs) as potential mediators. miRNAs are small non-coding RNAs that silence genes post-transcriptionally. Using RNA-seq, we have previously shown that ACSL4 alters the expression of several miRNA precursor transcripts. Here, we studied the expression of mature miRNAs and their precursors in response to changes in ACSL4 expression. Mature miRNAs sequencing (miRNA-seq) was performed using MCF-7 breast cancer cell model overexpressing ACSL4. The differential expression profile was analyzed by comparison with control cells using Log2 fold change > |1| cutoff. From the obtained miRNA signature, predictive analysis was performed to assess each miRNA's role in biological pathways, tissue expression, and associated physiological or pathological conditions, utilizing databases like KEGG Pathway, WikiPathways, and miRPathDB. Several miRNAs were selected for further validation by RT-qPCR. We focused on miR-99a-3p, which regulates DNA damage response, repair, and cell adhesion. We demonstrated that this miRNA is downregulated in breast cancer cell models with high ACSL4 expression and upregulated in models with low enzyme expression or activity ( $p < 0.05$ ). Similarly, its precursor and the 5' associated miRNA (miR-99a-5p) also show significantly decreased expression with increased ACSL4. Therefore, we describe and validate the miRNA profile altered by ACSL4. Our results suggest that miR-99a-3p may be one of the mediators of the effects of this enzyme on the cellular phenotype of breast cancer cells.

## P2 POSTERS

FECHA Y HORA: 19/11/2024 16:00-17:00 H

COORDINADORES: SANDRA GOMEZ MEJIBA,  
GISELA GIORGI, CLARA VENTURA

### 28. 028 THE EXHAUSTIVE ROAD TO ARRIVE AT A CDG DIAGNOSIS: WHAT HAPPENS IN ARGENTINA?

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Here we present not only a report of a cohort of Congenital Disorders of Glycosylation (CDG) patients but also a road map of countless

efforts to arrive at a CDG diagnostic in Argentina. The complexity of this algorithm requires coordinated efforts to overcome the obstacles of a fragmented system. During the last 20 years, a group of professionals has been encouraged to grow according to the emerging CDG classes. The objective is to communicate the multidisciplinary efforts necessary to detect CDG patients, to make appropriate health decisions and facilitates access to emergent or palliative therapies. Multisystem clinical presentations lead the healthcare and research professionals to work enhancing the methodological algorithm. Biomarkers, such as isoelectric focusing or capillary electrophoresis of serum transferrin, remain insufficient. It must be necessary to access powerful tools as high-performance liquid chromatography/mass spectrometry and genetic tests including massive sequencing to diagnose CDG (exome (WES) or genome (WGS) sequencing). Cell models are useful in identifying biological processes to assess protein functionality in glycosylation disorders. We report 15 patients, 3 by Sanger and 12 by massive sequencing (8 WES and 4 WGS): PMM2-CDG (n: 7), ALG2-CDG (n: 2), ALG1-CDG (n: 1), ATP6AP2-CDG (n: 1), SLC39A8-CDG (n: 1), MAN1B1-CDG (n: 1), CCDC115-CDG (n: 1), PIGA-CDG (n:1), CCDC115-CDG (n: 1) and a compounds ALG13/PIGN-CDG patient in study. The knowledge of the cell pathogenesis due to CDG is necessary when Variant of Uncertain Significance (VUS) is detected. We managed to establish two cell models (661W and HEK) to test it and to evaluate the follow-up in future therapies. DISCUSSIONS: The challenge is to engage families and professionals, to make evident a collective vision of the translational medical research necessary to provide responses to patients. This work proposes a new perspective on CDG teamwork in our region ( CONICET; FONCYT; UCC).

### 29. 323 REGULATION OF ANGIOGENIC FACTORS IN DERMAL PAPILLA CELLS CULTURED AS SPHERES

Cecilia Proietti, Ana María Gaitán, Nahuel Martínez, Julieta María Ceruti, Gustavo José Leirós, María Eugenia Balañá  
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The onset of the anagen phase involves the migration of HFSCs from the bulge to the base of the bulb and their differentiation into matrix cells. Anagen initiation requires the stimulation of a new vascular network to support the growth phase. Dermal Papilla Cells (DPCs) are implicated in both embryonic neogenesis and the hair follicle cycle. Therefore, DPC signaling plays a pivotal role in both processes. We have demonstrated that androgens decrease DPC inductivity on HFSC differentiation. Conversely, we have shown that culturing DPCs as spheres promotes the migration of endothelial cells and vascular network formation, as well as the expression of proangiogenic factors. In this study, we aimed to evaluate the action of androgens on the expression of angiogenic factors and investigate whether the increased angiogenic potential of DPCs cultured as spheres can be retained after successive passages. Our qPCR analyses indicate that androgens negatively regulate the expression of VEGF and FGF in DPCs cultured as spheres. On the other hand, the expression of both angiogenic factors increases when DPCs are cultured as spheres. Statistical analyses were performed using One-way ANOVA. Even though this increase is lost when DPCs are subsequently cultured as monolayer, culturing them as spheres restores the higher expression. These results suggest that passaged spheres retain an increased level of the main angiogenic factors despite DPC amplification as monolayer. We can speculate about the relevance of these results for translational medicine when large amounts of DPCs are needed. These results, combined with previous findings from the laboratory, allow us to speculate that androgens may alter the onset of anagen both by impairing HFSC differentiation and by generating an insufficient vascular network to support the growth phase.

### 30. 354 VINCULACIÓN ENTRE LA ARTRITIS REUMATOIDEA Y LA ENFERMEDAD PERIODONTAL. PARÁMETROS CLÍNICOS Y POLIMORFISMOS GENÉTICOS

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**Introducción:** La artritis reumatoidea (AR) es una enfermedad autoinmune sistémica y crónica del tejido conjuntivo. La enfermedad periodontal (EP) es una patología inmuno-infecciosa que afecta a los tejidos de soporte del diente. Se postula que los pacientes con AR presentan una mayor incidencia y severidad de EP que la población general. El gen FCGR2A funciona como un enlace crítico entre las respuestas inmunes humoral y mediada por células, algunos genotipos presentan una mayor producción de IL-1 $\beta$  y promueven las diferencias interindividuales en el riesgo para la EP. **Objetivos:** Estudiar la vinculación del polimorfismo de nucleótido simple (SNP) rs1801274 del gen FCGR2A con el diagnóstico periodontal en pacientes con AR e individuos control (C) sanos. **Materiales y Métodos:** Se reclutaron 33 pacientes con AR y 28 pacientes control. El diagnóstico periodontal se realizó confeccionando un periodontograma donde se registraron los índices periodontales en cada pieza dental mediante una sonda milimetrada. Para las PCRs punto final a partir de muestras de sangre se usaron primers específicos para el rs1801274. Los productos se secuenciaron mediante la técnica de Sanger. Para el análisis estadístico se utilizó el test de chi cuadrado. **Resultados:** Los grupos AR y C no presentaron diferencias significativas (DS) en relación a la presencia de EP sin embargo, los pacientes con AR presentaron mayor severidad de EP ( $p=0.02$ ). La distribución de los SNPs Wild Type (WT) y sus variantes AG y GG fue significativa entre ambos grupos ( $p=0.04$ ). La frecuencia del WT en relación a la severidad de la EP (estadios) entre el grupo C y AR no presentó DS; las variantes AG y GG fueron más frecuentes en los estadios más severos en el grupo AR,  $p=0.03$  y  $p=0.02$ , respectivamente. **Conclusión:** Como se esperaba los pacientes con AR presentaron mayor severidad de EP que los pacientes control. Los SNPs AG y GG están presentes en los estadios más severos de la EP ( III y IV) frecuentes en los pacientes con AR.

**31. 453 IMPACT OF TRPV4 INHIBITION ON MIGRATION DYNAMICS IN NORMAL AND TUMOR RENAL CELLS**

Paula Cerdan<sup>1,2</sup>, Natalia Beltramone<sup>1,2</sup>, Juan Martín Mucanena<sup>1,2</sup>, Claudia Capurro<sup>1,2</sup>, Valeria Rivarola<sup>1,2</sup>, Paula Ford<sup>1,2</sup>, Gisela Di Giusto<sup>1,2</sup>

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**Introduction:** TRPV4 is a calcium channel that plays a critical role in tumor endothelial cell migration by influencing actin remodeling. We hypothesize that modulation of TRPV4 may affect the migration of renal tumor cells derived from clear cell Renal Cell Carcinoma (ccRCC), the most common type of renal primary tumor. Also, it is known that cell migration involves complex intracellular processes, such as changes in morphology and actin fiber spatial arrangement. Although the pathophysiology of ccRCC is not yet fully understood, targeting these processes could significantly impact tumor progression. **Objective:** Our study aims to investigate the impact of TRPV4 modulation in normal and tumor renal cells, focusing on cell migration, morphology, and anisotropy. **Methods:** We used two renal cell models: HK-2, derived from normal human proximal epithelial cells, and 786-O, derived from human ccRCC cells. Cell migration was evaluated using wound healing assays with a selective TRPV4 antagonist (HC-067047, 10 $\mu$ M) or vehicle. Following the migration, cells were fixed and stained with Phalloidin to analyze cell morphology and anisotropy using the FibrilTool macro in ImageJ. **Results:** Our results showed that inhibition of TRPV4 reduced 786-O but not HK2 cell migration (%). 786-O: Control 44.91 $\pm$ 1.9, HC 35.99 $\pm$ 2.18,  $n=11$ ,  $p<0.01$ ; HK2: Control 16.08 $\pm$ 0.89, HC 15.85 $\pm$ 1.72,  $n=7$ , NS).

The same result was found for cell anisotropy (786-O: Control 0.23 $\pm$ 0.02, HC 0.07 $\pm$ 0.02,  $n=8-11$ ,  $p<0.001$ ; HK2: Control 0.24 $\pm$ 0.02, HC 0.23 $\pm$ 0.03,  $n=23$ , NS). Although, there were no changes in both cell types morphology between Control and HC groups (Round, 786-O: Control 0.48 $\pm$ 0.05, HC 0.39 $\pm$ 0.05,  $n=18-21$ , NS; HK2: Control 0.41 $\pm$ 0.04, HC 0.41 $\pm$ 0.04,  $n=23$ , NS). **Conclusion:** Our results demonstrate that while TRPV4 inhibition does not alter cell morphology, it reduces both stress fiber anisotropy and migration only of 786-O cells. This allows us to propose TRPV4 as a potential target for the treatment of ccRCC.

**P3 POSTERS**

FECHA Y HORA: 19/11/2024 16:00-17:00 H

COORDINADORES: AYELEN MARTIN, NICOLAS KOUYOUMDZIAN, RAYEN DE FAZIO

**32. 207 PRELIMINARY STUDY OF HLA-G EXPRESSION IN THE LIVER OF MALE C3H/S MICE**

Ayelen Fernández Blanco<sup>1</sup>, Elena B. Alegría Pérez<sup>1</sup>, Laura B. Andrini<sup>1</sup>, Natalia R. Lausada<sup>2</sup>, Félix J. Corrons<sup>1,3</sup>, Marcela N. García<sup>1</sup>.

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Human leukocyte antigen G (HLA-G) is an immune checkpoint belonging to the non-classical MHC I family, with soluble and membrane-bound variants. It promotes immune tolerance through interactions with its inhibitory receptors located on immune cells. Under normal physiological conditions, HLA-G expression is restricted to a few tissues, such as trophoblast, thymus, cornea, nail matrix, and pancreas. However, HLA-G is present in pathological contexts, such as tumors, although it has not been observed in healthy tissue surrounding tumors. In mice, we can find an HLA-G functional homolog named Qa-2. This study aims to determine the presence of the HLA-G homolog in the liver of normal and injured mice. Twelve male C3H/S mice were used. The right lateral lobe of the liver of young ( $n=4$ ) and adult healthy mice ( $n=4$ ), and adult mice with orthotopic graft of a spontaneous murine tumor was analyzed ( $n=4$ ). Immunohistochemistry (IHC) on liver sections was performed using a murine monoclonal antibody specific for HLA-G. Liver sections were evaluated as positive or negative for immunostaining and categorized based on the distribution of HLA-G staining as homogeneous or heterogeneous (focal). The IHC results showed positive staining for HLA-G in the livers of healthy animals with a homogeneous distribution pattern. Although HLA-G protein was expressed in some tumor cells of tumor-grafted mice, HLA-G expression in the surrounding (and apparently normal) liver tissue was homogeneously distributed. These findings suggest that normal liver tissue could express HLA-G as a tolerogenic mechanism to protect the organ against harmful and foreign substances. However, this expression should be taken into account in the presence of tumor metastasis since it would favor its development by generating an environment of immunotolerance. More studies are necessary to evaluate the importance of this result.

**33. 302 NA<sup>+</sup>/H<sup>+</sup> ION CHANNEL (NHE1) MOLECULAR STUDIES OF HEALTHY RENAL CELLS OF THE PROXIMAL EPITHELIUM AND CLEAR RENAL CARCINOMA CELLS**

Ana Celli<sup>1,2</sup>, Natalia Beltramone<sup>1,2</sup>, Claudia Capurro<sup>1,2</sup>, Gisela Di Giusto<sup>1,2</sup>, Paula Ford<sup>1,2</sup>, Valeria Rivarola<sup>1,2</sup>

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Although pH homeostasis is critical for cell survival, extracellular

acidosis is a hallmark of cancers. The NHE1 isoform of the Na<sup>+</sup>/H<sup>+</sup> exchanger would be associated with the adaptation of cancer cells to an acidic extracellular environment. However, our previous studies in healthy proximal cells showed that total NHE1 expression is similar in healthy and cancer cells. Neutralizing the acidic microenvironment with a mild alkali treatment did not change NHE1 expression. This work aimed to determine whether the role of NHE1, both in control and neutralized situations, is due to a change in the localization of NHE1 in different cell types. We used two renal cell models: HK2, derived from normal human proximal epithelial cells, and 786-O, derived from clear cells of human clear cell renal cell carcinoma. To neutralize the acidic microenvironment of cancer cells, we incubated both cell types for 72 h with 9.6mM NaOH. We analyzed the localization of NHE1 by immunofluorescence assays of NHE1 in the presence of wheat germ agglutinin (WGA) (plasma membrane marker). We used the Mander's coefficient as an index of intensity/plasma localization of NHE1. In control conditions, we observed a difference in the Mander's coefficient of the 786-O cells vs. the HK2 (Mander's, HK2: 0.6586 ±0.0655 vs 786-O: 0.9304 ±0.0137, p<0.001, n=18), indicating that, in 786-O cells, NHE1 localization is to a greater degree in the plasma membrane. The cells responded oppositely to the alkaline treatment: while the healthy cells increased their Mander's, in the cancerous cells, it decreased. We conclude that the role of NHE1 in adaptation to the acidic cancer microenvironment would be related to the localization of NHE1.

### 34. 306 MODULATION OF TRPV4 IN 3D SPHEROIDS: A POTENTIAL TARGET FOR CLEAR CELL RENAL CELL CARCINOMA TREATMENT

Juan José Sterba<sup>1,2</sup>, Natalia Beltramone<sup>1,2</sup>, Juan José Casal<sup>1,2</sup>, Claudia Capurro<sup>1,2</sup>, Valeria Rivarola<sup>1,2</sup>, Gisela Di Giusto<sup>1,2</sup>, Paula Ford<sup>1,2</sup>

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**Introduction:** Clear Cell Renal Cell Carcinoma (ccRCC) is a leading cause of cancer-related deaths worldwide. Previous studies have identified the TRPV channel family as potential biomarkers in various cancers, though their roles in ccRCC remain unclear. We previously demonstrated that inhibiting TRPV4 in 2D ccRCC cultures (CAKI-1 and 786-O cell lines) significantly reduced cell growth compared to normal kidney cells (HK2). **Objective:** This study aimed to explore TRPV4 modulation in 3D spheroids, which better simulate tumor-like *in vivo* conditions than traditional 2D cultures, providing more accurate insights into cell viability and treatment responses. **Methods:** We generated ccRCC spheroids from CAKI-1 cells using the hanging drop method, a scaffold-free approach. The spheroids were treated with a TRPV4 activator (4α-PDD, 5μM), inhibitor (HC-067047, 10 μM), or vehicle (DMSO), following two protocols: during and after spheroid formation. Spheroid size and morphology were assessed using ImageJ software and a custom deep learning algorithm developed in our lab. Additionally, we measured the proliferation halo after treatment over 11 days. **Results:** Spheroids formed with the TRPV4 activator had a significantly larger diameter than those treated with DMSO (285 ± 2.5 μm vs. 263 ± 2.9 μm, p<0.001), while spheroids treated with the inhibitor were smaller (250 ± 2.6 μm, p<0.01). When treatments were applied after spheroid formation, activator-treated spheroids exhibited faster growth, while inhibitor-treated spheroids grew more slowly. After 4 days of treatment, the proliferation halo increased 6-fold in spheroids treated with the TRPV4 activator, 1.9-fold in TRPV4-inhibited spheroids, and 3.1-fold in vehicle-treated spheroids. **Conclusion:** These findings suggest that TRPV4 activation promotes spheroid growth and cell proliferation, while its inhibition slows these processes. Modulating TRPV4 could represent a promising therapeutic strategy for ccRCC treatment.

### 35. 322 ABSENCE OF OSTEOPONTIN-PRODUCING MICROGLIAL CELLS AND STRUCTURAL ALTERATIONS IN

### THE RETINA OF AQUAPORIN-4 KNOCK-OUT MICE

María Azul Cocca<sup>1</sup>, Anabel Sevilano<sup>1</sup>, Francisco Mayo<sup>2</sup>, Nicolás Cutrera<sup>1</sup>, Claudia Capurro<sup>1</sup>, Miriam Echevarría<sup>2</sup>, Vanina Netti<sup>1</sup>

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Müller cells are the main type of glial cells in the retina, which provide structural and functional support to neurons. In these cells, Aquaporin-4 (AQP4), the most abundant water channel of the central nervous system (CNS), plays a key role in controlling the osmotic imbalance caused by neuronal activity. In the CNS of AQP4 knock-out (KO) mice, it was reported that there is an increase in Osteopontin (OPN) expression produced by microglial cells during postnatal development. OPN is a cytokine that promotes neuronal regeneration after injury, but its role in the retina and its association to AQP4 has not been fully explored. Thus, the aim of this work was to study OPN expression and retinal structure in AQP4-KO mice during postnatal development. Immunohistochemistry and hematoxylin-eosin staining of retinas of wild-type mice (WT) or with deletion of the *aqp4* gene (AQP4-KO) were performed. We quantified fluorescence intensity and the thickness of retinal layers. Results showed that AQP4 staining in WT mice was concentrated at the perivascular level and in Müller cells processes. In these animals, we observed the presence of OPN in cells with microglial morphology, mostly in the inner plexiform layer (IPL), the inner nuclear layer (INL) and the outer plexiform layer (OPL). This was confirmed by CD11b staining (microglial marker). These cells were absent in AQP4-KO mice. Histological analysis showed that the thickness of the outer nuclear layer (ONL) was similar between WT and KO (WT: 63.0±2.1 vs. KO: 60.2±2.0 μm), but INL thickness was reduced in AQP4-KO (WT: 60.5±2.6 vs. KO: 44.7±1.0 μm; p<0.001, n=3). We also observed a decreased expression of the intermediate filaments GFAP and Vimentin in the ganglionic cell layer (GCL), IPL and OPL of AQP4-KO. The observed alterations in retinal structure and the absence of OPN-producing microglial cells induced by the absence of AQP4 may impact on retinal function, evidencing the relevance of AQP4 during postnatal development.

### 36. 426 CHARACTERIZING THE ROLE OF SSRI ANTIDEPRESSANTS ON FKBP51 SUMOYLATION AND ACTIVITY

Gerson Smith Asti Tello<sup>1\*</sup>, Camila Mimura<sup>2\*</sup>, Victoria Ambrosino<sup>3</sup>, Angel Ramón Torres Mc Cook<sup>1</sup>, Facundo Claverie<sup>1</sup>, Vanina Giselle Velardo<sup>1</sup>, María Clara Sokn<sup>1</sup>, Eduardo Arzt<sup>4</sup>, Ana Clara Liberman<sup>1,2</sup>

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\*equally contributed.

Our laboratory has previously elucidated the critical role of FKBP51 SUMOylation in attenuating the Hsp90-mediated inhibitory influence on glucocorticoid receptor (GR) activity. Additionally, we have demonstrated that tricyclic antidepressants can reverse FKBP51 SUMOylation and restore GR function by directly targeting PIAS4, a pivotal E3 SUMO ligase in FKBP51 modification. Expanding on these findings, our investigation into the involvement of widely prescribed selective serotonin reuptake inhibitors (SSRIs) in this regulatory pathway revealed differential effects of antidepressants on FKBP51 SUMOylation. While tricyclic antidepressants suppress PIAS4 E3 ligase activity, fluoxetine, an SSRI, inhibits Ubc9 E2 activity. Building upon these observations, the present study demon-



strates that fluoxetine (FLX), akin to tricyclic antidepressants, disrupts the SUMO-dependent interaction among FKBP51, Hsp90, and GR both in vitro (percentage of inhibition of FKBP51 interaction in the presence of fluoxetine compared to vehicle: Hsp90= 29±2% & GR= 50±2%) and in vivo, particularly within the murine brain (percentage of inhibition of FKBP51 interaction in the presence of fluoxetine compared to vehicle: Hsp90= 70±1% & GR= 60±2%). Furthermore, we provide evidence that this inhibitory effect is essential for restoring GR transcriptional activity, as assessed by MMTV-luc reporter assays (Percentage of GR activity inhibition by dexamethasone in the presence of vehicle and fluoxetine: vehicle= 84±3% & FLX=55±2%) and qPCR analysis in primary astrocytes (Percentage of GR activity inhibition by dexamethasone in the presence of vehicle and fluoxetine, Sgk1: vehicle= 41±2% & FLX=35±1%; Erg2: vehicle= 53±3% & FLX=24±4%; Zbtb16: vehicle= 55±3% & FLX=40±2%). Notably, our data suggest a potential global inhibitory effect of fluoxetine on SUMOylation, as indicated by reduced general protein SUMO conjugation in both in vitro (percentage of inhibition of SUMO in the presence of fluoxetine compared to vehicle, SUMO1: FLX= 63±3%; SUMO2: FLX= 30±2%) and in vivo (percentage of inhibition of SUMO1 in the presence of fluoxetine compared to vehicle: FLX=52±5%) nickel purification assays.

**37. 434 ROLE AQP4 AND TRPV4 IN CYTOSKELETON REORGANIZATION AND THEIR EXPRESSION IN THE LAMELLIPODIA OF MIGRATING MÜLLER CELLS**

Nicolás Cutrera<sup>1</sup>, Azul Cocca<sup>1</sup>, Paula Ford<sup>1</sup>, Claudia Capurro<sup>1</sup>, Gisela Di Giusto<sup>1</sup>, Vanina Netti<sup>1</sup>

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Within the retina, the activation of glial Müller cells during injury leads to their proliferation, migration and differentiation into different cell types to restore tissue function. Our previous research showed that Aquaporin-4 (AQP4), the main water channel of the central nervous system, together with the calcium channel TRPV4, participate in cell volume regulation and the migration of Müller cells. Since cell migration depends on volume changes and cytoskeletal organization, the aim of this study was to evaluate the role of AQP4 in cytoskeleton remodeling of migrating Müller cells as well as its regulation by TRPV4 and the PI3K signaling pathway. MIO-M1 human Müller cells were exposed to 0.5 µM TGN-020 (AQP4 inhibitor), 0.5 µM Wortmannin (PI3K inhibitor), 1 µM HC067047 (TRPV4 inhibitor) or vehicle. Cell migration was evaluated by wound healing and cell adhesion, by a cell wash assay. AQP4 expression, F-actin fibers organization and Vimentin were evaluated by immunocytochemistry. AQP4 inhibition reduced cell migration by 30%. This was accompanied by a reduction in the anisotropy of F-actin fibers, which indicates its degree of organization (0.34±0.02 vs. 0.29±0.02, n=4, p<0.05) and the compactness of migrating Müller cells (0.41±0.02 vs. 0.36±0.02, n=3, p<0.05). TGN also increased the presence of Vimentin at the membrane by 25% (0.50±0.05 vs. 0.62±0.05, n=2, p<0.05), without changes in cell adhesion. PI3K inhibition reduced cell migration by 50%. The abundance and localization of AQP4 in the plasma membrane of lamellipodia was reduced by TRPV4 inhibition (control vs HC, intensity mean: 204±17 vs. 103±7; colocalization with membrane marker WGA: 0.75±0.02 vs. 0.60±0.02, n=3, p<0.001). We propose that AQP4 participates in Müller cell migration by facilitating cytoskeleton remodeling and changes in cell shape. This process is regulated by both TRPV4 and PI3K. The study of this process is essential for the development of new therapeutic strategies for retinal diseases.

**P4 - POSTERS BIOLOGÍA GENERAL / TRANSDUCCIÓN DE SEÑALES**  
FECHA Y HORA: 20/11/2024 11:30-12:30 H  
COORDINADORES: RUBIO MARIA FERNANDA,  
ROXANA SCHILLACI

**38. 341 NANOG GENE REGULATION BY AKT1 AND FOXA2 IN MOUSE EMBRYONIC STEM CELLS**

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Nanog is an essential pluripotency transcription factor (TF) whose expression decreases abruptly at the onset of differentiation during development and in embryonic stem cells (ESCs) in culture. We have previously demonstrated that the kinase AKT1 induces Nanog expression in a SUMOylation-dependent manner in mouse ESC (mESC); however, the mechanism involved remains unknown. Some evidence led us to speculate that the TF FOXA2 mediates this induction. FoxA2 is expressed in ESCs, increases its levels during differentiation, and is involved in the differentiation into various cell types. AKT1 phosphorylates FOXA2 promoting its nuclear exclusion in hepatocytes and cancer cells, yet there are no reports of this effect in ESCs. We hypothesize that in mESCs FOXA2 represses the expression of Nanog, and that AKT1 interferes with this repression by promoting the exit of FOXA2 from the nucleus. We also propose that the increase in FOXA2 levels contributes to the suppression of Nanog, crucial for initiating differentiation. In this work, by a reporter gene assay and FOXA2 overexpression, we found that this TF represses the Nanog promoter in mESCs. Remarkably, we also found that FOXA2 interferes with the induction exerted by AKT1 on this reporter, suggesting that FOXA2 could mediate this effect. We are currently setting the conditions for studying the effect of FOXA2 on the endogenous Nanog gene using RNA-FISH. Moreover, to explore the effect of AKT1 on the subcellular localization of FOXA2, we are analyzing, by FOXA2 immunofluorescence the effect of mCherry-AKT1 overexpression. We also generated an expression vector encoding FoxA2 fused to EGFP which will allow us to perform analyses in living cells. Additionally, we are studying the effect of AKT1 mutants with different SUMOylatability on FOXA2 subcellular localization. Elucidating this interplay would offer vital insights into the regulation of pluripotent stem cell fate, crucial for regenerative medicine and cancer therapeutics.

**39. 431 MITOFUSIN 2 EXPRESSION IS REGULATED BY SP1 TRANSCRIPTION FACTOR IN H295R HUMAN ADRENOCORTICAL CELLS**

Fabrizio Arona<sup>1</sup>, Martina Rodrigo<sup>1,2</sup>, Agustina Cianci Romero<sup>1</sup>, Katia Helfenberger<sup>1</sup>, Silvana Nudler<sup>1</sup>, María Mercedes Mori Sequeiros Garcia<sup>1,3</sup>, Yanina Benzo<sup>1,3</sup>, Cecilia Poderoso<sup>1,3</sup>.

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Mitochondrial function is essential for the signal transduction pathway of several hormones, including angiotensin II (Ang II) and adrenocorticotropin (ACTH) which stimulate aldosterone and glucocorticoids biosynthesis respectively, in adrenocortical cells. Steroidogenesis requires active, energized and polarized mitochondria. Previously, we had demonstrated that Ang II and ACTH/cAMP stimulate mitochondrial fusion in H295R human adrenocortical cells by increasing the expression of Mitofusin 2 (MFN2). MFN2 is a key protein involved in mitochondrial fusion of the outer mitochondrial membrane and it also plays a positive role in mitochondrial respiration and oxidative phosphorylation. Since MFN2 is crucial for hormone-stimulated steroidogenesis, the study of MFN2 expression regulation is highly relevant. The human MFN2 promoter has potential binding sites for different transcription factors, such as the

Specificity Protein 1 (Sp1). The aim of this study is to explore the relevance of Sp1 on MFN2 expression in H295R human adrenocortical cells. Using a plasmid containing the MFN2 promoter (pNL1.1-MFN2) lacking Sp1 binding sites, we observed a threefold lower luciferase activity compared to that observed in the longer construct containing these sites ( $p < 0.001$ ). Subsequently, we observed that Sp1 down-regulation using interference RNA resulted in a decrease in hormone-stimulated MFN2 expression, as determined by real time PCR ( $p < 0.01$ ) and western blot. We demonstrated that Ang II and 8Br-cAMP (cAMP second messenger permeant analogue) promotes mitochondrial activity in a time-dependent manner, as assessed by fluorescence microscopy using MitoTracker Red ( $p < 0.01$ ;  $p < 0.001$ ). Our results indicate that hormone stimulation enhance mitochondrial activity and that Sp1 is involved in the regulation of MFN2 expression in H295R adrenocortical human cells.

**40. 435 KSHV-INDUCED ONCOGENIC SIGNALING REGULATES RAC1 GENE EXPRESSION. INFLUENCE OF RAC1 MRNA ISOFORMS ON ITS FUNCTIONALITY AS AN ONCOGENE**

Mercedes Montani<sup>1,2</sup>, Victoria Nápoli<sup>1,2</sup>, Sofía Chemes<sup>1,2</sup>, Julián Naipauer<sup>1,2</sup>, Omar Coso<sup>1,2</sup>

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Kaposi's sarcoma (KS), an AIDS-defining cancer caused by the KS herpesvirus (KSHV), is a vascular sarcoma characterized by angiogenesis and spindle-cell proliferation. KSHV-encoded G protein-coupled receptor (vGPCR), expressed during the lytic phase of the viral cycle, can initiate KS-like tumors in mice. It has been found that endothelial cells expressing vGPCR show Rac1 upregulation. Moreover, inhibition of Rac1 activation in these cells diminishes tumorigenesis *in vivo*. Preliminary results indicated that alternative polyadenylation (AP) generates two mRNA isoforms in endothelial cells, and that vGPCR modulates the subcellular localization of Rac1. **Methods:** To investigate whether Rac1 subcellular localization, protein expression, and activation are associated with 3'UTR regulation, we engineered fluorescent vectors that allow us to over-express the different mRNA isoforms of Rac1 as fusion proteins with GFP. **Results:** Our results revealed that vGPCR enhances both the expression and activation of the shortest mRNA isoform generated by the proximal polyadenylation site. Additionally, we analyzed the influence of KSHV-induced signaling on the expression of the two variants of Rac1 mRNA generated by alternative splicing (AS), Rac1 and Rac1b. To assess the effect of viral-induced signaling on the expression of Rac1 isoforms by AS, we performed immunofluorescence against Rac1 and Rac1b in cells expressing a recombinant KSHV virus. We detected that Rac1 and Rac1b expression and localization are regulated differently during the latent and lytic phases of the KSHV viral cycle. **Conclusion:** we found that KSHV would influence Rac1 expression and activation levels by modulating the production of various mRNA isoforms resulting from alternative polyadenylation and alternative splicing. We plan to further investigate the pathways involved in vGPCR - Rac1 mRNA isoforms regulation and its cellular phenotypes.

**41. 467 CHARACTERIZATION OF SOME ENDOTHELIAL CELL FUNCTIONS FROM INDUCED PLURIPOTENT STEM CELLS DERIVED ENDOTHELIUM**

1 Daiana Martire Greco, 2 Guadalupe Amin, 2 Carolina Colli, 1 Federico Birnberg-Weiss, 1 Joselyn E. Castro, 1 Agustina Serafino, 1 Veronica Landoni, 1 Gabriela Fernandez, 1 Santiago Miriuka.

<sup>1</sup> Instituto de Medicina Experimental (IMEX-CONICET), Academia Nacional de Medicina, <sup>2</sup> Instituto de Neurociencias (INEU-CONICET), Laboratorio de Investigaciones aplicadas a Neurociencias (LIAN), FLENI-Escobar

The endothelium is a specialized epithelial tissue that lines the interior surfaces of blood vessels, lymphatic vessels, and the heart. It has a barrier function between blood, lymphatic vessels and surrounding tissues, regulates the vascular tone and has inflammatory responses. Endothelial cells derived from induced pluripotent stem cells (e-iPSC) have become an important area of research in regenerative medicine because they can be expanded indefinitely in culture, can be standardized and be obtained in large quantities, so they have a potential for use in regenerative medicine and the treatment of vascular injuries. Atherosclerosis is a chronic disease characterized by the buildup of plaques within the arterial walls, leading to the narrowing of arteries. This process impacts endothelial cells, which play a crucial role in vascular health. The aim of this work was to evaluate the functionality of e-iPSC in order to be used for experimental models of atherosclerosis. First, we differentiated iPSC (FN 2.1) into endothelial cells adding 10 ng/ml of VEGF to RPMI from day 3 to day 11. We observed on day 11 the maximum expression of endothelial markers CD31, CD34 and CD144 indicating the differentiated phenotype of the endo-iPSC. On day 11 we purified e-iPSC from the rest of the cells in the cultures dishes using magnetic beads, obtaining 60% of positive CD31+ cells before they passed through the columns with the magnetic beads. Also, we registered, by flow cytometry, that these purified cells can express ICAM-1 (percentage (%) ICAM-1:  $5.1 \pm 0.9$ ) and could form new tubules in a matrix of geltrex assay, indicating that they are capable to produce angiogenesis. Finally, we observed by immunofluorescence microscopy, the typical elongated morphology of endothelial cells with prolongations that communicate between other cells. In conclusion we could differentiate endothelial cells from iPSC and study some functional parameters that are necessary to characterized endo-iPSC.

**42. 530 SIGNIFICANCE OF FRACTAL DIMENSION ANALYSIS IN CORONARY ARTERIOLE STRUCTURAL CHANGES POST 5/6 NEPHRECTOMY IN RAT**

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centro de altos estudios en ciencias humanas y de la salud, UAI, national council of scientific and technical research (CONICET)

**Aim:** We investigate the significance of fractal dimension analysis in evaluating structural changes in coronary arterioles following 5/6 nephrectomy in rats. Chronic kidney disease (CKD) is known to exacerbate cardiovascular complications, with structural alterations in the coronary arteriolar walls being a critical factor. Coronary arterioles are crucial for the regulation of blood flow and pressure within the heart. Structural damage in these vessels can manifest as thickening of the arteriolar walls, increased collagen deposition, and reduced lumen diameter. **Material and Methods:** Seventy-two male Wistar rats were randomly assigned to three experimental groups. Two groups underwent 5/6 nephrectomy to induce CKD. One group (Nx5/6) received tap water ad-libitum, while the remaining group was treated with 40 mg/kg/day of Losartan (Nx5/6+L). The last group undergoing sham surgery (Sham). After six experimental months, the animals were sacrificed by anesthetic overdose. Coronary arterioles were harvested and subjected to histological analysis at various time points post-surgery. The fractal dimension of the arteriolar walls was computed using specialized image analysis software, enabling precise quantification of morphological alterations. The variables were expressed as mean $\pm$ SD and performed using the nonparametric Kruskal Wallis test and the Dunn multiple comparison test. **Results:** The Nx5/6 group demonstrated a significant reduction in the fractal dimension of the coronary arteriolar walls compared with the Nx5/6+L and Sham groups ( $1.523 \pm 0.06$  vs  $1.675 \pm 0.02$  and  $1.687 \pm 0.04$ ,  $p < 0.001$  respectively). Likewise, the Nx5/6 group showed an increase in lacunarity value ( $0.4512 \pm 0.04$ ) compared to the Nx5/6+L ( $0.3872 \pm 0.05$ ,  $p < 0.0001$ ) and Sham ( $0.4107 \pm 0.03$ ,  $p < 0.001$ ) groups. **Conclusion:** This study highlights the potential of fractal dimension analysis as a diagnostic tool for early detection of coronary microvascular changes in CKD patients, paving the way for targeted therapeutic interventions to mitigate cardiovascular risk.

**P5 - POSTERS BIOLOGÍA CELULAR Y MOLECULAR DE PROCESOS FISIOLÓGICOS Y PATOLÓGICOS - TRANSDUCCIÓN DE SEÑALES**

FECHA Y HORA: 21/11/2024 11:00-12:00 H

COORDINADORES: MARIELA URRUTIA, ALEJANDRA DUARTE, ADRIANA BURGUEÑO

**43. 239 MUTATIONS OF POST-TRANSLATIONAL MODIFICATION SITES ON SECURIN PTTG REVEAL CHANGES IN ITS PROTEIN STABILITY**

Manuel Fiz<sup>1</sup>, Joaquina Cagliero<sup>1</sup>, Florencia Herbstein<sup>1</sup>, Nicolas Ciancio de Giudice<sup>1</sup>, Sergio Senin<sup>1</sup>, Mariana Fuertes<sup>1,2</sup>, Eduardo Arzt<sup>1,2</sup>

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The proto-oncogene pituitary tumor-transforming gene (PTTG), first identified in pituitary neuroendocrine tumors, plays a key role in tumorigenesis through its overexpression and cell cycle regulation as securin. Previous studies have shown that PTTG abundance is influenced by specific post-translational modifications (PTMs). This work investigates how these PTMs affect PTTG degradation, stability and half-life, linked to the cell cycle and RSUME (a SUMOylation enhancer which stabilizes PTTG). We constructed PTM mutants targeting SUMO conjugation sites (K25R, K168R), ubiquitin domains ( $\Delta$ KEN-box,  $\Delta$ D-box), and phosphorylation residues (S165A, S183-S184A). Exploring the stability of the S165A mutant, a phospho-site for the mitotic kinase CDK1, we found that S165A stability mirrors that of the wt protein in asynchronous cells but increases in synchronized COS-7 cells in M phase (5  $\mu$ M nocodazole O.N.), with RSUME further enhancing stability. Ni<sup>2+</sup>NTA affinity chromatography for His-ubiquitin showed increased ubiquitination for the K25R and S165A mutants and decreased for the K168R and  $\Delta$ KEN-box mutants compared to wt PTTG. The known interaction between PTTG and RSUME was assayed by co-immunoprecipitation in COS-7 cells, showing that the SUMO mutants K25R and K168R continued interacting with RSUME. Moreover, cycloheximide treatment (100  $\mu$ g/mL) revealed that the  $\Delta$ KEN-box mutant exhibited an extended half-life when RSUME is present. In HeLa cells, a neuroendocrine tumor model, western blot analysis showed that reversine, an inhibitor of mitotic checkpoint, accelerates PTTG degradation in a dose- and time-dependent manner, highlighting the role of ubiquitination in PTTG stability. Finally, we concluded that PTMs regulate PTTG abundance. In particular, mitotic phosphorylation residue S165 reduces its protein stability, while SUMO lysines are important for maintaining stability and interactors, and ubiquitin domains control its degradation.

**44. 291 THE ROLE OF GLUCOCORTICOID RECEPTOR IN PANCREATIC PROGENITOR CELL DIFFERENTIATION AND BETA CELL GENESIS**

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Understanding the pancreatic developmental process is instrumental to tackle diabetes, allowing improvements of *in vitro* differentiation protocols aimed at producing functional  $\beta$ -cells for transplantation. Previous reports have shown that the Glucocorticoid Receptor (GR) controls the ultimate number of  $\beta$  cells that are produced, but suggest that it does not play a relevant role before the second transition in mouse. Here, we investigate the role of GR signaling from the earliest stages of pancreas development decisions in both mice and humans. To gain insights into the mechanism involved, here, we profiled the transcriptomic changes of *in vitro* derived pancreatic multipotent progenitor cells (MPCs) treated with Dexamethasone (Dex, a known GR agonist) or control (Ethanol) at the single-cell level (scRNA-seq). Analysis of these data, combined with the MPC epigenomic profile and scRNA-seq profiled in the human embryonic pancreas, revealed the modulation of genes with a known role in pancreas development such as *HES1*, as well as other transcripts with yet unknown functions. Further analysis suggests that activation of the GR in this model induces MPC differentiation to endocrine progenitors (EPs). In sharp contrast, a similar treatment with Dex in E11.5 embryonic mouse pancreatic explants (composed mostly of MPCs at this stage) induced cell commitment to the acinar fate. These results were supported by immunofluorescence and RT-qPCR ( $p < 0.05$ ) for key genes which were regulated by Dex in the human pancreatic differentiation model. Our results suggest that downregulation of *HES1* by the GR might be at the crossroad of other signaling pathways, which might allow understanding of the opposite results obtained in our human and mouse models used to study pancreatic progenitor developmental decisions.

**45. 353 ARRHYTHMOGENIC CARDIOMYOPATHY MODELING BY DIFFERENTIATION OF PKP2-KO AND PKP2-S140F EDITED iPSCs INTO CARDIOMYOCYTES**

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Arrhythmogenic cardiomyopathy (ACM) is an autosomal dominant hereditary disease characterized by the replacement of myocardium, with fibroadipose tissue, which can lead to progressive ventricular dysfunction. Mutations in desmosomal genes (PKP2, PKG, DSP, DSG, and DSC) are the most frequently found in patients with ACM, with PKP2 being the most common gene associated with the disease. An *in vitro* model of ACM using genetically edited induced pluripotent stem cells (iPSCs) in the PKP2 gene is proposed. For this study, we differentiated a PKP2 knock out (PKP2-KO) iPSCs line (pathogenic mutation), an iPSCs line with a missense mutation PKP2-S140F (uncertain meaning mutation) and a wild-type (WT) control into cardiomyocytes (CMs). We obtained fetal-like CMs after 21 days of differentiation and mature (post-natal-like) CMs after maturation protocol (2 weeks). In order to evaluate the adipogenic capacity of the mature CMs (a hallmark manifestation of ACM), they were exposed for 1 week to a pro-adipogenic medium and stained with oil red. Retained oil red was released, analyzed by optical density (OD) and normalized by cell density in each experimental group. We observed that the three iPSCs lines were able to differentiate into CMs but with different efficiency, obtaining  $11.4 \pm 4.4\%$ ,

13.6±5.7% and 18.9±1.6%, cTnT+ cells (cardiac marker) for PKP-KO, PKP2-S140F and WT, respectively. RT-qPCR analysis of day 21 CMs revealed alterations in desmosomal genes expression in the PKP-KO and PKP2-S140F with respect to the WT. Moreover, a tendency of higher lipid content was observed in PKP2-KO (OD 0.8) and PKP2-S140F (OD 1.0) CMs with respect to WT CMs (OD 0.7) after pro-adipogenic treatment. Additionally, the contraction capacity of the CMs will be measured and compared through video analysis. It is concluded that the PKP2-KO and the PKP2-S140 iPSCs lines were able to differentiate into CMs, with lower efficiency than the WT though, and exhibits pathological manifestations of ACM.

#### 46. 423 NEW INSIGHTS INTO FERROPTOSIS IN LEYDIG CELLS FUNCTION

Yanina Benzo, Melina Dattilo, María Agustina Raggio, M. Mercedes Mori Sequeiros García, María Susana Theas, Cristina Paz, Cecilia Poderoso and Paula Maloberti.

Male infertility is a growing worldwide problem mainly caused by the impairment of testosterone and sperm production. The testes are the site where spermatogenesis occurs and androgen synthesis, especially testosterone by Leydig cells (LCs). Excess of iron in the testes causes reproductive dysfunction by inducing oxidative stress. Ferroptosis, an iron-dependent type of cell death induced by oxidized phospholipids, is associated with infertility, particularly through cysteine deficiency and inhibition of glutathione peroxidase 4 (GPX4). Two well-characterized ferroptosis inducers are widely used to study ferroptosis: Erastin, which binds to the voltage dependent anion channel 2/3 (VDAC 2/3) and inhibits the anti-cystine/glutamate transporter and glutathione synthesis; and RSL3, which directly and selectively inhibits GPX4 activity. The aim of this work is to investigate ferroptosis in LCs since this process has not been studied in this type of cells. Using the mouse LC line MA-10 we assessed cell viability by MTT under different concentrations of Erastin and RSL3. We observed a dose-dependent effect on cell viability with a 30-40% decrease at 5µM Erastin and 10µM RSL3 ( $p < 0.05$ ). Interestingly, cell viability was dramatically reduced when primary cultures of rat interstitial cells were treated with the indicated concentrations of ferroptosis inducers. In MA-10 cells, we confirmed ferroptosis by lipid peroxidation determination using BODIPY C-11 ( $p < 0.05$ ). These effects were reduced when cells were simultaneously incubated with chorionic gonadotrophin hormone and ferroptosis inducers. Moreover, we evaluated the expression of genes involved in ferroptosis by RT-qPCR in ferroptotic MA-10 cells. We observed an increase in TfR1 (transferrin receptor 1) and VDAC2 ( $p < 0.01$ ) and a decrease in *acs4* (acyl-CoA synthetase 4) ( $p < 0.05$ ) expression. Therefore, these results highlight the importance of studying ferroptosis in LCs and suggest a protective role of hormone stimulation.

#### 47. 469 UTILIZING ATOMIC FORCE MICROSCOPY FOR THE OPTIMIZATION OF EXOSOME ISOLATION WITH FUNCTIONALIZED MAGNETIC PARTICLES

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Exosomes are nanometric membrane vesicles released by almost every type of cell. They contain proteins and nucleic acids from the originating cell, which can regulate signaling pathways in recipient cells. The exosomal cargo varies under pathological conditions, making these extracellular vesicles promising non-invasive biomarkers

for precision medicine, particularly in proliferative and neurodegenerative diseases. However, exosome isolation faces significant challenges, including cross-contamination and variability in the techniques used. Despite the availability of commercial kits, their high cost limits accessibility. The present work aims to optimize the atomic force microscopy (AFM) technique to monitor the development of a magnetic method based on functionalized magnetic particles for the specific recognition and isolation of urinary exosomes. For this purpose, four experimental conditions were analyzed by AFM: exosomes obtained by ultracentrifugation, exosomes incubated overnight with (3-Aminopropyl)triethoxysilane functionalized magnetic particles (APTES-MP), exosomes mixed with the particles without incubation, and particles alone. The results indicate that only when exosomes are incubated for prolonged periods with APTES-MP, aggregation of the extracellular vesicles is observed, likely due to electrostatic interactions between the positively charged particles and the vesicles. This finding suggests that AFM is a suitable tool for characterizing particle functionalization. Initially, the technique will allow us to ensure that the method does not generate nonspecific agglomerations, and subsequently to evaluate whether exosomes are specifically recognized after coupling a molecule to the particle surface. Currently, we are using an ultracentrifuged urine sample, which contains a complex mixture of other extracellular vesicles in addition to exosomes, highlighting the importance of specificity in functionalization for effective recognition.

#### 48. 485 H-RAS MODULATES STORE-OPERATED $Ca^{2+}$ ENTRY (SOCE) VIA DIRECT INTERACTION WITH ORAI1

Julieta Mansilla Ricarti, Lutz Birnbaumer and Sebastian Susperreguy

Institute of Biomedical Research (BIOMED UCA-CO-NICET), Catholic University of Argentina, Buenos Aires C1107AFF, CABA

The store-operated calcium entry (SOCE) channel is the primary  $Ca^{2+}$  entry pathway in non-excitabile cells, activated as a cellular response to replenish  $Ca^{2+}$  stores when endoplasmic reticulum (ER)  $Ca^{2+}$  levels are depleted. Depletion of ER  $Ca^{2+}$  leads to the translocation of STIM1 to ER-plasma membrane junctions, where it binds to and activates Orai1, the pore-forming subunit of the  $Ca^{2+}$  release-activated  $Ca^{2+}$  (CRAC) channel. Ras GTPases are molecular switches localized at the plasma membrane that alternate between an inactive GDP-bound state and an active GTP-bound state, which engages effector proteins to regulate various signal transduction pathways. In humans, three RAS genes encode four distinct isoforms: HRAS, NRAS, and two splice variants of the KRAS gene, KRAS4a and KRAS4b, which contain exons 4a and 4b, respectively. Our study focused on elucidating whether RAS plays a role in the SOCE mechanism. Experimental approach: To investigate the involvement of the proto-oncogene RAS in the SOCE mechanism, we first examined the effect of HRAS on SOCE by transfecting HEK293 cells with wild-type HRAS, the dominant-negative mutant HRAS-S17N, and the oncogenic mutant HRAS-Q61L, followed by analysis using the ratiometric Fura-2 assay. To further explore HRAS involvement in SOCE, we transfected HRAS and HRAS-S17N into Ras-deficient mouse embryonic fibroblasts (Ras-less MEFs). Finally, we co-transfected HEK293 cells with mCherry-tagged Orai1 and EGFP-tagged HRAS and analyzed the molecular interaction using Fluorescence Recovery After Photobleaching (FRAP) and FRET by acceptor photobleaching. Results: Our findings revealed that the expression of wild-type HRAS or the dominant-negative mutant HRAS-S17N reduces SOCE in a dose-dependent manner, whereas the oncogenic mutant HRAS-Q61L has no effect in transfected HEK293 cells. Moreover, both wild-type HRAS and HRAS-S17N prevent ORAI1-induced inhibition of SOCE, while HRAS-Q61L does not alter ORAI1-mediated inhibition of SOCE. In Ras-deficient MEFs, SOCE remains unaffected by the absence of RAS proteins; however, the expression of HRAS alone significantly reduces SOCE. Additionally, both HRAS and HRAS-S17N expression in Ras-deficient MEFs reduce SOCE in a dose-dependent manner. FRAP analysis showed that coexpression of Orai1 with HRAS increases the mobile fraction of HRAS and decreases its immobile fraction at the plasma membrane, while the mobility of Orai1 remains unchanged. FRET

analysis via acceptor photobleaching confirmed a direct interaction between Orai1 and HRAS in HEK293 cells co-transfected with Orai1-mCherry and HRAS-eGFP. Furthermore, the dominant-negative mutant HRAS-S17N also interacts with Orai1. Conclusions and implications: Our results demonstrate that HRAS is involved in the SOCE mechanism. Both HRAS and the dominant-negative mutant HRAS-S17N reduce SOCE in HEK293 and Ras-deficient MEF cells. FRET analyses indicate that these effects are mediated by the direct interaction of HRAS with Orai1. This study highlights the role of the small GTPase HRAS in modulating an ion channel through physical interaction with a core channel protein.

## CARDIOVASCULAR Y RESPIRATORIO

### P1 - POSTERS

FECHA Y HORA: 19/11/2024 11:00-12:00 H

COORDINADORES: VIRGINIA VANASCO, ROMINA HERMANN

#### 49. 080 15 YEARS OF EXPERIENCE OF THE INSTITUTIONAL AMYLOIDOSIS REGISTRY OF THE ITALIAN HOSPITAL OF BUENOS AIRES

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**Introduction:** Patient registries are organized systems of systematic data collection with an observational design to evaluate specific results in populations defined by a disease. The amyloidosis registry aims to obtain epidemiological data, support research on the genetic, molecular and physiological bases of the disease, establish a patient database for the evaluation of treatments and facilitate connections between patients, families and physicians. **Objectives:** To describe the occurrence, clinical characteristics, evolution and prognosis in the population of the Institutional Amyloidosis Registry. **Methods:** active cohort of consecutive incident cases of the different types of amyloidosis, since 2010 (CEPI 1675; NCT01347047). Data were systematically collected on demographic, clinical, diagnosis and treatment. All patients are followed annually for response to treatment and survival by specialized physicians. **Results:** Between January 2010 and July 2024, 426 patients diagnosed with amyloidosis were included. Of these, 37% had immunoglobulin light chain amyloidosis, 37% had transthyretin deposition amyloidosis, and 7% had serum protein A amyloidosis. Regarding baseline characteristics, the median age was 70 years (interquartile range (IQR) 59-80)) and 63% were men. The most affected organs were the heart (60%), kidneys (31%), and peripheral nervous system (23%). The median overall survival was 7.5 years (IQR 2.3 - not reached). The 3-year survival rate was 68% (62-73) and the 5-year survival rate was 58% (51-64%). **Conclusion:** This study provides information on amyloidosis in a cohort of patients enrolled in a 15-year institutional registry. The prevalence of different forms of amyloidosis and the multisystemic nature of amyloidosis highlight the complexity of the disease.

#### 50. 087 MITOCHONDRIAL METABOLISM OF H<sub>2</sub>O<sub>2</sub> AND NO IN

#### EARLY TYPE 1 DIABETES. EFFECT OF MELATONIN IN HEART

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Mitochondrial complex I dysfunction and H<sub>2</sub>O<sub>2</sub> and NO production rates enhancement have been postulated as prodromal signals of diabetic heart failure. Melatonin protects against cardiac dysfunction with its action being linked to mitochondrial function and dynamics modifications. The aim was to study the effect of melatonin against the cardiac mitochondrial dysfunction associated to short periods of hyperglycemia, making focus in H<sub>2</sub>O<sub>2</sub> and NO metabolism, in a type 1 diabetes model. Diabetes was induced by Streptozotocin (60 mg/kg, ip) in male rats. On day 3<sup>rd</sup> and until the day before sacrifice rats received a daily injection of melatonin (10 mg/kg day, ip). Animals were sacrificed 14 days-post STZ. Heart mitochondrial function was studied. Melatonin did not reverse the hyperglycemia developed in STZ-injected animals, but it prevented respiratory and energetic mitochondrial dysfunction, regularizing complex I activity. Melatonin avoided the H<sub>2</sub>O<sub>2</sub> production enhancement (90%) and it prevented the Mn-SOD activity decrease (15%) contributing to normalization of [O<sub>2</sub>]<sub>ss</sub> (73 pM) and [H<sub>2</sub>O<sub>2</sub>]<sub>2,ss</sub> (25 nM). Mitochondrial Se-GPx (38%) and catalase (350%) activities were higher in diabetic animals that received melatonin than in control group, contributing to sustain the [H<sub>2</sub>O<sub>2</sub>]<sub>ss</sub> within the physiological range (25 nM). The administration of melatonin to diabetic rats prevented NO and ONOO<sup>-</sup> production rates rise (50%, 240%), and led to mitochondrial [O<sub>2</sub>]<sub>ss</sub> and [NO]<sub>ss</sub> preservation (73 pM, 9 nM). The reactive species concentrations maintenance within physiological values avoided the enhancement of lipid oxidation (48%) and protein nitration (35%) observed in the mitochondrial matrix of diabetic animals. The administration of melatonin to diabetic animals prevented cardiac mitochondrial dysfunction normalizing the redox condition. Melatonin treatment at an early stage of diabetes could delay the onset of heart failure that occurs after much longer hyperglycemia in diabetic patients.

#### 51. 356 SILDENAFIL TREATMENT INHIBITS FIBROBLAST ACTIVATION IN VIVO AND IN VITRO

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Sildenafil (S) is broadening its clinical uses. Na<sup>+</sup>/H<sup>+</sup> exchanger (NHE1) hyperactivity is a hallmark of severe cardiac pathologies. It was shown that chronic S treatment reduced NHE1 activity in hypertensive rats (SHR), therefore reducing cardiac hypertrophy and fibrosis. This work was aimed to evaluate whether S affects fibroblast activation. SHR and 3T3 fibroblast culture lines were used for the experiments. 4 month old SHR were treated with 100mg/Kg/day S for 3 months (drinking water, n=4) to test possible *in vivo* anti-fibrotic effects of S. Non-treated SHR were used as control (n=4). S treatment did not alter blood pressure but reduced cardiac hypertrophy (LVW/BW in mg/g: control 2.69±0.04; S 2.52±0.05, p<0.05), extracellular collagen (%: control 1.82±0.01; S 1.38±0.01, p<0.05), and myocardial stiffness evaluated in left ventricle papillary muscles (g/mm<sup>2</sup>: control 1.17±0.21, S 0.41±0.19, p<0.05). In agreement, a decreased matrix metalloprotease activity was detected by zymography of cardiac homogenates (AU: control MMP2 10190±1820, S 3178±718, p<0.05; control MMP9 10770±2140, S 2660±1226, p<0.05). In line, TGFβ and aSMA expression showed a tendency to be reduced in S-treated group (%: TGFβ control 100±11, S 78±9; aSMA control 100±20, S 88±3). 48hs of incubation with 10<sup>-8</sup>M Angiotensin II (AngII) was used to stimulate 3T3 cells to mimic SHR condition. Another group of cells received 5mM S during the final 24hs. AngII promoted a not significant tendency to increase ROS that was

reverted by S (% from control: 207±23 AngII; 137±19 S). Migration assay (scratch test) showed increased closing in AngII-treated cells, which was canceled by S (%): control 8±2, AngII 19±4; S 5±2 (p<0.05). NHE1 activity (epifluorescence of BCECF) followed a similar pattern (dpH/dt in s<sup>-1</sup>) control 0.016±0.001, AngII 0.021±0.001, S 0.016±0.002 (p<0.05). In conclusion, the results suggest a potent antifibrotic effect of S *in vivo* and *in vitro* by directly targeting fibroblast activation process.

## 52. 429 REMOTE ISCHEMIC PRECONDITIONING ATTENUATES EARLY AND LATE MYOCARDIAL POSTINFARCTION VENTRICULAR REMODELING

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Remote ischemic preconditioning (rIPC) reduces infarct size in a models of myocardial infarction (MI) with reperfusion, however the effect of rIPC on post-infarction ventricular remodeling is controversial. The aim was to evaluate the effect of rIPC on early (7days) and late ventricular remodeling (28 days) in a model of MI without reperfusion. Male FVB mice were underwent to MI without reperfusion and recovered for 7 or 28 days. In other group, a rIPC protocol (3 cycles of 5 min ischemia/reperfusion in the left lower limb) was performed before MI. The sham group were undergoing a thoracotomy without MI. The same procedures were performed in thioredoxin-1 dominant negative mutant mice, which were recovered for 7 days post-MI. We measured infarct size, ventricular function, MMPs 9 activity and Trx-1 expression in ventricular tissue and calculated the expansion index. While oxidative damage to macromolecules, TNF- $\alpha$ , IL-6 and IL-10 were measured in serum. At 28 days we evaluated ventricular function and collagen volume. At 7 days, MI and rIPC groups had the same infarct size, however rIPC improved systolic function (p<0,05) and attenuated ventricular expansion (p<0,05). rIPC significantly reduced MMP-9 activity, proteins and lipids oxidation, as well as TNF- $\alpha$  and IL-6 levels (p<0,05). IL-10 was increased in rIPC compared with MI group (p<0,05). Additionally, rIPC increased the Trx-1 expression in the MI remote area (p<0,05). Meanwhile, in the transgenic mice the rIPC cardioprotection was completely abolished, in terms of ventricular function, oxidative stress and inflammatory response. Finally, at 28 days, rIPC decreased collagen volume in the MI remote zone and improved ejection fraction (p<0,05). In conclusion, rIPC increases Trx-1 ventricular expression, prevents the development of ventricular early and late remodeling and congestive heart failure through a mechanism related to a reduced oxidative stress and inflammatory response, independently of the infarct size.

## 53. 436 EFFECT OF TOFACITINIB AND TOCILIZUMAB TREATMENT ON Lp(a) LEVELS IN PATIENTS WITH RHEUMATOID ARTHRITIS

Leonardo Gómez Rosso<sup>1</sup>, Maximiliano Martín<sup>1</sup>, Eliana Botta<sup>1</sup>, Florencia Pierini<sup>2</sup>, Osvaldo Cerda<sup>3</sup>, Ezequiel Lozano Chiappe<sup>1</sup>, Gustavo Citera<sup>3</sup>, Belén Davico<sup>1</sup>, Ignacio Gandino<sup>2</sup>, María Saéz<sup>2</sup>, Amanda Yañez<sup>1</sup>, Javier Rosa<sup>2</sup>, Enrique Soriano<sup>2</sup>, Fernando Brites<sup>1</sup>.

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**Introduction:** In rheumatoid arthritis (RA) patients, unconventional factors, instead of traditional ones, play a key role increasing cardiovascular risk (CVR). Among them, lipoprotein(a) [Lp(a)] is considered an independent and causative CVR factor capable of displaying atherogenic, thrombotic and inflammatory effects. **Objectives:** To assess the impact of two antiinflammatory biological agents, tofacitinib and tocilizumab, on Lp(a) levels in RA patients.

**Methods:** The study included 37 women with active RA, 21 treated with tofacitinib (janus kinase inhibitor) and 16 with tocilizumab (IL-6 receptor inhibitor), and evaluated before and after 3 and 6 months. Disease activity was assessed using the Disease Activity Score (DAS-28), and the levels of high-sensitivity C reactive protein (hsCRP), lipids and lipoproteins, and Lp(a) were measured by standardized methods. Data were analyzed using parametric and non-parametric tests for paired samples. **Results:** The group treated with tofacitinib showed a significant reduction in DAS-28 after 3 months, which was maintained after 6 months of treatment. hsCRP concentration showed a significant decrease only at 6 months, while the lipid and lipoprotein profile, as well as Lp(a) concentration did not show significant differences after treatment. On the other hand, patients treated with tocilizumab showed significant changes at 3 months in DAS-28 and in hsCRP levels, which were maintained at 6 months. Additionally, these patients exhibited an increase in low density lipoprotein cholesterol at 3 months and in total cholesterol at 6 months of treatment initiation. Interestingly, Lp(a) levels decreased significantly at 3 months and remained unchanged at 6 months. **Conclusion:** Although both biological agents proved to be effective upon disease activity and inflammation, only tocilizumab significantly reduced Lp(a) levels, thus evidencing that the beneficial effect was selectively related to the pathway involving inhibition of IL-6 receptor.

## 54. 446 NHE1 AND MITOCHONDRIAL DYSFUNCTION

Rayen De Fazio, Joshua Godoy Coto, María C. Ciancio, Alejandro Orłowski, Ernesto A. Aiello, Carolina Jaquenod De Giusti

*Centro de Investigaciones Cardiovasculares – CONICET – UNLP – Facultad de Ciencias Médicas*

**Introduction:** Mitochondria dysfunction is linked to various diseases, such as heart failure and diabetes. NHE1, a key alkalizing mechanism in the heart, is hyperactive in heart conditions like diabetic cardiomyopathy. Although NHE1 is a membrane-bound exchanger, it has been detected in the mitochondria of rats, and its inhibition modulates their response to Ca<sup>2+</sup>. **Objectives:** understanding the consequences of increased mitochondrial NHE1 expression. **Methods:** Mitochondria were isolated from ob/ob mice.  $\Delta\psi_m$  was measured with Rhodamine 123. PTP opening was assessed using the CRC assay with CsA as an inhibitor. Mitochondrial Ca<sup>2+</sup> content was determined using Fluo-3 and their morphology was evaluated by TEM. HEK293T cells were transfected with pmitoNHE1.  $\Delta\psi_m$  was quantified as JC-1 fluorescence. PTP opening was measured by calcein release. Mitochondrial Ca<sup>2+</sup> content was determined by co-transfection with a ratiometric Ca<sup>2+</sup> indicator. ATP levels and mitochondrial ROS production were evaluated in transfected cells and isolated mitochondria using a commercial ATP assay kit and H2DCFDA indicator, respectively. Data were analyzed with Student's t-test or two-way ANOVA (means  $\pm$  SEM). **Results:** Mitochondria from ventricular tissue of ob/ob mice showed increased NHE1 expression. This was linked to altered mitochondrial Ca<sup>2+</sup> handling, as indicated by reduced CRC, reversible by specific NHE1 inhibition. Mitochondria also exhibited lower Ca<sup>2+</sup> content, increased PTP sensitivity, membrane hyperpolarization, elevated ROS production, and reduced ATP. *In vitro* NHE1 overexpression led to similar effects. **Conclusions:** Our results show a strong link between mitochondrial NHE1 overexpression and alterations in  $\Delta\psi_m$ , Ca<sup>2+</sup> content, ROS production, and ATP levels. While no DRP-1 changes were observed *in vivo*, previous TEM studies revealed smaller mitochondria in ob/ob mice, suggesting increased mitochondrial fission.

## P2 - POSTERS

FECHA Y HORA: 19/11/2024 16:10-17:10 H

COORDINADORES: MARIA MARTA AMARAL, LAURA VALDEZ, VERONICA DE GIUSTI

## 55. 265 CoCl<sub>2</sub> AND ARTERIAL FUNCTION: WHAT IS GOING ON?

Gutierrez Christopher<sup>1</sup>, Matias Ramirez<sup>1</sup>, Santiago Gullino<sup>1</sup>,

Agustina Mansisor<sup>1</sup>, Tomás Little<sup>1</sup>, Rocío Castilla<sup>1</sup>  
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Recent studies from our laboratory, conducted using an isolated organ model, have demonstrated that 230  $\mu\text{M}$   $\text{CoCl}_2$  can act as a cardioprotective agent during cardiac ischemia/reperfusion by reducing the calcium overload that occurs during reperfusion. Given the strong interplay between vascular processes and cardiac function, we aimed to study the effect of  $\text{CoCl}_2$  on arterial function. For this purpose, rings of thoracic aorta and mesenteric arteries were obtained and evaluated in an isolated organ bath for isometric contraction in response to norepinephrine (NA), phenylephrine (PHE), and KCl. The addition of 230  $\mu\text{M}$   $\text{CoCl}_2$  to the arteries did not alter the baseline tension. However, the response of both aortic and mesenteric rings to cumulative additions of NA in the presence of  $\text{CoCl}_2$  produced a biphasic curve with a maximum contraction value similar to the control (99.35 $\pm$ 0.46% of the control), but followed by a decrease in tension ( $p < 0.001$ ). The addition of more calcium to the medium did not reverse the loss of tension, and the loss was not evident with another vasoconstrictor such as KCl. The relaxing effect was not due to late activation of  $\beta_2$ -adrenergic receptors as the response to PHE was similar to NA. Endothelium-free rings showed less loss of tension in response to PHE (23.35 $\pm$ 4.75% vs. 0.1 $\pm$ 0.07%), but when contractility was analyzed in rings without endothelium in the presence of another ring with endothelium, the loss of tension increased. The presence of L-NAME did not prevent the loss of tension. Inactivation of catecholamines was evident since the artery incubated with medium collected from another artery exposed to PHE and  $\text{CoCl}_2$  did not produce contraction. The addition of ascorbic acid to the incubation medium prevented the decrease in tension. Washing out  $\text{CoCl}_2$  restored the normal response to catecholamines. In conclusion:  $\text{CoCl}_2$  induces inactivation of catecholamines through oxidation, facilitated by an endothelial factor, without altering cellular function.

**56. 272 BRIEF COBALT CHLORIDE EXPOSURE PROVIDES CARDIOPROTECTION FOLLOWING ISCHEMIA-REPERFUSION INJURY IN INFANT RATS**

Tomás Little<sup>1</sup>, Christopher Gutierrez<sup>1</sup>, Matías Ramirez<sup>1</sup>, Santiago Gullino<sup>1</sup>, Agustina Mansisor<sup>1</sup>, Patricia Bonazzola<sup>1</sup>, Rocío Castilla<sup>1</sup>

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Ischemia-reperfusion (I/R) disturbs  $\text{Ca}^{2+}$  homeostasis. Cobalt chloride ( $\text{CoCl}_2$ ) inhibits  $\text{Ca}^{2+}$  currents in cardiac muscle.  $\text{CoCl}_2$  administered during reperfusion reduces I/R-induced damage, which correlates with improved cellular calcium handling. Infant rat hearts, with different dependence on plasma  $\text{Ca}^{2+}$  for myocyte function, showed similar results. Knowing the primary effect of  $\text{CoCl}_2$  may occur early in reperfusion, we assessed whether administering  $\text{CoCl}_2$  only during the first 5 min of reperfusion could also protect the heart from I/R injury. Additionally, we evaluated the impact of  $\text{CoCl}_2$  on aortic contraction. Ventricles from 6-8 day old Wistar rats were isolated, perfused arterially, suspended, and connected to an isometric force transducer, placed in an organ bath at 37°C, stimulated at 3 Hz, and subjected to either 45 min of ischemia followed by reperfusion with 0.23 mmol/L  $\text{CoCl}_2$  for 5 min or reperfusion with control Krebs solution. Mechanical parameters were assessed. Arterial contractility was evaluated by measuring isometric tension in aortic rings that were incubated with 0.23 mmol/L  $\text{CoCl}_2$  for 5 min, followed by  $\text{CoCl}_2$  removal and stimulation with cumulative doses of phenylephrine (PHE).  $\text{CoCl}_2$  treatment increased post-ischemic contractile recovery (developed tension at 40 min: control: 26.4 $\pm$ 1.7%,  $\text{CoCl}_2$ : 38.7 $\pm$ 5.0%,  $p < 0.05$ ). Diastolic tension or the maximum rates of contraction and relaxation were not affected. Additionally, the extent of ventricular damage was reduced by  $\text{CoCl}_2$  (control: 63.33 $\pm$ 3.63%,  $\text{CoCl}_2$ : 36.17 $\pm$ 5.05%,  $p < 0.001$ ).  $\text{CoCl}_2$  acted as a negative inotropic, decreasing developed tension in non-ischemic hearts by 61.65 $\pm$ 6.57%, with recovery of 84.2% after 5 min of  $\text{CoCl}_2$  washout. 5 min of  $\text{CoCl}_2$  exposure did not alter aortic basal tension or the PHE response. **Conclusion:** 5-minute administration of  $\text{CoCl}_2$  in

reperfusion provides cardioprotection in infant hearts without affecting arterial responses to PHE.  $\text{CoCl}_2$  shows clinical potential as a cardioprotective agent.

**57. 329 EXERCISE AND TRX-1 OVEREXPRESSION: STRATEGIES TO MITIGATE ISCHEMIA AND REPERFUSION INJURY IN MIDDLE-AGED MICE**

Eugenia Godoy Olazar<sup>1</sup>, Virginia Perez<sup>1,2</sup>, Verena Franco Riveros<sup>1,2</sup>, Fernando Garcia<sup>1</sup>, Bruno Buchholz<sup>1,2</sup>, Verónica Casanova<sup>3</sup>, Carla Greco<sup>3</sup>, Eliana Cicale<sup>3</sup>, Tamara Zaobornyj<sup>4</sup>, Verónica D'Annunzio<sup>1,2</sup>.

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Previously, we demonstrated that voluntary wheel running and thio-redoxin-1 overexpression (Trx1) reduce infarct size by acute ischemia/reperfusion (I/R) in young mice. However, in middle age the cardioprotection conferred by Trx1 is abolished. This study aimed to investigate if voluntary exercise could restore the cardioprotective effect of Trx1 in middle-aged mice. We used hearts from wild-type mice (Wt), transgenic mice overexpressing Trx1, and mice with a dominant negative mutant of Trx1 (DN). They were divided into exercise (E) and sedentary (S) groups and housed in cages with running wheels for 8 weeks. Afterwards, hearts were subjected to 30 min of I and 120 min of R (Langendorff technique). Body weight, caloric intake, and running distance were recorded. The heart rate variation was evaluated through ECG with atropine and was expressed as % change in cardiac frequency relative to basal levels and infarct size was determined using triphenyl tetrazolium staining. We performed a two-way ANOVA, data was expressed as mean $\pm$ SEM and  $p < 0.05$  was considered statistically significant.  $n = 5$  in each group. Training was confirmed through the evaluation of heart rate variation (Wt-E: 32.2 $\pm$ 1.1 vs Wt-S: 28.5 $\pm$ 1.1 %,  $p < 0.05$ ) and voluntary exercise showed a cardioprotective effect by reducing infarct size in Trx1 mice (Wt-E: 51.9 $\pm$ 1.7 vs. Trx1-E: 28.6 $\pm$ 5.2,  $p < 0.05$ ) but not in Wt or DN mice (DN-E: 52.5 $\pm$ 3.9). The average running distance of training were similar (Wt-E: 4.6 $\pm$ 0.3; Trx1-E: 3.5 $\pm$ 0.8; DN-E: 3.8 $\pm$ 0.8 km/week) and changes in body weight remained comparable throughout the assessment period in both E and S groups. However, caloric intake was significantly increased in all E groups compared to all the S groups (Wt-E: 25.9 $\pm$ 0.9; Trx1-E: 26.8 $\pm$ 0.8; DN-E: 27.4 $\pm$ 0.7 vs Wt-S: 21.4 $\pm$ 0.8; Trx1-S: 23.3 $\pm$ 0.8; DN-S: 24.1 $\pm$ 0.7 Kcal/g,  $p < 0.05$ ). In conclusion, 8 weeks of voluntary wheel running significantly reduces infarct size in middle-aged transgenic mice overexpressing Trx1 restoring its cardioprotective effect.

**58. 437 DOES ANGIOTENSIN-(1-7) SIGNALING UNDERLINE THE CARDIOPROTECTIVE EFFECTS OF APELIN IN THE HYPERTROPHIED MYOCARDIUM OF SPONTANEOUSLY HYPERTENSIVE RATS?**

Andrés J. Medina<sup>1</sup>, Charles Smith<sup>1</sup>, Claudia Caldiz<sup>1</sup>, Mariela M. Gironacci<sup>2</sup>, Erica Pereyra<sup>1</sup>, Irene L. Ennis<sup>1</sup>, Alejandra M. Yeves<sup>1</sup>.

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The renin-angiotensin system and apelin and its receptor (APJ) play a crucial role in the pathophysiology of hypertension. The angiotensin-converting enzyme type 2 (ACE2) catalyzes the conversion of angiotensin II (Ang II) into angiotensin-(1-7) (Ang-(1-7)), counteracting Ang II effects. Apelin and Ang-(1-7) exert cardioprotective effects through APJ and Mas (MasR) receptors, respectively. However, evidence that demonstrate the interaction between these two system is lacking. **Objective.** To determine whether chronic exogenous administration of apelin upregulates the myocardial ACE2/Ang-(1-

7)/MasR axis in the myocardium of spontaneously hypertensive rats (SHR). **Methods.** Adult SHR received apelin (1mg/kg/day) or physiological solution (control) through osmotic minipumps for 14 days. We measured: 1) morphometric parameters, 2) myocardial Ang II and Ang-(1-7) levels by radioimmunoassay, 3) AT1R, MasR, APJ, AKT, mitochondrial superoxide dismutase (SOD2), GP91phox content by Western-blot, and 4) ACE2 and citrate synthase (CS) activities by colorimetric assay. Results were expressed as mean  $\pm$  SEM and *t* test was used ( $P < 0.05$ ). **Results.** Apelin showed a tendency to increase the expression of APJ and phosphorylation of AKT and to improve the redox balance by increasing the expression of SOD2 and decreasing the catalytic subunit of NADPH oxidase. The myocardial CS activity was significantly higher in response to apelin infusion than control, without significant changes in its expression (CS activity: Apelin:  $121 \pm 3\%$ , vs Control:  $100 \pm 5\%$ ; CS/Na-KATPase: Apelin:  $253 \pm 74\%$ , vs Control:  $100 \pm 13\%$ ). Interestingly, myocardial Ang II and Ang-(1-7) levels were unchanged by apelin infusion, which was consistent with the lack of modification in ACE2 activity and expression induced by apelin. Furthermore, MasR, and AT1R content was also not modified by apelin. **Conclusion.** Our preliminary data suggest that apelin improved mitochondrial and redox status with no change in the protective axis cardiac ACE2/Ang-(1-7)/Mas pathway. We could not disregard that both systems may interact at the receptor level.

#### 59. 442 POTENTIAL PROTECTIVE ROLE OF GALECTIN 1 ON CARDIAC DAMAGE IN ANGIOTENSIN II- INDUCED HYPERTENSION

Juan Fleischer, Ignacio Moglie, Victoria Mainero, Francisco Alvarado, Leonardo Cacciagiú, Vanessa Touceda, Agustina Vidal, Gabriel Rabinovich, Verónica Miksztoicz, Germán E. González.

Galectin-1 (Gal-1), a proto-type member of a family of  $\beta$ -galactoside-binding lectins, plays a crucial role in immunoregulation. However, its role and mechanism of action in hypertension-induced target organ damage (TOD) remain unclear. We hypothesized that, in angiotensin II (ANG II)-induced hypertension, genetic deletion of Gal-1 would impair left ventricular (LV) remodeling by enhancing myocardial hypertrophy, fibrosis, oxidative stress, and metalloproteinase (MMP) activity. To test this, we induced hypertension in wild-type C57BL/6J and Gal-1 knockout (KO) male mice ( $n = 4-5$ /group) by infusing ANG II ( $3 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  sc) for 4 weeks. We assessed: 1) systolic blood pressure (SBP) by plethysmography, 2) myocardial hypertrophy and fibrosis through histology, 3) Antioxidant enzymes activity (catalase and superoxide dismutase), and 4) Type 2 and 9 metalloproteinases (MMP-2 and MMP-9) by gelatinolytic zymography. **Results (X $\pm$ SD):** ANG II significantly increased SBP (mmHg) from  $122 \pm 5$  and  $112 \pm 6$  to  $158 \pm 14$  and  $162 \pm 11$  in wild-type and Gal-1 KO mice, respectively ( $p < 0.05$ ). Heart weight/body weight (mg/g) ratios also increased in both hypertensive strains ( $p = \text{NS}$ ), while myocardial fibrosis (%) was higher in hypertensive Gal-1 KO mice ( $5 \pm 2$  vs.  $9 \pm 4$ ,  $p = 0.06$ ). The antioxidant effect of catalase was significantly reduced from  $37 \pm 6$  (A.U.) in hypertensive control mice to  $32 \pm 7$  (A.U.) in hypertensive Gal-1 KO mice. Expression of MMP-2 and 9 were similar among groups. **Conclusion:** These results suggest that, while Gal-1 is not associated with hypertension or myocardial hypertrophy in ANG II-induced hypertension, it may play a protective role against TOD by preventing myocardial fibrosis and oxidative stress. Further studies are needed to fully understand the role of Gal-1 in TOD-associated inflammation in hypertension.

#### 60. 508 ROLE OF THE AUTONOMIC NERVOUS SYSTEM IN THE CARDIAC ELECTRICAL CHANGES AND LEFT VENTRICULAR FUNCTION INDUCED BY CEREBRAL ISCHEMIA AND REPERFUSION IN MICE

Ignacio P Barbieri<sup>1</sup>, Verena Franco-Riveros<sup>1,2</sup>, Agustina Schirripa<sup>1</sup>, Verónica Casanova<sup>3</sup>, Bruno Buchholz<sup>1,2</sup>.

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**Objective:** To determine whether left ventricular (LV) dysfunction and ECG changes induced by cerebral ischemia (I) and reperfusion (R) are modified in mice with cardiac sympathetic hyperactivity and whether these changes improve with the administration of donepezil (DPZ). **Materials and Methods:** A middle cerebral artery occlusion was performed for 60 min, followed by 24h of R, in male transgenic mice overexpressing the Gs-alpha protein (TG) and in wild-type controls (WT). ECGs were performed at 60 min of I and 24h of R, and echocardiograms were conducted after 24h of R. DPZ (an acetylcholinesterase inhibitor) was administered 15 min before I. **Results:** In WT animals, a slight decrease in ejection fraction (EF) was observed (I/R:  $65.9 \pm 1.5\%$  vs. SHAM:  $74.3 \pm 0.8\%$ ,  $p < 0.01$ ) and in shortening fraction (SF) (I/R:  $36.2 \pm 1.6\%$  vs. SHAM:  $43.6 \pm 1.6\%$ ,  $p < 0.05$ ), which improved with DPZ (EF:  $82.8 \pm 0.3\%$ ,  $p < 0.01$ ; SF:  $45.3 \pm 0.3\%$ ,  $p < 0.01$ ). In the TG group, the decrease in EF was (I/R:  $66.5 \pm 1.4\%$  vs. SHAM:  $73.0 \pm 1.2\%$ ,  $p < 0.01$ ), which improved with DPZ ( $75.2 \pm 1.4\%$ ,  $p < 0.01$ ). In WT mice, the QTc interval was prolonged at 60 min of I ( $143.8 \pm 5.2\text{ms}$ ,  $p < 0.01$ ) compared to baseline ( $125.1 \pm 4.3\text{ms}$ ), and even more so at 24h of R ( $170.3 \pm 5.8\text{ms}$ ,  $p < 0.01$ ). DPZ prevented the prolongation during I ( $133.0 \pm 3.3\text{ms}$ ) but not at R (24h:  $197.3 \pm 4.3\text{ms}$ , baseline:  $129.1 \pm 6.3\text{ms}$ ,  $p < 0.01$ ). The same occurred in TG mice with DPZ (baseline:  $143.8 \pm 5.0\text{ms}$ , 60min:  $132.2 \pm 8.1\text{ms}$ , 24h:  $155.9 \pm 7.7\text{ms}$ ;  $p < 0.05$ ). In TG mice, the TpTe interval was prolonged during I ( $16.52 \pm 0.9\text{ms}$ ,  $p < 0.05$ ) and even more so at 24h of R ( $21.4 \pm 1.9\text{ms}$ ,  $p < 0.01$ ) compared to baseline ( $10.8 \pm 1.4\text{ms}$ ). DPZ reduced this alteration during I ( $12.2 \pm 1.1\text{ms}$ ) but not at R (24h:  $21.7 \pm 2.7\text{ms}$ ,  $p < 0.01$ ) compared to baseline ( $17.7 \pm 1.7\text{ms}$ ). **Conclusion:** Cerebral I causes changes in ventricular repolarization, which worsen during R and are accompanied by mild systolic LV dysfunction. DPZ prevents the decline in LV function and ECG changes during I, but this electrical improvement does not persist during R.

#### P3 - POSTERS

FECHA Y HORA: 21/11/2024 11:00-12:00 H

COORDINADORES: CAROLINA JAQUENOD DE GIUSTI, LUIS GONANO, VERENA FRANCO RIVEROS

#### 61. 181 ISCHEMIC POSTCONDITIONING IN HEARTS SUBJECTED TO ISCHEMIA-REPERFUSION: ROLE OF AMP ACTIVATED PROTEIN KINASE (AMPK) IN ITS EFFECT ON MITOCHONDRIAL STATUS

María de las Mercedes Fernández Pazos<sup>1,2</sup>, Victoria Evangelina Mestre Cordero<sup>1,2</sup>, Violeta Gomez Peracca<sup>1</sup>, Lucía Safer<sup>1</sup>, Federico Reznik<sup>1</sup>, María Gabriela Marina Prenches<sup>1,2</sup>, Romina Hermann<sup>1,2</sup>

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In previous studies, we began to evaluate the role of AMPK, a key enzyme in the regulation of cellular energetic metabolism, in the beneficial effects of ischemic postconditioning (IPostC), a potent cardioprotective strategy. We evidenced that IPostC improved contractile function, decreased infarct size, and enhanced ATP synthesis rate, these effects being reverted when AMPK was inhibited. However, further studies are essential to deepen our understanding of the molecular mechanisms involved in IPostC. The aim of this study was to investigate the involvement of AMPK in the effects of IPostC in mitochondria from Langendorff perfused rat hearts subjected to Ischemia (I) - Reperfusion (R). Isolated hearts from female Sprague-Dawley rats (220-270 g) were perfused and subjected to 25 min I-60 min R. IPostC (6 cycles, 10 sec R-10 sec I) was carried out at the onset of R. Compound C (CC, 20  $\mu\text{M}$ ) was added in the first 5 min of R, to inhibit AMPK. Mitochondrial structure, number, and area were analyzed by electron microscopy. To assess mitochondrial function, mitochondrial membrane potential ( $\Delta\Psi\text{m}$ ), respirato-



ry complexes activity, and calcium retention capacity (CRC) were measured. To evaluate autophagy, LC3II-/LC3-I ratio and p62 were analyzed by Western Blot. ANOVA, n=8/group. IPostC preserved mitochondrial structure and increased the number of mitochondria/ $\mu\text{m}^2$  ( $p<0,05$  vs Control), which was prevented in the presence of CC.  $\Delta\Psi\text{m}$  was preserved by IPostC ( $p<0,05$  vs Control), effect not significantly affected by CC. IPostC also improved complex I-III and II-III activities and CRC ( $p<0,05$  vs Control), and CC addition reversed these effects. LC3-II/LC3-I ratio was increased and p62 levels decreased at 30 min R in IPostC ( $p<0,05$  vs Control), both effects were overturned by CC. These results suggest that IPostC promotes the preservation of mitochondrial structure and function, and that AMPK may be involved, at least partially, in these beneficial effects.

## 62. 330 EXPLORING THE EFFECTS OF MAMMARY TUMORAL CELLS ON CARDIAC PATHOGENESIS

Camila Galeota Satini<sup>1</sup>, Jorge Omar Velez Rueda<sup>1</sup>, Aldana Schey<sup>2</sup>, Alejandro Urtreger<sup>2</sup>, Veronica De Giusti<sup>1</sup>, Valeria Martinez<sup>1</sup>

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<sup>2</sup>Instituto de Investigación "Angel H. Roffo"

**Introduction.** Cardiovascular diseases and breast cancer in women share common risk factors. Molecular mediators facilitate communication between neoplastic cells and cardiomyocytes, which could alter the cardiac function. However, the impact of breast cancer on cardiac function remains unexplored. We hypothesized that breast cancer promotes cardiac dysfunction through the production of reactive oxygen species (ROS) and the activation of the  $\text{Na}^+/\text{H}^+$  exchanger (NHE). **Methods.** H9C2 cardiomyocytes were incubated with conditioned DMEM from MDA-MD-231 breast cancer cells and treated with angiotensin II (An II). HACAT epithelial cells served as control. ROS production and NHE activity were measured. Female BALB/c mice (8 weeks old, n=6) were subcutaneously inoculated in the left axillary flank with the LM3 cell line. Following tumor development, the animals were euthanized and the hearts were removed. ROS generation, the GSH/GSSG ratio and nitric oxide (NO) production were assessed. Western blot was performed to measure the expression of NHE, gp91<sup>phox</sup>, and p47 subunits. Additionally, the activity of metalloproteinases MMP2 and MMP9 was evaluated. **Results.** Conditioned DMEM from MDA-MD-231 cells increased NHE activity in H9C2 cells, whereas the conditioned medium from HACAT cells did not induce any changes. Similarly, ROS production was elevated. These parameters were enhanced by Ang II. In hearts extracted from tumor-bearing mice, cardiac ROS levels and TBARS were significantly increased compared to non-tumor-bearing mice, while the GSH/GSSG ratio decreased. NO levels also decreased. Protein expression analysis revealed increased levels of NHE, gp91<sup>phox</sup>, and p47 subunits, as well as heightened MMP2 and MMP9 activity. **Conclusion.** Breast cancer is associated with cardiac dysfunction which is at least partly related to dysregulation of the cardiomyocytes' alkalizing mechanism and redox state.

## 63. 338 "PROTON OSCILLATIONS IN THE DYADIC SPACE AND MITOCHONDRIAL MATRIX OF CARDIAC MYOCYTES"

Sofía Ciarrocchi, Delfina Gallo, Romina A Di Mattia, Ernesto A Aiello, Alejandro Orłowski

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**Introduction:** Intra and extracellular pH regulation is essential for the normal physiological function of all cells and tissues and, specifically in heart, it is a prerequisite for both its electrical and mechanical activity. Oxidative cellular metabolism produces protons that are exported by the mitochondrial matrix to the cytosol and then recaptured for the production of ATP. **Aim:** To study proton microdomains that occur beat-to-beat in cardiomyocytes using a proton sensor fluorescent protein. **Methods:** We fused pHluorin2 with canonical targeting signals to the endoplasmic reticulum, mitochondrial matrix (mt) and junctional cleft of cardiac myocytes (cleft-targeted pH sensors were generated by fusing pHluorin2 with FKBP12.6). We

generated an adeno-associated virus (AAV9) to express pH sensors in cardiac myocytes. AAV9-pHluorin2-mt and AAV9-pHluorin2-FKBP were injected in rats. After 28 days, cardiac myocytes were isolated and pH sensor expression was confirmed by immunofluorescence. We measured fluorescence intensity in isolated cardiomyocytes using confocal microscopy. **Results:** pHluorin2 was expressed in a striated pattern, and colocalization with RyR was demonstrated using immunofluorescence. The colocalization of pHluorin2-mt and MitoTracker showed the correct expression of the sensor in the mitochondrial matrix. When we stimulated cells electrically, we observed a beat-to-beat decrease in fluorescence intensity in those cardiomyocytes expressing pHluorin2-FKBP, but pHluorin2-mt showed an increase in fluorescence intensity. **Discussion:** The proton dynamics in cardiac cells are poorly understood. We generated a fluorescent pH sensor targeting different cellular microdomains as a tool for proton microdomain studies. The acidic pH transient observed in cardiomyocytes expressing pHluorin2-FKBP could be related to ATP consumption during cell contraction. Conversely, we observed alkaline pH transients when we express pHluorin2-mt that could be explained by the oxidative phosphorylation activity in cellular metabolism.

## 64. 339 IMPACT OF GENETIC MODIFICATION OF MYOCARDIAL CANNABINOID RECEPTORS IN THE TREATMENT OF CARDIOVASCULAR PATHOLOGIES WITH CANNABIS

Delfina Gallo<sup>1</sup>, Sofía Ciarrocchi<sup>1</sup>, Romina A. Di Mattia<sup>1</sup>, Juliana C. Fantinelli<sup>1</sup>, Luisa González Arbelaez<sup>1</sup>, Paula Blanco<sup>2</sup>, Alejandro Orłowski<sup>1</sup>, Ernesto A. Aiello<sup>1</sup>

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**Introduction:** Chronic marijuana use induces structural, mechanical, and electrical cardiac dysfunctions. Most studies agree that these deleterious effects are derived from the action of tetrahydrocannabinol (THC) and/or cannabidiol (CBD) on CB1r, resulting in lower contractility and blood pressure. **Aims:** Based on the differences observed in the literature regarding the role of these receptors, we aimed to investigate the role of differential activation of receptors in models of cardiovascular pathologies and their respective controls, by using adeno-associated viral vectors to differentially silence CB1r or CB2r (AAV9-shCB1r or AAV9-shCB2r). **Materials and methods:** We administered AAV9-shCB1r and its respective control virus, AAV9-shControl, via intracardiac injection into 4- to 5-day-old male Spontaneous hypertensive rats (SHR) and performed a series of studies to assess cardiac hypertrophy, blood pressure, and ischemia/reperfusion response. Data are expressed as mean  $\pm$  standard error of the mean and were compared using Student's t-test. **Results:** Four months after injection, we found an increase in systolic, diastolic, and mean blood pressure in rats that were treated with AAV9-shCB1. In addition, after the ischemia-reperfusion protocol, we found that rats injected with AAV9-shCB1 showed a smaller infarct size after staining with triphenyltetrazolium chloride (TTC) salts, and also showed a higher developed pressure. **Conclusion:** Based on the results obtained so far, we can conclude that CB1 silencing increases both systolic and diastolic blood pressure, as well as mean arterial pressure, which is consistent with what has been described so far, where it has been established that CB1 activation is hypotensive. The mechanism by which this increase in blood pressure occurs remains unclear. On the other hand, we conclude that CB1 silencing decreases infarct size and improves systolic function after ischemia-reperfusion injury.

## 65. 355 GENDER-AFFIRMING ESTROGEN THERAPY (ET) IN A HYPERTENSIVE RAT MODEL OF TRANSGENDER FEMALE (T) LEADS TO A COMPLEX STRUCTURAL AND FUNCTIONAL CARDIAC SCENARIO

Escudero DS, Martínez VR, Colareda GA, Pis Diez M, Verzoletto B, Lofeudo JM, Vélez Rueda JO, Portianski EL, Pérez NG, Díaz RG.

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ET is used to align secondary sex characteristics of T to their gender identity. T have greater cardiovascular risk than cisgender females and males, but it is unknown whether it is related to ET. This study was aimed to characterize cardiac effects of ET in an animal model of T in spontaneously hypertensive rats (SHR). 3-month-old male SHR were assigned to gonadectomy (G) for 3 months; T therapy (G plus last month ET); or SHAM (S), N=6-8/group. Estradiol (10µg/0.2ml/rat, SC) was given every 4 days to mimic estrous peaks. Blood Pressure (BP) was monitored during treatment. Echocardiography was assessed before sacrifice. After sacrifice, hypertrophic and contractile parameters, collagen content, oxidative stress and protein expressions were determined. BP was similar in all groups. Testosterone deprivation decreased LV mass, effect canceled by ET (mg/mm: S 29±1; G 24±1; T 29±2, p=0.05). Systolic wall stress followed the same tendency but h/r ratio did not change. G promoted a decrease in cardiac fibrosis partially reverted by ET (% total collagen: S 1.5±0.006; G 0.5±0.001; T 0.7±0.001, p<0.05). Isolated left ventricle papillary muscles from T showed lower basal developed force: (g/mm<sup>2</sup>) S 2.7±0.6; G 1.8±0.6; T 1.1±0.6, p<0.05. A tendency to increase Ca<sup>2+</sup> handling protein expressions (SERCA2A, NCX) was observed in T cardiac samples. G decreased reduced/oxidized glutathione ratio, while effect that was reverted by ET: S 3.2±0.2; G 2.0±0.4; T 3.0±0.3, p<0.05. In agreement, TBARS and reactive oxygen species followed an opposite tendency. ET increased cardiac NO: (%) S 1.82±0.18; G 1.55±0.07; T 2.33±0.08 (p<0.05), effect that was accompanied by a tendency to increase eNOS expression. These preliminary results suggest that ET in T promotes a complex mosaic of effects including restoration of the cardiac hypertrophic pattern, lower contractility, and similar oxidative stress with increased NO production. Further experiments are needed to shed more light upon these issues.

## ENDOCRINOLOGÍA

### O1 COMUNICACIONES ORALES

FECHA Y HORA: 21/11/2024 11:00-12:00 H

LUGAR: SALA DE CÁMARA

COORDINADORES: CORA CYMERING, MARTA TESONE

#### 66. 085 MECHANISM OF ACTION INVOLVED IN THE ANTIOXIDATIVE EFFECT OF ESTRONE ON RAT UTERUS

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Estrone is one of the major estrogens produced by ovaries, and in slight amount by extragonadal synthesis. Previously in our lab., we reported that in rat uterus (Ut) E<sub>1</sub> counteracts the inflammatory and oxidative stress induced by obesity. The aim of this work was to study the mechanism of action involved in the antioxidant effect displayed by E<sub>1</sub>. To that end, Ut slices were isolated from female Wistar rats fed with high-fat diet for 10 weeks, and in vitro exposed to 10 nM E<sub>1</sub>. The influence of the hormonal impact during estrous cycle was ruled out using bilaterally ovariectomized rats (OVX-Ob). The estrogen exhibits a positive effect on the balance antioxidant (nitric oxide, NO) / pro-oxidant (ROS) production. After E<sub>1</sub> treatment (4h), an enhancement in NO synthesis (130% above control P<0.001), and a reduction in ROS release (H<sub>2</sub>O<sub>2</sub> 30% below control, P<0.01) in Ob-Ut slices, were detected. To assess whether the effect of E<sub>1</sub> on H<sub>2</sub>O<sub>2</sub> generation depends on its ability to enhance NO synthesis, L-NAME (cNOS inhibitor) and L-NIL (iNOS inhibitor) compounds were employed. In the presence of L-NAME, the reduction on ROS production elicited by E<sub>1</sub> was blocked; meanwhile L-NIL did not alter the hormonal action. The involvement of estrogen receptors ER or GPER was tested using ICI182780 or G15 compounds respectively.

The reduction in H<sub>2</sub>O<sub>2</sub> production was blunted by the presence of ICI182780 but not by G15 (2033 ±24; 2972± 18; 1873 ±11 nmol H<sub>2</sub>O<sub>2</sub>/mg prot; E<sub>1</sub> vs E<sub>1</sub>+ICI; E<sub>1</sub>+G15 respectively). Instead, G15 suppressed the increase in NO production elicited by E<sub>1</sub>; meanwhile ER antagonist did not modify it. E<sub>1</sub> acts by its own and not via conversion to estradiol, since preincubation with Equilin (100-10 µM), an specific inhibitor of 17HSD, did not alter the hormonal action, either on NO synthesis stimulation or ROS release reduction (p< 0.05). In summary, in Ob-Ut, E<sub>1</sub> "per se" elicits an antioxidative effect, through a mechanism that involves ER, GPER, and cNOS participation.

#### 67. 145 HEMIN TREATMENT PROTECTS THE ADRENAL GLAND FROM IR DAMAGE IN A RAT SEPSIS MODEL

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Adrenal insufficiency (low glucocorticoid production) can lead to adrenal crisis and death if not identified and treated in time. Rats fed sucrose rich diets (SRD) for several weeks mimic certain traits of human IR and exhibit lower basal corticosterone levels. The aim of the present study was to analyze the antioxidant and anti-inflammatory effect of hemin on the adrenal cortex of SRD-treated rats subjected to a ligation and cecal puncture procedure (CLP), the gold standard model for sepsis. Previous results indicate that SRD-treated animals have a diminished glucocorticoid response to CLP, and that hemin treatment prevents this effect. In the present study adult male Wistar rats were randomly distributed in three groups: Control (C), SRD and SRD+H. Rats in SRD groups received 30% sucrose in the drinking water for 12 weeks while the last group received hemin for the last 2 weeks (15 mg/kg i.p every 48 h). All the animals were subjected to CLP surgeries during the 12<sup>th</sup> week. As expected, HO-1 expression levels were increased in the SRD+H group while inflammatory markers as TNFα and iNOS were also higher in this group (p<0.05 vs SRD). In addition, M2 phenotype macrophage markers such as IL-10 and MRC1 showed a significant increase in the group that received hemin (p<0.001 vs. SRD or C). Apoptotic parameters such as the BAX/BCL2 ratio were higher in both SRD-treated groups (p<0.05 vs. C) and not different between SRD-groups. Histological examination of H&E-stained tissues showed a greater area of vascularization in the adrenal cortex in both SRD groups compared to controls. eNOS and VEGF mRNA levels were higher in the SRD+H group (p<0.01 vs SRD). In summary, although CLP triggered changes in adrenocortical inflammatory and apoptosis markers that could affect its function, treatment with hemin appears to improve the response to inflammatory stimuli and to induce an angiogenic response.

#### 68. 270 THE CONCENTRATION OF LEAP2 IN HUMAN MILK AND INFANT PLASMA IS POSITIVELY ASSOCIATED WITH ADIPOSITY AND BODY WEIGHT IN THE FIRST YEAR OF LIFE

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**Objectives:** 1 – to assess liver-expressed antimicrobial peptide 2 (LEAP2) presence in human milk and putative associations with infant body weight and adiposity in the first year of life. 2- To evaluate the impact of maternal weight status on LEAP2 concentration. 3 – To explore the relationship between infant plasma LEAP2 and body weight and adiposity. **Methods:** Prospective cohort observational study assessing LEAP2 concentration in plasma and milk from lactating women with normal weight (n=26) or overweight/obesity (OW/OB, n=26) at six months postpartum and in infant plasma. Multiple linear regression analysis was used to test examining associations with metabolic and anthropometric variables at 6 months and 1 year. Regression coefficients presented with 95% confidence interval and significant a value  $p < 0.05$ . Effect size reported as Cohen's  $f^2$ . LEAP2 expression in milk fat globules and single-cell-RNA-sequencing datasets was evaluated **Results:** LEAP2 was detected in all milk samples and was positively associated with infant triceps ( $p=0.022$ ,  $f^2=1.25$ ) and subscapular ( $p=0.008$ ,  $f^2=0.68$ ) skinfolds at 1 year old. Maternal LEAP2 was positively associated with insulin ( $p=0.005$ ,  $f^2=0.30$ ) and pre-pregnancy body mass index (BMI) ( $p=0.040$ ,  $f^2=0.17$ ) and negatively associated with gestational weight gain ( $p=0.008$ ,  $f^2=0.25$ ) and postpartum weight retention ( $p=0.036$ ,  $f^2=0.15$ ). Maternal LEAP2 was higher in plasma ( $p=0.039$ ), but not milk of lactating women with OW/OB. Infant plasma LEAP2 was positively associated with weight ( $p=0.004$ ,  $f^2=0.63$ ), BMI ( $p=0.049$ ,  $f^2=0.37$ ), and weight-for-length ( $p=0.024$ ,  $f^2=0.35$ ) z-scores at 1 year old, predominantly in males. No evidence of LEAP2 mRNA expression was found in mammary cells. **Conclusions:** Milk LEAP2 is a bioactive component that plays a role in infant fat accretion in the first year of life. While maternal LEAP2 responds to weight change in pregnancy and lactation, infant plasma LEAP2 might be involved in body weight regulation in early life.

**69. 308 SERUM FSH IN COMBINATION WITH TESTICULAR SERTOLI CELL BIOMARKERS ACCURATELY DISTINGUISH BETWEEN CONGENITAL HYPOGONADOTROPIC HYPOGONADISM AND SELF-LIMITED DELAYED PUBERTY IN ADOLESCENT MALES: A PROSPECTIVE, LONGITUDINAL, NESTED CASE-CONTROL STUDY**

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**Background:** Delayed puberty is a common concern in males, with distinguishing between self-limited delayed puberty (SLDP) and congenital hypogonadotropic hypogonadism (CHH) posing a significant challenge. Commonly used endocrine tests, particularly those focusing on stimulated luteinising hormone (LH) or testosterone levels, often fail to provide a definitive diagnosis. Given that follicle-stimulating hormone (FSH) action on Sertoli cells leads to testicular enlargement and increased secretion of anti-Müllerian hormone (AMH) and inhibin B, and that the FSH-Sertoli cell axis function is detectable during normal childhood and early puberty, we hypothesised that assessing serum levels of FSH, AMH and inhibin B could be informative in differentiating between SLDP and CHH. **Objective:** To assess whether serum levels of FSH, AMH, and inhibin B can effectively distinguish between SLDP and CHH. **Methods:** We conducted a prospective, nested case-control study within a cohort of male adolescents presenting with delayed puberty. Baseline serum reproductive hormone levels were compared to identify potential biomarkers predictive of CHH. Participants were followed prospectively until a final diagnosis was established using gold standard criteria (age 18 years or  $\geq 4$  years after achieving a testicular volume of 4 mL). **Results:** Among 65 participants who completed the follow-up, 33 were diagnosed with SLDP and 32 with CHH. Serum FSH, AMH, and inhibin B demonstrated superior diagnostic accuracy compared to LH and testosterone in distinguishing

between SLDP and CHH. FSH (IU/L) x inhibin B (ng/mL)  $< 92$ , and FSH (IU/L) x AMH (pmol/L)  $< 537$  showed high sensitivity ( $> 93\%$ ), specificity ( $\geq 92\%$ ), predictive values ( $> 92\%$ ), and positive likelihood ratio ( $> 12$ ) for CHH. **Conclusion:** The combination of serum FSH with inhibin B or AMH is highly predictive for accurately differentiating between SLDP and CHH in male adolescents.

**70. 384 IDENTIFICATION OF DYSLIPIDEMIA IN A HEALTHY POPULATION OF CHILDREN AND ADOLESCENTS AT THE HEALTH SERVICE OF TALCAHUANO, CHILE**

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**Background:** Cardiovascular diseases (CVD) are the main cause of mortality worldwide. They develop mainly from atherosclerosis, a chronic and inflammatory disease that begins in childhood and progresses until its clinical onset in adulthood. Cardiovascular risk factors (CVRF), such as dyslipidemia, promote atherosclerosis and CVD. Thus, the early diagnosis and treatment of CVRF is essential to reduce CVD. In children and adolescents (CA), dyslipidemia is usually screened in obesity or a history of first-degree relatives with dyslipidemia or early CVD. However, dyslipidemia could not be diagnosed in patients that do not meet these criteria. **Aim:** to identify the prevalence of dyslipidemia in CA at the Talcahuano Health Service, Chile. **Materials and methods:** observational, descriptive, retrospective and cross-temporal study, using a database from the Clinical Laboratory of the Hospital Las Higueras, Talcahuano. It included patients (0-19 years old) between January 1st, 2019 and December 31th, 2021. Exclusion criteria: incomplete lipid profile, samples from emergency, intensive care and palliative care units; patients with hypothyroidism, diabetes, nephrotic syndrome, rheumatoid arthritis, HIV or pregnancy. Abnormal lipid parameters were identified using the reference values of the Ministry of Health. **Results:** 2,184 patients were included ( $11.6 \pm 4.8$  years old; 42.2% boys and 57.8% girls). 62.7% had hypertriglyceridemia; 53.4% high total cholesterol; 53.4% elevated LDL-C; and 41.2% low HDL-C. 56.1% of boys and 53.6% of girls showed dyslipidemia, with atherogenic dyslipidemia and isolated hypertriglyceridemia being the most prevalent. Boys aged 17 to 19 years and girls aged 0 to 2 years and 17 to 19 years showed the highest frequency of dyslipidemia. **Conclusions:** This work showed the high prevalence of dyslipidemia in apparently healthy children and adolescents and highlights the importance of universal screening in this population, following international recommendations.

**P1 - POSTERS**

FECHA Y HORA: 19/11/2024 11:00-12:00

COORDINADORES: LUZ ANDREONE, MARIEL NUÑEZ

**71. 042 GALECTIN 1, GALECTIN 3 AND TGFb1 ARE UPREGULATED IN THE LIVER OF MICE LACKING PULSATILE GROWTH HORMONE ACTION**

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A link between growth hormone (GH) and cancer has been established. Transgenic mice overexpressing GH (GH-Tg) exhibit a high incidence of hepatocellular carcinoma (HCC), while GH receptor

knockout (GHR-KO) mice are cancer resistant. The glycan binding proteins galectin (GAL) 1 and 3 are involved in liver tumorigenesis in humans, their expression is low in normal hepatocytes but increase in tumors. We previously found that GAL1 and 3 are upregulated in the liver of GH-Tg mice exposed to high continuous GH levels, as well as in GHR-KO mice deficient in GH action, suggesting that normal pulsatile GH secretion helps maintain low galectin expression in the adult liver. This study aimed to evaluate if transforming growth factor b1 (TGFb1), a cytokine implicated in HCC development and known to induce GAL1 and 3 expression, contributes to the changes observed in the abundance of these galectins in the liver of GH-Tg and GHR-KO mice. TGFb1 hepatic expression was analyzed by RT-qPCR. GAL1 and 3 expression was assessed by RT-qPCR and Western-blotting. GH-Tg mice of 2-3 months (with preneoplastic liver pathology) and 10-13 months (with liver tumors) were studied. In the case of GHR-KO mice, 2-3 months old animals were analyzed. Normal littermates served as controls. Statistical analysis was performed by Student's t-test or two-way ANOVA. TGFb1 liver expression was higher ( $P<0.05$ ) in GH-Tg ( $n=13$ ) and GHR-KO ( $n=5$ ) mice compared to aged-matched normal littermates in both sexes. In normal mouse liver, TGFb1, as well as GAL1 and 3 expression, was higher ( $P<0.05$ ) in females than in males ( $n=13$ ). These results suggest that TGFb1 may underlie the increased liver expression of GAL1 and 3 in conditions lacking pulsatile GH action. Both TGFb1 and GAL1 have dual roles in HCC with antitumorigenic activity in early stages and protumorigenic effects in later stages, which could explain their increased liver expression in both HCC-prone (GH-Tg) and HCC-resistant (GHR-KO) mice.

- 72. 216 EXPOSURE TO LOW DOSES OF GLYPHOSATE AFFECTS LONG BONE DEVELOPMENT IN JUVENILE RATS**  
Martin, A(1); Fernandez, MC(1); Lombarte, M (2); Cruz, ME(1); Brun, LR (2); Zeni, S(3); Pennisi, P(1).  
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Glyphosate is an herbicide used in agriculture for weed control. The chronic effects of the herbicide at low doses have been controversial. Its potential to act as an endocrine disruptor affecting longitudinal growth during postnatal development is unknown. **OBJECTIVE:** To analyze the impact of exposure to low doses of glyphosate during early postnatal life on the development of long bones. **METHODS:** Female (8/16) and male (8/16) S-Dawley rats were treated daily from days 30 to 70 with 1 mg/kg/day of glyphosate (EPA reference dose). On day 71 blood was collected. Bones (hind legs) were harvested for macroscopic, microscopic, biomechanical and RNAseq analysis. SaOS-2 cells were cultured in differentiation with/without glyphosate added (10, 50, 100 ng/ml) for matrix deposition and expression profiling studies on days 7, 14, 21, 28 and 35. **RESULTS:** No differences were observed in body weight, renal or hepatic parameters, cortisol, E2, T or T3 between control and treated animals of both sexes. Tibias' weight and length were similar in treated versus control animals. RNAseq studies showed no differentially expressed genes in females under glyphosate treatment. In treated males, 54 genes were differentially expressed compared to controls (gene groups related to proteolysis, disassembly and organization of the extracellular matrix, collagen catabolic processing, and osteoblast differentiation). BMD was decreased ( $0.268\pm 0.006$  vs  $0.253\pm 0.002$ g/cm<sup>2</sup>  $p<0.03$ ), while Stiffness and Ultimate Load were higher ( $S:211\pm 14$  vs  $285\pm 23$ N;  $UL:78.3\pm 1.5$  vs  $84.9\pm 3.2$ N/mm,  $p<0.05$ ) in treated males vs control. In vitro, SaOS2 cells exhibited significantly reduced mineralized matrix deposition ( $0.704\pm 0.018$  vs  $0.301\pm 0.169$  mMd7;  $3.12\pm 0.2$  vs  $2.27\pm 0.10$  mMd14,  $p<0.05$  ANOVA) under glyphosate, with no differences in proliferation or apoptosis. **CONCLUSION:** Exposure to low doses of glyphosate alters the quality of developing bone in juvenile male rats, likely through modification of matrix deposition in

developing bone.

- 73. 280 CORTISOL LEVELS AFTER DEXAMETHASONE SUPPRESSION TEST IN HYPERTENSIVE PATIENTS WITH NON-FUNCTIONING ADRENAL INCIDENTALOMAS**  
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High blood pressure (HBP), is usually linked to the discovery of either functioning or non-functioning adrenal incidentalomas (AI). The 1 mg dexamethasone suppression test (DST), is considered the "gold standard" for detection of subclinical cortisol hypersecretion in these cases. The purpose of this study was to evaluate the relationship between circulating cortisol levels after DST (Fdex) and HBP in patients with non-functioning AI. Thirty-four patients with AI and cortisol values considered normal after DST ( $< 1.8$  ug/dl) were studied. Another 34 subjects without evident adrenal pathology were used as controls (C). Blood pressure (BP) levels were recorded in both groups. HBP was defined as systolic BP  $\geq 140$  and/or diastolic BP  $\geq 90$  mmHg. All subjects received 1 mg oral dexamethasone at 11.00 pm and, at 8 am the following day, blood was drawn for Fdex. Both, patients and C, were divided in 2 groups: subjects with Fdex levels from 0 to 0.9 ug/dl and from  $>0.9$  to 1.8 ug/dl. Fdex was determined by RIA. Statistics were performed using Stata statistical software version 11,  $p < 0.05$  was considered significant. Results: HBP was found in 23 AI (47.8% with Fdex values  $>0.9$  ug/dl) and 16 C (12.5% with Fdex values  $> 0.9$  ug/dl),  $p= 0.141$ . HBP was not associated with Fdex levels  $> 0.9$  ug/dl in AI or C. Interestingly normotensive IA had lower Fdex values,  $p<0.001$ . Conclusions: Fdex was not related to higher BP. The evaluation of a larger number of subjects and the inclusion of other parameters, are needed to comprehensively assess the connection between adrenal function and different comorbidities commonly found in AI.

- 74. 363 PROBIOTIC ADMINISTRATION ON TYPE 1 DIABETES PREVENTION**  
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Type 1 diabetes (T1D) is characterized by impaired insulin secretion. In recent years, the number of T1D patients worldwide has increased significantly, suggesting the involvement of multiple factors in T1D onset. Gut microbiota is the group of microorganisms (commensal, symbiotic and pathogenic) that reside in our gut. In animal models of T1D and in T1D patients, reduced microbial diversity and altered microbial composition have been found. Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host. Certain lactic acid bacterial strains are recognized as beneficial and are widely used in the food industry. In addition, these strains could be used as vehicles for the delivery of products in the gut mucosa. One of this strain is *Lactococcus lactis* NCDO2118. It was used for the expression of cytoplasmatic (*L.lactis* HSP65-Cyt) or secreted (*L.lactis* HSP65-Sec) *Mycobacterium leprae* 65-kDa HSP (Hsp65). Hsp65 protein belongs to the family of HSP60 chaperons, with high homology between different taxa and plays an important role in the regulation of inflammation. Therefore, the aim of this work is to determine the effect of the administration of *L.lactis* NCDO2118, *L.lactis* HSP65-Sec and *L.lactis* HSP65-Cyt in T1D. For this purpose, the bacterial strains were administered in the drinking water of females NOD/ShiJ mice (a model of autoimmune T1D) at 12 weeks of age, prior to T1D onset and were monitored for T1D development. The results showed a non-significant difference in T1D incidence between the different treatments (long-rank Test,  $p=0.2169$ ,  $n=10$  per group). Since probiotic administration can improve gut barrier, gut permeability was assessed and no difference was detected between groups (Kruskal-Wallis Test,  $p=0.55$ ,  $n=10$  per group). These probiotics, at the time of administration were not able to reduce T1D onset. Fur-

ther studies are needed to test different initiation ages or different probiotic strains.

#### 75. 415 INHIBITION OF AUTOPHAGY PREVENTS SPEXIN-INDUCED WHITENING OF ADIPOSE TISSUE

Patricia Castro<sup>1</sup>, Carolina Garraza<sup>1</sup>, Ana Alzamendi<sup>1</sup>, Maria Amanda Rey<sup>1</sup>, Sabrina Gambaro<sup>1</sup>, Andrés Giovambattista<sup>1</sup>  
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Spexin (SpX) is an adipokine that inhibits the browning process in white adipose tissue (WAT). Recently, we showed that SpX favors WAT whitening with a positive modulation of autophagic markers. In this line, the aim of this study was to evaluate if SpX whitening modulation is inhibited using chloroquine (Cq). C57BL/6J male mice were exposed for 7 days at 4°C and then returned to room temperature for 24h. They were divided into four groups: ones treated or not with SpX (ip. 29 µg/kg, SpX-W and Ctr-W (PBS)) and others treated with Cq or both SpX and Cq (ip 60mg/kg, Cq-W and SpXCq-W). Body weight (BW) and caloric intake (CI) were daily recorded. At the end of the protocol, plasma was collected for Triglycerides (TG) measurement; Epididymal AT (EAT) and Inguinal AT (IAT) were dissected and weighted. IAT was also processed for qPCR gene expression (ucp1; thermogenic marker and pink; autophagic marker) and for mitochondrial DNA (mitDNA) analysis. Two-way ANOVA was used to determine variable (Cq and SpX) and interaction (Cq x SpX) contribution. When significant, post-test was applied. No significant differences were observed in CI, but there was a trend towards higher consumption by Cq groups (Cq-W and SpXCq-W). These latter groups showed a significant decrease in BW during whitening process, regardless of SpX treatment ( $P_{Cq} < 0.01$ ). Plasma TG levels and EAT mass were not significantly altered. IAT mass was significantly increased in SpX-W vs. Ctr-W ( $P_{Cq \times SpX} < 0.05$ ). Ucp1 gene expression revealed a significant increase in both Cq group ( $P_{Cq} < 0.05$ ) being higher than Ctr-W and SpX-W. For pink an increase was observed in all groups vs. Ctr-W ( $P_{Cq \times SpX} < 0.05$ ). The mitDNA content was decreased only in SpX-W vs. all the other groups ( $P_{Cq \times SpX} < 0.05$ ). The findings suggest that Cq treatment inhibits the effect of SpX on WAT whitening process. In conclusion, the positive modulation of whitening by SpX appears to be mainly through the activation of the autophagy process.

#### P2 - POSTERS

FECHA Y HORA: 20/11/2024 16:10-17:10

COORDINADORES: NORA SARACO, FERNANDO DOMINICI

#### 76. 003 EFECTOS DE LA EXPOSICIÓN CRÓNICA DEL DISRUPTOR ENDÓCRINO NONILFENOL SOBRE LA REGULACIÓN NEUROENDOCRINA DEL EJE REPRODUCTOR EN RATAS MACHO DE 45 Y 70 DÍAS

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El disruptor endócrino Nonilfenol (NP) utilizado en productos como conservas de alimentos y cosméticos presenta acción estrogénica y antiandrogénica. Hemos demostrado que la exposición a dosis de 100 mg/kg/d (NP100) en ratas macho desde la postlactancia hasta la adultez disminuye testosterona plasmática, aumenta liberación "ex vivo" hipofisaria de FSH y LH y disminuye el epitelio germinal testicular. El objetivo fue estudiar los parámetros neuroendocrinos posibles de ser responsables de estos cambios. Para ello se estudió la liberación "ex vivo" de la hormona liberadora de gonadotropinas (GnRH) y sus principales neurotransmisores reguladores aminoácidos: estimulador (aspartato) e inhibidor (GABA) en ratas macho expuestas a NP 100 mg/kg/d (v.o.) desde el día 21 (postdestete) hasta los días 45 y 70 de edad (n= 10-12). Explantes de hipotálamo anteriomedial extraídos al momento del sacrificio fueron incubados "ex vivo" en medio de Earles en condiciones estables de temperatura y gaseado. Se consideró significativa una  $p < 0.05$ . La exposición provoca a los 45 días de edad un aumento en la lib-

eración de GnRH (Control:  $2.63 \pm 0.40$ , NP100:  $6.19 \pm 1.6$   $p < 0.05$ ), descenso de la liberación de GABA (Control:  $268.86 \pm 56.4$ , NP100:  $70.48 \pm 14.30$   $p < 0.005$ ) sin cambios en la liberación de aspartato. Mientras que a los 70 días de edad se observó aumento de la liberación "ex vivo" de GnRH (Control:  $0.92 \pm 0.14$ , NP100:  $3.68 \pm 0.71$ ,  $p < 0.004$ ), aumento del aspartato (Control:  $639.85 \pm 138.90$ , NP100:  $1087.24 \pm 108.54$   $p < 0.0005$ ) y disminución del GABA (Control:  $205.07 \pm 44.00$ , NP 100:  $66.81 \pm 6.78$   $p < 0.04$ ). El aumento en la liberación de GnRH sería causado a los 45 días por descenso del tono inhibitorio y a los 70 días por aumento del tono estimulador y descenso del tono inhibitorio de aminoácidos reguladores; pudiendo ser estos mecanismos responsables de cambios en la liberación de gonadotropinas descriptos en trabajos anteriores.

#### 77. 235 TRABECULAR BONE AND CALCIUM BALANCE ARE NEGATIVELY ALTERED BY SODIUM INTAKE. GLP-1 AGONISTS AS THERAPEUTIC OPTION

Vanessa Touceda<sup>1,2</sup>, Leonardo Cacciagüé<sup>2,3</sup>, Melina Sosa De Lucca<sup>2</sup>, Ignacio Moglie<sup>1</sup>, Valeria Sánchez<sup>4</sup>, Agustina Vidal<sup>4</sup>, Paola Finocchietto<sup>5</sup>, German E. González<sup>1</sup>, Verónica Miksztoiwicz<sup>1,2</sup>

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Recently, it has been suggested that excessive salt intake could affect bone tissue and Liraglutide (L), a GLP-1 agonist, could be a potential therapeutic option. Aim: to study the impact of consuming a high-salt diet (HSD) and the effect of L treatment on bone histomorphometry and calcium balance in an animal model. Methods: Male C57BL/6 mice (8 weeks old) were divided in Control (C, n=10) fed with standard diet, and HSD (n=17) fed a diet with 8% NaCl for 15 weeks. Then, both groups were subdivided according to subcutaneous administration of L (200µg/kg/day) or vehicle for 5 weeks. Body weight and caloric intake were registered weekly. Blood pressure (BP) was assessed by plethysmography, and 24-hour urine was collected in metabolic cages at the end experience to analyse calcium, phosphate and sodium excretion. Animals were euthanized and serum was obtained to evaluate calcemia, phosphatemia, sodium and alkaline phosphatase activity (ALP), among others. Femurs were removed and weighed. Tibia length was measured to calculate femur/tibia index. Cortical and trabecular bone volume (cBV and tBV, %) were determined in sections stained with haematoxylin-eosin. The study was approved by the Ethic Committee of BIOMED. Results: HSD presented higher BP than C ( $p < 0.01$ ) and it decreased in HSD+L (vs HSD,  $p < 0.01$ ). In HSD a significant decrease in Femur/Tibia index (vs C,  $p < 0.001$ ) and in tBV% (vs C,  $p < 0.001$ ) was observed. tBV% was higher in HSD+L compared to HSD ( $p < 0.05$ ). No differences in cBV% were observed between groups. Serum ALP activity was increased in HSD (vs C,  $p < 0.01$ ), as well as calciuria and natriuria (vs C,  $p < 0.05$  and  $p < 0.01$ , respectively). %tBV was inversely associated with calcium excretion ( $r = -0.67$ ,  $p = 0.022$ ). Conclusion: Chronic salt intake alters femur mass and trabecular bone volume, partially due to an increase in calcium excretion. Liraglutide administration could be considered as a therapeutic option for bone loss associate with salt consumption.

#### 78. 495 INSULIN RESISTANCE IN ADOLESCENTS WITH OVERWEIGHT AND OBESITY IN LA PLATA

Claudia Melillo<sup>1</sup>, Paola Prener<sup>1</sup>, Inés Canullan<sup>1</sup>  
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In Argentine, according to latest National Nutrition and Health Survey, 41% girls, boys and adolescents between 5 and 17 years are overweight or obese. Both entities are associated with risk of developing cardiovascular disease. On the other hand, there is a growing

recognition of the role played by insulin resistance (IR) and inflammation in the pathogenesis of diabetes mellitus type 2. The objective of this work is to evaluate parameters of IR and inflammation in adolescents with different body mass index (BMI). **Materials and Methods:** a cross-sectional descriptive study was carried out on 122 adolescents, of both sexes divided into groups according to BMI, low weight and normal weight (G1; n: 60) and overweight and obesity (G2; n: 62), with ages between 12 and 18 years and similar distribution. Anthropometric and biochemical parameters were evaluated: glycemia, lipid profile, C-reactive protein (CRP) by autoanalyzer and insulin by chemiluminescence immunoassay (Access Beckman), in venous blood and after 12 hours of fasting. HOMA, QUICKI and Triglycerides-Glucose (TyG) indices were calculated. **Results:** No significant differences were observed between insulin levels and IR rates between both sexes. G2 showed a significantly higher mean insulin level than G1 ( $14.20 \pm 7.10$  vs  $5.50 \pm 3.70$  mIU/L). Hyperinsulinemia occurred in 15% of G2 and only one case in group 1 (consensus cutoff:  $\leq 17$   $\mu$ U/ml). The mean IR HOMA, QUICKI and TyG indices were significantly higher in G2 adolescents ( $p < 0.05$ ). TyG parameter reflected the increase in triglycerides in G2, 35% of adolescents had fasting triglycerides above the desirable level. CRP average level was higher in G2 compared to G1 ( $p < 0.05$ ). Only 15% of G2 showed altered CRP. We conclude that the periodic evaluation of IR parameters in overweight and obese adolescents will allow early intervention in order to prevent the development of metabolic, cardiac and vascular alterations.

#### 79. 509 miRNAs AND DOPAMINE D2 RECEPTOR IN PROLACTINOMAS: NEW PERSPECTIVES TO IDENTIFIED BIOMARKERS OF RESPONSE A DRUGS

Evelyn N. Prodan<sup>1,2</sup>, Emilia Maticich<sup>1</sup>, Erika Werner<sup>1</sup>, Agustina Chimento<sup>1,3</sup>, Sofía Perrone<sup>1</sup>, Florencia Pascual<sup>5</sup>, Silvia Berner<sup>4</sup>, Damasia Becú-Villalobos<sup>5</sup>, Carolina Cristina<sup>1,2</sup>.

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Pituitary tumors (PT) represent about 15-20% of intracranial tumors. MicroRNAs (miRNAs) are highly conserved and their expression is dysregulated in cancer. This small RNA can act as oncogenes (oncomiRs) or tumor suppressors genes. miRNA profiling studies have shown that deregulation of miRNA expression may modulate sensitivity to targeted therapies and could be also associated with an increased risk of tumor recurrence. This study aimed to evaluate the expression of *in silico* preselected intratumoral miRNAs and Dopamine D2 Receptor (*D2R*), as measure of response to cabergoline, in human PT obtained from surgery and in *D2R* female knockout mouse (*D2RKO*), a prolactinoma model. The expression of miR-15b-5p and miR-26a-5p was evaluated by Stem-loop RT-PCR and the expression of *D2R* was measured by quantitative real-time PCR (RT-qPCR). The levels of miRNAs were normalized to 5s or SNORD44 and the expression of *D2R* was normalized to *GAPDH* as internal reference gene. The statistical significance of parametric data was tested by ANOVA, while statistical significance of non-parametric data was tested by Kruskal-Wallis test. In PT, we found lower expression levels of miR-15b-5p in prolactinoma samples compared to other tumor histotypes, while miR-26a-5p showed homogeneous expression among tumor subtypes (NF (n=19); GH (n=8); ACTH (n=6); PRL (n=4); \* $p=0,0197$ ) (NF (n=19); GH (n=8); ACTH (n=6); PRL (n=4); ns). *D2R* mRNA expression showed no differences between different tumor histotypes and we did not find any correlation between miRNAs and the *D2R* expression. Interestingly, we found that miR-15b-5p was downregulated in the pituitaries of *D2RKO* mice compared with normal pituitaries (n=6,4; WT and *D2RKO*; \* $p: 0,0095$ ) while miR-26a-5p didn't show significant differ-

ences between genotypes in the prolactinoma model, (n=7,5; WT and *D2RKO*; ns). Our results suggest the potential of miRNAs as resistance and malignancy biomarkers in prolactinomas.

## FARMACOLOGÍA

### P1 - POSTERS

FECHA Y HORA: 19/11/2024 11:00-12:00

COORDINADORES: MARIELA PÉREZ, MARÍA LAURA RUIZ

#### 80. 066 PRESCRIPTION OF NON-INSULIN ANTIDIABETICS DRUGS TO PATIENTS AFFILIATED WITH SOCIAL SECURITY CHACO-CORRIENTES, 2019-2023

Isabel Hartman<sup>1</sup>, María Teresa Rocha<sup>1</sup>, Joaquín Burgos<sup>1</sup>, María Eugenia Horna<sup>1</sup>, Sergio Daniel Morales<sup>1</sup>, Lorena dos Santos<sup>1</sup>.

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**Objective:** To estimate the quality of non-insulin antidiabetic drug (NIATD) prescriptions among outpatients with type 2 diabetes mellitus. **Methods:** We conducted an observational, cross-sectional study involving patients diagnosed with type 2 diabetes mellitus who are beneficiaries of a social security institute in Chaco-Corrientes. The study covered the period from 2019 to 2023. The variables assessed included anthropometric data (age and sex) and prescription quality. We used the Use Potential Level Quality Indicators (UPLI), which measure the proportion of specific active ingredients consumed relative to the total consumption within the anatomical therapeutic category (other NIATDs). An UPLI value of >88% indicates high-quality prescribing. Defined Daily Doses (DDD) were used as the measurement unit, as established by the World Health Organization's Collaborating Center for Drug Statistics Methodology. The analysis focused on institutional pharmacy records for all NIATD dispensations. Data were entered into an Excel spreadsheet and analyzed using Epi-Info-7. Descriptive statistics were employed for data validation. **Results:** A total of 21,828 NIATD dispensations were analyzed for 855 patients. The average age of the patients was 61 years (SD: 10 years), with 58% being male. The percentage of metformin prescriptions relative to other NIATDs was 98.94% in 2019. However, this percentage declined in subsequent years: 83.18% in 2020, 78.53% in 2021, 85.96% in 2022, and 87.02% in 2023. **Conclusion:** The results suggest that metformin remains the preferred and rational choice for most prescribers, aligning with its status as the first-line treatment for type 2 diabetes mellitus due to its favorable benefit/risk/cost ratio. The observed decrease in UPLI over the past four years highlights the need for continued monitoring and opportunities to enhance the quality of NIATD prescribing practices.

#### 81. 100 DEVELOPMENT OF ANTIOXIDANT SILICA-NANOPARTICLES

Francisco Gualdieri<sup>1</sup>, Exequiel Giorgi<sup>1,2</sup>, Gabriela Rocha<sup>1,2</sup>, Ruben Iacono<sup>1</sup>, Martin Desimone<sup>3</sup>, Mauricio De Marzi<sup>1,2</sup>, Liliana N. Guerra<sup>1,2</sup>.

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Silica nanoparticles (SiNPs) are a possible approach to deliver antioxidant molecules into cells. We aim to develop antioxidant SiNPs using vegetable extracts enriched in polyphenols. SiNPs were prepared by Stöber method. We obtained spherical SiNPs, size of  $110 \pm 21$  nm (ANP) and  $376 \pm 67$  nm (BNP). SiNPs were homogeneous population (dynamic light scattering analysis) with negative potential Z. A portion of the SiNPs were positized with APTES. Extracts (EXT) from *Baccharis articulata* (C), *Peumus boldus* (B) and *Citrus sinensis* (O) were obtained from a sample of 30 mg dried leaves/mL heated at 70°C for 14 min. EXT or gallic acid were adsorbed on 8 mg/mL SiNPs by constant agitation at 25°C for 24h. We

determined: antioxidant capacity by inhibition of DPPH radical assay (AC, %), polyphenol concentration by Folin method using gallic acid as standard (Pph, mg/mL). In EXT we obtained AC: 51.0±1.3 (C), 71.3±8.2 (B), 71.6±22.0 (O); Pph: 0.57 (C), 3.19 (B), 0.60 (O). After NPs treatments with the EXT, all of the NPs showed similar adsorption of Pph in C and O treatments independent of size or charge, 28% and 30% respectively (no significant difference,  $p=0.7$ ). These rates are lower than adsorption of 0.4 mg/mL gallic acid on the NPs: 37% (ANP-), 68% (ANP+), 83% (BNP-), 79% (BNP+). In the case of B, both ANP and BNP(+) adsorbed near 50% Pph; BNP(-) adsorbed 59% Pph, this result was significantly higher than 4 mg/ml gallic acid adsorbed in BNP(-) which rendered 44% ( $p<0.05$ ); in the other NPs, Pph adsorption was: 50% (ANP-), 45% (ANP+), 50% (BNP+). NPs had no AC, the highest AC was observed for ANP(+) treated with EXT, with a maximum for B of 72.00±0.01 in concordance with higher adsorption of Pph compared to C and O; O showed the lowest AC in all of the NPs evaluated, with a mean of 30.25±1.38. We concluded that NPs could conserve EXT AC activity when EXT were adsorbed independently of the concentration of Pph adsorbed. However, we observed a higher AC in complex NP(+) with EXT

## 82. 170 USE OF WATCH GROUP ANTIBIOTICS BY THE WORLD HEALTH ORGANIZATION IN INPATIENTS AT A REFERRAL HOSPITAL. CORRIENTES, 2023-2024

Horna María Eugenia<sup>1</sup>, Kairuz María Mileva<sup>1</sup>, Morales Sergio Daniel<sup>1</sup>, Hartman Isabel<sup>1</sup>, Rocha María Teresa<sup>1</sup>, Pomares Damián<sup>1</sup>, dos Santos Antola Lorena<sup>1</sup>.

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To promote rational use of antibiotics (ATB), the World Health Organization (WHO) offers evidence-based guidelines for antibiotic selection, in the AWARe guide. The aim of the study was to characterize the ATB considered of the watch group (WHO) prescribed to hospitalized patients. This is a Drug utilization study, observational, descriptive, cross-sectional, of the prescription-indication type. All adult patients admitted (n=136) to the general ward of a referral hospital who received systemic antibiotics (without prior antibiogram), period December 2023-June 2024. Variables analyzed: age, sex, diagnosis, ATB prescribed, to determine the rationality of the prescription the WHO AWARe guide was used. Unit of analysis: clinical records. The Epi Info program was used for statistical analysis. Mean age: 53 years, SD:18; female sex: 67 (54%). 124 patients (91%) received watch group antibiotics: ceftriaxone 35%, ciprofloxacin 28%, vancomycin 13%, piperacillin-tazobactam 13%, clarithromycin 10%, cefotaxime 0.5% and ceftazidime 0.5%. The diagnoses were: respiratory pathologies (community-acquired, hospital-acquired and aspiration pneumonia) 31%; skin and soft tissues (cellulitis, abscess, erysipelas, necrotizing fasciitis, surgical site infection, skin and soft tissues) 30%; urinary pathologies (acute pyelonephritis, kidney abscess, sepsis secondary to perinephric abscess) 20%; digestive pathologies (spontaneous peritonitis, secondary peritonitis, diverticulitis, cholecystitis) 8%; bacteremia associated with hemodialysis catheter, meningitis, meningoencephalitis, superinfected arterial and venous ulcers 6%; bone pathologies (septic arthritis, osteomyelitis) 5%. Conclusion: following the AWARe guide, most prescriptions were rational, except for the use of ciprofloxacin as monodrug in patients with diabetic foot, kidney abscess and diverticulitis, and ciprofloxacin plus clindamycin in pyelonephritis.

## 83. 172 EVALUATION OF THE SAFETY AND EFFICACY OF IMMUNE SERA IN CHILDREN

Guillermo Keller<sup>1,2</sup>, Guillermo Temprano<sup>1</sup>, Claudio Bone<sup>1</sup>, Adolfo De Roodt<sup>1</sup>, José Christian Dokmetjian<sup>1</sup>.

<sup>1</sup> Instituto Nacional de Producción de Biológicos (INPB, AN-LIS-Malbran). <sup>2</sup> Universidad de Buenos Aires, Facultad de Medicina, Centro de Vigilancia y Seguridad de Medicamentos.

Introduction: Accidents linked to snakebites and arthropod stings are treated based on the use of immune sera (F(ab')<sub>2</sub> equine anti-venoms). There is little data on the safety of their administration in childhood. Aim: to provide safety information in children on the use of antisera for the treatment of accidents with poisonous animals,

over the course of 5 years at the national level. Methods: accidents with venomous animals are mandatory reporting in our country. All cases are uploaded to an integrated national Argentine health information system, along with data linked to the characteristics of the event, health history, treatment performed, evolution, and outcome. The data were analyzed, maintaining the anonymity of the patients and compared with the case list of adverse reactions reported using common data such as date, location, and type of accident. Results: A total of 40,182 reports were found, which were classified by age into Children (10,515, 26%), Adolescents (2,434, 6%), Adults (24,046, 60%), older people (2,706, 7%), and NS (481, 1%). Among pediatrics, the cases were: 730 (7%) spider stings (303 *Loxosceles*, 295 *Latrodectus*, 12 *Phoneutria*, 120 NE); 600 (6%) snakebites (542 *Bothrops*, 20 *Crotalus*, 9 *Micrurus*, 29 NE), 9,062 (86%) scorpion stings, and 123 cases of Lepidopterism (*Lononia*). Antivenom was used in 2133 (20%) cases (*Loxosceles* 96, *Latrodectus* 114, *Phoneutria* 4, *Crotalus* 8, *Bothrops* 331, unspecified snake 3, *Tityus* 1577). The patients who received the antisera did not have comorbidities. The subsequent evolution showed that all patients who received antiserum were discharged, with complete resolution of the poisoning symptoms, without sequelae. Three cases with poor evolution were identified, corresponding to lack of administration of the antiserum (2) or late administration. Conclusions: Antisera show adequate safety profile in children. The greatest risk is linked to lack of efficacy due to delayed administration.

## 84. 478 NR112 GENE, ANTIRETROVIRALS AND PORPHYRIA CUTANEA TARDA ONSET IN HIV INFECTED INDIVIDUALS: BIOINFORMATIC APPROACH

Priscila Pagnotta<sup>1,2</sup>, Johanna Zuccoli<sup>3</sup>, Viviana Melito<sup>1,3</sup>, Victoria Parera<sup>3</sup>, Ana María Buzaleh<sup>3</sup>

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Porphyria Cutanea Tarda (PCT) can be hereditary or acquired (A-PCT) with a high association between A-PCT and HIV infection. We previously observed experimentally that genetic variants of *ABCB1* and *GST* would contribute to its onset. *NR112* encodes PXR that regulates *ABCB1* and *CYP3A4* expression. The aim was to evaluate *in silico* the influence of *NR112* variants in PCT onset in HIV infected individuals in relation to antiretroviral treatments and drug metabolizing and transporting systems. Six SNVs (rs12721613, rs2472677, rs12721607, rs12721608, rs72551372 and rs72551374) and gnomAD, PharmGKB, Gene Expression Omnibus, UniProt, GenBank, PreADMET and SwissADME databases were considered. Free Wilson equations were used to design possible therapeutic alternatives. Population study demonstrated SNVs allele frequency in Controls varied between different geographic regions. A allele of rs72551372 was associated with an increased basal transactivation of *CYP3A4* promoter reporter gene. T allele of rs2472677 was associated with toxicity to Efavirenz therapy. We observed that individuals treated with protease inhibitors (PI) compared to those receiving non-nucleoside reverse transcriptase inhibitors (NNRTI) showed under expression of *ABCB1* (FC=0.83;  $p\text{ adj}<0.05$ ) and differential expression of 17 ABC (65% overexpressed) and 21 CYP (71% overexpressed) (GSE44228). We found derivatives of Thiophene[3,2]pyrimidine that could be a therapeutic alternative for NNRTI and derivatives of (S)-tetrahydrofuran-tertiary amine-acetamide for PI with differential action on P-gp and CYP. We conclude that antiretrovirals, and particularly Efavirenz, alter drug metabolizing and transporting system, and genetic variants of *NR112* could contribute to PCT onset in HIV infected individuals in relation to antiretrovirals therapy due to hepatotoxicity. It is important to continue exploring experimentally variants of *NR112* gene to be applied in personalized medicine.

## 85. 523 DEVELOPMENT AND IN VITRO CHARACTERIZATION OF NANOSTRUCTURED LIPID CARRIERS LOADING WITH CANNABIDIOL AND STIRIPENTOL AS A POTENTIAL THERAPEUTIC STRATEGY FOR DRAVET SYNDROME

Sebastian Scioli-Montoto<sup>1</sup>, Eduardo Martín Lobos<sup>1</sup>, Yamil Cecilia Chain<sup>2</sup>, German Abel Islan<sup>3</sup>, Alan Talevi<sup>1</sup>, María Esperanza Ruiz<sup>1</sup>

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Dravet syndrome is a severe drug-resistance type of epilepsy that can occur in the first year of life in previously healthy children. One of the main problems in the treatment of this syndrome is that drugs acting on sodium channels (phenytoin, carbamazepine, oxcarbazepine, lamotrigine, etc.) cannot be used since they exacerbate seizures in these patients. Recently, only Stiripentol (STP) has emerged as a specific treatment for this syndrome. However, it has a very low and variable bioavailability, and a high plasma protein binding which could lead to interactions. In recent years cannabidiol (CBD) has shown certain effectivity over refractory epilepsy. Nevertheless, CBD has a low water solubility, low bioavailability and a variable pharmacokinetic, needing high doses to achieve a therapeutic effect. A strategy capable of overcoming both drugs problems is their incorporation within nanostructured lipid carriers, capable of crossing different biological barriers and thus allowing a better arrival of these drugs to their site of action, improving their bioavailability and decreasing their adverse effects. The objectives of this work were to develop and characterize, in terms of particle size, Z potential, polydispersity index (Pdl) and percentual entrapment efficiency (%EE), nanostructured lipid carriers containing STP and CBD and to study their release kinetics. Nanoparticles were prepared using homogenization by ultrasonication technique. Particle size, Z potential and Pdl were measured using a Z-sizer, while in vitro release study was carried out by the dialysis bag method. The developed formulation averaged particle sizes, Pdl and Z potential between 174 to 190 nm, 0.184 to 0.250 and -11.63 to -6.60 mV, respectively and also showed a high %EE (over 99%). On the other side, drugs showed a prolonged release for up to 24 hs. Although many more studies are needed, these nanoparticles containing STP and CBD are a promising tool for the treatment of Dravet syndrome.

## P2 - POSTERS

FECHA Y HORA: 20/11/2024 16:10-17:10

**COORDINADORES: SILVINA STELLA MARIS VIL-LANUEVA, FEDERICO MONCZOR, GUERRA LILIANA NOEMÍ**

### 86. 057 INSULIN-LIKE GROWTH FACTOR 1 BINDING PROTEIN 2 (IGF2BP1) UP-REGULATION BY GENISTEIN IN HEPG2/C3A. IMPACT ON CHEMORRESISTANCE AND MOLECULAR MECHANISM

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IGF2BP1 is an oncofetal RNA binding protein expressed in tumors, which is associated to chemoresistance in some types of cancers. Genistein (GNT) is an isoflavone present in soy that has estrogenic activity. Previously, we demonstrated that GNT 10 $\mu$ M increases IGF2BP1 protein expression in HepG2/C3A. Aim: to study the molecular mechanism of IGF2BP1 up-regulation by GNT and its impact on sorafenib (Sfb) cytotoxicity. Methods: HepG2/C3A were incubated with GNT 10 $\mu$ M or vehicle (DMSO) for 48h, mRNA levels were measured by RT-qPCR. Cells were transiently transfected with scramble (SCR; Control group) or siRNA targeting IGF2BP1 (siRNA), and 24h after transfection, treated with GNT 10 $\mu$ M or DMSO for 48h. IGF2BP1 knock-down efficiency and GNT effect were evaluated by western blotting. For IGF2BP1 inhibition assay, cells were incubat-

ed with 5 $\mu$ M of BTYNB, an IGF2BP1 inhibitor, for 72h. Twenty-four h after BTYNB addition, GNT 10 $\mu$ M or DMSO were added to the medium for 48h. IGF2BP1 expression was evaluated by western blotting. Additionally, Sfb cytotoxicity (0-150 $\mu$ M, 16h) was evaluated in HepG2/C3A cells pre-incubated with DMSO (Control), BTYNB 5 $\mu$ M or BTYNB 5 $\mu$ M and GNT 10 $\mu$ M by MTT assay. All results are presented as mean $\pm$ SEM, n=3, \*p<0.05 vs SCR C or Control, #p<0.05 vs SCR GNT. IGF2BP1 mRNA levels remain unchanged after GNT treatment. The induction of IGF2BP1 protein by GNT 10 $\mu$ M was prevented by IGF2BP1-siRNA (36 $\pm$ 11%#). Besides, IGF2BP1 induction by GNT 10 $\mu$ M was prevented by BTYNB. MTT assay showed a significant reduction of Sfb IC50 by BTYNB treatment (44 $\pm$ 4 $\mu$ M\*). However, IGF2BP1 inhibition could not impair the significant increase in IC50 by GNT 10 $\mu$ M (BTYNB 5 $\mu$ M + GNT 10 $\mu$ M group: 103 $\pm$ 16 $\mu$ M\*). These results suggest that IGF2BP1-increased expression by GNT could be due to translational mechanisms. IGF2BP1 is involved in its own induction by GNT. GNT-induced resistance to Sfb occurs independently of IGF2BP1.

### 87. 064 THE BISPHOSPHONATE ALENDRONATE PROTECTS VASCULAR ARCHITECTURE UNDER STRESS CONDITIONS INDUCED BY OBESITY

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Alendronate (ALN) is a bisphosphonate used as first line therapy for bone-related diseases. Indeed, various extrasosseous effects have been reported. We previously demonstrated a favorable vascular action of ALN through the inhibition of cellular events that trigger atherosclerosis disease. The final stage of atheroma formation is vascular calcification (VCa). In turn, as a defense mechanism, vascular system induces neovascularization. Obesity is a risk factor that promotes VCa and impairs angiogenic process, so that, we investigated the effect of ALN on VCa and angiogenesis under stress conditions induced by obesity. To that end, thoracic aorta was isolated from female Wistar rats fed with a high-fat diet (27%) or standard diet (4% fat) for 10 weeks. Primary cultures of endothelial cells (EC) and vascular smooth muscle cells (VSMC) were performed. VSMC treatment with 10  $\mu$ M ALN reduced the expression of osteogenic markers RUNX2 and TNAP (92 and 69% vs. control, respectively, p<0.05) and matrix mineralization (38% vs. control, p<0.05, alizarin staining) induced by osteogenic medium. ALN treatment induced a marked reduction in VCa in aortic explants isolated from obese rats (24% vs. control, p<0.05, Von Kossa staining). In order to evaluate angiogenesis, EC proliferation (MTT assay) and capillary formation (tube formation assay) were studied. EC derived from obese rats showed lower proliferation rate and capillary formation than lean rats (51 and 26% respectively, p<0.05). Treatment with ALN reversed these results: ALN increased cell growth (13% vs. control, p<0.01), and stimulated tube formation (18% vs. control, p<0.05). The pro-angiogenic effect is mediated by VEGF, since the presence of a VEGF receptor antagonist (1  $\mu$ M SU5416), completely suppressed tube formation induced by ALN. In conclusion, ALN could exert a potential beneficial action on events that compromise vascular architecture through the downregulation of VCa and the impairment of arterial remodeling.

### 88. 175 EVALUATION OF THE EFFECT OF NON-SPECIFIC TREATMENTS ON ACCIDENTS WITH VENOMOUS ANIMALS THAT OCCURRED OVER 5 YEARS IN ARGENTINA

Micaela Loñ1, Carlos Sehn1, Ignacio Racedo Mogli1, Valentina Romero1, Juana Farinelli1, Guillermo Keller1,2.

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Introduction: Accidents linked to venomous animals (snakebite, arachnidism, Ionomiasis) are a potentially fatal pathology, which



requires specific treatments (therapeutic serums) with precise indications. The use of non-specific treatments (corticosteroids, analgesics, antibiotics, antiseptics) is often counterproductive and could mask warning symptoms to indicate a specific treatment. Aim: to evaluate the effect of non-specific treatments on the evolution of patients who suffered accidents with venomous animals. Methodology: A database with records of accidents reported in the last 5 years was evaluated. The frequency of fatal outcomes was analyzed versus the presence or absence of non-specific treatments. The risk associated with an unfavorable outcome was calculated. Results: Of a total of 40,182 accidents with venomous animals (3,847 snake-bites, 35,771 spider bites, 564 Ionomiasis), 1,995 patients (4.96%) received non-specific measures. These were associated with a clinically unfavorable outcome and/or death in 105 cases. The risk of poor outcome was quantified [RR (95%CI)] for NSAIDs 2.07 (1.70-2.52), antibiotics 16.94 (13.59-21.13), corticosteroids 13.10 (10.87-15.80), antiseptics 4.90 (3.88-6.21). Conclusions: Symptomatic treatment based on NSAIDs, corticosteroids, antiseptics and/or antibiotics is associated with a significant risk of unfavorable outcome.

**89. 263 IDENTIFICATION AND CHARACTERIZATION OF NOVEL INHIBITORS OF G PROTEIN-COUPLED RECEPTOR KINASE 5 (GRK5)**

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GRK5 is overexpressed in failing hearts causing increased desensitization of beta-adrenergic receptors ( $\beta$ ARs), deficit in cardiac contractility, and failure progression, validating GRK5 as a therapeutic target for heart failure. We have previously identified potential inhibitors of GRK5 through docking-based virtual screening. Accordingly, we chose and purchased 12 compounds to evaluate them in biological activity assays by intracellular real-time cAMP determination. We used HEKT-H187 cells that overexpress the mTurquoise2-EP-AC-cp173VenusVenus fusion protein, a biosensor for cAMP levels. H187 cells co-transfected with GRK5 and  $\beta$ ARs were pre-treated with 10  $\mu$ M isoproterenol (ISO) for 15 min and then stimulated with 10  $\mu$ M ISO, in the presence of the potential GRK5 inhibitors. AUC (area under the curve) values of 10 min residual response were determined. We observed that the positive control Amlexanox (commercial GRK5 inhibitor) at a concentration of 1  $\mu$ M increased AUC, as did our compounds Z38, Z53, Z68, and Z92 at a concentration of 10  $\mu$ M ( $P < 0.05$ ) indicating an increased residual response and reduced receptor's desensitization. Additionally, for Z05, we also observed a sustained cAMP response, indicating a delay in the desensitization process. All compounds were further evaluated in the presence of CMPD101 and KT5720 (inhibitors of GPCR phosphorylation by GRK2 and PKA respectively). In these conditions, the inhibitory properties of the hits were enhanced showing a reversion of GRK5-mediated desensitization. Beyond evidences respect to cardiovascular disease, a literature review highlights the abnormal activity of GRK5 in other chronic degenerative diseases, including rheumatoid arthritis, and neurodegenerative disorders such as Alzheimer's and Parkinson's. In this context, our inhibitors warrant further characterization, as they present themselves as promising tools for the treatment of chronic degenerative diseases in which GRK5 plays a crucial role.

**90. 420 ENDOTHELIN 1 (ET-1) INDUCES ENDOPLASMIC RETICULUM STRESS IN PC-12 CELLS THROUGH ETA AND ETB RECEPTORS**

Fabian A Innamorato Costas<sup>1</sup>, Guadalupe Alvarez<sup>1</sup>, Nicolas Giangreco<sup>1,2</sup>, Ana Paula Courreges<sup>1</sup>, Clarisa Marotte<sup>1</sup>, Liliana G Bianciotti<sup>1,3</sup>, Marcelo S Vatta<sup>1,2</sup>.

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The neuronal norepinephrine (NE) transporter (NET) is responsible for NE clearance from the synaptic cleft and its impairment is associated with diverse several cardiovascular diseases. Increasing evidence supports that the endoplasmic reticulum stress (ERE) is involved in the pathogenesis of cardiovascular disorders. We previously reported that blockade of endothelin receptors (ETA and ETB) with a dual antagonist decreases hemodynamic parameters and ERE markers in the adrenal medulla of salt-dependent hypertensive rats. The aim of the present study was to unveil the underlying mechanisms of these effects in PC12 cells exposed to ET-1 for 48h. The expression of NET, tyrosine hydroxylase phosphorylated forms, Bak, Bax, Bcl2 and ERE markers were determined by western blot, mRNA NET levels by real time PCR, apoptosis and necrosis by flow cytometry and NE uptake by a radiometric method. Results showed that ET-1 increased non glycosylated NET expression as compared with glycosylated NET and decreased NET mRNA levels and NE uptake. ET-1 increased tyrosine hydroxylase activity evaluated by the expression of phosphorylated TH at Ser 19 and 40 sites. The expression of ERE markers and the proapoptotic proteins Bak and Bax were enhanced by ET-1 whereas Bcl-2 was decreased. ET-1 reduced cell viability and increased apoptosis, and necrosis evaluated by flow cytometry. Treatment with specific ETA (BQ610) and ETB (BQ788) antagonists showed that both receptors mediated ET-1 effects. The finding that ET-1 increases non-glycosylated NET suggests that misfolded and non-functional proteins accumulate in the endoplasmic reticulum leading to ERE and apoptosis in PC12 cells. The observed ET-1 effects were mediated by ETA and ETB receptors.

## FISIOLOGÍA

### O1 COMUNICACIONES ORALES

FECHA Y HORA: 19/11/2024 16:00-17:00 H

LUGAR: ANEXO (STREAMING)

COORDINADORES: CLAUDIA CALDIZ

**91. 210 CHRONIC GREEN COFFEE INTAKE RESTORES THE CARDIOVASCULAR AND METABOLIC CHANGES INDUCED BY THE METABOLIC SYNDROME**

María Cristina Paredes Carbajal<sup>1</sup>, Fernando Ortega Paz<sup>1,2</sup>, Omar Chainani Herrera Tolentino<sup>1,3</sup>, Irais Martínez Vázquez<sup>1</sup>, Fernando Escandón Sarmiento<sup>1</sup>, Oscar Alberto López Canales<sup>1</sup>.

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The intake of green coffee (GC) has garnered attention for its potential cardiovascular benefits, primarily attributed to its rich content of bioactive compounds, particularly chlorogenic acids. These compounds are known for their antioxidant properties, which would significantly mitigate cardiovascular risk factors, especially in high-risk individuals such as metabolic syndrome (MS) patients. However, its effects on *in vitro* vasomotor responses have not yet been elucidated. This study aimed to assess the impact of chronic GC intake on the reactivity of the aortic rings of adult male rats with SM. Twenty-four male rats weighing between 100-110 g were randomly assigned into four groups: group A (control), group B (MS), group C (GC control), and group D (MS-GC). Groups A and B received water *ad libitum*, while groups C and D received a green coffee solution (6 g/L) *ad libitum*. Groups A and C were fed a standard diet, while groups B and D were fed a high-fructose-fat diet for eight weeks. Blood Pressure, food, water intake, and weight were measured daily in all groups. At the end of the treatment, blood samples were taken, and biochemical parameters were evaluated. The animals were

then euthanized to remove the thoracic aorta and perform dose-response curves to phenylephrine and carbachol. The results showed that chronic consumption of green coffee tends to lower blood pressure as well as glycemia and triacylglycerides levels compared to the control. The contractile response to phenylephrine is increased in the MS group, and endothelial dysfunction is developed by decreasing the response to carbachol. These deleterious effects are reversed by the chronic consumption of green coffee. In conclusion, chronic GC intake reduces the increase in the main components of MS, such as changes in blood pressure, glucose, and triglycerides, as well as improves vascular function in vitro, reducing contractility and improving vasodilation.

**92. 211 CHRONIC COFFEE INTAKE EXACERBATES FUNCTIONAL CARDIAC DAMAGE CAUSED BY ISCHEMIA-REPERFUSION EVENT IN A MODEL OF METABOLIC SYNDROME**

Omar Chainani Herrera Tolentino<sup>1,2</sup>, Fernando Ortega Paz<sup>1,3</sup>, Irais Martínez Vázquez<sup>1</sup>, Fernando Escandón Sarmiento<sup>1</sup>, María Cristina Paredes Carbajal<sup>1</sup>, Oscar Alberto López Canales<sup>1</sup>.

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Coffee is one of the most consumed aromatic beverages worldwide. It has been determined that its components can vary depending on the type of coffee, caffeinated or decaffeinated, as well as the way it is prepared. In some clinical studies, it has been observed that chronic coffee consumption can prevent the increase in some components of metabolic syndrome (MS), such as glucose and triglycerides; however, there is still controversy about its beneficial effect on cardiovascular function. The aim of this study was to evaluate the effect of chronic consumption of regular and decaffeinated coffee on the biochemical and cardiovascular components of MS. Thirty male Wistar rats fed a high fructose, high fat diet (HFFD) for 8 weeks were divided into three groups: A) HFFD + water (SM), B) HFFD + normal coffee (SM+CR), and C) HFFD + decaffeinated coffee (SM+CD). At the end of treatment, SM components such as blood pressure, glucose, triglycerides, and cholesterol were assessed. After euthanasia, cardiac functionality tests were performed in the Langendorff device before and after a period of ischemia-reperfusion (10–10 minutes). Regular and decaffeinated chronic coffee intake significantly decreased glucose and triglyceride levels compared to the SM control. The cardiac functionality tests demonstrated that after an ischemia-reperfusion event, the cardiac contractility (LVDP and +dPTmax) and cardiac relaxation (LVEDP and -dPTmax) were significantly altered, while the Rate Pressure Product (RPP) declined significantly after the ischemia-reperfusion event in the hearts treated with the two types of coffee compared to the SM group. In conclusion, regular and decaffeinated chronic coffee intake improves the biochemical profile in a model of metabolic syndrome. However, it induces a significantly lower recovery of cardiac inotropic and lusitropic function after an ischemia-reperfusion event with decreased global work of the heart.

**93. 296 UNRAVELING ENERGETIC DYSFUNCTION IN CARDIOMETABOLIC HEART FAILURE: INSIGHTS FROM TRANSCRIPTOMICS AND MITOCHONDRIAL COMPLEXOME PROFILING TO CARDIOMYOCYTE METABOLISM**

Abraham Méndez-Fernández<sup>1</sup>, Luis A. Luévano-Martínez<sup>1</sup>, Alfredo Cabrera-Orefice<sup>2</sup>, Ángel Fernández-Mora<sup>1</sup>, Felipe Salazar-Ramírez<sup>1</sup>, Bianca Nieblas-León<sup>1</sup>, Noemí García<sup>1</sup>, Víctor Treviño<sup>1</sup>, Guillermo Torre-Amione<sup>1</sup>, Gerardo García-Rivas<sup>1</sup>

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Cardiovascular Physiology, Goethe University, Frankfurt am Main, Germany.

**Background.** Heart failure remains a leading cause of death worldwide. Of note, cardiometabolic heart failure with preserved ejection fraction (HFpEF) can be viewed as the heart response to a systemic metabolic disruption, typically secondary to obesity. In the current study, transcriptomic data suggested impairment of HFpEF heart mitochondria, later confirmed by functional assays and proteomic complexome profiling. **Methods.** HFpEF mice were exposed to a high-fat diet and L-NAME during eight weeks. The transcriptome of the heart was determined through next-generation-based RNA-seq. In-gel activity assays were performed following BN-PAGE of solubilized heart mitochondria. Metabolism of ventricular cardiomyocytes was explored via high-resolution respirometry. Mitochondrial complexome profiling and peptide acetylation were assessed via mass-spectrometry. **Results.** HFpEF animals were characterized by visceral adiposity, hypertension, pulmonary congestion and overt diastolic dysfunction. RNA-seq revealed 1535 differentially-expressed genes (DEGs; FC>2, FDR<0.05) in the HFpEF heart. Interestingly, 146 DEGs (142 down-regulated in HFpEF) were contained in the GO-CC “mitochondrion” term, predominantly in respiratory complexes I, IV, and V. Respirometric analysis pointed at a compromised spare respiratory capacity of HFpEF myocytes (CTRL 2.3±0.23, HFpEF 1.6±0.12) along with a 1.5-fold increase of leak respiration. In-gel activity of complex V decreased to 0.88 times the CTRL in solubilized mitochondrial membranes of the HFpEF heart. Mass spectrometry revealed hyper-acetylation mostly affecting complex V subunits. A t-test was used for HFpEF vs. CTRL comparisons. Multiple testing correction was done by the FDR method for differential expression and high-throughput proteomic analyses. **Conclusion.** The present findings suggest impaired mitochondrial bioenergetics in the heart of a mouse model of cardiometabolic HFpEF, probably due to ATP-synthase subunits hyper-acetylation.

**94. 298 SIGNIFICANT ALTERATIONS IN THE EXCITATION-CONTRACTION COUPLING IN VENTRICULAR CARDIOMYOCYTES OF A NOVEL CARDIOMETABOLIC HFPEF MODEL**

Abraham Méndez-Fernández<sup>1</sup>, Leandro Díaz-Zegarza<sup>2</sup>, Ángel Fernández-Mora<sup>1</sup>, Felipe Salazar-Ramírez<sup>1</sup>, Alejandro Aiello<sup>2</sup>, Guillermo Torre-Amione<sup>1</sup>, Gerardo García-Rivas<sup>1</sup>

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**Background.** Cardiometabolic heart failure with preserved ejection fraction (HFpEF) accounts for half of the total cases of heart failure and its prevalence is forecasted to increase in the forthcoming future. However, HFpEF remains understudied, partly due to the only recent development of *bona fide* preclinical models. The current study aimed to characterize the excitation-contraction coupling process in a novel mouse model of HFpEF. **Methods.** HFpEF animals consisted in mice fed with a high-fat diet and exposed to L-NAME for eight weeks. Controls (CTRL) were fed with a standard chow diet. Action potentials and L-type Ca<sup>2+</sup> current of ventricular cardiomyocytes were recorded via patch clamp. Ca<sup>2+</sup> transients and the sarcoplasmic reticulum Ca<sup>2+</sup> content were measured in Fluo-4-loaded myocytes by confocal microscopy imaging. The T-tubule system was studied in micrographs of di-8-ANNEPS-loaded myocytes. **Results.** HFpEF myocytes exhibited a dramatic enlargement of the duration of the action potential (APD<sub>90</sub> was 2.2 times higher than CTRL). Interestingly, the amplitude of Ca<sup>2+</sup> transients was slightly higher, explained by a higher sarcoplasmic reticulum Ca<sup>2+</sup> content with unvarying L-type Ca<sup>2+</sup> current. Of physiological relevance, HFpEF myocytes were characterized by a delayed Ca<sup>2+</sup> reuptake (increased T50), paralleled by a slower re-lengthening rate. Finally, the release of Ca<sup>2+</sup> occurred in a less synchronic manner during HFpEF Ca<sup>2+</sup> transients, despite the T-tubule system regularity and density were both preserved. A t-test was used for HFpEF vs. CTRL

comparisons. **Conclusion.** Ca<sup>2+</sup> dynamics of cardiomyocytes of a preclinical model of HFpEF suggested an impairment of cytosolic Ca<sup>2+</sup> reuptake, which may underlie diastolic dysfunction at the left ventricle level.

## O2 (40 MIN.) COMUNICACIONES ORALES

FECHA Y HORA: 20/11/2024 11:50-12:50 H

LUGAR: ANEXO (STREAMING)

COORDINADORES: ALICIA KLECHA,  
ROMINA HERMANN

### 95. 166 EFFECT OF DIAZOXIDE AND MODERATE-INTENSITY EXERCISE ON MITOCHONDRIA FUNCTION ISOLATED FROM SKELETAL MUSCLE DURING OBESITY

Jessica Ortega Pérez<sup>1</sup>, Koré Montserrat Moreno Calderón<sup>1</sup>, Elizabeth Sánchez Duarte<sup>2</sup>, Alfredo Saavedra Molina<sup>1</sup>, Christian Cortés Rojo<sup>1</sup>, Alain R. Rodríguez Orozco<sup>3</sup>, Rocío Montoya Pérez<sup>1</sup>

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Obesity is a chronic disease characterized by an excessive accumulation of adipose tissue that harms the health of the individual who presents it. It is associated with an imbalance between energy intake and expenditure. Various factors trigger this disease, mainly poor nutrition and a sedentary environment. It is known that during obesity, the skeletal muscle is structurally and metabolically affected due to a decrease in its contraction force, capacity to support fatigue, and cellular damage, which are associated with inadequate mitochondrial function. Therefore, alternative treatments with pharmacological and non-pharmacological importance, such as diazoxide and exercise, have been reported to have positive effects on mitochondrial functionality by improving antioxidant defense, oxidative phosphorylation, regulating the volume of the mitochondrial matrix favoring the membrane potential, the reduction in the generation of reactive species, as well as mitochondrial dynamics, contributing to the reduction of metabolic and oxidative stress. However, the interaction between both treatments is still unknown. Hence, in this present work, the respiration and mitochondrial swelling of mitochondria isolated from the skeletal muscle of male Wistar rats fed a high-calorie diet (8 weeks), administered retroperitoneally with diazoxide (14 days), and exercised at a moderate intensity (8 weeks) were evaluated. **Aims:** Evaluate the effect of diazoxide and moderate-intensity exercise on the function of mitochondria isolated from skeletal muscle during obesity. **Specifics 1.** Determine the effect of diazoxide and moderate-intensity exercise on the respiration of mitochondria isolated from the skeletal muscle of obese rats. **2.** Evaluate the effect of diazoxide and moderate-intensity exercise on the swelling of mitochondria isolated from the skeletal muscle of obese rats. **3. Materials and methods: 1.1 Biological material** The biological material used during this experimental method consists of 9-week-old male Wistar strain rats with an initial weight between 250-300 g, maintained in controlled conditions with food and water ad libitum. The experimental groups were divided into 8 different groups (n=8): control (C), diazoxide (DZX), exercise (EJER), obese (HFD), HFD + DZX, HFD + EJER, EJER + DZX, and HFD + EJER + DZX. Obesity was induced by a high-fat diet composed of 50% Rodent<sup>®</sup> brand commercial food, 25% vegetable butter, and 25% animal butter for eight weeks. Likewise, a moderate-intensity exercise protocol was performed 5 days a week for 8 weeks. Diazoxide was administered retroperitoneally at a pharmacological dose of 35 mg/kg for a period of fourteen days. **3.2 Skeletal muscle removal** At the end of the different experimental treatments, the groups were sacrificed by decapitation, and dissection was carried out to extract the different muscles that make up the lower extremities. After their extraction, they were suspended in Medium 1 for mitochondria ex-

traction. **3.3 Isolation of mitochondria** Mitochondria were isolated from previously extracted skeletal muscle through homogenization and differential centrifugation. The muscle was suspended in medium 1 (100 mM KCl, 50 mM Tris-HCl, 5 mM MgCl<sub>2</sub>, and 1 mM EDTA, pH 7.2) and fragmented with constant cuts. Additionally, 7.5 µL of protease (Trypsin) was added to be homogenized. Once homogenized, it was centrifuged at 600 g for 10 min at 4 °C. The supernatant was recovered and centrifuged at 14,000 g for 10 min at 4 °C. The supernatant was discarded, and the pellet was resuspended in medium 2 (100 mM KCl, 50 mM Tris-HCl, 1 mM MgCl<sub>2</sub> and 0.2 mM EDTA, pH 7.2) and bovine serum albumin (BSA) (1mg/mL) and centrifuged at 7000 g for 10 min at 4 °C. Finally, the final pellet was resuspended with 400 µL of medium 2 for subsequent testing. **Oximetry:** The oxygen consumption rate was determined after the isolation of the mitochondria, determining the basal state (state 4) and the phosphorylation state, which consists of the addition of a substrate, glutamate + malate (10 mM), and ADP (0.45 mM) (state 3), for this, the mitochondria were resuspended in a respiration medium (250 mM sucrose, 10 mM KH<sub>2</sub>PO<sub>4</sub>, 1 mM EGTA, 10 mM Tris, pH 7.4) with 0.3% BSA on a Clark-type electrode coupled to a YSI 5300 biological oxygen monitor with PC interfaces for data acquisition. The respiratory consumption rate (RCR) was obtained after the oxygen consumption of states 3 and 4. **Mitochondrial swelling:** Swelling was determined by adding Calcium to isolated mitochondria, using a spectrophotometric method with a reduction in absorbance at 520 nm. Mitochondria at a concentration of 0.3 mg/ml protein were suspended in a swelling medium (120 mM KCl, 10 mM Tris-HCl, 5 mM K<sub>2</sub>HPO<sub>4</sub>, 20 mM MOPS). Finally, the opening of the permeability pore was induced with CaCl<sub>2</sub> (60 µM) to be measured spectrophotometrically. **4. Results:** The data obtained at the end of the experimental treatments showed how the administration of a high-fat diet reduced oxidative phosphorylation activity by up to 50% in the RCR and a significant increase in swelling of the mitochondrial matrix of the HFD group, compared to the control group. However, pharmacological and non-pharmacological treatments, exercise, and diazoxide increased the activities by 20-25% compared to the control group. On the other hand, the results show that the interaction between both treatments and the progress of obesity can generate changes in mitochondrial function of up to 50% in RCR and a statistically significant reduction in matrix swelling compared to the HFD group. **Conclusions:** The administration of a diet rich in fat leads to the deterioration of mitochondrial functionality, showing high levels of swelling of the mitochondrial matrix, which generates an imbalance in the membrane potential and thus affects oxidative phosphorylation, reflected in the decrease in the respiratory quotients obtained through mitochondrial respiration concerning the control group. However, exercise and diazoxide are treatments that increase the activity of mitochondria isolated from skeletal muscle in pathological conditions, increasing mitochondrial respiration and energy production and allowing statistically significant changes in respiratory ratios. However, the interaction between both treatments shows a better response than their control groups, which is why it is associated with a better response to muscle function.

### 96. 427 ACTIVATION OF ANGIOTENSIN II TYPE 2 RECEPTOR (AT2R) INDUCES AUTOPHAGY IN CILIATED RENAL TUBULAR CELLS

Tomás Rivabella Maknis, María Fernanda Fussi, Alejandro Pedro Pariani, Cristian Favre, Sara María Molinas, María Cecilia Larocca.

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**Introduction:** AT2R activation preserves the integrity of primary cilia on tubular cells during renal ischemia/reperfusion (IR) injury, eliciting a nephroprotective effect. Primary cilia activate autophagy by inhibiting the mammalian target of rapamycin (mTOR) pathway. Cilia-deficient tubular cells are more susceptible to acute kidney injury, partly due to the suppression of nephroprotective autophagy. **Objectives:** To evaluate the effect of AT2R activation on autophagy in renal tubular cells and the putative role of primary cilia in this effect. **Methods:**

MDCK cells were cultured under conditions that promote epithelial polarity. Twenty-four hours before lysis or fixation, cells were preincubated with angiotensin II (AGII, 1 $\mu$ M) or with AT2R-specific agonists, buloxibutid (also known as C21) (Bu, 1 $\mu$ M) or CGP42112A (CG, 0.1 $\mu$ M). Levels of p-mTOR and of the early marker of autophagy activation LC3-II were analyzed by Western blot. Formation of autophagic vacuoles was assessed in cells preincubated with LysoTracker (1:1000) and chloroquine (25 $\mu$ M). Cells were then fixed and stained for acetylated  $\alpha$ -tubulin to identify primary cilia. The area of acidic compartments was analyzed by confocal microscopy. Statistical analysis was performed using t-test or ANOVA for multiple group comparisons. Results are expressed as mean  $\pm$  SE. \* $p$ <0.05 versus control (C). Results: The AT2R agonist CG decreased p-mTOR levels (C: 1.00 $\pm$ 0.06; CG: 0.39 $\pm$ 0.12\*;  $n$ =3). AGII and both AT2R agonists increased LC3-II levels (C: 1.00 $\pm$ 0.10, AGII: 1.36 $\pm$ 0.17\*, CG: 1.41 $\pm$ 0.12\*, Bu: 1.56 $\pm$ 0.22\*;  $n$ =4). Confocal microscopy analysis showed that AT2R activation increased the area of autophagic vesicles in ciliated (Ci) but not in non-ciliated (NCi) MDCK cells (C-Ci: 0.61 $\pm$ 0.13; Bu-Ci: 0.81 $\pm$ 0.07\*; C-NCi: 0.77 $\pm$ 0.09; Bu-NCi: 0.86 $\pm$ 0.10;  $n$ =1). Conclusion: Our results demonstrate that AT2R stimulation promotes autophagy activation in MDCK cells, with the pro-autophagic effect being significant specifically in ciliated cells.

**97. 479 DETECTION OF FLUORESCENT BIOSTRUCTURES IN CERVICAL MUCUS DURING THE WOMAN'S FERTILITY WINDOW: A NOVEL FINDING**

Alejo Chacón<sup>1</sup>, María Belén Peralta<sup>2,3</sup>, Florencia Aguilar<sup>2</sup>, Guillermo Ignacio Valentín Kerz<sup>2</sup>, Sebastián Tschaggeny<sup>4</sup>, Ingrid Paul<sup>2</sup>, Melisa María del Luján Velázquez<sup>2,3</sup>, and Gastón Leonardo Miño<sup>1,5</sup>

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The biophysical composition of cervical mucus undergoes cyclical changes, which are reflected in the way that it crystallizes *in vitro*: as fern-like structures during the fertile (estrogenic) stage and as free crystals in the infertile (progestagenic) stage. We propose that the variations in crystallographic patterns, observed using bright-field microscopy, are correlated with changes in the sample's fluorescence during the female cycle. This study aimed to establish optimal imaging conditions for cervical mucus smears and to explore the possibility of detecting new information associated with the microscopic variations in the sample's autofluorescence signal throughout the woman's fertility window. The number of volunteer women that met the inclusion criteria was ten. The samples of cervical mucus were obtained at different times during the fertile window of the enrolled woman and were extended over a microscope cover slide using the "spread it out" technique. Brightfield and fluorescence microscopic images were acquired using an Olympus IX83 inverted microscope by using TRITC (585/42), DAPI (450/50) and FITC (530/43) filters. Image processing and analysis were carried out using ImageJ software. We observed distinct fluorescence emissions from the mucus, varying based on the structures visualized under different filters. These emissions were particularly pronounced within specific biostructures. Our results indicate that cervical mucus exhibits a specific emission spectrum, suggesting a particular composition and a characteristic arrangement of biostructures that are directly linked to the observed signals. By building on this knowledge, we can develop a more comprehensive understanding of the physical and optical changes in mucus throughout the cycle and explore their potential implications for reproductive health.

**O3 COMUNICACIONES ORALES**

FECHA Y HORA: 20/11/2024 16:10-17:10 H

LUGAR: ANEXO (STREAMING)

COORDINADOR: TOMAS RIVABELLA MAKNIS

**98. 078 GALECTIN-1 AND ITS ROLE IN CELL VOLUME REGULATION: INSIGHTS INTO AQP8 PERMEABILITY**

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Galectin-1 (GAL-1) is a widely expressed  $\beta$ -galactoside-binding protein that is up-regulated in hepatocarcinoma cells. It binds to various glycosylated proteins on the cell surface promoting different effects. Aquaporins (AQPs) are transmembrane glycosylated proteins that transport water through the cell membrane, with AQP8 being an important AQP in hepatocytes. When hepatic cells are incubated in hyposmotic medium (Hypo), they initially swell and then loose water through AQPs, returning to their initial volume. This study aimed to investigate GAL-1's role in cell volume regulation (RVD) in hepatocarcinoma cells and its potential impact on cell water transport through AQP8. We used video microscopy with calcein-AM-loaded HepG2 cells for RVD40 measurements. To examine if GAL-1 modulates AQP8 water permeability, the AQP8 sequence was subcloned into a TTS plasmid, and cRNA was *in vitro* synthesized and injected into *Xenopus laevis* oocytes for water transport measurements. Results showed that cells overexpressing GAL-1 (GAL) increased their volume when incubated in Hypo but remained swollen. After 40 min in Hypo, significant differences were observed in RVD: 66.5 $\pm$ 3.55 (HepG2, control,  $n$ =6) and 30.8 $\pm$ 5.92 (GAL,  $n$ =7) ( $p$ <0.05). In oocytes measurements 5 ng of cRNA yielded good expression, as indicated by measurements of oocyte osmotic water permeability values (Pf=(191.8 $\pm$ 57.30)  $\times 10^{-4}$  cm/s). Oocytes expressing AQP8 incubated in the presence or absence of GAL-1 (400  $\mu$ g/ml) showed no statistical differences in Pf between control conditions and the presence of GAL-1 ((105.6 $\pm$ 28.78 vs 105.8 $\pm$ 29.79)  $\times 10^{-4}$  cm/s,  $n$ =38). We also removed the oocytes vitellin membrane to determine if it was impairing interaction between GAL-1 and AQP8, but again, results showed no significant differences. In conclusion, while GAL-1 affects cell volume regulation in Hypo, our preliminary data suggest that this effect is not due to a direct regulatory action on AQP8 transport function.

**99. 177 STUDY OF THE REWARD-RELATED BEHAVIORS UNDER CALORIE RESTRICTION: ROLE OF AGOUTI-RELATED PROTEIN-EXPRESSING NEURONS AND GROWTH HORMONE SECRETAGOGUE RECEPTOR**

Cassano Daniela Alejandra<sup>1</sup>, Barrile Franco<sup>1</sup>, Reynaldo Mirta<sup>1</sup>, Ferreira Nathalia<sup>2</sup>, Cornejo María Paula<sup>1</sup>, Fideles Silva Higor<sup>2</sup>, Rorato Rodrigo<sup>2</sup>, Schioth Helgi<sup>3</sup>, Perelló Mario<sup>1,2</sup>.

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**Introduction:** Ghrelin is a stomach-derived hormone that acts via growth hormone secretagogue receptor (GHSR). GHSR has ligand dependent and independent actions and is highly expressed in Agouti-related protein (AgRP) expressing neurons located in the hypothalamic arcuate nucleus (ARH). Ghrelin rises during energy deficit condition, and leads to the activation of AgRP neurons. GHSR and AgRP neurons are known to modulate reward-related behaviors but the neurobiological bases enhancing the rewarding value of food under energy deficit remain poorly understood. **Objectives:** We studied the role of AgRP neurons and GHSR in the enhancement of reward-related behaviours in calorie-restricted (CR) mice. **Methods:** Male mice were fed with the 40% of their daily food intake for 5 days and daily exposed to a non-caloric sweetener solution,

saccharine, for 4 hours before each meal. **Results:** We characterized the ghrelin-GHSR system and we found that wildtype mice under CR exhibit an increase in the GHSR mRNA levels in the ARH, an increase in ghrelin plasma levels and an increase in saccharine intake. Using two transgenic mouse model in which GHSR is either not expressed or mutated to reduce GHSR ligand independent activity, we found that GHSR is required for the enhancement of reward-related behavior under CR. Using designer receptor exclusively activated by designer drugs technology we, 1) selectively inhibited AgRP neurons and found a reduction of CR-induced enhancement of saccharine intake, and 2) selectively activated AgRP neurons in *ad libitum* fed mice and found an increase in saccharin intake. **Conclusion:** In conclusion, GHSR expression and activation of AgRP neurons are required for the enhanced saccharine intake during CR.

**100. 397 ALTERATIONS IN THE PLASTICITY OF SYMPATHETIC SYNAPSES ARE PART OF THE DYSAUTONOMIAS ASSOCIATED WITH METABOLIC SYNDROME AND BINGE EATING DISORDER**

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The autonomic nervous system (ANS) participates in and regulates the functioning of the internal environment. Dysautonomias, or disorders of the ANS can be found in stress, hypertension (HT), metabolic syndrome (MS), and binge eating disorder (BED). We have described dysautonomias in stress and HT. MS is a disorder associated with dysfunction in glucose assimilation, insulin resistance, obesity, and cardiovascular diseases. BED is defined as the uncontrolled intake of large amounts of food in a short time. In this study, we examined dysautonomias associated with MS and BED models induced in 8-week-old male Wistar rats. The MS model was generated by administering a 30% sucrose in drinking water for 8 or more weeks. BED was induced by dietary changes through restriction and administration of highly caloric food. We also tested whether exercise mitigates the dysautonomia of BED by including a rotating wheel in the cages. After inducing the models, we studied synaptic transmission and plasticity determined by long-term potentiation (LTP) expression in the sympathetic ganglion. Additionally, we assessed the presence and segregation of the neurotransmitters acetylcholine (ACh) and GABA through detection of VACHT and GAD67, markers of these neurotransmitters. In the MS model, we found a reduction in LTP expression, and changes in the presence and in the colocalization/segregation balance of ACh and GABA, thus, the presence of ACh and its segregation with GABA were reduced. In the BED model, there was a decrease in LTP expression, an increase in both GABA expression, and segregation. These changes in BED were reversed with exercise. These data indicate that dysautonomias associated with MS and BED include alterations in synaptic plasticity and neurotransmitter presence and distribution. Exercise mitigates the changes occurring in BED. These results may enhance the understanding of the pathophysiological changes occurring in these conditions.

**101. 401 GUT MICROBIOTA AND PREBIOTICS: ROLE IN MODULATING THE MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 2 (MRP2) IN AN EXPERIMENTAL MODEL OF DIET-INDUCED OBESITY**

Zecchinati F<sup>1</sup>, Ricardi L.L<sup>1</sup>, Arana M.R<sup>1</sup>, Perdomo V.G.<sup>2,3</sup>, Gomez Burich A.G<sup>1</sup>, Luquita M.G<sup>1</sup>, García F<sup>4</sup>, Blancato V.S<sup>5</sup>, Villanueva S.S.M<sup>1</sup>

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**Introduction.** Mrp2 is an ABC transporter that regulates the absorption of dietary toxicants and drugs orally ingested, thus acting as an essential component of the intestinal biochemical barrier. Recently, we observed that standard diet enriched with 40% kcal (HFD) for 8 weeks, reduced the expression and activity of intestinal Mrp2 in mice. **Objectives.** To evaluate the role of gut dysbiosis and the prebiotic inulin (5 % w/w, 2 weeks) in the modulation of intestinal Mrp2 in an experimental obesity model induced by HFD administration. **Methods.** Proximal jejunum from C57BL/6 mice was removed to study paracellular permeability by non-everted intestinal sacs, and mRNA expression, by real-time PCR. Plasma and stool samples were collected for endotoxin assay and 16S rRNA sequencing, respectively. Statistical analyses were performed using one-way ANOVA followed by the post hoc Tukey-test and results were expressed as % of control (C). **Results.** The amount of the non-permeable macromolecule FD-4 in the mucosal compartment significantly decreased (-48%) in HFD group, while inulin cotreatment restored to C values ( $p<0.05$ ,  $n=4$ ). Plasma of HFD mice showed a positive value in the endotoxin assay. In addition, elevated IL-6 mRNA levels found in HFD group (+335%) were normalized in cotreated group ( $p<0.05$ ,  $n=4$ ). Inulin administration revert downregulation of Mrp2 mRNA expression (-91%) induced by fat diet ( $p<0.05$ ,  $n=4$ ). Increased ratio of *Firmicutes/Bacteroidetes* in HFD group (+141) was normalized after prebiotic administration ( $p<0.05$ ,  $n=3$ ). Moreover, fat diet completely abolished *Akkermansia* genus, while in cotreated animals this bacterial population was recovered. **Conclusion.** We demonstrated that altered intestinal microbiota is a key factor in the Mrp2 regulation under obese conditions. In addition, the use of prebiotics is of particular clinical relevance as it avoids Mrp2-dependent barrier disruption and the consequent drug-toxicological impact.

## GASTROINTESTINAL

### P1 - POSTERS

FECHA Y HORA: 19/11/2024 16:00-17:00

COORDINADORES: BARROSO ISMAEL, BASIGLIO CECILIA, TORIANO ROXANA

**102. 012 ROLE OF ENDOGENOUS BILIRUBIN GENERATION ON HEPATIC REDOX STATUS IN BILE-DUCT LIGATION INJURY**

Pamela L. Martín<sup>1</sup>, Geraldine L. Hillotte<sup>1</sup>, Eleonora Pezzarini<sup>2</sup>, Enrique J. Sánchez Pozzi<sup>1</sup>, Marcelo G. Roma<sup>1</sup>, Cecilia L. Basiglio<sup>1,2</sup>

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2. Área Bioquímica Clínica; Fac. Ciencias Bioquímicas y Farmacéuticas, UNR

**Introduction:** Bilirubin (BR) is an antioxidant biliary pigment derived from heme metabolism initiated by hemoxygenase 1 (HO1). During cholestasis, the buildup of toxic bile acids (BAs) causes mitochondrial damage, leading to increased reactive oxygen species (ROS). **Objectives:** To evaluate the effect of HO1 inhibition on the antioxidant protection afforded by BR in obstructive cholestasis. **Methods:** Male Wistar rats were subjected to 7-day bile-duct ligation (BDL,  $n=10$ ) or sham surgery (Sh,  $n=8$ ). HO1 was inhibited with Zn(II) protoporphyrin IX (PP, 25 mg/Kg b.w., i.p.), 24h before BDL (PP+BDL,  $n=10$ ) or Sh (PP+Sh,  $n=8$ ). **Results:** As media $\pm$ SD, and analyzed by Kruskal-Wallis' test. Plasma BR levels (mg/dl) were higher in BDL than in PP+BDL (8.46 $\pm$ 1.70 vs 3.90 $\pm$ 2,  $p<0.01$ ), confirming HO1 inhibition. The increase in plasma BAs ( $\mu$ mol/L) was lower in PP+BDL compared to BDL (116 $\pm$ 14 vs 67 $\pm$ 25,  $p<0.05$ ), likely due to the lower BR-mediated induction of the sinusoidal BA extrusion pump, Mrp3, as evidenced by its lower mRNA levels (%Sh: 140 $\pm$ 10 vs 191 $\pm$ 50). Total oxidant status ( $\mu$ mol H<sub>2</sub>O<sub>2</sub> Equiv/g prot) increased in the BDL group (13.0 $\pm$ 7.1 vs 5.7 $\pm$ 1.5,  $p<0.05$ ), and this

increase was even higher in the PP+BDL group (15.5±6.0, p<0.05 vs BDL). Lipid peroxidation (nmol MDA/mg prot) was higher in the PP+BDL group compared to that in the BDL group (7.0±0.6 vs 5.4±0.3, p<0.05); intrahepatic protein carbonyl levels paralleled that of lipid peroxidation. Glutathione peroxidase activity decreased in both BDL and PP+BDL, as compared to Sh and PP+Sh (p<0.05), while that of catalase and superoxide dismutase was similar between groups; these enzymatic activities could have returned to normal values 7 days after BDL. Finally, total antioxidant status (μmol Trolox™ Equiv/mg prot) was lower in BDL than in control groups, while PP+BDL showed the lowest values (0.11±0.03 vs 0.08±0.02, p<0.05). **Conclusion:** Inhibiting BR production worsens obstructive cholestasis by weakening liver antioxidant defenses.

### 103. 101 FENOFIBRATE ENHANCES THE ANTICHOLESTATIC ADAPTIVE RESPONSE IN ESTROGEN-INDUCED CHOLESTASIS

Geraldine Hillotte<sup>1</sup>, Maria Valeria Razori<sup>1</sup>, Pamela L. Martin<sup>1</sup>, Anabela C. Medeot<sup>1</sup>, Cecilia L. Basiglio<sup>1</sup>, Marcelo G. Roma<sup>1</sup>  
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Estrogens trigger cholestasis during pregnancy and oral contraceptive use in susceptible women. 17α-ethinylestradiol (EE) is a model estrogen that mimics this condition. In cholestasis, a spontaneous adaptive response occurs to limit hepatocellular accumulation of toxic bile salts (BSs), consisting of inhibition of BS synthesis via cyp7a1 downregulation and delivery of BSs to kidney for alternative urinary excretion, via upregulation of Mrp3 (main basolateral BS export pump) and downregulation of Ntcp (main BS uptake system). The selective PPARα agonist Fenofibrate (FF) is used to treat certain human cholestatic liver diseases, in part due to its ability to modulate these anticholestatic mechanisms. Therefore, in this study, we ascertained whether FF can enhance the spontaneous adaptive response in EE-induced cholestasis. For this purpose, Male Wistar rats were randomly assigned into the following groups: i) Control (C), ii) EE (5 mg/kg/day, i.d., 5 days), iii) FF (200 mg/kg/day, p.o., 7 days), and iv) EE+FF. FF normalized BS hepatic levels, which had been elevated by EE (+52%\*). Both EE and FF independently downregulated cyp7a1 expression at both mRNA (-50%\* and -56%\*) and protein (-57%\* and -58%\*) levels, respectively, and they were further decreased in the EE+FF group (-73%\* and -71%\*, respectively). Both EE and FF increased Mrp3 mRNA levels (+244\* and +265%\*, respectively), and this increase was even higher in the EE+FF group (+391%\*). Ntcp expression was repressed by both EE and FF when administered alone (-58%\* and -59%\*, respectively), and this repression persisted in the EE+FF group (-60%\*). This was associated with urinary (+283%#) BS levels in the EE+FF group compared to the EE group (\*p<0.05 vs. C; #p<0.05 vs. EE). We concluded that FF reinforces the adaptive response in EE-induced cholestasis by further inhibiting BS synthesis and by promoting BS elimination through urine, via both Mrp3 induction and Ntcp downregulation.

### 104. 183 ESTRADIOL 17β-D-GLUCURONIDE-INDUCED NOX2 ACTIVATION IS NOT DIRECTLY DEPENDENT ON CHANGES IN ITS PLASMA MEMBRANE MICRODOMAIN EXPRESSION

Alen A. Litta<sup>1</sup>, Gimena Salas<sup>1</sup>, Julieta Marrone<sup>1</sup>, M. Cecilia Larocca<sup>1</sup>, Fernando A. Crocenzi<sup>1</sup>.

<sup>1</sup> Instituto de Fisiología Experimental (IFISE)- CONICET, Rosario, Santa Fe, Argentina

The NADPH oxidase 2 (NOX2) complex possess two membrane subunits, NOX2 and p22phox. We found that estradiol 17β-D-glucuronide (E17G) elevates intracellular ROS, which participate in the internalization of canalicular transporters such as Mrp2, in a completely NOX2-dependent manner. In non-hepatic cells, NOX isoforms are mainly inactive in *raft* microdomains, being activated by maneuvers leading to its mobilization to *non-raft* microdomains. We demonstrated that E17G-induced endocytosis of Mrp2 and Bsep depends on their mobilization from *raft* to *non-raft* microdomains. Our aim is to evaluate whether E17G induces a similar redistribution of NOX2 from *raft* to *non-raft* microdomains leading to its further activation. Isolated hepatocytes were obtained from Wistar rat livers

by collagenase perfusion. Then, 18-h primary cultured hepatocytes (CPH) were incubated for 15 min with E17G (200 μM) or its vehicle (DMSO; C). Then, highly purified plasma membranes were treated with Triton X-100 buffer to obtain detergent-soluble (*non-raft*) and detergent-insoluble (*raft*) fractions; the proportion of NOX2 expressed in both microdomains was determined by Western Blot. To evaluate the effect of *raft* disruption in NOX-dependent ROS production, CPH were treated with methyl-β-cyclodextrin (MβCD, 10 mM, 30 min) to deplete membrane cholesterol, or its vehicle (DMSO). Then, fluorescence of the redox probe DCF was measured to assess ROS as a surrogate parameter of NOX activation. E17G increased the percentage expression of NOX2 in the *non-raft* fraction (45±11 C vs 66±9 E17G, mean±SEM, n=3, p<0.05). Disruption of *rafts* with MβCD did not modify NOX activity (U/mg prot: 4.7±1.0 C vs 4.8±1.8 E17G, mean±SEM, n=4). Thus, although E17G induces an enrichment of NOX2 in *non-raft* fractions, disruption of *raft* microdomains with MβCD does not increase NOX2 activity. This suggests that E17G-induced mobilization of NOX2 from *raft* to *non-raft* microdomains is not necessary or sufficient to induce its activation.

### 105. 227 INFLAMMATORY PROFILE IN ESTROGEN-INDUCED CHOLESTASIS

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Estrogen-induced cholestasis is a common condition affecting susceptible women. Ethinylestradiol (EE) treatment to rodents, a model of this pathology, induces an increase in inflammatory cytokines which may contribute to cholestasis. Previously, we established that Dexamethasone (Dex) mitigated estrogen-induced cholestasis and reduced the estrogen-mediated elevation of TNF and IL-1β mRNA in hepatocytes and in non-parenchymal cells (NPCs), which include Kupffer cells, Ito cells and endothelial cells. Kupffer cells are the main responsible for cytokine synthesis within the liver. These cells can exhibit either a pro-inflammatory (M1) or an anti-inflammatory/regulatory (M2) profile, with iNOS and Arginase serving as markers. Another inflammation biomarker is COX2, an enzyme that can amplify the inflammatory response through prostaglandins synthesis. Aim: to assess the inflammatory profile in ethinylestradiol cholestasis. Methods: Male C57BL/6 mice were randomly assigned to: (a) Control group (vehicle) or (b) EE group (10 mg/kg/day, s.c., 5 days). Hepatocytes and NPCs were obtained by collagenase perfusion and stored at -70°C for subsequent RT-qPCR and Western Blot analyses. Results (Mean±SEM, n=3. \* p<0.05 vs. Control): In NPCs, mRNA expression of iNOS, a marker of the pro-inflammatory M1 phenotype, increased in EE-treated mice (Control: 1.0±0.4; EE: 2.2±0.3\*), while mRNA expression of Arginase-I, associated with the anti-inflammatory M2 phenotype, decreased (Control: 1.0±0.2; EE: 0.7±0.1\*). Protein expression of COX2 was increased in EE group compared to the control group, in hepatocytes (Control: 100±17; EE: 727±109\*) and tend to increase in NPCs (Control: 100±5; EE: 144±60). These findings suggest that EE treatment induces a hepatic inflammatory condition characterized by a predominance of inflammatory M1 macrophages, increased synthesis of TNF and IL-1β, and upregulation of COX2.

### 106. 233 SPECIFIC MITOCHONDRIAL ANTIOXIDANT PREVENTS AQUAPORIN 8-INDUCED BILIARY CHOLESTEROL EXCRETION IN MICE

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In the hepatocyte, aquaporin 8 (AQP8) present in the inner mitochondrial membrane (mtAQP8) can function as a porixiporin, facilitating the diffusive efflux of hydrogen peroxide. Diverse experimental evidence suggests a key role of hydrogen peroxide in hepatic cholesterol metabolism through the induction of sterol regulatory

element-binding protein (SREBPs). Cholesterol is mainly excreted into bile via the canalicular cholesterol transporter ABCG5/8, which are SREBP2 target genes. Our previous results in mice expressing human AQP8 (hAQP8) in mitochondria show increased expression of SREBP2 and ABCG5, and also a marked increase in the biliary excretion of cholesterol. **Aim:** to study whether the mechanisms of action of hAQP8 on biliary cholesterol excretion in mice involve mitochondrial hydrogen peroxide transport. **Methods:** Male C57BL/6 mice were transduced with AdhAQP8 adenovector by retrograde intrabiliary infusion to induce hepatic mitochondrial expression of hAQP8. At 24 and 48 h, the specific mitochondria-targeted antioxidant MitoTempo (2.5 mg/kg body weight i.p.) was administered. After 72 h, the gallbladder was ligated, the common bile duct was cannulated and bile was collected. **Results:** MitoTempo prevented increased cholesterol excretion in hAQP8-expressing mice by around 46% ( $p=0.008$ ,  $n=4$ ) without affecting mitochondrial hAQP8 protein expression. MitoTempo also reduced mtAQP8-induced total hepatic SREBP2 expression by about 45% ( $p=0.05$ ,  $n=3$ ), and the expression of ABCG5, by around 70% ( $p=0.045$ ). **Conclusion:** These observations suggest that AQP8, via mitochondria-derived H<sub>2</sub>O<sub>2</sub>, plays a role in SREBP2-controlled hepatocyte cholesterol biliary excretion by modulating the canalicular expression of ABCG5/8.

## P2 - POSTERS

FECHA Y HORA: 20/11/2024 11:30-12:30

**COORDINADORES: CAGNONI ALEJANDRO JAVIER, ATORRASAGASTI FERNANDEZ MARIA CATALINA, DONOSO ADRIANA SUSANA**

### 107. 015 INFLUENCE OF AGE ON THE PROGRESSION OF MASLD: SEEKING HIGH-VALUE PREDICTIVE BIOMARKERS OF DISEASE SUSCEPTIBILITY AND PROGRESSION

Lucía Lameroli Mauriz<sup>1</sup>, Andrea Scelza<sup>2</sup>, Miranda Orellano<sup>2</sup>, Juan Bayo<sup>1</sup>, Esteban Fiore<sup>1</sup>, María Jose Cantero<sup>1</sup>, Barbara Bueloni<sup>1</sup>, Mailin Casadei<sup>1</sup>, Dolores Martin<sup>1</sup>, Guillermo Mazzolini<sup>1</sup>; Luz Andreone<sup>2</sup>; Catalina Atorrasagasti<sup>1</sup>.

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Metabolic dysfunction-associated steatotic liver disease (MASLD) ranges from steatosis, steatohepatitis, fibrosis, and can progress to cirrhosis and liver cancer. It is more prevalent in older individuals, who frequently experience severe symptoms. There are no reliable markers to diagnose or predict disease progression. This study aims to examine the impact of ageing on MASLD to establish criteria for stratification and identify predictive biomarkers of progression. Male C75BL/6N mice were divided into 4 groups: 2-month-old mice (young, Y) fed with control diet (Y-CD), or high fat western diet (Y-WD) and 10-month-old mice (middle-aged, MA) fed with CD (MA-CD) or WD (MA-WD). For 20 weeks, weight and blood glucose levels were tracked. Then, liver tissue was collected for histological staining, gene expression analysis by qPCR and RNAseq. Differentially expressed genes and biological processes were identified via gene ontology (GO) analysis in RStudio. Mice on the WD gained more weight than those on the CD, with a greater effect in young mice. Liver mass relative to total body mass increased significantly only in MA-WD compared to MA-CD. MA mice became insulin resistant regardless of diet, while WD also caused insulin resistance in young mice. Serum ALT, AST, and LDH levels were increased in MA-WD mice. Disease scoring showed that WD had a greater effect on MA mice. A different distribution pattern of hepatic lipid droplets was observed between Y-WD and MA-WD. Ageing promoted fibrosis, worsened by WD. GO analysis revealed that ageing modulated hepatic circadian rhythm processes. Interestingly, WD modulated different biological processes in Y and MA mice. In MA-WD mice, downregulated genes were linked to energy metabolism, while upregulated genes were associated with inflammation. These findings

enhance our understanding of MASLD progression and suggest potential biomarkers and therapeutic targets for better future patient stratification and management.

### 108. 109 THE IMPACT OF THE ABSENCE OF KIR6.2/K-ATP CHANNEL ON LIVER REGENERATION AFTER PARTIAL HEPATECTOMY

Lautaro Daniel Hesse<sup>1</sup>, Marina Cecilia Vera<sup>2,3</sup>, Carla Gabriela Comanzo<sup>1,2,3,4</sup>, Lucía Oviedo Bustos<sup>1</sup>, Nicolás Francisco Palma<sup>1,4</sup>, Anabela Cecilia Ferretti<sup>1,4</sup>, María Paula Ceballos<sup>1</sup>, Ariel Darío Quiroga<sup>1,4</sup>, María de Luján Álvarez<sup>1,4</sup>  
1 Instituto de Fisiología Experimental (IFISE), Facultad de Ciencias Bioquímicas y Farmacéuticas (FCByF), CONICET, Universidad Nacional de Rosario (UNR). 2 Área Bioterio Central, FCByF, UNR. 3 Universidad Abierta Interamericana. 4 Área Morfología, FCByF, UNR.

Liver regeneration (LR) after two-thirds partial hepatectomy (PH) in rodents has emerged as a useful model to study the unique ability of the liver to regenerate. Numerous signals contribute to normal LR, with hepatic ATP reserves playing a crucial role. Liver expressed-Kir6.2/K-ATP channels are essential in cellular responses to protect tissue under stress and injury. Previous results from our group showed that Kir6.2 knockout mice (Kir<sup>-/-</sup>) exhibit lower liver/body weight ratio than control mice after PH at all stages of LR, indicating that Kir6.2 could play a role in the process. **Aim:** to analyze the role of Kir6.2 in LR after PH, focusing on the balance proliferation/apoptosis. **Methods and results:** Male C57/B6 wild-type (WT) and Kir<sup>-/-</sup> mice were subjected to PH. Studies were performed at 48 and 96 h (proliferation stage). H&E staining of liver tissue showed that the absence of Kir6.2 did not affect the liver histoarchitecture. Hepatic levels of proliferation markers PCNA and Cyclin D1 (by western blot, WB) were significantly decreased in Kir<sup>-/-</sup> (48 h: PCNA: -62%; Cyclin D1: -57%; 96h: PCNA: -45%; Cyclin D1: -47%). Apoptotic index (AI, determined by WB as Bax/Bcl-xl ratio) was increased in Kir<sup>-/-</sup> (48h: AI: WT: 3.3±0.6; Kir<sup>-/-</sup>: 5.3±0.7\*; 96h: AI: WT: 1.1±0.1; Kir<sup>-/-</sup>: 1.4±0.3). \* $p<0.05$  vs. WT. In conclusion, our data indicate that the Kir6.2 protein seems to be involved in LR after PH in mice and its absence affects the normal course of the process, due to less proliferation and higher apoptotic cell death. A deeper understanding of the mechanisms governing LR is essential for preventing liver function loss and developing new therapeutic strategies.

### 109. 314 GALECTIN-1 CONTRIBUTION TO STEMNESS IN HEPATOCELLULAR CARCINOMA CELLS

Lorena R García, Luciana Sarrias, María T Elola, María V Espelt, Johanna G Miquet, María F Troncoso  
Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Química y Físicoquímica Biológicas (IQIFIB), Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina.

Cancer stem cells (CSCs) are a specialized subset within a diverse population of cancer cells, known for their enhanced pro-malignant properties. In hepatocellular carcinoma (HCC), CSCs are linked to tumor recurrence, metastasis, resistance to radio- and chemotherapy, and poor prognosis. Our previous work showed that galectin-1 (GAL1) promotes HCC progression, metastasis and chemoresistance, and identified CD13, a liver CSC (LCSC) marker, as a novel GAL1 receptor on hepatic sinusoidal endothelial and HCC cells. This study aimed to investigate whether GAL1 contributes to CSC properties (stemness) in HCC. We performed an *in silico* analysis using RNA-seq data from 365 liver cancer patients in The Cancer Genome Atlas (TCGA) to examine the correlation between GAL1 expression and LCSC markers. CSC subpopulations were isolated from human HCC HepG2 cells based on their ability to form non-adherent spheres. Wild type (WT), GAL1-overexpressing (HepG2-GAL1) and mock (M) cells were cultured in anti-adherent wells in serum-free DMEM with B27 and epidermal growth factor. Under these growth differentiation-inhibiting conditions, spheres formed after 11 days were photographed, quantified, and measured. GAL1 protein levels in non-adherent spheres (NAS) and parental cells

(PC) were assessed by Western blotting.  $P < 0.05$  was considered significant. High GAL1 mRNA expression in HCC tumors correlated with LCSC markers CD90 (Spearman: 0.52,  $P = 2.63 \times 10^{-26}$ ; Pearson: 0.41,  $P = 4.42 \times 10^{-16}$ ) and CD44 (Spearman: 0.28,  $P = 7.11 \times 10^{-8}$ ; Pearson: 0.29,  $P = 2.62 \times 10^{-8}$ ), indicating considerable and moderate correlations, respectively (UALCAN and cBioPortal platforms). No correlation was found with CD133, EpCAM, or CD13. GAL1 protein levels showed a tendency to increase in WT-derived NAS respect to PC ( $n = 2$ ). HepG2-GAL1-derived NAS increased both in number (3-fold) and area (188±39%) with respect to M-derived NAS (Student's *t*-test). In conclusion, GAL1 overexpression may enhance stemness in HCC cells.

#### 110. 321 PROTECTIVE EFFECT OF QUERCETIN (Q) ON BILE FLOW AND MRP2 EXPRESSION IN A FRUCTOSE-INDUCED RAT MODEL OF METABOLIC SYNDROME (MS)

Igor Costa de Lima<sup>1</sup>, Geraldine L. Hillotte<sup>1</sup>, Agustina Fazzi<sup>1</sup>, Diego Taborda<sup>1</sup>, Enrique J. Sánchez Pozzi<sup>1</sup>, Ismael R. Barosso<sup>1</sup>

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**Introduction:** Metabolic syndrome (MS) is a metabolic disorder characterized by obesity, hypertension, dyslipidemia, and insulin resistance, resulting in elevated oxidative stress and inflammation. Bile secretion, a liver-produced regulated process, is affected by inflammatory cytokines and reactive oxygen species (ROS). Previous experiments demonstrated decreased bile flow and impaired expression and activity of Mrp2, a canalicular organic anion transporter, in rats with a MS model. **Aim:** To assess the protective effect of Q, a flavonoid with antioxidant and anti-inflammatory properties, in a rat model of MS induced by fructose. **Methodology:** Adult male Wistar rats (220-250 g, 70 days old) were divided into four groups: control (C;  $n = 10$ ), fructose (F;  $n = 10$ ), fructose+Q group (Q+F;  $n = 4$ ) and Q group (Q;  $n = 3$ ). The F group received 10% w/v fructose in drinking water for 8 weeks, while the Q+F group additionally received quercetin (15 mg/kg, ip, 3 times/week) for the last 15 days of fructose treatment. The Q group received quercetin only. A surgical procedure catheterized the common bile duct and femoral vein with PE10 and PE50 polyethylene tubing, respectively. Bromosulphophthalein (BSP) was injected via femoral to evaluate Mrp2 activity. Bile was collected for 55 minutes, and bile flow assessed gravimetrically. Mrp2 expression was analyzed by western blot from liver homogenates using primary antibodies against Mrp2. **Results** (% of Control group): Bile flow: C: (100±3); F: (71±3)\*; Q: (102±2); F+Q: (87±2)\*. Mrp2 expression was, C: 100±14; F: 77±4\*; Q: 77±18; F+Q: 96±8, respectively. Mrp2 transport activity: C: 100±14, F: 47±20, Q: 155±42, F+Q: 80±22 \* Statistically different from C, ( $p < 0.05$ ); \* Statistically different from C and F, ( $p < 0.05$ ). **Conclusion:** Q treatment ameliorates the decrease in bile flow induced by fructose in the rat MS model, associated with an increased MRP2 expression. Its anti-inflammatory and antioxidant properties could be relevant in these effects.

#### 111. 400 HUMAN INTESTINAL SPHEROIDS AS A MODEL SYSTEM FOR STUDYING ABC TRANSPORTERS

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The human Caco-2 monolayer culture is a valuable *in vitro* model for studying intestinal barrier function, with a particular focus on the role of ATP-binding cassette (ABC) proteins in mediating drug transport. However, 3D *in vitro* models, which better replicate the complex *in vivo* environment due to their ability to capture tissue-specific architecture and cell-cell interactions, have become increasingly important. Even so, these models still require characterization. **Objectives:** To generate a Caco-2 spheroid model using the Liquid Overlay technique and evaluate the ABCC2 and ABCB1 protein

expression. **Methods:** Caco-2 spheroids were formed by seeding 10.000 cells/well onto 96-well plates coated with 1.5% agarose. After 4 and 7 days of culture, spheroid diameter was measured, and cell viability was assessed by MTT assay and LDH activity. ABCC2 and ABCB1 protein expression was analyzed using Western blotting (WB). Statistical analyses were performed using the *t*-test. **Results:** Caco-2 spheroids exhibited reproducible diameter of 866±34  $\mu$ m after 4 days of culture and 1041±65  $\mu$ m after 7 days, with a statistically significant difference between the two-time points ( $p < 0.05$ ,  $n = 10$ ). Color responses of MTT formazan formed in spheroids on day 7 showed a significant increase (+35%) compared to day 4 ( $p < 0.05$ ,  $N = 10$ ). No significant differences in LDH activity in supernatants were detected between groups. WB analysis revealed a significant rise in MRP2 protein expression at day 7 (+20%) compared to day 4 ( $p < 0.05$ ,  $n = 3$ ). Similarly, ABCB1 protein expression increased throughout the culture period ( $n = 2$ ). **Conclusion:** We generated viable Caco-2 spheroids using the low-cost Liquid Overlay technique. ABC transporter expression began early and consistently within 4 days of culture and increased by day 7, as cell differentiation progressed. This reproducible *in vitro* model provides a valuable system for studying intestinal ABC protein regulation as well as assessing drug substrate transport

#### 112. 514 STABLE ISOTOPES FOR THE DIAGNOSIS OF *Helicobacter pylori* INFECTION

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The Laboratory of Stable Isotopes Applied to Biology and Medicine has been operating at the Department of Physics, School of Pharmacy and Biochemistry, UBA, since 2002. In this lab, we conduct mass spectrometry measurements of the breath Carbon Isotope Ratio (CIR) for diagnosing *H. pylori* infection using the <sup>13</sup>C-Urea Breath Test (<sup>13</sup>C-UBT). This methodology has been used by our group in various research projects and technology transfer services. The objective of this work was to evaluate the sensitivity and specificity of the <sup>13</sup>C-UBT in relation to histopathological diagnosis of *H. pylori*. We included results from adult patients (18-70y) who participated in research protocols conducted by our laboratory between 2011 and 2019. These patients were referred to the Esophagus-Stomach Section of the Hospital de Gastroenterología "Dr. Carlos Bonorino Udaondo" for upper gastrointestinal endoscopy. The presence of *H. pylori* was assessed through histopathology of gastric biopsies from the antrum and body, and by the <sup>13</sup>C-UBT, measuring the CIR with a Mass Spectrometer coupled with a Gas Chromatographer (Finnigan-MAT GmbH, ThermoQuest Corp., Bremen, Germany), determining the Delta Over Baseline. A total of 154 patients were included for the evaluation of <sup>13</sup>C-UBT versus histopathology for the initial diagnosis of *H. pylori* infection, achieving a sensitivity of 93.02% and specificity of 95.59%. Additionally, the performance of <sup>13</sup>C-UBT for post-treatment control of the infection was assessed in 46 patients, yielding a sensitivity of 94.44% and a specificity of 100%. The <sup>13</sup>C-UBT is a highly sensitive and specific method for both initial diagnosis and post-treatment control of *H. pylori* infection. The measurement of CIR using a more modern mass spectrometer, which our laboratory currently has, could further improve these parameters.

## GENÉTICA

### O1 COMUNICACIONES ORALES

FECHA Y HORA: 19/11/2024 16:00-17:00 H

LUGAR: SALA DE CÁMARA

COORDINADORES: LILIANA ROSSETTI, ANA MARÍA BUZALEH, CARINA RIVOLTA



**113. 154 GENETIC CHARACTERIZATION OF 21-HYDROXYLASE DEFICIENCY COHORT BY LONG READ SEQUENCING**

Aldana Claps<sup>1</sup>, Emilio Kolomenski<sup>2</sup>, Franco Fernández<sup>3</sup>, Natalia Macchiaroli<sup>2</sup>, Marina L Ingravidi<sup>2</sup>, Marisol Delea<sup>4</sup>, Cecilia Fernández<sup>5</sup>, Tania Castro<sup>1</sup>, Julieta Laiseca<sup>1</sup>, Laura Kamenetzky<sup>2</sup>, Melisa Taboas<sup>1</sup>, Liliana Dain<sup>1,2</sup>

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We used 3<sup>rd</sup> generation sequencing Oxford Nanopore Technology (ONT) Long Reads (LR) to analyze samples from a well characterized 21-hydroxylase deficiency cohort. Previously, we analyzed 12 samples by ONT LR to validate and compare with our current diagnostic algorithm. We now extend our study by analyzing a total of 34 samples, 28 already genotyped and 6 new samples studied for the first time double blind using ONT LR and our current algorithm in parallel. We amplified the centromeric and the telomeric RCCX (*STK19 (RP)-C4-CYP21-TNX*) modules in 2 different amplicons of 8,5 kb -amplicon A & B- spanning the active *CYP21A2-TNXB* genes and *CYP21A1P-TNXA* pseudogenes, respectively. Samples were sequenced using MinION FlowCell R9.4.1 or PromethION FlowCell R10.4.1, following ONT recommendations. We developed custom Python scripts based on the recommended ONT pipelines for the analysis. The read depth was >400X for all the amplicons and the number of genomic variants (GVs) ranged from 17-106 in amplicon A and 3-66 for B in MinION sequencing and 12-80 in amplicon A and 15-55 for B in PromethION sequencing. GV's founded in *CYP21A2* were concordant for both approaches. However, ONT LR allowed us to complete GV's that are not detected in some samples using other methods. For example, 5/20 alleles macroconverted or chimeric genes lacked variants commonly expected to be involved in this type of rearrangements. Among them, a *TNXA/TNXB* chimera lacked the p.G111fs GV. In addition, LR sequencing allowed to determine with a higher resolution the regions converted in the chimeras and macroconversions and the *cis/trans* locations of the GV's. We also found a novel p.H403fs\*5 in one simple virilizing patient, 4 novel GV's in *CYP21A1P* and 10 novel GV's in *TNXA* not previously reported in population databases. Our study highlights the importance of ONT LR sequencing as a better approach to diagnosis, and the genomic characterization of the RCCX modules in our population.

**114. 241 COEXISTENCE OF TWO RARE GENETIC DISEASES IN PATIENTS WITH COMPLEX PHENOTYPES REVEALED BY NEXT GENERATION SEQUENCING**

María Esnaola Azcoiti<sup>1,2</sup>, Paula Scaglia<sup>1,2</sup>, Agustín Izquierdo<sup>1,2</sup>, Romina Armando<sup>3</sup>, Ana Keselman<sup>2</sup>, Nora Sanguineti<sup>2</sup>, María Gabriela Ropelato<sup>2</sup>.

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Next-generation sequencing (NGS) enables rapid screening of multiple candidate genes, useful for the precise molecular diagnosis of rare diseases (RD), especially when clinical diagnosis is blurred. As NGS became more widely used, the possibility of detecting the coexistence of two independent genetic diseases on the same patient has increased. Aim: to bring attention to the possibility of finding 2 different RD in the same patient. NGS was performed in a NextSeq500 (Illumina) with TruSightOne kit (Illumina) or Inherit-

ed Diseases Panel (Agilent). Variants were classified according to ACMG/AMP criteria. Patient 1 (female, 7 mo) presented with overgrowth (height +3,3 SD), advanced bone age and macrocephaly, facial dysmorphism, broad thumbs, hypermetropia, developmental delay, and hypotonia. The presumptive diagnosis was Weaver Syndrome (WS). NGS results showed two heterozygous *de novo* variants, one likely pathogenic (LP) in *EZH2* related to WS, and one pathogenic (P) in *PTEN*, associated with PTEN Hamartoma Tumor Syndrome (PHTS). Patient 2 (male, 7y) presented at birth with facial dysmorphism, hypotonia, neurodevelopmental delay, cryptorchidism, and small hands. At 4y episodic abdominal pain, vomiting, diarrhea, arthralgia, and arthritis. Laboratory tests showed high PCR levels and leukocytosis with neutrophilia. KBG Syndrome in combination with an autoinflammatory syndrome were suspected. NGS revealed two heterozygous *de novo* P variants: one in *ANKRD11* explains KBG syndrome while a variant in *TNFRSF1A* is associated with TNF Receptor-Associated Periodic Syndrome (TRAPS). These cases illustrate the importance of bearing in mind the possibility of the co-concurrence of two different RD in a single patient, and the utility of NGS large panels or exome in these scenarios. Deep phenotyping is critical for the suspicion of these situations, the selection of the most appropriate diagnostic technique, and the successful identification of disease cause.

**115. 312 RASOPATHIES: MOLECULAR DIAGNOSIS BY NEXT GENERATION SEQUENCING USING A CUSTOM GENE PANEL**

Paula Alejandra Scaglia<sup>1,2</sup>, Ana Keselman<sup>2</sup>, María Esnaola Azcoiti<sup>1,2</sup>, Agustín Izquierdo<sup>1,2</sup>, Romina Armando<sup>3</sup>, Florencia Villegas<sup>3</sup>, María del Carmen Fernández<sup>3</sup>, Nora Sanguineti<sup>2</sup>, Débora Braslavsky<sup>2</sup>, Andrea Arcari<sup>2</sup>, Ignacio Bergadá<sup>2</sup>, Claudia Arberas<sup>3</sup>, Rodolfo Alberto Rey<sup>1,2</sup>, María Gabriela Ropelato<sup>1,2</sup>.

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RASopathies comprise a group of genetic syndromes caused by gain of function of the Ras/MAPKinase signaling pathway. At least 10 different overlapping phenotypes related to defects in more than 20 genes have been described. Etiological diagnosis allows personalized follow up, adequate treatment and genetic counseling. **Aim:** To implement molecular diagnosis for patients with clinical characteristics suggestive of Noonan syndrome or other RASopathies by next generation sequencing (NGS) using a custom gene panel. **Materials and methods:** We designed a custom gene panel (Twist) for the diagnosis of pediatric diseases including 23 genes with high or moderate evidence of being associated to RASopathies. NGS was performed in a NextSeq500 (Illumina). Variants were classified according to ACMG/AMP criteria following ClinGen RASopathy Expert Panel recommendations. Sanger sequencing was used to confirm variants and for family segregation analysis. We included 33 index cases (20 boys, 13 girls; median age 8.9 years, range 0.2-19.3) and 42 relatives. All the patients were evaluated by pediatric endocrinologists and clinical geneticists. **Results:** We found 24 different heterozygous pathogenic or likely pathogenic variants in 27/33 patients (81.8%, positive cases) in the following genes *PTPN11* (12, 42% of cases, 10 different variants), *NF1* (5), *MAP2K1* (2) and *CBL*, *HRAS*, *KRAS*, *LZTR1*, *RAF1*, *RIT1*, *SHOC2*, *SOS1* (1 each). Segregation studies showed 67% of *de novo* variants and confirmed diagnosis in 8 heterozygous affected relatives. Variants included 2 frameshifts, 1 splicing, 2 missense; only 1 variant of uncertain significance was novel. **Conclusions:** Our results are similar to those reported in the literature, being Noonan syndrome caused by *PTPN11* defects the most prevalent. Even if there are hotspot regions in many of the affected genes, due to high locus and allelic heterogeneity and significant phenotypic overlap, NGS is the most adequate option for the study of RASopathies.

#### 116. 357 ANALYSIS OF ARRAY-CGH STUDY IN PATIENTS FROM ARGENTINA: PERIOD 2023-2024

Laiseca, Julieta<sup>1</sup>; Claps, Aldana<sup>1</sup>; Solari, Andrea<sup>1</sup>; Castro, Tania<sup>1</sup>; Delea, Marisol<sup>2</sup>; Dain, Liliana<sup>1,3</sup>; Taboas, Melisa<sup>1</sup>

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Copy number variants (CNVs) represent a significant cause of genetic diseases, including autism and intellectual disability (ID). Array-CGH (A-CGH) has been one of the main methodologies used worldwide for their analysis, with a diagnostic yield of 15-20%. In this work, we present the results obtained at the National Center of Medical Genetics (CNGM) after applying A-CGH in DNA samples from patients recruited in the period 2023-2024. A total of 92 patients with ID, developmental delay with or without autism, with significant dimorphisms, with or without major anomalies and a previous normal karyotype were included. A-CGH was performed with the SurePrint G3 ISCA V2 platform (8x60K, Agilent Technologies) and the results were analyzed using the Cytogenomics software. We found 20 pathogenic (P) and 1 likely pathogenic (LP) CNVs in 19 patients. Except for the LP, the CNVs found explained the patient's phenotypes. The diagnostic yield of the analyzed sample was 19.6% (18/92). Among the 20 P CNVs, 18 were deletions and 2 were gains. We also found 19 CNVs classified as uncertain (VUS) in 19 patients. Two patients presented 2 P CNVs each, and 2 had also 1 VUS. The most frequent P CNVs found were 3 deletions in 22q11.21 ranging from 254.8 Kb to 2689 Kb and 2 deletions at 17q11.2 containing the *NF1* gene related to Neurofibromatosis Syndrome. The diagnostic yield in our study was similar to the yield reported in the literature worldwide. The results obtained emphasize the importance of using this technology in patients with this type of pathology that otherwise would remain undiagnosed.

#### 117. 419 DEEP PHENOTYPING AND NEXT-GENERATION SEQUENCING (NGS) IN CONGENITAL HYPOGONADOTROPIC HYPOGONADISM: DIAGNOSTIC YIELD IN ISOLATED AND SYNDROMIC CASES

Lourdes Correa Brito<sup>1</sup>, Gabriela Sansó<sup>1</sup>, Agustín Izquierdo<sup>1,2</sup>, Paula A. Scaglia<sup>1,2</sup>, María Esnaola Azcoiti<sup>1,2</sup>, Bárbara Casali<sup>1,2</sup>, Sebastián Castro<sup>1</sup>, Jimena Lopez Dacal<sup>1</sup>, Sofía Suco<sup>1</sup>, Franco Brunello<sup>3</sup>, Mariela Urrutia<sup>1</sup>, Marianela Maier<sup>4</sup>, Florencia Villegas<sup>4</sup>, Claudia Arberas<sup>4</sup>, Analía Freire<sup>1</sup>, Ana Kesselman<sup>1</sup>, Andrea Arcari<sup>1</sup>, Ignacio Bergadá<sup>1</sup>, Romina P. Grinspon<sup>1</sup>, María Gabriela Ropelato<sup>1,2</sup>, Rodolfo A. Rey<sup>1,2</sup>.

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Congenital hypogonadotropic hypogonadism (CHH), which may go unnoticed until pubertal age, may present isolated or associated with malformations in non-reproductive organs and/or neurodevelopmental disorders, *i.e.* syndromic. NGS technologies have allowed the identification of genetic variants, confirming the etiological diagnosis and broadening known clinical phenotypes. Objective: To evaluate the diagnostic yield of NGS in children and adolescents with CHH clinically diagnosed after deep phenotyping. Methods: this cross-sectional study included patients with clinical diagnosis of CHH referred to the Division of Endocrinology of a tertiary pediatric

hospital, between 2008 and 2023. After deep phenotyping, using HPO terms, patients were categorized into: isolated CHH (iCHH), hyposmic CHH (hCHH), normosmic syndromic CHH (nsCHH) and hyposmic syndromic CHH (hsCHH). Genetic analysis was performed using NGS (whole exome or gene panels). Variants were classified using ACMG/AMP guidelines and ClinGen SVI WG recommendations. Only pathogenic and likely pathogenic variants were considered causative. Results: Causative variants were identified in 18/39 (46%) patients (34 male/5 female). The diagnostic yield was 42% in iCHH, 33% in hCHH, 56% in nsCHH and 67% in hsCHH. The affected genes were: iCHH: *ANOS1*, *FGFR1*, *GNRHR*, *PROK2* and *PROKR2*; hCHH: *ANOS1*, *PROK2*, *PROKR2* and *WDR11*; nsCHH: *CHD7*, *FGFR1* and *TUBB3*; and hsCHH: *CHD7*, *FGFR1*, *HESX1*, *SIN3A* and *SOX10*. Conclusion: The overall diagnostic yield, attaining approximately half of the cases, meets the highest rates reported in the literature. Deep phenotyping was probably crucial. *ANOS1*, *PROK2* and *PROKR2* are shared in iCHH and hCHH. nsCHH and hsCHH were associated with pleiotropic genes, such as *CHD7*, *SOX10*, *SIN3A* and *TUBB3*. Finally, *FGFR1* was identified in patients with both isolated and syndromic forms. This highlights the complexity of the clinical spectrum in CHH, which justifies the careful evaluation and reverse phenotyping.

#### P1 POSTERS

FECHA Y HORA: 19/11/2024 11:00-12:00 H

COORDINADORES: CECILIA FERNÁNDEZ, CARLOS DAVID BRUQUE, PAULA BUONFIGLIO

#### 118. 071 FREQUENCY AND CHARACTERISTICS OF DOUBLE IGHV REARRANGEMENTS IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

Carmen Stanganelli<sup>1</sup>, Juana Cabrera<sup>1</sup>, Cecilia Rodríguez<sup>2</sup>, Silene Silvera<sup>2</sup>, Andrea Bender<sup>3</sup>, Evangelina Agrielo<sup>3</sup>, Irma Slavutsky<sup>4</sup>

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The IGHV (*immunoglobulin heavy variable*) mutational status, a robust prognostic marker in chronic lymphocytic leukemia (CLL) allows to stratified patients into two groups: mutated (M) (<98% homology to the germline) or unmutated (U) (≥98%), with different outcome. Most of cases usually develop a monoclonal disease but a subgroup carries double productive IGHV rearrangements (DPR). The aim of this study was to evaluate the frequency and characteristics of DPR CLL patients in an Argentine cohort. A total of 1138 CLL patients (704 men; mean age: 65.8 years, range: 23-100 years; 58% M and 42% U) were analyzed. RT-PCR and bidirectional sequencing were performed. The presence of stereotyped receptors was evaluated. The study was approved by the Institutional Ethics Committee. All individuals provided informed consent. A total of 21/1138 (1.8%) cases showed DPR, 13 with concordant mutational status (7 double M and 6 double U), 8 discordant were observed. In addition, 24/1138 cases with one productive (P) and 1 non-productive (NP) rearrangement (22 with concordant mutational status (14 U and 8 M) and 2 discordant cases), were detected. The most represented VH families were VH3>VH4>VH1, with over-representation of the VH2, VH5 and VH7 families in the DPR group. The most frequent combination of families in the DPR was VH1+VH3 and VH3+VH4 (6 each). VH2-5 gene was over-represented in DPR cases (p=0.04). Two patients with DPR presented stereotyped receptors. Two cases studied twice showed molecular clonal evolution. To our knowledge, this is the first analysis of DPR in CLL from Latin America. A similar frequency of IGHV DPR carriers to those described in the literature (~2%) as well as family distribution were observed. The co-occurrence of concordant mutational status could indicate the presence of common factors acting during maturation and selection, such as the presence of a common progenitor or reactivity to shared epitopes.

**119. 130 ZEBRAFISH AS AN *IN VIVO* MODEL TO CHARACTERIZE THE PATHOGENICITY OF *IGF1* VARIANTS IDENTIFIED IN PATIENTS WITH SEVERE SHORT STATURE**

Macarena Recalcatti<sup>1</sup>, Lucía Salatino<sup>2</sup>, María Celia Fernández<sup>1</sup>, Ayelén Martín<sup>1</sup>, Horacio Domené<sup>1</sup>, Patricia Pennisi<sup>1</sup>, Paola Plazas<sup>2</sup>, Sabina Domené<sup>1</sup>.

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The insulin-like growth factor 1 (IGF1) is highly conserved among vertebrates and plays a central role in pre- and postnatal growth. IGF1 deficiency due to homozygous pathogenic variants in the *IGF1* human gene leads to intrauterine and postnatal delay of growth sometimes associated with deafness and mental retardation. Our objective was to develop a functional assay to determine the pathogenicity of two genetic variants (p.Ser35Cys y p.Tyr60His) identified in *IGF1* using the zebrafish as an animal model. Although both patients share a severe delay in growth, the patient with p.Ser35Cys has a cognitive development close to normal and normal hearing, while the patient with p.Tyr60His has mental retardation and deafness. We performed knockdown assays using *igf1* morpholinos (MOs) and overexpression assays using human WT *IGF1* mRNA and variants, microinjecting these molecules into zebrafish embryos followed by phenotype scoring at 48 hours post fertilization. Variants were introduced into human *IGF1* cDNA by site-directed mutagenesis. MO-mediated *igf1* knockdown resulted in embryos with pericardial edema (51%), yolk edema (38%), and tail malformation (32%). On the other hand, overexpression of human WT *IGF1* mRNA resulted in embryos with cyclopia (37%), pericardial edema (52%), yolk edema (54%), and tail malformation (43%). Finally, overexpression of variants p.Ser35Cys y p.Tyr60His resulted in embryos without cyclopia, pericardial edema (7, 0%), yolk edema (17, 5%), and tail malformation (10, 0%). In conclusion, our preliminary study showed the pathogenic nature of both *IGF1* variants. Moreover, overexpression studies showed that variant p.Ser35Cys retained minimal biological activity while variant p.Tyr60His resulted practically inactive. Rescue assays using both WT and *IGF1* variants mRNAs remain to be performed to evaluate the effectiveness of each variant in to rescue MO-mediated *igf1* knockdown phenotypes.

**120. 240 MOLECULAR CHARACTERIZATION OF THE BREAKPOINTS IN *F9* DELETIONS IN THREE UNRELATED FAMILIES WITH HAEMOPHILIA B**

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**Introduction:** Haemophilia B (HB) is an X-linked recessive coagulopathy caused by deleterious variants in the *F9*. *F9* large deletions (LD) cause 3-5% of severe cases of HB. **Aim:** To characterize the LD breakpoints and to estimate the molecular mechanisms involved in three unrelated families with severe HB (F1/F2/F3). **Methods:** Schemes of case-specific bipartition-tagging PCR systems were designed for each family. SNP-array analysis was performed in F3 to estimate the extragenic extension of the LD. Long range-PCR amplifications were achieved by bridging the nearest 5' and 3' positives PCR tags. Sanger sequencing spanning the breakpoints allowed characterization of their sequences and their eventual molecular features. 25bp-windows around the LD breakpoint events were bioinformatically analysed using the ChrX RefSeq (NC\_000023.11). The analysis involved the detection of repetitive elements (RE), recombinogenic motifs, 2dary DNA and non-B-DNA structures. **Results:** F1 showed a deletion of 231bp involving 76bp

of *F9*-exon 8 g.139561369\_139561599del. F2, a LD of 6.5kb spanning from *F9*-exon 8 3'-UTR to near extragenic non-coding regions g.139562041\_139568612del. F3, a complex LD of 1.26Mb encompassing the entire *F9* and *MCF2*, *ATP11C*, *CXorf66*, *LOC389895*, *SOX3* genes g.[139298179\_140557203del;140557390\_140564347delinsAG]. F1/F2 showed microhomologies of 2(TA)3(AAC) bp and F3, a micro-insertion of 2bp (AG). Breakpoint analysis revealed motifs associated with DNA instability (i.e., Topoisomerase I, Translin-binding site, DNA polymerase-alpha pause site, Murine MHC deletion hotspot, Ig heavy chain class switch and Jurka hexanucleotides), RE (i.e., LINE and MER) and 2dary DNA structures at breakpoints. **Conclusion:** The evidence is consistent with the model of Microhomology-Mediated-Break Induced Replication model in cases F1 and F2, although Alternative End Joining model cannot be excluded. F3 complex event fits with the model of Fork Stalling and Template Switching.

**121. 344 HEALTH-RELATED QUALITY OF LIFE IN TRANSTHYRETIN AMYLOIDOSIS: PATIENT-REPORTED OUTCOMES FROM THE REGISTRY OF PEOPLE WITH AMYLOIDOSIS**

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**Introduction:** The impact of systemic amyloidosis on health-related quality of life (HRQoL) can be measured by patient-reported outcomes. **Objective:** To explore HRQoL in patients with systemic amyloidosis, using data from the Registry of People with Amyloidosis. **Methods:** A cross-sectional study was conducted including consecutive subjects with variant-type transthyretin deposition amyloidosis (ATTRv) included in the Registry of People with Amyloidosis between 2021-2023. Participants with other types of amyloidosis were excluded. HRQoL was assessed using the EuroQoL 5D-3L questionnaire, self-administered in REDCap. Patients' HRQoL results were compared with those of the general population of Argentina. **Results:** During the study period, 46 patients completed the EQ-5D-3L questionnaire. Of these, 24 had ATTRv and were included in the analysis, while 22 were excluded due to a diagnosis of AL amyloidosis. The median age was 51 years, and most participants were women. The Val30Met mutation was the most frequent. Regarding HRQoL, 19 participants (79;95%CI 58-93%) reported moderate or severe pain or discomfort, 16 (67%; 95%CI 45-84%) experienced problems walking, 15 (63%; 95%CI 41-81%) had problems carrying out daily activities, 13 (21%; 95%CI 7-42%) had problems in personal care and 12 (50%; 95%CI 29-71%) had anxiety or depression. Regarding the comparison with the general population, it was observed that subjects with the disease presented a significantly higher proportion of moderate or severe problems compared to the general population of Argentina in all domains evaluated ( $p < 0.001$ ). **Conclusions:** ATTRv amyloidosis has a significant negative impact on HRQoL, underlining the importance of assessing patient-reported outcomes as key indicators in comprehensive patient care.

**122. 371 BEYOND THE BASICS: EXPLORING *STRC*-RELATED HEARING LOSS, A LESSER-KNOWN CAUSE OF HEARING LOSS IN ARGENTINA**

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4- División Genética del Hospital de Clínicas "José de San Martín".

5- Tercera Cátedra de Farmacología, Facultad de Medicina, Universidad de Buenos Aires.

Genetic hearing loss (HL) affects around 1:500 newborns and is primarily non-syndromic (70%). It is an heterogeneous condition, involving 124 genes. Most cases are attributed to *GJB2* and *GJB6* variants. One lesser-known cause of moderate HL with autosomal recessive inheritance is *STRC* gene alterations. This gene is in a tandem duplicated region with the *STRCP1* pseudogene. Large deletions are the most common type of *STRC* mutations, which may also involve the adjacent *CATSPER2* gene, crucial in sperm motility, leading to Deafness-Infertility Syndrome. While some studies in patients have reported a frequency of 5%, its prevalence in Argentina remains unclear. In this study, we aimed to investigate the frequency of *STRC* alterations in a moderate HL cohort from Argentina. A total of 105 patients were tested. Homozygous deletions were detected by amplifying a non-polymorphic marker located exclusively between *STRC* and *CATSPER2*. Heterozygous variants were studied by MLPA. Long-Range allele-specific PCR and Sanger sequencing were used to detect SNVs. Seven of 105 unrelated cases resulted with causative *STRC* variants (6.7%). Some cases had homozygous deletion of *STRC* and *CATSPER2* while others exhibited compound heterozygous deletion of *STRC* in trans with causative SNVs. The complexity of this region was shown in our findings, with one case presenting a deletion of *STRC* and duplication of the *STRCP1* pseudogene, suggesting a possible mechanism of gene conversion. *STRC*-related hearing loss typically presents as moderate and stable, so the diagnosis could bring relief to patients concerning the evolution of the pathology as well the *STRC-CATSPER2* loss modifies the clinical diagnosis of the patients to a syndromic status. This highlights the importance of accurate diagnosis for providing patients with proper genetic counseling. Our study serves as a significant precedent in the analysis of *STRC*-associated hearing loss in Argentina.

### 123. 445 CHROMATINOPATHIES: PATHOGENIC COPY NUMBER VARIANTS IN GENES ASSOCIATED WITH CHROMATIN REMODELING

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Chromatinopathies (CPs) are neurodevelopmental disorders caused by pathogenic variants in chromatin-modifier genes (called epigenes, genes that regulate epigenome). Currently, 179 syndromes are classified as CPs. General clinical characteristics include neurodevelopmental delay and intellectual disability, seizures, facial dimorphism, growth and feeding problems. Aim: to describe a cohort of pediatric patients with pathogenic copy number variants (CNVs) that encompass genes associated with chromatin remodeling processes. Patients and Methods: We selected cases from 291 neurodevelopmental disorder (NDD) patients studied with array-CGH with pathogenic CNVs that encompass genes associated with chromatin remodeling processes. DNA samples of patients were studied using GenetiSure Cyto 8x60K Platform, and the results were analyzed with Cytogenomics v5.0.2 software provided by Agilent. The detected CNVs were classified using the ClinGen CNV pathogenic calculator for applying ACMG criteria. Cases and Results: We found 6 patients, 3 girls and 3 boys, with a median age: 10.8 y (range:

4m to 14 y). The most common overlapping features include neurodevelopmental phenotypes as ID, or motor delay, dysmorphism and growth abnormalities (growth retardation or overgrowth). CNV size between 86.7 kb to 5.5 Mb with 5 deletions and 1 duplication. The CNVs include the following dominant epigenes: ARIBD1B, SAMRCB1, PDH6, EHMT1, DNMT3A and MECP2 genes. Conclusions: We present a new entity recently reported in the literature, which represents 2% of our population of NDD patients studied by array-CGH. We emphasized the importance of reporting patients with CNVs that encompass epigenes to consider the complex relationships between genetic variants and phenotypes for each syndrome and to expand the clinical spectrum of chromatinopathy.

### 124. 465 MOLECULAR AND CLINICAL INSIGHTS INTO LAMA2-RELATED DYSTROPHIES IN AN ARGENTINEAN PAEDIATRIC COHORT

María Eugenia Foncuberta<sup>1</sup>, Soledad Monges<sup>2</sup>, Isabel Cristina Pareja Betancur<sup>2</sup>, Sofía Corbaz, Silvina Gómez Montoya<sup>2</sup>, Giovanna Aschettino<sup>3</sup>, Francisco Martín García<sup>3</sup>, Fabiana Lubieniecki<sup>4</sup>, Luis Pablo Gravina<sup>1</sup>

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LAMA2-related dystrophies present a continuous clinical spectrum, from severe congenital muscular dystrophy type 1A (MDC1A) to milder late-onset forms of limb-girdle muscular dystrophy (LGM-DR23), both inherited in an autosomal recessive manner. MDC1A is one of the most common congenital muscular dystrophies worldwide. The aim of this study is to describe the spectrum of LAMA2 pathogenic variants and their correlation with the phenotype in a paediatric cohort. We studied 22 unrelated patients (15 males, 7 females) and one sibling (1 male) using a customized NGS panel for neuromuscular disorders that includes genes associated with muscular dystrophies. Molecular diagnosis was achieved in 22 patients; in one patient only a single pathogenic variant in the LAMA2 gene was identified. In total, 14 different variants were detected among the 22 unrelated patients, including 2 novel variants. Small variants accounted for 86% of the total variants (37/43), whereas copy number variations were observed in 14% (6/43). Among the detected variants, 40 were protein-truncating variants and 3 were missense. The five most frequent variants that represented 76.8% of the total alleles were: c.3085C>T (44.2%), c.4992\_4996del (9.3%), exon 3-4 deletion (9.3%), c.2049\_2050del (7%), and c.6145A>T (7%). A clear association with congenital muscular dystrophy was observed in patients homozygous or compound heterozygous for truncating variants. Of the three patients compound heterozygous for a truncating and a missense variant, two presented with MDC1A and one presented with LGMDR23. The inclusion of probes between introns 2 and 4 in the NGS panel enabled the characterization of exon 3-4 deletion breakpoints and facilitated the development of a GAP-PCR for carrier testing. In conclusion, this study contributes to the characterization of pathogenic variants in the LAMA2 gene in Argentinean patients and to the optimization of the molecular diagnostic algorithm for LAMA2-related dystrophies.

## P2 POSTERS

FECHA Y HORA: 20/11/2024 11:30-12:30 H

COORDINADORES: VIVIANA DALAMON, FLORENCIA GILIBERTO

### 125. 016 RISK OF SUDDEN DEATH: COHORT ANALYSIS OF GENETIC AND CLINICAL OUTCOMES

Luis Enrique Gómez<sup>1,2</sup>, Mercedes Saenz<sup>2</sup>, Ana Leticia Fernandes Gomes<sup>1</sup>, Héctor Martín Cifuentes<sup>1</sup>, Sofía de La Fuente<sup>1</sup>, Romina Soledad Navarro<sup>1</sup>, Agustín Izquierdo<sup>1,3,4</sup>, Monica Paola Bellazzi<sup>1</sup>, Guillermo Corró<sup>1</sup>, Marisol Delea<sup>1</sup>, Carlos Da-

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**Introduction:** Cardiogenetics (CG) examines a variety of cardiac disorders that impact the heart's structure and function, leading to an elevated risk of heart failure and sudden cardiac death. This study aims to analyze a cohort of patients within the public health system between 2021 and 2024. It will focus on evaluating their genetic profiles, clinical characteristics, and therapeutic outcomes. **Material and Methods:** The cohort included 110 patients and relative (80 probands), with a mean age of 43 years, who presented with various forms of genetic cardiomyopathies, including hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, and channelopathies. Genetic testing was performed on all patients at the Hospital de Alta Complejidad El Calafate SAMIC and Fernandez Hospital, to identify pathogenic variants in genes related to GC. Additionally, clinical symptoms, treatments received, and short- and long-term outcomes were recorded. **Results:** The results showed that 61.6% of patients had genetics variants in MYH7 (22.2%), MYBPC3 (8.1%), TTN (7.1%), PDLIM3 (2%), RAF1 (2%), DSP (4%), SCNA5 (2%), RBM20 (2%), TNNT2 (1%), KCNQ1 (<1%), KCNH2 (<1%) and LMNA (<1%), and those with variants in DSP, RBM20 and LMNA, had a worse prognosis in Major Adverse Cardiovascular Events (MACE). Managing patients with GC remains challenging due to variability in clinical presentation and treatment response. **Conclusion:** This study underscores the importance of comprehensive genetic evaluation in patients with CG, enabling a more personalized approach to their management. Identifying specific variants could guide therapeutic decisions and improve long-term outcomes. Future research should focus on validating these findings and developing targeted therapies that address the underlying causes of genetic cardiomyopathies.

**126. 162 DESIGN AND APPLICATION OF A NEW PROTOCOL FOR THE DETECTION OF VARIANTS BY NEXT GENERATION SEQUENCING (NGS) IN PATIENTS WITH HEMOPHILIA A (HA) AND B (HB).**

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Hemophilia is an X-linked coagulopathy caused by defects in the F8(HA) an F9(HB) genes. **Aims:** Design, develop, adjust and validate a protocol for the detection, characterization and interpretation of variants by NGS technology in patients with HA and HB. **Materials and methods:** DNA genotyping involved PCR amplification of all the relevant regions of F8 and F9 in 16 and 4 amplicons. DNA libraries were prepared from the amplicons with Illumina DNA prep kit and sequenced on Illumina MiSeq platform. Sequence mapping and variant calling format (VCF) file analysis were performed using BWA, GATK Best Practices pipeline and the platform Franklin (pro-

vided by Genoox), respectively. Validation: four patients with HA and four with HB genotyped by standard PCR-CSGE-Sanger, were studied by NGS. In addition, five patients with presumptive diagnosis of HA and three with HB with undetermined genetic diagnosis by standard method were analyzed. **Results:** Validation: concordance was obtained in all eight samples analyzed, including the detection of variants previously associated to patients' phenotype and other benign SNVs. Undetermined cases: the causal variant was identified in 2/5 HA patients and in 3/3 HB cases. The average depth and coverage on target (depth $\geq$ 20) were 3781/99.7% and 3611/99.5% for F8 and F9 genes respectively. The presence of artifacts and common errors in sequencing were identified, 50% due to sequencing and alignment errors in repetitive regions and homopolymers and 50% owed to primers SNVs. **Conclusion:** A new protocol for the detection of variants in F8 and F9 genes by NGS was developed and validated with excellent results in depth and coverage. The introduction of this technology increases the coverage analyzed, extending the study to intronic regions and improve the success rate in genetic diagnosis compared to PCR-CSGE-Sanger.

**127. 217 EPIGENETIC INSIGHTS INTO SPORADIC COLORECTAL CANCER USING EPICV2 BEADCHIP: A FIRST IN-LINE ANALYSIS OF AN EIGHT-GENE CIMP-LINKED PANEL**

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Aberrant DNA methylation (*DNAm*), like CpG island methylator phenotype (CIMP) in colorectal cancer (CRC), is common in all cancers. We analyzed methylation in the CGI region (CpG island and shore with 2kb flanks) of a CIMP-linked 8-gene panel on 63 paired CRC mucosa (N) and tumor (T) samples, using EPICv2 and the SeSAME R pipeline. CGI coordinates (GRCh38) for each gene were defined according to UCSC, and all EPICv2 probes within CGI region were selected to score methylation ( $\beta$ -value) for each probe. The mean  $\beta$  ( $\bar{x}\beta$ ) was calculated for each gene using all CGI probes in N/T samples. A gene was classified as hypermethylated if  $\bar{x}\beta$  in tumor crossed a threshold [average( $\bar{x}\beta$ \_Normals)+ $\Delta\beta$ ] set for each gene-specific CGI.  $\Delta\beta$  (significant threshold) was defined to avoid hypermethylation in mucosa when comparing matched N/T methylation levels for each EPIC probe. Since *DNAm* increases with age, we assessed methylation levels in each mucosa specimen relative to diagnosis age. A moderate positive correlation was found for *IGF2*, *CDKN2A*, and *RUNX3* ( $r=0.5, 0.56, 0.42$ ; and  $p<0.001, <0.0001, <0.019$ ; respectively; Pearson Test/Hochberg FWER correction), showing aging increases methylation of CIMP markers in mucosa tissue. No age correlation was found for *MLH1* CGI, but  $\bar{x}\beta$  in N/T pairs perfectly aligns with MMR status by IHC: 95.5% dMMR samples (21/22) showed hypermethylation in T ( $\bar{x}\beta=0.51, \max=0.85, \min=0.18$ ) and N  $\bar{x}\beta=0.034$ ; pMMR samples are completely unmethylated in both tissues (T  $\bar{x}\beta=0.04$ ; N  $\bar{x}\beta=0.03$ ). We determined CIMP status using the Ogino panel: 44% (28/63) of samples were CIMP(+), with only one CIMP-low. Interestingly, 95.5% (21/22) of dMMR cases were CIMP-high. CIMP status by MLPA (N=47) strongly correlated with EPIC results ( $r=0.828$ ;  $p<0.001$ ). Sensitivity (0.96, 0.88-0.99) and Specificity (0.88, 0.69-0.97) confirm our EPIC-derived CIMP efficiency in reproducing MLPA (reference method) outcomes. Our findings confirm aberrant *DNAm* and CIMP play crucial roles in CRC.

**128. 232 CONTRIBUTION OF LARGE REARRANGEMENTS IN BRCA1/2 GENES AND CHEK2 110DEL C ALLELE VARIANT TO THE DEVELOPMENT OF BREAST/OVARIAN CANCER IN UNSELECTED ARGENTINIAN POPULATION**  
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Purpose: Among women in Argentina, the most common cancer is breast cancer with 21,631 new cases and 6436 deaths per year. The ovarian cancer is fifteenth in frequency. The contribution of cancer-related large genomic rearrangements (LGRs) of the BRCA1/BRCA2 genes and the 1100delC allelic variant in the CHEK2 gene has not yet been widely studied in our population. Methods: LGRs in the BRCA1/BRCA2 genes and the CHEK2 1100delC variant were analyzed using the MLPA technique in 85 unselected Argentinian breast cancer/ovarian cancer patients. Results: A total of 85 women, 69 with BC, 15 with OC and 1 with BC and OC were studied. The mean age at diagnosis of BC was 41,9 years (range 22–65), OC 44,4 years (range 31-60) and BC+OC 38 years. 61 of 85 patients (71,8%) were premenopausal. Of those with known ER/HER2 status, 39 of 67 patients (58.2%) presented with ER-positive BC, and 14 patients (20.9%) were HER2-positive. 19 patients (28.4%) were recorded as having triple-negative BC. MLPA analysis revealed 8 different pathogenic variants, 7 LGRs in the BRCA1 gene and the CHEK2 1100 delC in eleven out of 85 (12,9%) patients. Among the LRGs, 6 were exons 1-2, 3, 10, 13, 15, 19 deletions and one exons 20-22 duplication. Only one patient presented the CHEK2:c.1100delC variant. Two of 15 OC patients (13,3%) and 9 of 69 BC patients (13,0%) presented a pathogenic LRGs. Deletions of exons 1-2 and 15 were recurrent in our series and were found in three (30%) and in two of the 10 (20%) LRGs patients, respectively. Conclusions: LRGs in the BRCA1 gene contributed significantly to the burden of pathogenic genetic variants responsible for the development of breast and ovarian cancer in our serie. The low frequency of the CHEK2 1100delC underlines the importance of considering ethnic background before offering a genetic test. It would be appropriate to consider the routine inclusion of assays for LGRs for BRCA1 and BRCA2 in unselected breast/ovarian cancer population.

**129. 452 CELLULAR CHARACTERIZATION OF THE EXON13\_15DUP OF LDLR ASSOCIATED WITH FAMILIAL HYPERCHOLESTEROLEMIA**

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Familial Hypercholesterolemia (FH) is an autosomal dominant genetic alteration associated with a high rate of early cardiovascular disease. The leading cause is related to mutations in the gene that codes for the LDL receptor (LDLR); One of the most frequent mutations identified in the Chilean population is the duplication of exons 13-15 (exon13\_15dup) of the LDLR, classified as a variant of uncertain significance (VUS). Aim. Characterize the functional effect of exon13\_15dup-LDLR variant associated with FH through ex vivo assays. Methodology. A descriptive study. Exon13\_15dup variant patients were recruited. A blood sample was collected to perform cellular and molecular analysis. CD14+ mononuclear cells were purified and differentiated into macrophages with GM-CSF. LDLR expression levels were evaluated by flow cytometry and data processing with FlowJo. Co-localization of LDLR with cellular structure marker proteins: calreticulin, LAMP-1, and clathrin was performed by immunocytochemistry and analyzed by confocal microscopy. Visualization and analysis were performed using confocal microscopy, ImageJ, and PRISM. Results. 4 heterozygous index cases carrying exon13\_15dup were recruited. The exon13\_15dup mutation increases LDLR expression levels at the macrophage membrane ( $p < 0.0001$ ). Cellular localization of the variant presented an increased and decreased colocalization with calreticulin ( $p < 0,05$ ) and clathrin ( $p < 0,01$ ), respectively. Conclusion. This work represents the first functional evidence of exon13\_15dup-LDLR effect. This variant produces high levels of LDLR expression and modifies the LDLR cellular localization, contributing to the understanding of the pathophysiology of FH.

**P3 POSTERS**

FECHA Y HORA: 20/11/2024 16:10-17:10 H

COORDINADORES: FIORELLA BELFORTE, LOURDES CORREA BRITO

**130. 372 UNDERSTANDING THE COMPLEXITIES OF HEARING LOSS DIAGNOSIS WITH WHOLE EXOME SEQUENCING: SYNDROMIC OR NOT?**

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Hearing loss (HL) is the most common disorder in 1:500 newborns. Most cases are monogenic, non-syndromic (80%), and with recessive inheritance (75%) with more than 100 genes reported. Additionally, there are over 400 syndromes associated with HL that include extra clinical signs, each linked to a few candidate genes. Whole-exome sequencing (WES) offers a thorough and cost-effective diagnostic tool by analyzing all genes simultaneously. Additionally, WES has the advantage of revealing syndromes where extracochlear signs have not yet developed, allowing for earlier detection and management. The aim of this study was to identify the genetic variants in 50 Argentine patients with clinical diagnosis of non-syndromic HL. DNA was extracted from blood samples. An *in silico* panel of 224 genes was applied from WES data after excluding variants in the most frequently mutated genes *GJB2*, *GJB6*, *MT-RNR1*, and *STRC*. Variants were filtered and classified based on HL Variant Curation Expert Panel and ACMG guidelines. Disease-causing variants were found in 16 known genes in 20 out of 50 patients, with 4 out of 26 variants being novel. Five non-syndromic HL cases had causative variants in genes associated with syndromic forms including Usher, Perrault, Waardenburg, and Heimler syndromes. Variants in *MYO7A* and *USH2A* led to clinical follow-up for visual defects. Two cochlear-implanted male siblings had *LARS2* variants with an initially overlooked syndrome affecting only females (premature ovarian failure). Further analysis of a patient, prompted by her sister's dental defects, led to the detection of *PEX6* variants associated with Heimler syndrome (retinitis, enamel hypoplasia, and nail abnormalities). These results highlight the fine line between syndromic and non-syndromic HL, emphasizing the benefits of thorough clinical follow-up as well as executing a wide genetic study for accurate medical counseling and improved therapeutic outcomes, refining patient quality of life.

**131. 391 CHALLENGES IN THE DESIGN OF A TARGET miRNA AND GENE EXPRESSION SYSTEM IN BACULOVIRAL VECTORS TO INDUCE PROLIFERATION OF MATURE HUMAN CARDIOMYOCYTES DERIVED FROM INDUCED PLURIPOTENT STEM CELLS**

Julia María Halek<sup>1</sup>, Joaquín Smucler<sup>1</sup>, María Agustina Scarafía<sup>1</sup>, Denisse Saulnier<sup>1</sup>, Federico Hernán Zacca<sup>1</sup>, María Belén Palma<sup>1</sup>, Alberto José Crottogini<sup>2</sup>, Jorge Alejandro Simonin<sup>3</sup>, Mariano Nicolás Belaich MN<sup>3</sup>, Santiago Gabriel Miriuka S1, Ariel Waisman<sup>1</sup>

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Cardiovascular disease is the leading cause of death worldwide,

with ischemic heart disease being widespread. Postnatally, cardiomyocytes lose their ability to proliferate, and the heart's limited regeneration is insufficient to repair an injury. Developing models and therapies to enhance cardiomyogenesis is essential to reduce the need for heart transplants. Objective: Development of a novel system to overexpress the proliferation promoting hsa-miR-25 and the cardioprotective factor PITX2C by a baculoviral vector in human cardiomyocyte cell cycle arrest model. Two microRNA expression systems were tested: a **conventional** one using the genomic sequence of the microRNA cluster as an intron, PITX2C, and GFP-coupled 2A under the CMV promoter, and a more **innovative** one using the mature microRNA sequence under U6 (sense) and H1 (antisense) promoters, with mCherry under CMV. Transfections were performed in HEK293T cells and human induced pluripotent stem cells (hiPSC), with expression assessed by RT-qPCR. The innovative system in HEK293T cells did not significantly overexpress the microRNA of interest compared to basal levels. In contrast, the conventional system was able to overexpress the construct in HEK293T and hiPSC cells. Preliminary results indicate that the conventional expression system is the most promising for subsequent production of the baculoviral vector and testing of the gene therapy in the mature human cardiomyocyte model derived from iPSC.

**132. 440 DISCOVERING A NOVEL VARIANT IN HEREDITARY COPROPORPHYRIA: DIAGNOSTIC INSIGHTS**

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Hereditary Coproporphria (HCP) is a rare autosomal dominant disorder caused by mutations in the *CPOX* gene, leading to neurological and/or dermatological symptoms. Effective management requires accurate diagnosis, biochemical control, and strict adherence to treatment. We present the case of a 39-year-old male diagnosed with HCP, showing the critical role of hemin therapy and ongoing maintenance treatment. The patient, with a history of urolithiasis and significant familial medical history, initially experienced abdominal pain requiring opioid analgesics. He later suffered generalized tonic-clonic seizures and neurological decline, prompting comprehensive diagnostics including neuroimaging and biochemical tests, initially suggesting acute intermittent porphyria (AIP). A key diagnostic feature was dark urine. Laboratory findings revealed elevated urinary aminolevulinic acid (ALA), porphobilinogen (PBG), and total urinary and fecal porphyrins, with coproporphyrin predominance. Genetic analysis identified a novel pathogenic variant (c.200\_204del p.(Thr67LysfsTer36)) in the *CPOX* gene, confirming HCP. Although this variant is novel, it meets pathogenic criteria, being absent in control databases and segregating with disease in two affected relatives. Hemin therapy led to significant clinical improvement and decreased levels of urinary ALA and PBG. Nevertheless, recurrent symptoms were linked to poor adherence to folic acid, vitamin B, and glucose supplements. Long-term data revealed fluctuating urinary porphyrin and PBG levels related to treatment adherence. This case points out hemin therapy's importance in managing acute HCP episodes and the need for patient adherence to treatment protocols. The case also highlights the necessity of genetic diagnosis for familial screening and counseling and stresses the value of genetic research in understanding HCP pathogenesis.

**133. 513 FROM POLICY TO PRACTICE: ADVANCEMENTS IN THE MANAGEMENT OF RARE DISEASES**

Érica Johanna Caramello<sup>1</sup>, María Gabriela González<sup>1</sup>, Andrea Rossi<sup>1</sup>, Fernanda Saravia<sup>1</sup>, Marisol Delea<sup>1</sup>, Maximiliano Meló<sup>2</sup>, Rosa Sánchez<sup>2</sup>, Paula Ponzone<sup>3</sup>, Guillermo Corró<sup>1</sup>, Macarena Martínez<sup>1</sup>, Emiliana Francavilla<sup>1</sup>, Carlos David Bruque<sup>1</sup>, Sabrina Soledad Fernández<sup>1</sup>.

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Introduction: Rare diseases (RD) affect 1 in 2000 people and can affect up to 8% of the global population. Almost 80% of these conditions are genetically derived and 75% out of them are manifested in childhood. Diagnosis, when done, is frequently delayed impacting the quality of life for patients and their families. Our aim is to develop a RD Unit (UHEPF) to ensure the diagnosis and treatment of RD as quickly as possible, facilitating access to appropriate care and continuous follow-up. This includes strengthening the information system to ensure service quality and raising awareness among the public and healthcare professionals through an integrated, multidisciplinary approach. Materials and Methods: The National Law N°26.689 and the National Program for RD have been crucial to improve the management of these conditions. The UHEPF implemented a local patient registration system using a QR code and an email for consultations. Additionally, tools such as ORPHANET and FINDZEBRA were utilized for patient support and diagnostic standardization. The UHEPF has trained its team in using the SISA platform and in interdisciplinary consultation and has provided advice on high-cost medications. Results: A total of 100 patients were registered locally, and 60 patients with definitive diagnoses were added to the SISA platform. There were 276 consultations with 190 patients under multi and interdisciplinary follow-up. The UHEPF has also participated in awareness events and planned community activities. Social media networks were created and collaboration with patient associations. Conclusions: The establishment of the UHEPF has led to significant progress in patient registration, staff training, diagnosis, acquisition of high-cost medications, and raising awareness about RD. Despite these advancements, it is essential to continue enhancing awareness and educate the healthcare system on these conditions to further improve the quality of life of the patients and families.

**134. 520 INTEGRATIVE EVALUATION OF GUT METAGENOMIC BIOMARKERS IN ULCERATIVE COLITIS ASSOCIATED WITH SECRETORY IGA GLYCOSYLATION IN THE CONTEXT OF TRANSKINGDOM COMMUNICATION NETWORKS**

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BACKGROUND: The human gut microbiome is the set of microorganisms that generally coexist in a commensal symbiotic relationship with the host. Elucidating interkingdom communication networks is a challenge in the dynamic context of dysbiosis. Considering that secretory IgA (sIgA) controls the composition and function of the gut microbiome, and the dysbiosis observed in ulcerative colitis (UC), we studied if changes in sIgA glycosylation could be associated with an altered microbiome in an Argentine cohort. METHODS: Patients with active UC and in remission were compared with non-UC individuals. Microbial DNA was extracted from stool samples to study microbiome structure and diversity by shotgun sequencing technology (Illumina). We analyzed the population structure for each of the 5 kingdoms, the differential taxa and metabolisms (ANCOM-BC) and calculated the core microbiota. SIgA was obtained by sequential centrifugation and affinity chromatography from 250 mg of stool samples. Lectin-blot of purified sIgA were performed and logistic regression analysis allowed identifying possible biomarkers be-

tween groups. RESULTS: SlgA is desialylated in patients with active UC, a pattern not observed in the non-UC and remission groups. Microbiome studies show differential taxa between the groups. In active UC, *Desulfovibrio oralis* and *Methanobacterium paludis* were dysregulated, while *Pseudomonas sp.*, *Afonbuvirus intestinihomini* and *Natribaculum sp.* were altered in remission. Core microbiota and functional pathways were also analyzed, finding different exclusive core microorganisms with different metabolic capabilities between groups. CONCLUSIONS: We provide new insights into the relevance of slgA glycosylation in modulating microbiome profiles and its participation in interkingdom communication at different stages of UC, allowing for an integrative association of local changes in gut microbial diversity with slgA glycosylation.

### 135. 532 GENETIC AND BIOCHEMICAL CHARACTERIZATION OF ARGENTINE PATIENTS WITH GLYCOGEN STORAGE DISEASES TYPE VI AND IX

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**Background:** Glycogen storage diseases (GSDs) are a group of inherited metabolic disorders characterized by deficiencies in glycogen metabolism. GSD VI and GSD IX involve defects in glycogen phosphorylase and its regulatory proteins, leading to glycogen accumulation and subsequent clinical manifestations. This study aims to elucidate the genetic and biochemical profiles of GSD VI and IX in a cohort of Argentine patients. **Methods:** We conducted a comprehensive evaluation of 21 patients diagnosed with GSD VI or IX through a combination of genetic analysis and biochemical assessments. Genetic testing was performed by sanger sequencing or next-generation sequencing (NGS) to identify mutations in the genes associated with these conditions, including PYGL (for GSD VI) and PHKA2, PHKB, and PHKG2 (for GSD IX). Biochemical assays measured enzyme activity in red blood cells. **Results:** Biochemical assays confirmed significantly reduced phosphorylase beta kinase activity in GSD IX patients. Genetic analysis revealed pathogenic variants in the PYGL gene in 5 patients with GSD VI, including two novel mutations. In the 14 patients diagnosed with GSD IX, genetic changes were identified in PHKA2 (3 patients), PHKB (1 patient), and PHKG2 (2 patient), with 5 non previously described pathogenic variants. **Conclusions:** This study underscores the genetic heterogeneity of GSD VI and IX and highlights the importance of genetic and biochemical analyses for accurate diagnosis and management. The identification of novel mutations contributes to the expanding mutation spectrum for these disorders and provides valuable information for genetic counseling and potential therapeutic interventions. Future research should focus on correlating specific genotypes with clinical phenotypes to enhance understanding of disease variability and progression.

#### P4 POSTERS

FECHA Y HORA: 21/11/2024 11:00-12:00 H

COORDINADORES: ALEJANDRA DUARTE, MARIANA FUERTES

### 136. 155 LOW FREQUENCY GENES ASSOCIATED WITH HOLOPROSENCEPHALY

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Holoprosencephaly (HPE) is a rare complex brain malformation characterized by incomplete division of the forebrain, affecting both the most anterior portion of the brain and the face, causing neurological manifestations and dysmorphisms of varying severities. The prevalence is 1/10,000 live born and stillborn, and 1/250 conceptions. HPE is classified in 3 classical forms: lobar, semilobar, and alobar, according to their severity. HPE may be associated with certain syndromes or chromosomal abnormalities (Smith-Lemli-Opitz syndrome, Hartsfield syndrome, trisomy 13). In addition, *SHH*, *ZIC2*, *SIX3*, *GLI2*, *FGF8*, *FGFR1* are commonly affected in isolated HPE. We have previously analyzed 3 patients with HPE, 1 isolated with a pathogenic variant in *SHH*, and 2 with other congenital anomalies with pathogenic CNVs, one overlapping the *SHH* gene and other with a deletion at 16p12.2. We now report 2 additional patients with HPE and other clinical characteristics with a normal karyotype and array-CGH, analyzed by whole-exome sequencing using the Agilent SureSelect Human All Exon V6 kit (Agilent Technologies). An *in-silico* selection of candidate genes for variant analysis was developed in-house based on Human Phenotype Ontology. Variants were interpreted using American College of Medical Genetics and Genomics (ACMG) guidelines, and the GRCh38 genome was used as the reference sequence. We found a novel p.E694Q variant in the *KMT2D* gene in one of the patients and the p.S747T in *SEMA3E* in the other. Both genes were associated mainly with Kabuki and Charge Syndrome, respectively. The variants were confirmed by Sanger sequencing in the probands and the study of parental samples is currently in progress. Up to date, only 3 patients with Kabuki and 2 with Charge syndromes presenting HPE have been reported in the literature. Therefore, our findings provide additional knowledge of the relationship of these genes and brain development, and grants information for the classification of variants.

### 137. 278 GENETIC SCREENING AND BIOINFORMATICS ANALYSIS IN ARGENTINIAN PATIENTS WITH CONGENITAL HYPOTHYROIDISM

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Congenital hypothyroidism (CH) is one of the most frequent neonatal endocrine and metabolic diseases and affects approximately 1:2,000–1:4,000 infants worldwide. The thyroid peroxidase (TPO) is the crucial enzyme in thyroid hormone synthesis. Defective TPOs are one of the main genetic factors leading to CH. Two patients with clinical and biochemical data compatible with CH due to iodine organification blocks were analyzed in the present study by using high-throughput sequencing (whole exome sequencing) and bioinformatics analysis. 30 pathogenicity predictor programs were used (SIFT, SIFT4G, Polyphen2 HDIV, Polyphen2 HVAR, LRT, Mutation taster, MutationAssessor, FATHMM, Provean, VEST4, MetaSVM, MetaLR, MetaRNN, M-CAP, REVEL, MutPred, PrimateAI, DEOGEN2, BayesDeladdAF, BayesDelnoAF, ClinPred, LIST-S2, VARI-



TY R, VARIETY ER, EVE, Alphamissense, CADD, DANN, FATHMM-MKL y FATHMM-XF). The variants were classified according to the American College of Medical Genetics and Genomics. The putative biological function of detected variants in TPO gene was evaluated by homology modeling using the Swiss Model program, which was based on the 5zuz PDB. The PDB obtained was studied through the ChimeraX and Swiss PDB viewer programs. Patient 1 showed the novel variant c.1758C>A, p.His586Gln inherited from her father and the c.920A>C, p.Asn307Thr, from her mother, both variants in TPO gene. Additionally Patient 1 carries the c.1231C>T, p.Pro411Ser in GNAS gene; the c.1061C>T, p.Thr354Met in KCNJ18 gene and the c.424G>A, p.Val142Ile in TTR gene. All variants in Patient 1 were heterozygous. Patient 2 carries the c.1682C>T, p.Thr561Met inherited from his father and the c.1784G>A, p.Arg595Lys, from his mother, both variants in TPO gene. The findings obtained will facilitate the genetic counseling and prognosis of patients with CH and will be helpful for clarifying the molecular mechanism underlying CH pathogenesis.

**138. 385 BIOCHEMICAL AND STRUCTURAL CHARACTERIZATION OF EXON13\_15DUP VARIANT ASSOCIATED WITH FAMILIAL HYPERCHOLESTEROLEMIA**

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Introduction. Familial Hypercholesterolemia (FH) is the most common hereditary disorder of lipid metabolism, mainly caused by mutations in the LDL receptor (LDLR) gene and confers a high cardiovascular risk. One of the most frequent mutations identified in Chile is the duplication of exons 13-15 (exon13\_15dup) of the LDLR. However, the biochemical and molecular characterization of this variant is unknown. Aim. Biochemical and structural characterization of exon13\_15dup variant associated with Familial Hypercholesterolemia. Methodology. A descriptive study of recruitment by convenience. Lipid profile and molecular analysis were performed in a patient with exon13\_15dup variant. The LDLR expression was analyzed in macrophages differentiated from CD14+ monocytes from peripheral blood and plasma by western blot. The spliced sequence of the duplication was analyzed by RT-PCR and Sanger sequencing. To evaluate the impact of exon 13\_15dup on the structure of LDLR, we performed molecular modeling with MODELLER (version 10.4), molecular dynamics analysis with Desmond software and cluster analysis by Maestro. Results. 7 heterozygous index cases with exon13\_15 dup variant was recruited. The LDL-C average levels were 254±58 and 269±103 mg/dL in children and adults, respectively. Different LDLR bands were detected by western blot in plasma between exon13\_15 dup variant and WT subjects. PCR results show that the exon13\_15dup mutation generates a frameshift and an early stop codon, altering the structure of the LDLR and its interaction with the plasma membrane. Conclusion. The exon13\_15dup variant generates an increase in total cholesterol and LDL-C levels, a product of the presence of a truncated protein, with the absence of the transmembrane and cytosolic domain, which would promote its export to the plasma, notably altering its functionality in the metabolism of the cholesterol.

**139. 388 APPLICATION OF AN OPPORTUNITY-BASED SCREENING FOR DETECTION OF PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA IN A HIGHLY COMPLEX HOSPITAL CENTER**

Mariana Opazo (1), Fabiola Ramírez (1), Benjamín Catalán (1), Catalina Martínez (1), Isabel Muñoz (2), Katia Sáez (3), Claudia Radojkovic (1), Andrea Sánchez (1).

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Introduction. Familial Hypercholesterolemia (FH) is a common hereditary metabolic disease, associated with high levels of LDL-C and a high rate of mortality due to early cardiovascular disease (CVD). The most frequent causes are produced by mutations in the LDL receptor and PCSK9 gene. In Chile, there is no epidemiological data on FH; however, a prevalence of 1:220 is estimated. The identification of FH is carried out through validated algorithms, such as the Dutch Lipid Clinic Network (DLCN), which provides a probability of FH. General objective. Identify patients with FH through the application of an opportunity-based screening model in the clinical laboratory of a highly complex hospital center. Methodology. Cross-sectional descriptive observational study. Lipid profiles were obtained from the clinical laboratory database between 2019-2021. All records with LDL-C≥190 mg/dL and triglycerides<200 mg/dL were identified. The exclusion criteria were pregnancy, secondary hypercholesterolemia, neoplasia, severe cognitive impairment, and death. The final categorization was carried out by applying the DLCN criteria. The patients with the highest scores were sequenced. The numerical variables were represented by measures of central tendency and dispersion and the categorical variables were represented by frequency and percentage. The normality of the variables by the Kolmogorov-Smirnov test. Results. A total of 13,806 lipid profiles were analyzed. We selected 358 patients with LDL-C 190 mg/dL. The DLCN score allowed us to identify 5, 4, and 325 patients with certain, probable, and possible HF, respectively. 4/6 patients were identified with FH by genetic analysis. Conclusions. The application of this pilot program of opportunistic screening allowed the identification and genetic diagnosis of patients with FH, contributing to the early diagnosis and preventive treatment of CVD.

**140. 407 FUNCTIONAL CHARACTERIZATION OF D47N-LDLR MUTATION ASSOCIATED WITH FAMILIAL HYPERCHOLESTEROLEMIA THROUGH EX VIVO ASSAYS**

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Familial Hypercholesterolemia (FH) is an autosomal dominant genetic alteration associated with a high rate of morbidity and mortality due to early cardiovascular disease. The leading cause is related to mutations in the gene that codes for the LDL receptor (LDLR); one of the most frequent mutations in Chile is the D47N, classified as a variant of uncertain significance (VUS). Aim. To characterize the functional effect of D47N-LDLR mutation associated with FH through ex vivo assays. Methodology. Descriptive study. Peripheral blood mononuclear cells were obtained from patients with D47N-LDLR mutation. CD14+ cells were purified and differentiated into macrophages with GM-CSF. LDLR expression levels were evaluated by flow cytometry and data processing with FlowJo. For LDLR cellular localization, immunofluorescence was used with antibodies against LDLR and cellular structural marker proteins: calreticulin, LAMP-1, and clathrin. Visualization and analysis were performed using confocal and super-resolution microscopy. For LDL transport assays, macrophages were cultured in a standard growth medium with 10% delipidated serum for 4 hours and incubated with 20 µg/mL of LDL-FITC at different times. Visualization and analysis were performed using confocal microscopy, ImageJ, and a t-student test. Results. D47N mutation increases LDLR expression levels at the macrophage membrane (96.9 vs. 13.0; p<0.0001). Cellular localization of the D47N variant presented a significantly decreased colocalization with clathrin concerning WT subjects. For LDL transport assays, this mutation changes the uptake levels of LDL-FITC. Conclusion. The presence of the D47N mutation determines high levels of LDLR expression and modifies the localization and function, suggesting that the D47N mutation can be a pathogenic variant of class 3.

**141. 438 ANALYSIS OF VARIATION SPECTRA OF 12 GENES ASSOCIATED WITH THYROID DYSHORMONOGENESIS IN A PILOT COHORT OF CHILDREN WITH HYPERTHYROTROPINEMIA**

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Context: Lower TSH cutoffs for congenital hypothyroidism (CH) have led to an increased detection of isolated hyperthyrotropinemia (HTT), characterized by elevated TSH levels with normal free thyroxine (FT4) levels. HTT may be the expression of a mild persistent thyroid failure due to genetic abnormalities of thyroid structure or function, however, the screening of genes associated with thyroid dysmorphogenesis (TDH) and TSHR has been limited in this setting. Objective: To evaluate the contribution and molecular spectrum of variants in 12 TDH causative genes (TG, TPO, DUOX1, DUOX2, DUOX1, DUOX2, SLC5A5, SLC26A4, SLC26A7, IYD, GNAS and TSHR) in a pilot cohort of HTT. Materials and methods: HTT was defined by elevated serum TSH levels (9-20 uU/ml in neonates up to 3 months of age, 5-20 uU/ml in children) with normal age-appropriate FT4 levels. Ten patients (4 males and 6 females) underwent targeted next-generation sequencing (tNGS) or whole exome sequencing (WES). Data were analyzed for single nucleotide variants (SNVs), short insertions/deletions, noncanonical splice site (NCSS) variants, and copy number variants (CNVs). Pathogenicity was analyzed according to ACMG/AMP and ClinGen standards. A systematic review was conducted for gene curation. Results: Eight monoallelic SNVs affecting TG, TPO, TSHR, and DUOX2 genes were identified in 5 subjects. Two likely pathogenic (LP) variants and 1 variant of uncertain significance (VUS) were found in TSHR, 1 VUS and 1 pathogenic (P) variant in TG, 1 LP and 1 VUS in TPO, and 1 P variant in DUOX2. Molecular diagnosis was established based on zygosity, variant pathogenicity, and phenotypic specificity. A definitive molecular diagnosis was reached in 3 cases. Two additional cases were classified as probably solved or ambiguous and the remaining 5 patients as unsolved. No clinically significant CNVs or NCSS were found. Discussion: Genetic screening of 12 TDH-related genes revealed variants in a limited number of HTT cases, suggesting the presence of additional, yet unexplored genetic mechanisms, warranting further research to enhance the understanding of this condition.

#### 142. 493 ROLE OF NR1I2 GENE VARIANTS IN ACUTE INTERMITTENT PORPHYRIA ONSET

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Acute Intermittent Porphyria (AIP), an Acute Hepatic Porphyria, is a metabolic disease due to an inherited Porphobilinogen deaminase (PBG-D) deficiency. Enzyme activity reduction is not enough for AIP onset which is triggered by several factors, including therapeutic drugs. Genetic variants in other genes like cell metabolizing (CYP) and transport system (ABC) could have a role in Hepatic Porphyrias onset. NR1I2 gene encodes for the transcription Pregnane X Receptor (PXR): NM\_003889.4:c.22-7659C>T intronic variant NM\_003889.4:c.52G>A, NM\_003889.4:c.79C>T, NM\_003889.4:c.106G>A, NM\_022002.3:c.196C>T in exon 2, and NM\_003889.4:c.418G>A and NM\_003889.4:c.488A>G in exon 4

variants affect the expression of ABC drug transporters and CYP3A4. The aim was to perform a joint role analysis of these PXR SNVs in AIP triggering. Cohorts studied: Control, symptomatic AIP (S-AIP) and latent AIP (L-AIP). PCR direct sequencing and PCR-RFLP were used for genotyping. S-AIP allelic frequency for c.196C>T (0.13) was lesser than control (0.29, p<0.01) and L-AIP (0.27, p<0.05). A different genotypic profile was observed: S-AIP showed a minor value in heterozygosity (26%) vs Control (45.5%, p<0.01) and L-AIP (48.5%, p<0.01); TT was in a very low/null value (Control= 6%, S-AIP=0%, L-AIP=3%). No different allelic or genotypic profile was observed among S-AIP, L-AIP and control groups for c.-22-7659C>T, c.52G>A, c.79C>T, c.106G>A, c.418G>A and c.488A>G SNVs. In conclusion, the higher presence in latent patients of c.196C>T would suggest that T could be a protector allele, probable related to a minor demand of heme from the regulatory pool in the liver, due to the downregulation of PXR on the transcription of CYP3A4. Some NR1I2 gene variants could be considered as possible modulators in the pharmacological induction of AIP.

## HEMATOLOGÍA

### P1 POSTERS

FECHA Y HORA: 20/11/2024 16:10-17:10 H

COORDINADORES: ANA CLAUDIA GLEMBOTSKY, PAOLA ROXANA LEV, ROMINA EUGENIA MALTANERI

#### 143. 021 EFFECT OF *Ligaria cuneifolia* INFUSION ("Argentine mistletoe") ON PLASMATIC CHOLESTEROL, ERYTHROCYTE MEMBRANE CHOLESTEROL AND THE BLOOD FLUIDITY MEASURED AT HIGH FLOW RATE IN DYSLIPIDEMIC PATIENTS

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*Ligaria cuneifolia* (Lc) is a hemiparasitic plant which infusion is used to increase blood fluidity and decrease plasma cholesterol levels. Objectives: to analyze the effect of Lc infusion on plasma cholesterol levels (Cho) and red blood cells deformability at high shear rate, estimated by the erythrocyte rigidity index (RI) in patients with plasma cholesterol>200mg/dl. Methods: blood samples were collected for basal determinations (C) from 10 patients aged 50±15 years old attending the Cardiology Service at the Hospital Provincial Centenario. Each patient received lyophilized extract of Lc in tea bags to be taken for a month time. After 31 days, blood samples were again obtained (TLc). We assessed in plasma: Total Cho, HDLCho and LDLCho by colorimetric methods. In blood, we evaluated relative blood viscosity (RBVs) with rotational viscometer, erythrocyte membrane cholesterol (MCho) extracting lipids from the membrane of lysed red blood cells. RI was determined by a filtration method, with nucleopore membranes. Statistical analysis was performed using Wilcoxon test. Results: Median and confidence interval (CI 95%). Cho: C: 230 (205-278) vs. TLc: 230 (200-251) ns; HDLCho: C: 59 (42-84) vs TLc: 63.5 (35-76) ns; LDLCho: C: 185 (140-239) vs. TLc: 169 (135-215) \*; Blood: (RBVs) (cP): C: 2.79 (2.5-3.38); TLc: 2.99 (2.2-3.35) ns. MCho C: 0.9 (0.80-1.10) TLc 0.65 (0.5-0.9) \*\*; RI C: 14.38 (7.94-20.97) TLc: 12.24 (7.07-19.09) \* (\* p<0.05 vs C; \*\* p<0.01 vs C; ns: non significant vs C). Conclusion: in the patients studied, treatment with Lc generated a significant decrease in LDLCho, without promoting alterations in RBVs. At the cellular level, a decrease in MCho and RI was observed, im-

proving erythrocyte deformability at the level of the microcirculation. In addition, considering that high LDLcho plasma levels are related to atherosclerosis developing, the importance of these results lies in considering *Lc* feasible to be used for the prevention of cardiovascular diseases.

**144. 040 ESCHERICHIA COLI ALPHA HEMOLYSIN INCREASES RED BLOOD CELL SEQUESTRATION IN A MICROFLUIDIC DEVICE MIMICKING THE SPLEEN'S RED PULP**

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Uropathogenic *Escherichia coli* (UPEC) is a major cause of urinary tract infections, with alpha-hemolysin (HlyA) being a key virulence factor. The presence of HlyA correlates with the occurrence of pyelonephritis and sepsis, where HlyA can enter the bloodstream and bind to circulating red blood cells (RBCs). While hemolysis by HlyA is well-documented, its non-lytic effects on RBCs, such as increased cytosolic calcium, ATP release, and cell volume expansion have been less studied. We developed a microfluidic device based on a design proposed by Picot et al. (2015). This device features channels with slits of 5, 4, 3, and 2 micrometers, specifically designed to capture poorly deformable RBCs. The 2-micrometer slit mimics the red pulp fenestrations of the spleen, enabling quantification of RBC retention under pathological conditions. First, we investigated which HlyA concentrations elicited non-lytic alterations in RBCs loaded with Fluo-4, finding that between 3 and 8 ng/mL HlyA increased cytosolic calcium in over 60% of the cell population after 30 minutes at 37°C, without causing hemolysis. Treatment with 8 ng/mL HlyA significantly increased RBC sequestration in the 4, 3, and 2-micrometer slits, doubling the number of sequestered cells in comparison with a control without toxin. In contrast, 3 ng/mL significantly increased sequestration only in the 2-micrometer slit increasing, leading to a 50% increase in the number of sequestered RBCs. The non-acylated precursor of HlyA, ProHlyA, did not elevate cytosolic calcium or induce sequestration in the device. We conclude that treatment with non-lytic concentrations of HlyA induces mechanical retention in the slits of the microfluidic device. In vivo, this retention may facilitate the clearance of HlyA-bound RBC by spleen macrophages, thereby preventing vascular hemolysis during UPEC infections. Future experiments will elucidate the role of cell deformability and purinergic signaling in the HlyA-induced retention of RBCs.

**145. 045 ALTERED ANDROGENIC PATHWAY IN *in vitro* TESTOSTERONE STIMULATED PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA**

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Chronic Lymphocytic Leukemia (CLL) is the most common leukemia in our society, affecting mainly elderly males. It is characterized by proliferation and accumulation of mature CD5 positive B cells in blood, bone marrow and lymphatic tissues. Because CLL males have higher disease incidence and worse prognosis than women, the possible role of sex steroids in CLL has been studied. Regarding this, we evidenced diminished mRNA expression of enzymes associated with testosterone (T) metabolism, such as 5 alpha reductase (5 $\alpha$ -R) 1 and 3, in PBMC of CLL patients when compared to healthy controls (HC). Given this, the aim of our work was to evaluate if *in vitro* stimulation with T could reverse those effects in CLL patients. PBMC from HC (n=5) and CLL patients (n=6), males ages 40 - 90, were separated from whole blood using Ficoll gradient, and cryopreserved for further use. After thawing and vitality count, 5\*10<sup>5</sup> cells/mL were incubated with pokeweed mitogen (PWM) with or without exogenous T (PWM+T). Negative controls consisted of cells

with dimethylsulfoxide (DMSO). After incubation, cells were harvested, RNA was obtained with Trizol reagent and reverse transcribed to cDNA. Real-Time PCR was performed using L19 as housekeeping gene. Statistical analysis was carried out using R software. We found that PWM alone or with T increased mRNA expression of 5 $\alpha$ -R 1 in HC PBMC compared to DMSO, but with no significant differences between treatments. Adding T to PWM treated HC PBMC decreased 5 $\alpha$ -R 3 mRNA expression, yet with no significant differences between treatments. In CLL PBMC, mRNA expression of both enzymes was significantly lower than in HC PBMC, in all culture conditions (5 $\alpha$ -R1 p<0.05; 5 $\alpha$ -R3 p<0.001), and did not differ from its negative control. These results show that in CLL PBMC, exposure to exogenous stimulus does not activate the 5 $\alpha$ -R androgenic pathway. This could imply the existence of alterations in androgen receptor expression or function in individuals with CLL.

**146. 055 STUDY OF LEUKEMIC STEM CELLS (LSCS) IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS IN TREATMENT-FREE REMISSION (TFR)**

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CML patients receiving long-term tyrosine kinase inhibitor (TKI) therapy who achieve a sustained and deep molecular response (DMR) may discontinue treatment under strict molecular monitoring. Published data showed that dipeptidylpeptidase IV (CD26+) is a specific marker for CML leukemic stem cells (LSCs), expressed on the CD45+/CD34+/CD38- cellular fraction but not detected in other *BCR::ABL1* negative myeloid pathologies. Relapse after TKI discontinuation is likely due to the persistence of LSCs, which are transcriptionally quiescent and not detectable through conventional RNA studies. This study aimed to investigate the role of CD26+ LSCs in molecular relapses following TKI discontinuation. Peripheral blood samples from 20 CML patients in TFR (14 males and 6 females) were analyzed for the presence of CD26+ LSCs using flow cytometry (CD45/CD34/CD38/CD26 panel) and for *BCR::ABL1* transcript quantification by real-time PCR. Both analyses were performed simultaneously on the same sample. The median age at the time of therapy discontinuation was 59 years (range: 22–87). The first-line treatments were Imatinib, Nilotinib, or Dasatinib in 15, 4, and 1 cases, respectively. The median duration of treatment before discontinuation was 9.4 years (range: 5.1–18 years), and the median time off treatment was 0.8 years (range: 0.2–3.1 years). Five patients (25%) experienced early molecular relapse between 2.5 and 6 months after stopping TKIs, requiring immediate resumption of therapy due to the loss of Major Molecular Response (MMR) (*BCR::ABL1*  $\geq$ 10%). Among the five relapsed patients, one had detectable CD26+ LSCs, two did not present CD26+ LSCs despite molecular relapse and two were not evaluated. The remaining 15 patients continued in TFR, 2 in MMR and 13 in DMR (2/13 had detectable CD26+ LSCs without molecular relapse). Although the sample size is small, the data suggest that the presence of LSCs may not be directly associated with the loss of TFR.

**147. 063 EXPRESSION OF RELB-CONTROLLED GENES IDENTIFY HODGKIN AND REED-STERNBERG (HRS) CELLS IN SINGLE CELL TRANSCRIPTOME OF A CLASSIC HODGKIN LYMPHOMA PATIENT AND UNVEILS MACROPHAGES AS THE MAIN PARTNER IN HRS-IMMUNE CELLS COMMUNICATION WITHIN THE MICROENVIRONMENT**

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Hodgkin AND Reed Sternberg (HRS) cells are a classic Hodgkin Lymphoma (cHL) hallmark. We previously demonstrated the alternative NF $\kappa$ B arm (RelB/p52 mediated) critical role in HRS survival through expression arrays (induced vs uninduced shRelB) in the human cHL UH01 cell line. HRS account for 1% of the tumour being the rest immune cell types including T cells and macrophages with suppressive profiles. We used our expression array to evaluate differentially expressed genes (DEGs), functional genomics and network enrichment using conventional transcriptomic tools (limma R package, GO, Kegg, Reactome database, cytoscape). We aimed to identify a HRS cluster in a 19 years old cHL patient sRNAseq dataset. We hypothesized genes downstream RelB could be used to identify HRS. We selected 37 DEGs from our microarray that are known to affect HRS functions to identify a HRS cluster. We conducted a DEGs analysis of HRS cluster vs the rest of cell types DEGs analysis with Seurat 5.0. We considered eight main cell types identified as HRS (2.7%), macrophages (8.9%), Naïve CD4T (17%), memory CD4T (5.5%), effector CD4T (25.5%), CD8 NKT (20.5%), plasmacytoid dendritic (0.9%) and B cells (19%) according to the immune cells expression profile scType database. We further explored cognate receptor-ligand interactions among all eight clusters using CellPhoneDB 3.1 database. The highest number of HRS cells' interactions was with macrophages, CD8 NKT and CD4T effectors. We next explored all interactions HRS cells-macrophages through network clustering which included integrins and TAM receptor family-GAS6, involved in macrophages immune suppressive profile. Critical HRS functions shRelB affected in UHO1 cell line was used to identify HRS in a cHL patient scRNAseq. This showed that RelB controls expression of genes that provide the HRS identity in the milieu of inflammatory cells. The cHL microenvironment appears strongly influenced by macrophages controlled by a small HRS cells population.

**148. 159 SYNTHESIS AND METABOLISM OF SEX HORMONES IN LEUKOCYTES OF WOMEN WITH SYNDROME ANTI-PHOSPHOLIPID**

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Antiphospholipid syndrome (APS) is an autoimmune disease characterized by thrombo-inflammatory processes. There is a known sex bias in the incidence and clinical course of autoimmune diseases, with sex hormones proposed as important mediators. In this study, we investigate sex hormone synthesis in leukocytes from women with APS. We focus on aromatase (Aro), an enzyme involved in estrogen (E) synthesis, and the 5-alpha-reductase isozymes 1 (5 $\alpha$ R-1) and 3 (5 $\alpha$ R-3), involved in the metabolism of testosterone (T) to its active metabolite, dihydrotestosterone. Peripheral blood samples were collected from 25-45 years old women with APS (n=6) and healthy controls (HC; n=15). Peripheral blood mononuclear cells (PBMC) and polymorphonuclear cells (PMN) were separated by Ficoll gradient. RNA was isolated with Trizol reagent and reverse transcribed to cDNA. Real-time PCR was performed to quantify Aro, 5 $\alpha$ R-1 and 5 $\alpha$ R-3 levels. Relative expression levels were compared between groups using L32 as a housekeeping gene. In addition, the PBMC/PMN expression ratio for each target was calculated and statistically compared between groups. There was no expression of Aro in any cell type or patient group. APS was associated with lower levels of 5 $\alpha$ R-1 in PMN (p<0.05) and 5 $\alpha$ R-3 in PBMC (p<0.05) when compared to HC. In APS, the PBMC/PMN ratio for 5 $\alpha$ R-1 expression was higher than in HC (p<0.05), while no significant change was observed for 5 $\alpha$ R-3 expression. In conclusion, no evidence of E synthesis was found in leukocytes from HC and APS women. Regarding T metabolism, expression of the two reductase isozymes was

cell-type-specific. In APS, an imbalance in reductase expression was observed. Whether this is a cause or consequence of the disease is a key question to be answered. Our findings suggest a potential protective role of androgens in leukocytes, whose impairment could be associated to APS. Identifying precise mechanistic drivers of this disease is necessary to improve therapeutic outcomes.

**149. 266 VALIDATION OF NOVEL HUB GENES EXPRESSION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME AND SF3B1 MUTATIONS**

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Myelodysplastic syndromes (MDS) with pathogenic variants in the splicing factor (SF) *SF3B1* (*SF3B1*mut) are a recognized subtype associated with ring sideroblasts, good outcome and response to Luspatercept. Using bioinformatic approaches and publicly available RNA-seq data, we identified novel differentially expressed hub genes related to mitochondrial pathways and telomeric maintenance in *SF3B1*mut MDS patients. The aim was to validate the differential expression of the hub genes *NDUFA8*, *RBM25*, *MRRF* and *ACD* in *SF3B1*mut MDS patients. Total RNA from peripheral blood or bone marrow samples was collected from 50 MDS patients (21 *SF3B1*mut, 29 SF-wild type [wt, *SRSF2*wt and *U2AF1*wt]) and 42 healthy controls (HC). Gene expression was evaluated by quantitative real-time PCR and comparative method 2<sup>- $\Delta$ CT</sup> relative to *GAPDH* and *HPRT1*. Statistical analyses were performed using R version 4.4.1, p-values <0.05 were considered significant. These hub genes were significantly downregulated in MDS compared to HC (Mann-Whitney test p<0.0001). *SF3B1*mut patients presented a higher expression of *NDUFA8* (p=0.019), a lower of *RBM25* (p=0.043) and *ACD* (p=0.015) compared with SFwt, without a differential expression of *MRRF*. A principal component (PC) analysis of the expression levels discriminated *SF3B1*mut and SFwt patients from HC (explained variance: 77.9%; PC1: 54.7%, PC2: 23.2%) and clustered *SF3B1*mut within MDS patients (explained variance: 69.9%; PC1: 40.9, PC2: 29.0%). This last multivariate analysis highlighted *MRRF* as the major PC1 contributor (48.8%). These results validate the differential expression of hub genes that discriminate MDS patients from HC, and *SF3B1*mut within the MDS cohort. *MRRF*, the mitochondrial ribosome recycling factor, was key to distinguish *SF3B1*mut patients, despite no differential expression was observed among patients. Expression levels of these novel hub genes may provide substantial clues for the diagnosis and pathogenesis of the MDS-*SF3B1* subtype.

**150. 307 HEMOLYTIC DISEASE OF THE FETUS AND NEWBORN DUE TO ANTI-D IN A PREGNANT WOMAN WITH RHD DBT VARIANT**

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The D blood group antigen (Rh system) is the most clinically significant after the ABO system antigens. Partial RhD variants exhibit incomplete expression of the epitopes, differing enough from normal RhD to elicit the production of allo-anti-D, although this occurs ex-

ceptionally. We present a case of severe Hemolytic Disease of the Fetus and Newborn (HDFN) associated with the partial DBT variant. Case Report: BB: Born via cesarean section. Apgar: 3/8. Blood group O RhD(+), Direct Antiglobulin Test (DAT): Positive (++++). Diagnosis: erythroblastosis with anemia and jaundice. The treatment included RBC transfusion, platelet transfusion, exchange transfusion (one blood volume), intravenous immunoglobulin, and phototherapy. Discharged on day 7. MM: G2 C2. She had not been previously studied. She was typed immediately postpartum due to suspected fetomaternal immunohematological incompatibility and the need to transfuse the baby. Her RBCs showed a normal agglutination pattern with the routinely used monoclonal anti-D reagents, classifying her as Blood Group O RhD(+). Unexpectedly, her serum revealed the presence of an IgG class antibody with anti-D specificity. The DAT and serum agglutination test of the patient's RBCs with her own serum were negative. The D antigen was tested with a panel of monoclonal antibodies from the "ID-Partial RhD Typing Set" (BIORAD) directed against different epitopes of the RhD antigen, identifying a DBT phenotype in the mother. Molecular typing of RHD and RHCE genes was performed on DNA samples by PCR-SSP, identifying the DBT2 allele and confirming serological results.

Discussion: The anti-D identified in the pregnant woman carrying the partial DBT variant was responsible for severe HDFN. This case highlights certain limitations in the strategy universally implemented for the serological typing of the D antigen in patients and underscores the importance of strict adherence to prenatal immunohematological study protocols.

## INFECTOLOGÍA Y PARASITOLOGÍA

### P1 - POSTERS

FECHA Y HORA: 19/11/2024 11:00-12:00

COORDINADORES: SARACINO M. PRISCILA,  
AMARAL M. MARTA

#### 151. 086 CHROMOSOMAL DNA AND THE DNA-PROTECTING PROTEIN DPS ARE RELEASED INTO DOUBLE-MEMBRANE VESICLES BY ENTEROAGGREGATIVE ESCHERICHIA COLI IN STATIONARY PHASE

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Enteroaggregative *Escherichia coli* (EAEC) is a primary enteric pathogen that produces diarrhea in humans. While studying its ultrastructure, we made the serendipitous observation that bacteria were releasing extracellular vesicles different from those already described. Our hypothesis was that those vesicles could contain bacterial DNA. The purpose of this work was to study vesicles using ultrastructural, biochemical and molecular biology methods. The 17-2 bacterial strain was cultured in 2xYT broth at 37°C and aliquots were taken from 1 h post-inoculation until 7 days pi. At this point, bacteria were in late stationary phase. Cells were removed by centrifugation at 500 xg and filtration through 0,45 nm membranes. Vesicles contained in the supernatant were purified employing exoEasy affinity columns (Quiagen). The homogeneity of suspensions was determined by nanoparticle tracking analysis (NTA). Transmission electron microscopy (TEM) using thin sections and negative staining was employed to describe vesicles ultrastructure. Chromosomal DNA was characterized by Random Amplification of Polymorphic DNA-PCR (RAPD) and the Kleinschmidt method, and proteins were characterized by two-dimensional polyacrylamide gel electrophoresis and mass spectrometry. Dps was detected by Western blotting. Results indicated that EAEC produced classic outer membrane vesicles but also 100-120 nm width, double membrane vesicles with electron-dense cores containing chromosomal DNA, several proteins and the DNA-protecting protein Dps. The main finding was that these vesicles were observed only in late stationary bacterial growth phase. Our conclusions are that EAEC releases a poorly known kind

of vesicles composed of the outer and the inner membrane, containing chromosomal DNA together with a protein that protects DNA. This could be a mechanism of bacterial gene transmission after long periods of incubation that diminishes the eventual DNA damage due to extracellular-induced bacterial stress.

#### 152. 126 COMPREHENSIVE POSTMORTEM ANALYSIS OF COVID-19-RELATED ADULT MORTALITY IN BUENOS AIRES

Pablo Pakciarz<sup>1</sup>, Paola De la Iglesia<sup>2</sup>, Sebastián Esperante<sup>1,3,4</sup>, Javier Cotignola<sup>3,5</sup>, Ayelén Toro<sup>3,5</sup>, Damián Álvarez-Paggi<sup>1,3,4</sup>, Mauricio Caballero<sup>1,3</sup>.

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The pandemic associated with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that caused the outbreak of the coronavirus disease 2019 (COVID-19), has been a major public health challenge worldwide. This virus causes mild upper respiratory infection in most of the population; however, there's a 20% of people that develop pneumonia, respiratory failure or ARDS, which can lead to death. Understanding the possible mechanisms of death due to COVID-19 is key to planning and designing preventive and therapeutic strategies in the near future. We established a cross-sectional study aimed at identifying cause of death and possible molecular mechanisms of adults who died from SARS-CoV-2 using a combined approach of histopathology and microbiology assays in target organs obtained by Minimally Invasive Tissue Sampling (MITS), a novel autopsy technique. 17 patients were enrolled. Hypertension (11/17) and diabetes (8/17) were the most frequent comorbidities. Frequent histological findings were diffuse alveolar damage (DAD) (13/17), organizing pneumonia (8/17), capillaritis (14/17), liver steatosis (12/17), liver mild sinusoidal inflammation (14/17) and hemophagocytosis 9/14. 10/17 patients had coinfection and 6 had histological evidence of bacterial coinfection in the lung (one lung abscess, 5 cases of exudative pneumonia). Dead patients had significantly higher titers of neutralizing antibodies than controls ( $p < 0.05$ ). RNA extraction was successfully performed, and gene expression profiles were available from NanoString. KIR3DL3 expression was higher in the brain, liver, and lungs of dead patients. MITS can be safely performed and can be a useful tool in assessing superimposed infections in the context of long hospitalizations both for histological and genomic analysis. Immune complexes do not seem to be relevant in COVID-19 related deaths. KIR3DL3 was highly expressed in different organs.

#### 153. 158 RELATIONSHIP BETWEEN EMERGING SPECIES OF CANDIDA SPP. AND ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES IN PATIENTS WITH RHEUMATOID ARTHRITIS AND PERIODONTITIS

Murciano Roxana Mariel<sup>1</sup>, Felkar Selene Aldana<sup>1</sup>, María Isabel Brusca<sup>1</sup>, Garzón María Laura<sup>1</sup>, Morales Celina<sup>2</sup>, Maso Mariana<sup>3</sup>, Virginia Jewtuchowicz<sup>1,3</sup>

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Periodontitis (P) is characterized by the loss of alveolar bone around the tooth. It is a dysbiotic ecological niche where opportunistic pathogens such as *Candida* spp. develop. P contributes to the pathogenesis of rheumatoid arthritis (RA), as both are chronic inflammatory diseases. It has been documented that the microbiota in P induces the production of anti-cyclic citrullinated peptide (anti-CCP) antibodies, which are increased in RA. **Objective:** To study the prevalence of *Candida* spp. in P and its relationship with anti-CCP and anti-inflammatory treatment in patients with RA. **Materials and Methods:**

From 87 adult patients with RA (12 males and 75 females), the Disease Activity Score for rheumatoid arthritis (DAS-28) was obtained and compared with P and *Candida* spp. culture from oral and periodontal samples in 44 patients. Yeasts were identified by mass spectrometry and PCR. Percentages and Confidence Intervals were calculated with a 95% confidence level (CI 95%) using Epi Info. **Results:** 43% (CI 95%: 0.267-0.567) of patients with high or moderate active RA (mean DAS-28: 4.01 to 6.03) presented P, while only 20% with low or mild active RA (mean DAS-28: 2.32 to 2.94). In P, a 13.6% (CI 95%: 0.052-0.274) of subgingival *Candida* was found. *C. albicans* was predominant in both sites. Other emerging species such as *C. glabrata* were also found. In this preliminary study, no relationship was found between *Candida* spp. and anti-CCP. **Conclusions:** Periodontitis is common in patients with RA, especially in those with high or moderate activity. Although the subgingival niche in patients with active RA is suitable for the development of *C. albicans*, its prevalence was lower than in individuals without RA, which could be due to the anti-inflammatory treatment they receive. We emphasize the importance of periodontal control in patients with RA, as despite the anti-inflammatory treatment they receive, they are at risk of suffering disseminated infections from the periodontal focus.

**154. 173 EVALUATION OF PATIENTS IN A FEBRILE UNIT DURING A DENGUE OUTBREAK: DEVELOPMENT OF A LOCAL SCREENING STRATEGIES**

Alejandro Aymar<sup>1</sup>, Guadalupe Verneti<sup>1</sup>, Patricia Delnero<sup>1</sup>, Pablo Manjarín<sup>1</sup>, Carlos Hurtado<sup>1</sup>, Guillermo Keller<sup>1,2</sup>.  
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**Introduction:** In the 2023-2024 period, dengue cases reached a historic peak that significantly increased consultations to the health system, requiring cost-efficient algorithms to detect patients at risk. **Aim:** Detect symptoms and determinations that allow adequate screening of the population that requires special treatment and early hospitalization. **Methods:** A special unit was used to care for febrile patients. All cases were recorded in an electronic file, along with the symptoms of presentation, time of evolution, and determinations made. The file was linked by identification codes with evolution data allowing the identification of subsequent hospitalizations, event reports, new consultations, and fatal outcomes. **Results:** 2741 patients had 3905 consultations in 90 days. 797 (20%) patients were confirmed for dengue (antigen/PCR). 39 (1% of consultations) required hospitalization (5 positive dengue, 11 negative dengue, and 36 for problems that did not require testing). Additionally, 4 identified patients were admitted without previously outpatient consultation (3 with a diagnosis of severe dengue, and 1 with multiple trauma and incidental finding of dengue; of which 2 had a fatal outcome). Patients with positive dengue were associated ( $P < 0.05$ ) with lower counts of: white blood cells (5318 vs. 4197), platelets (257,000 vs. 175,000) and higher SGOT (57 vs. 33) and SGPT (55 vs. 36) at the first consultation. The best screening determinations for dengue were identified as the combination of platelets  $< 200,000$  and white blood cells  $< 6,000$  (sensitivity of 86% and specificity of 78%). The analysis of the markers of unfavorable evolution (hospitalization, hemorrhage and death) showed that the most sensitive indicator at the first consultation of these patients is severe thrombocytopenia  $< 50,000$  (sensitivity 100%, specificity 75%). **Conclusions:** During Dengue outbreak simpler screening determinations can be useful until the appropriate testing are available.

**155. 433 SOLUPLUS® NANOMICELLES AS ADJUVANT FOR SHIGA TOXIN TYPE 2 IMMUNIZATION**

Angie Muñoz<sup>1,2</sup>, Claudia Guerrero<sup>1,2</sup>, Analia Lopez Diaz<sup>1,2</sup>, Daniel Giron Reyes<sup>1,2</sup>, Diego Chiapetta<sup>3,4,5</sup>, Marcela Moreton<sup>3,4,5</sup>, Flavia Sacerdoti<sup>1,2</sup>.

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Shiga toxin type 2 (Stx2) is the main virulence factor of Shiga toxin-producing *Escherichia coli* (STEC) and is responsible for developing hemolytic uremic syndrome (HUS). Nanocarriers, such as polymeric nanomicelles (NM) are promising technologies for the enhancement of transcutaneous immunization in vaccine formulations. Soluplus® is an amphiphilic polymer that spontaneously forms nanomicelles in aqueous solutions. In this work, we propose that NM may be a good adjuvant for the induction of neutralizing anti Stx2 antibodies that may protect against HUS. For that, purified Shiga toxin type 2 B subunit (Stx2B) was associated with NM by stirring. Then, three BALB/c mice groups (n=3-6) were immunized with three doses of antigen separated by 15 days each: NM, Stx2B, NM-Stx2B and Stx2B. Blood was collected from the lateral saphenous vein at 0, 30 and 45 days after the first dose (d30 and d45). Antibody titers (anti Stx2B-Ab) were evaluated by ELISA. After immunization, all the mice groups were challenged with a 100 lethal dose of Stx2 (LD100). Food intake, weight and survival were registered daily for eight days. The NM-Stx2B group showed a significant increase in anti-Stx2B-Ab at d45 (\*\*p<0.01). On the contrary, the Stx2B group did not exhibit this increase. Freund-Stx2B immunized mice showed a significant titer of anti-Stx2B-Ab at d30 and d45 (\*\*p<0.001). Furthermore, NM-Stx2B immunized mice showed mild weight loss until 4 days after Stx2 injection with a total weight recovery after 8 days of Stx2 challenge. Mice immunized only with Stx2B showed a similar behavior as the NM-Stx2B group but with a lower survival rate (80% vs 100 %). All the mice immunized with NM died after 4 days of toxin challenge. These preliminary results showed that NM may potentiate the Stx2B immune response. Low levels of anti-Stx2B-Ab may be protective against Stx2 since Stx2B immunized mice survived to a Stx2 DL100 dose. Further studies may be needed to clarify the mechanism of protection and the possible enhancement of response due to NM.

**156. 501 DENGUE VIRAL DISEASE: FRACTAL ANALYSIS FOR PREDICTIVE PURPOSES IN THE ARGENTINE REPUBLIC**

Fabrizio Nazareno Trabachino<sup>1,3</sup>, María Delamorclaz<sup>1,3,4</sup>, Juan Ignacio Vansteenkiste<sup>1,2,3,4</sup>, Micaela Alaniz<sup>1,3,4</sup>; José Octavio Carloni<sup>1,3,4</sup>, Nicolás Giraudo<sup>1,3,4</sup>, Cielo Ailén Pérez Obeid<sup>1,3,4</sup>, Nerea, Vila<sup>1,3,4</sup>, Juan José Negri González<sup>1,4</sup>; María Clara Civetta<sup>1,3,4</sup>, Juan Pablo Trabachino<sup>1,3,4</sup>, Damián Lerman Tenenbaum<sup>1,3, 4</sup>, Jorge Luis Molinas<sup>1,2,3,4</sup> & María Eugenia Cabral<sup>1,3,4</sup>,  
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Dengue viral disease (DVD), it is currently the most important arbovirus at the Americas and finds in the Argentine Republic (AR) the suitable conditions to develop its epidemic cycle (EC) during the epidemiological weeks (EW) between the months of November to the end of the following year. The EVD would reproduce a fractal rhythm feasible to be analyzed by mathematical algorithms that determine Fractal Dimension (FD) and Predictive Determination Coefficient (R<sup>2</sup>). It was decided to analyze the temporal frequency of DVD in the AR by Higuchi's Algorithm (HA) with predictive purposes. Observational, longitudinal, retrospective study. The positive cases (CP) of DVD were considered according to what was published in bulletins of the Ministry of Health of the Nation, considering from the 2017 cycle to April 2024, excluding cases outside the epidemiological period. HA was applied to PC weekly registry. FD and R<sup>2</sup> were obtained by EC and Pearson correlation coefficient (r) was determined by fractal parameters. Results by EC: 2017-2018: FD = 0,38, R<sup>2</sup> = 0,43; EC: 2018-2019: FD = 0,25, R<sup>2</sup> = 0,08; EC: 2019-2020: FD = 0,7, R<sup>2</sup> = 0,3; EC: 2020-2021: FD = 0,92, R<sup>2</sup> = 0,77; EC: 2021-2022:

FD = 0,91, R<sup>2</sup> = 0,69; EC: 2022-2023: FD = 2.35, R<sup>2</sup> = 0.88; EC: 2023-2024: DF = 1.36, R<sup>2</sup> = 0.88. Correlation:  $r = 0,91$  ( $p < 0,0307$ ). Conclusion: HA shows that DVD vector at present finds the environmental conditions for its development, if these conditions persist, it will find a favorable niche for its growth in the EC 2024-2025.

## P2 - POSTERS

FECHA Y HORA: 21/11/2024 11:00-12:00

COORDINADORES: GOMEZ MEJIBA SANDRA,  
RUBINSTEIN GUICHON MARA ROXANA

### 157. 049 INTERFERON GAMMA DEFICIENCY AS A KEY INDICATOR OF SEVERE DENGUE AND A TARGET FOR THERAPEUTIC INTERVENTION

Agustina Sabater<sup>1,2,3</sup>, Ines Achinelli<sup>1,2</sup>, Paula Perbolianachis<sup>4,5</sup>, Javier Cotignola<sup>1,2</sup>, Elba Vazquez<sup>1,2</sup>, Gonzalo Moratorio<sup>4,5</sup>, Juan Manuel Carballeda<sup>6</sup>, Geraldine Gueron<sup>1,2</sup>, Ayelen Toro<sup>1,2</sup>

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In 2024, more than 11 million suspected cases of Dengue Virus (DENV) infection were registered across the American continent, and, in Argentina, its incidence is 12 thousand cases per million inhabitants. DENV generates mild to severe symptoms, such as Dengue Hemorrhagic Fever (DHF), and fatal cases even in individuals without risk factors; however, there is currently no specific treatment for Dengue. Given the critical role of IFN- $\gamma$  in immune defense against DENV infection, we propose that deficiencies in this pathway are linked to more severe clinical symptoms. We first evaluated the relevance of IFN- $\gamma$  signaling pathway in Dengue Fever (DF) patients by analyzing the GSE174482 dataset (n=40), which contains transcriptomics data from peripheral blood mononuclear cells (PBMC) gated by IFN- $\gamma$  expression ( $p < 0.001$ ). Multidimensional scaling analysis clearly distinguished IFN- $\gamma$  positive and negative subpopulations. Furthermore, differential expression analysis demonstrated distinct transcriptional profiles, confirming the global role of the IFN- $\gamma$ -mediated response in DENV infection. Additionally, we investigated the association between IFN- $\gamma$ -mediated response and disease severity by exploiting the GSE215835 dataset (n=11), which contains transcriptomics data from PBMCs of patients with classic DF and DHF. We observed decreased IFN- $\gamma$  expression in patients with severe DHF compared with DF patients ( $p = 0.0172$ ). Gene Set Enrichment Analysis (GSEA) revealed "Response to Interferon Gamma" as one of the most enriched pathways in DHF vs DF differential expression analysis (adj.p=0.035). Gene Set Variation Analysis (GSVA) followed by unsupervised clustering of IFN- $\gamma$  categories efficiently grouped patients according to disease severity. In conclusion, our results demonstrate that IFN- $\gamma$  pathway is relevant in the context of DENV infection and its downregulation is associated with disease severity, positioning IFN- $\gamma$  pathway as a potential therapeutic target for managing DENV infection.

### 158. 284 SPECTRAL FLOW CYTOMETRY (SFC) AND NANOPARTICLE TRACKING ANALYSIS (NTA) OF STX2-CARRYING MICROVESICLES: INVESTIGATING THEIR CYTOTOXIC EFFECTS ON HUMAN GLOMERULAR ENDOTHELIAL CELLS (HGEC)

Fernando Gomez<sup>1,2</sup>, Claudia Guerrero<sup>1,2</sup>, Daniel Girón Reyes<sup>1,2</sup>, Marcela Cuher<sup>3,4</sup>, Soledad Collado<sup>5</sup>, Plácida Baz<sup>5</sup>, Ariel Billordo<sup>5</sup>, Alejandro Ballestracci<sup>6</sup>, Roxane Piazza<sup>7</sup>, Fla-

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Microvesicles (MVs: 10-1000 nm) are involved in physiological and pathological conditions, such as Hemolytic Uremic Syndrome caused by Shiga toxin (Stx)-producing *Escherichia coli* (STEC-HUS). Recently, we detected circulating MVs carrying Stx2 (MVs-Stx2) in STEC-HUS patients. This study aimed to characterize MVs generated under *in vitro* conditions using SFC and NTA and evaluate their potential cytotoxicity on HGEC viability, in the presence or not of Stx2 receptor inhibitor, Eliglustat (EG). For MVs-Stx2 obtention, peripheral blood samples of healthy donors were stimulated with 200 ng/mL Stx2 for 1 h at 37°C under gentle shaking. Unstimulated and stimulated blood samples were ultracentrifuged and washed with PBS to obtain an enriched suspension in MVs-Ctrl or MVs-Stx2, respectively. For SFC, MVs were labeled with Annexin V-FITC, and an anti-Stx2 antibody detected Stx2 in MVs. MVs-Stx2 cytotoxicity was analyzed, by neutral red uptake assay, on HGEC pretreated or not with 1  $\mu$ M EG for 24 h and then incubated for 72 h with MVs-Stx2 (5.3x10<sup>9</sup>) washed five times to deplete free Stx2 traces. MVs showed a concentration of 2.6 x10<sup>11</sup>/mL with four different population sizes: 162 (65%); 259 (15%); 304 (13%); 360 nm (6%) by NTA and a Zeta Potential of -7.87 mV. MVs-Stx2 showed a concentration of 1.7 X10<sup>11</sup>/mL, five different population sizes: 201 (43%); 278 (18%); 315 (18%); 156 (13%) nm, and a Zeta Potential of -5.01 mV. SFC results indicated that 31.0 $\pm$ 2.7% of the total MVs were Stx2 positive (MVs-Stx2, n=2). Also, MVs-Stx2 significantly decreased HGEC viability (34.4  $\pm$  1.2 % vs 97 $\pm$  4% MVs-Ctrl, n = 3). Finally, EG prevented (55%) the MVs+Stx2 cytotoxicity (viability % = EG+MVs-Stx2: 70.6 %  $\pm$  5.4 % vs MVs + Stx2: 34.4  $\pm$  1.2 %, n = 3,  $p < 0.05$ ). MVs-Stx2 were obtained *in vitro* in a considerable percentage compared to total MVs and exhibited different numbers and population sizes. Moreover, we demonstrated for the first time the MVs-Stx2 cytotoxicity on HGEC and its prevention by EG.

### 159. 287 ENZYME-LINKED IMMUNO SORBENT ASSAY (ELISA) FOR SHIGA TOXIN DERIVED FROM MICROVESICLES DETECTION

Claudia Guerrero<sup>1,2</sup>, Angie Muñoz<sup>1,2</sup>, Daniel Giron Reyes<sup>1,2</sup>, Fernando Gomez<sup>1,2</sup>, Roxane, Piazza<sup>3</sup>, María Marta Amaral<sup>1,2</sup>, Flavia Sacerdoti<sup>1,2</sup>.

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Shiga toxin-producing *Escherichia coli* gastrointestinal infection may develop Hemolytic Uremic Syndrome (HUS). Detection of STEC or Stx2 is an important issue to have a definitive diagnosis. It has been described that Stx2 may be transported to the target cells through microvesicles (MVs-Stx2). Previously we detected MVs-Stx2 in HUS patients by flow cytometry. This work aimed to develop an ELISA for Stx2-MVs detection in order to propose it as a routine complementary tool for STEC infection diagnosis. MVs-Stx2 were obtained

by stimulating normal blood with purified Stx2 (200 ng/mL) for 1 h at room temperature (RT). Then, plasma was obtained by blood centrifugation and further centrifuged to obtain platelet-poor plasma (PPP). Finally, MVs were obtained after ultracentrifugation of 200  $\mu$ L of PPP at 23,000 g for 20 min at RT. Total obtained MVs were treated with different detergents to release Stx2 (Triton 0.075%, Tween 10%, guanidinium chloride 200 mM, saponin 0.5%). ELISA was performed in 96 well plates coated with an anti-Stx2 mAb. Mvs-Stx2 samples treated or not with detergents were incubated for 1 h at RT. Then, a rabbit anti-Stx2 pAb was incubated for an additional hour. Detection was revealed with a secondary antibody conjugated with HRP and OPD substrate was used. A standard curve was performed with purified Stx2 (0.073-300 ng/mL) and negative controls (blood without Stx2 stimulation) were used to determine the limit detection (LD) of the technique. We obtained a concentration of Stx2 50 ng/mL in MVs from Stx2 stimulated blood treated only with saponin 0.5%. The detection limit of the method was set at 6.7 ng/mL. Further technique optimization will be necessary to detect concentrations above the LD obtained in this ELISA model for detection in suspicious STEC infected patients. Improving sensitivity by increasing the volume sample, using chemiluminescence and streptavidin-biotin system for detection would be necessary to render this method proper for HUS diagnosis.

**160. 342 TOWARDS PRECISION MEDICINE FOR PEOPLE LIVING WITH HIV-1. ANALYZING THE EMERGENCE OF ARV RESISTANCE FROM MINOR VIRAL VARIANTS (NGS VS SANGER)**

de La Fuente Sofía<sup>1</sup>, Castro Gonzalo M.<sup>2</sup>, Sánchez Loria Julián<sup>3</sup>, Delea Marisol<sup>1</sup>, Bruque Carlos D.<sup>1</sup>, Corró Guillermo<sup>1</sup>.

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**Introduction:** Highly Active Antiretroviral Therapy (HAART) resistance viral variants can significantly reduce the effectiveness of available treatments and increase the risk of therapeutic failure. The early detection of these kinds of variants may direct the therapy scheme to the most effective treatment. The global prevalence of transmitted resistance to antiretroviral drugs (ARV) is estimated within the range of 10% to 17% among people living with HIV-1 who are ARV treatment naïve. Non-nucleoside reverse transcriptase inhibitors (NNRTI) resistance-associated mutations are found in approximately 5.9% of these individuals, while mutations conferring resistance to NRTIs or protease inhibitors are transmitted in around 1.3%. Our aim was to analyze the relevance of minority variants not detected by the gold standard method (Sanger sequencing) and their role in the development of resistance to HAART in the short or medium term. **Material and Methods:** In this study, we examined 18 samples of living with HIV with viral loads >1000 copies/mL. Viral RNA was extracted from plasma using Quick RNA Viral kit (Zymo), and then sequenced using MiSeq Illumina (by RT/PT and IN fragments as the gold standard method. The sequencing data were aligned to the HXB2 HIV reference genome, and variants were analyzed using Stanford University HIV Drug Resistance Database. **Results:** Our preliminary results reveal an excellent correlation between methodologies, showing higher sensitivity when sequences were obtained by NGS compared with SANGER. The analysis also revealed the presence of resistance variants <20%, that could not be found by the gold standard SANGER sequencing method. We are still analyzing the risk of resistance emergence in those patients. **Conclusion:** We developed a method to assess HAART resistance using NGS for the Public Health System, which has higher sensitivity than SANGER and can alert to the risk of resistance emergence in both naïve and treated patients.

**161. 463 EVALUATION OF A NOVEL M-ANTIGEN ELISA FOR THE DIAGNOSIS OF HISTOPLASMOSIS IN IMMUNOCOMPROMISED PATIENTS IN SANTA FE - ARGENTINA**  
Julián Abud<sup>1,2</sup>, Matías Cabeza<sup>3</sup>, Manuela Loaiza-Oliva<sup>3</sup>, Gui-

lermo García-Effron<sup>3</sup>, Horacio Rodríguez<sup>1,2</sup>.

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*Histoplasma capsulatum* (*H. capsulatum*), the dimorphic fungus responsible for histoplasmosis, is a prevalent opportunistic pathogen, particularly in immunocompromised individuals such as those with HIV, post-transplant patients, and those with oncohematological diseases. Histoplasmosis is the most common endemic mycosis in Argentina and is widespread in Latin America. The World Health Organization (WHO) recommends antigen detection in urine due to its high sensitivity and rapid diagnosis. However, few commercial kits are available in our country, they are expensive, and all are based on the detection of membrane polysaccharides, which exhibit cross-reactivity with other related fungi. Protein M-based immunoassays present a promising alternative for the specific and accurate diagnosis of histoplasmosis. Our study aimed to develop an ELISA targeting the M glycoprotein of *H. capsulatum*. We established a first prototype anti-M sandwich ELISA (EIA anti-M) and evaluated its functional parameters, including a detection limit of 0.5 ng/ml, a linear range of 0.15 – 0.01  $\mu$ g/ml, sensitivity of  $4.7 \times 10^{-3}$  Abs.ml/ng and specificity. The performance of this ELISA was compared with the commercial Clarus Histoplasma GM EIA/IMMY kit, using 156 urine samples from immunocompromised patients, including those with recent transplant (n=124), HIV (n=22), and leukemia with aspergillosis (n=10). Of the samples tested, 149 were categorized as negative and 7 as positive by our ELISA, with 3 positives confirmed by the IMMY kit, showing a 96% concordance between the two methods. These findings suggest that M antigen detection immunoassays could offer a rapid and accurate diagnostic tool for histoplasmosis. Further validation with a larger sample size and confirmed histoplasmosis cases is necessary to confirm these promising results.

## INMUNOLOGÍA E INFLAMACIÓN

### P1 - POSTERS

FECHA Y HORA: 19/11/2024 16:10-17:10

COORDINADORES: ADA BLIDNER, SANDRA GOMEZ MEJÍA, ROSANNA RAMHORST

**162. 143 THE TUMOR MICROENVIRONMENT AFTER BNCT: PRO OR ANTITUMORAL RESPONSE AND BYSTANDER EFFECT IN COLORECTAL CANCER**

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**Abstract:** Radiotherapy (RT) can induce an immune response by modulating the tumor microenvironment (TME), but little is known about the effect of particle RT in this regard. Boron Neutron Capture Therapy (BNCT) is a particle therapy based on the concentration of <sup>10</sup>B in the tumors followed by the irradiation with neutrons triggering the reaction <sup>10</sup>B(n, $\alpha$ )<sup>7</sup>Li, causing short range damage. The effectiveness of photon RT for colorectal cancer (CRC) is sometimes limited by the lateral scattering of the beam. BNCT is an alternative



since it allows higher doses of radiation in the tumor and less in the nearby organs. Aim: 1) Identify components of the TME after BNCT that could condition immune system response. 2) Study Bystander influence on the proliferation and migration of not irradiated cells. Material and Methods: HT29 colorectal carcinoma cells were incubated with BPA (50ppm) and irradiated with neutrons. Cytosolic dsDNA detection by immunofluorescence and INF $\beta$ , Galectin1, IRF1, Nox1 and Nox5 expression by qPCR were performed. Culture mediums from irradiated cells were used as conditioned mediums (CM) to study the bystander effect on migration and proliferation. Results: Cytosolic dsDNA increased in cells BNCT treated ( $p < 0.001$ ). INF $\beta$  highest levels were at 8 Gy+BPA ( $p < 0.001$ ). Galectin 1 expression was similar to controls in BNCT treated cells. IRF1 mRNA increased at 3 and 8 Gy -BPA ( $p < 0.05$ ,  $p < 0.01$ ). Nox1 increased at 8 Gy +/- BPA ( $p < 0.05$ ) and Nox5 increased at 8 Gy+BPA ( $p < 0.05$ ). CM of 8 Gy+BPA induced cells migration of not irradiated cells ( $p < 0.05$ ) but had no effect on cells proliferation. Conclusions: BNCT increase cytosolic DNA and through INF $\beta$  activates cGAS-STING pathway, exerting anti-tumor responses. Galectin1 decrease could be a positive response. Persistence of NADPH induction represents long term damage which could be either beneficial or detrimental. The bystander effect on cell migration demonstrates the impact of RT out of the field of irradiation.

**163. 215 ORAL HEALTH LINKED TO PHYSICAL FITNESS AND MUSCLE INJURIES IN SOCCER PLAYERS**

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Introduction: Studies regarding the link between sports and oral health revealed that high performance athletes have poor oral health, which can lead to impaired physical condition and predispose them to injuries. Objective: Analyze the link between oral health, physical fitness (VO<sub>2</sub> and muscle strength), and the incidence of muscle injuries in professional male soccer players. Methodology: The project was approved by the Odontology Bioethics Committee, with informed consent. Twenty professional male soccer players aged 18-22 from Club Newell's Old Boys were evaluated. Dental evaluations were carried out by the Community Periodontal Index Treatment Needed (CPITN) and periodontogram. In addition, a deadlift strength test (kg) and a VO<sub>2</sub> max test (mL/min<sup>-1</sup>/kg<sup>-1</sup>) were executed. Then, a medical record review about non-contact muscle injuries throughout the season was carried out. Statistical analysis was performed using SPSS software, and the Student's t test was applied to compare groups (mean  $\pm$  SD). Hedges' g was used to estimate the effect size. Results: According to a dental exam, eleven players had poor or very poor oral health; five players had one or more gums bleeding (25%); and five players had one or more lost teeth (25%). CPITN was 0-1 in nine players (group 1) and 2-3 in eleven players (group 2). Only one player missed a match due to a dental problem. We found significant differences in noncontact muscle injuries (2.33  $\pm$  1.22 for group 1 and 3.8  $\pm$  1.39 for group 2; \*  $p < 0.05$ ), with a large effect size ( $g = 1.114$ ). Overload and cramp assays showed the greatest differences between the groups. In the case of the deadlift (97.13  $\pm$  17.76 vs. 96.88  $\pm$  9.28) or VO<sub>2</sub> max (54.41  $\pm$  1.74 vs. 54.62  $\pm$  2.41), no significant differences were observed. Conclusions: These preliminary findings suggest that poor oral health is a factor in the potential risk of noncontact muscle injuries in soccer players, while physical performance and strength were not affected.

**164. 244 PULMONARY NEUTROPHILIC INFLAMMATION IN MICE FED AN OBESOGENIC DIET**

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A dietary chronic positive-energy balance causes low-grade systemic inflammation. Neutrophilic inflammation (NI) is the homing and activation of neutrophils in tissues with local release of myeloperoxidase (MPO) and generation of HOCl causing chlorinating stress, a marker of NI. Herein we hypothesized that a dietary chronic positive energy balance can cause pulmonary NI. To test this we fed, ad libitum, 2 groups of male C57 mice ( $n=32$  each) for 24 weeks with either an obesogenic diet (ObD) consisting of rodent chow containing a 22% bovine fat (483 kCal/100g) and 10% fructose in drinking water (3.9 Kcal/ml), or a control diet (CoD) containing rodent chow (329 Kcal/100g) and tap drinking water. Weight gain, blood pressure, insulin resistance, and adiposity index were higher in ObD compared to CoD. Plasma concentrations of total cholesterol, triglycerides, glutamic-oxaloacetic transaminase (GOT), and glutamic-pyruvic transaminase (GPT) were higher in ObD compared to CoD mice. Compared to the lungs of CoD, ObD mice had a higher number of BALF neutrophils, content, and activity of MPO, chlorotyrosine-modified proteins, inducible nitric oxide synthase, and IL-6. Interestingly, when instilled with bacterial lipopolysaccharide or dead *Pseudomonas aeruginosa*, ObD mice had higher MPO content and activity, and chlorinated proteins than CoD mice. These data corroborate a higher content and activation of neutrophils in the lung of ObD mice than in the CoD mice. A chronic dietary positive energy balance causes pulmonary neutrophilic inflammation in mice.

**165. 246 THE NITRONE SPIN TRAP 5,5-DIMETHYL-1-PYRROLINE-N-OXIDE REDUCES HEPATIC NEUTROPHILIC INFLAMMATION AND INSULIN RESISTANCE IN A MOUSE MODEL OF DIET-INDUCED OBESITY**

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A chronic positive-energy balance causes low-grade systemic inflammation in diet-induced obesity (DIO) models. This causes the activation of the expression of inflammatory biomarkers, such as inducible nitric oxide synthase (iNOS), inflammatory cytokines, and adhesion molecules for neutrophils in tissue microvasculatures. In this study, our objective was to determine whether the nitron 5,5-dimethyl-1-pyrroline N-oxide (DMPO) is capable of reducing hepatic neutrophilic inflammation, and systemic inflammation; and consequently improving insulin sensitivity in a DIO mouse model. To achieve this goal, we fed male C57 mice a control diet (CoD, rodent chow and tap water) or a hypercaloric diet (ObD, 22% bovine fat + 10% fructose in drinking water) for 24 weeks. Food and water intake, body weight, and caloric intake were measured weekly. At the end of the dietary regime, serum IL-6, iNOS, nitrotyrosine formation (a marker of NO-induced oxidative stress), chlorotyrosine (a marker commonly used as a measure of neutrophilic inflammation), and MPO content were assessed by ELISA in liver homogenates. Mice fed the ObD for 24 weeks showed lower food intake than the CoD group, but higher water with fructose consumption, resulting in a higher total caloric intake, which was reflected in increased body weight, and higher epididymal fat content, adiposity index, and insulin resistance (glucose tolerance test). Additionally, ObD mice showed higher IL-6 concentration in serum, suggesting a state of chronic systemic low-grade inflammation and insulin resistance. Mice injected with DMPO (2.5  $\mu$ mol/mice/day for 7 days) or saline, a week before the end of the regimen, showed decreased hepatic inflammation, as well as improved insulin sensitivity, as compared to those mice injected with saline. By blocking the expression of neu-

trophil adhesion molecules and pro-inflammatory cytokines, DMPO can reduce hepatic neutrophilic inflammation and improve insulin resistance in obesity.

**166. 248 THE NITRONE 5,5-DIMETHYL-1-PYRROLINE-N-OXIDE PREVENTS PULMONARY NEUTROPHILIC INFLAMMATION AND GENOTOXICITY IN A MOUSE MODEL OF ACUTE RESPIRATORY DISTRESS SYNDROME**

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Lipopolysaccharide (LPS), is a component of the outer membrane of Gram-negative bacteria, such as *Pseudomonas aeruginosa* (*Pa*) that causes redox changes and inflammatory NF- $\kappa$ B-controlled gene expression. In the pulmonary microvasculature, the latest controls the expression of adhesion molecules which can slow down neutrophil migration and further activation (a process known as pulmonary neutrophilic inflammation, PNI). During PNI, myeloperoxidase (MPO) is released and can be taken up by surrounding airway epithelial cells, where it can oxidize chloride anions to HOCl. HOCl is a powerful oxidant that can damage the genomic DNA by free radical-operated mechanisms. Herein we aimed to test in vivo whether the nitrone 5,5-dimethyl-1-pyrroline N-oxide (DMPO) can prevent DNA-centered radical formation in C57 male mice exposed to dead *Pa*. After 24 h of oropharyngeal aspiration of *Pa* ( $5 \times 10^7$  cells), we found a marked PNI, as assessed by a large infiltration of neutrophils, MPO (content and activity), and chlorotyrosine-modified proteins in the BALF and lung parenchyma. We also found increased expression of ICAM-1, inducible nitric oxide synthase, and content of nitrotyrosine-modified proteins in the lung parenchyma, as well as increased pro-inflammatory cytokines in serum. These changes were blocked when DMPO (2.5  $\mu$ mol/mice) was administered 1h before the exposure to *Pa*. DMPO also trapped protein- and DNA radicals in the lung parenchyma forming protein- and DNA-nitron adducts (immuno-spin trapping technique). These data indicate that DMPO reduces *Pa*-induced genotoxicity and proteotoxic stress. Taken together, these data are consistent with an effect of DMPO interfering with the LPS-triggered signaling causing inhibition of the expression of genes under the transcriptional control of NF- $\kappa$ B, thus blocking PNI. The nitrone spin trap can serve as a novel structural platform for the design of novel drugs to reduce death associated with sepsis and genotoxic damage to the airways.

**167. 250 ASSESSMENT OF IMMUNE-RELATED RHEUMATOLOGICAL (IrAEs) EVENTS IN A MONOVALENT ONCOLOGY CENTER IN ARGENTINA. EXPERIENCE OF THE INSTITUTE OF ONCOLOGY ÁNGEL H. ROFFO'S RHEUMATOLOGY DEPARTMENT**

Collado María Victoria<sup>1</sup>, Gandur Natalia<sup>2</sup>, Ranuncolo Stella Maris<sup>3</sup>, Cugliari Silvana<sup>4</sup>, Castro Mónica<sup>5</sup>, Cuartero Viviana<sup>6</sup>, Cáceres Valeria<sup>7</sup>, Serrano Marcela<sup>8</sup>, Casas Parera Ignacio<sup>9</sup>, Cinat Gabriela<sup>10</sup>, Zerga Marta<sup>11</sup>.

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Immunotherapy drugs, named as Immune Checkpoint Inhibitors (ICI), block checkpoint proteins from binding with their corresponding partner proteins. The activation of the immune system can lead to the emergence of de novo autoimmune manifestations or reactivation of pre-existing autoimmune diseases, known as immune-related adverse events (IrAEs). We aimed to describe the IrAEs features in

oncological patients treated with ICIs, determining the oncological treatment continuation following their development and investigating whether pre-existing rheumatologic disease (PRD) was the reason for not initiating the ICI treatment. A total of 159 oncology patients received ICIs at the Roffo Institute during 2019-2022. A descriptive and retrospective study with 40/159 referred to the Rheumatology Department was performed. 16/40 experienced rheumatologic IrAEs (IrAEs). The IrAEs treatment was as follows 5/16 received non-steroidal anti-inflammatory drugs, 11/16 corticosteroids (5/11 required additionally Disease-Modifying Antirheumatic Drugs). The outcome was complete response (9/16), partial response (2/16) and no response (5/16). As a consequence 7/16 patients (44%) were able to continue the ICI therapy, 3/16 (19%) experienced a temporary suspension and 6/16 (37%) a permanent discontinuation. Most of the IrAEs observed were mild to moderate. Only a myositis case was a severe life-threatening IrAE. Combined treatment with anti-CTLA-4 and anti-PD-1 posed a higher risk of more severe IrAEs. In conclusion, 1 in 4 ICI exposed patients presented rheumatologic symptoms. Rheumatological assessment prior to ICIs contributed to identify unknown rheumatic conditions. The follow-up by rheumatologists and oncologists facilitated the treatment of exacerbations in PRD and de novo IrAEs, attempting to allow concurrent ICI treatment. Distinguishing between IrAEs and rheumatological manifestations not associated with ICIs allowed to restrict the use of corticosteroids to the IrAEs cases.

**168. 537 DYSREGULATION OF THE COMPLEMENT SYSTEM IN CHILDREN WITH POST-INFECTIOUS GLOMERULONEPHRITIS (PIGN)**

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**Background:** Post-streptococcal glomerulonephritis (PSGN) is the main cause of acute glomerulonephritis in pediatrics and develops after nephritogenic throat or skin infections. Although its clinical course usually resolves within weeks, few patients show an atypical form with persistent hypocomplementemia and kidney damage. **Aim:** To assess alterations in the complement system in children with PSGN. **Methods:** 11 patients were enrolled. We measured C3/C4 levels by nephelometry, CH50/AH50 by hemolytic assays and C3Nef/PDC by functional assay. Atypical-persistent clinical course was characterized by an accelerated deterioration of renal function. **Results:** Patients (5 females, 6 males), median age: 9 years [2-15], median follow-up: 35.3 $\pm$ 8.4 weeks [12-100], median ASTO: 866.8 $\pm$ 180 UI/mL [165-2123], negative for antinuclear antibody test. 8/11 patients showed self-limited evolution and recovered within 8 weeks, median urinary protein:creatinine ratio (UPCR): 2.2 $\pm$ 0.5 mg/mg [0.5-12.2], transient C3 hypocomplementemia (median: 22.6 $\pm$ 4.8 mg/dL), normal C4 levels (median: 18.4 $\pm$ 1.7 mg/dL) and C3Nef transitionally positive (3/8 patients). Furthermore, they evidenced functional alteration in alternative complement pathway, with median AH50 of 46.4 $\pm$ 6.9 minutes, and normal classical pathway (median CH50 59.7 $\pm$ 8.1 U/mL). They showed a median normal glomerular filtration rate (GFR) of 75.5 $\pm$ 6.4 ml/min/1.73m<sup>2</sup>. 3/11 cases, all females, presented a torpid evolution after 6 months of follow-up, median UPCR: 8.6 $\pm$ 2.9 mg/mg [5.1-12.2], C3 hypocomplementemia (median: 18.7 $\pm$ 8.2 mg/dL), normal C4, with median AH50 greater than 60 minutes and C3Nef/PDC temporarily positive. Patients with atypical PSGN had a GFR that decreased to 20 ml/min/1.73m<sup>2</sup>. **Conclusions:** The patients with worst evolution showed functional time-persistent complement alterations according with constant low GFR and higher proteinuria. These findings suggest that complement dysregulation could be linked to genetic alterations in the alternate pathway.

## P2 POSTERS

FECHA Y HORA: 20/11/2024 16:10-17:10 H

COORDINADORES: YANINA LANGLE,  
SACERDOTI FLAVIA

## 169. 077 IMMUNE BIOMARKERS THAT DIFFERENTIATE ACTIVE TUBERCULOSIS IN CHILDREN

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The diagnosis of *Mycobacterium tuberculosis* (*Mtb*) infection is very challenging in the pediatric population. In children, clinical manifestations of tuberculosis (TB) are often non-specific and disease is usually pauci-bacillary, making it very difficult to achieve the microbiological diagnosis of active TB. Moreover, both tuberculin skin test (TST) and IFN- $\gamma$  release assays (IGRAs) have been shown to have limited usefulness in pediatric cases. Thus, improved tests to diagnose *Mtb* infection are required. In the present work, we assessed the distinct expression of immune biomarkers against *Mtb* specific antigens during active TB in children. BCG-vaccinated healthy controls (HC) and TB patients between 0-14 years old were recruited for this study. Whole blood was stimulated with the *Mtb* specific antigen CFP-10-ESAT-6 fusion protein (PF) at 2.5  $\mu\text{g/ml}$  during 24 hours. Afterwards, IFN- $\gamma$  and IP-10 production were tested in plasma by ELISA. Furthermore, we used PF at 2.5  $\mu\text{g/ml}$  to sensitize ELISA plates and then measured IgG specific antibodies in unstimulated plasma samples. Our results showed that IFN- $\gamma$  levels produced against PF allowed to differentiate TB patients from HC ( $p < 0.001$ , Mann-Whitney test). Accordingly, ROC analysis indicated 100% sensitivity, 100% specificity and a cut-off=66pg/ml. In a similar manner, the secretion of IP-10 in response to PF showed significantly higher levels in TB as compared to HC ( $p < 0.01$ , Mann-Whitney test). Consequently, ROC analysis indicated 100% sensitivity, 100% specificity and a cut-off =123pg/ml. Finally, anti-PF IgG levels allowed to distinguish TB patients from HC ( $p < 0.05$ , Mann-Whitney test) with ROC analysis showing 64.71% sensitivity and 95% specificity and a cut-off D.O.=0.32. In summary, our present results indicate that the amounts of IFN- $\gamma$  and IP10 produced against PF together with the levels of Ig-G anti-PF antibodies might be important tools to identify active TB in the pediatric BCG-vaccinated population.

170. 247 A PRELIMINARY STUDY ABOUT THE CYTOTOXICITY AND PRO-INFLAMMATORY PROPERTIES OF NANODIAMONDS AND TiO<sub>2</sub> NANOPARTICLES

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Nanodiamonds (ND) are carbon nanoparticles (NPs) with unique optical, chemical, and biological properties that make them applicable in medicine, pharmaceuticals, and personal care products. Therefore, before naming NDs as nanomaterials of biomedical interest more studies are necessary to clarify the interaction with living systems, including cytotoxicity and pro-inflammatory outcomes. Macrophages are ubiquitous cells in all tissues and central cells in the innate immune system. The measurement of the cytotoxic properties of nanomaterials on primary macrophage culture is the first step in testing further biological effects. Furthermore, the inflammatory priming of peritoneal macrophages can be directly studied by measuring the radical nitric oxide (\*NO) production as nitrites in the culture medium. Therefore, in this study, we aim to test the cytotoxic

and pro-inflammatory properties of TiO<sub>2</sub> NPs and ND. To accomplish our goal, we isolated mouse peritoneal macrophages (PM) from peptone-elicited male 7-week-old BALB/c mice. Monolayers of PM were cultured in clear 12-well plates for 24h in DMEM-high glucose medium containing 10% fetal bovine serum and 1% penicillin/streptomycin (complete medium, CM). After this incubation cell monolayers were rinsed and incubated in CM containing different concentrations of TiO<sub>2</sub> NPs and ND: 50, 100, and 200  $\mu\text{g/ml}$ . After 24h of incubation, nitrite concentration in the medium was measured (Griess assay). Cell viability was measured using the MTT assay. Neither TiO<sub>2</sub> NPs nor ND caused significant cytotoxicity under the incubation conditions tested. However, TiO<sub>2</sub> NPs caused a dose-dependent increase in \*NO production suggesting that TiO<sub>2</sub> NPs and ND NPs caused pro-inflammatory priming of PM. Data gathered from this study suggest that although nanomaterials cannot cause cytotoxicity under these conditions, their proinflammatory properties should be investigated before their application to living systems

## 171. 245 IN SILICO SCREENING AND IN VIVO TESTING OF NITRONE COMPOUNDS TO REDUCE INSULIN RESISTANCE IN A MOUSE MODEL OF DIET-INDUCED OBESITY

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Obesity is the result of a chronic positive energy balance that results in circulating free fatty acids (FFAs) which can activate macrophages triggering adipose tissue (AT) inflammation. Together with AT and muscle, the liver is one of the main organs that control systemic insulin sensitivity. Both, FFAs and AT-derived inflammation mediators are responsible for systemic inflammation and insulin resistance (IR), respectively, in obesity. Nitro compounds can act as agonists of the Nrf2 pathway facilitating the storage of FFAs as triglycerides, thus reducing AT- and systemic-inflammation and thus improving IR. Computational chemistry can predict leading structures of a certain specific biological activity, thus guiding experimental research. In this research, we aimed to screen using in silico methods, synthetic nitro compounds that can activate the Nrf2 signaling pathway. Interestingly, our in silico studies predicted that the nitro compound LQB122 has a similar Nrf2 agonist activity to sulforaphane—a well-known Nrf2 signaling agonist. Our in vitro studies showed that LQB122 did not cause toxicity in 3T3.L1 cells. Then, we aimed to test the compound in a diet-induced obesity mouse model for its anti-inflammatory and insulin-sensitizing effects. To achieve this goal, we fed male C57 mice with a control (CoD, rodent chow, and tap water) or an obesogenic (ObD, 22% bovine fat + 10% fructose in drinking water) diet for 24 weeks. At 23 weeks, a group of CoD and ObD were injected (i.p.) with either saline or saline containing the LQB122 nitro (0.125nmol/50ul/g) for the remaining week of the dietary regime. The nitro did not cause significant oxido-inflammatory or metabolic changes in CoD mice, however, LQB122 reduced hepatic neutrophilic inflammation and systemic inflammation, and improved insulin sensitivity in the ObD mice. LQB122 can serve as a structural platform for the design of novel mechanism-based drugs to reduce IR and maybe other obesity-associated metabolic abnormalities.

## 172. 294 IN VITRO AND IN VIVO EVALUATION OF NOVEL RAC1 INHIBITOR 1A-116 FOR THE TOPICAL TREATMENT OF SKIN DISORDERS

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RAC1 is a small GTPase that plays a crucial role in regulating various cellular processes. Its hyperactivation has been implicated as a key mediator of epidermal dysfunction, cell hyperproliferation and inflammatory disorders, such as psoriasis and atopic dermatitis (AD). In this study, we evaluated 1A-116, a RAC1 inhibitor initially developed as an antitumor agent, now being investigated for the first time as a topical therapeutic aimed at targeting the aberrant activation of RAC1 in a range of skin conditions. *In vitro* studies using HaCaT (human keratinocytes) and HDF (dermal fibroblasts) to assess the effects of 1A-116 on cell viability were conducted. Treatment with 1A-116 resulted in a significant reduction in cell viability ( $P < 0.05$ ) in both cell lines, without inducing skin corrosion or irritation, as confirmed by EpiSkin™ Small Model SCT. Additionally, 1A-116 significantly decreased total reactive oxygen species (ROS) production ( $P < 0.05$ ) and inhibited the secretion of pro-inflammatory cytokines, including IL-6, IL-1 $\beta$ , IL-17A, IL-4, and TNF- $\alpha$ . To further assess the skin penetrability of 1A-116, *ex vivo* testing was performed using human skin tissue showing a good skin penetrability profile. Additionally, two *in vivo* models were established to evaluate the efficacy of 1A-116. An atopic dermatitis (AD) model was used to assess the activity of 1A-116, showing a significant anti-inflammatory effect *in vivo* and the reduction in pro-inflammatory cytokines expression as seen *in vitro*. Furthermore, using a psoriasis-like dermatitis model we evaluated different topical formulations of the compound, observing that the ointment formulation of 1A-116 significantly reduces epidermal thickness. The comprehensive evaluation of 1A-116 across multiple models underscores its potential as a therapeutic agent for skin disorders driven by RAC1 hyperactivation. These findings pave the way for further research into the efficacy of 1A-116 in treating inflammatory skin conditions.

### 173. 316 IgY TECHNOLOGY - AN ALTERNATIVE METHOD FOR THE PRODUCTION OF OPHIDIAN ANTIVENOM AGAINST CROTALUS DURISSUS TERRIFICUS (ARGENTINEAN RATTLESNAKE)

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Snakebite envenoming is caused by the injection of a mixture of toxins, and represents an example of a pathology whose effective treatment is the administration of antivenoms based on sera or plasma from hyperimmunised large animals (horses). An alternative to mammalian polyclonal sera is the use of egg yolk antibodies because of their advantages in terms of animal welfare and lower production cost. The aim of this study is to produce an IgY antivenom against *Crotalus durissus terrificus* venom on a pilot scale, using more simplified purification methods. A group of laying hens ( $n=2$ ) was immunised i.m. with 80  $\mu$ g of whole *C. d. terrificus* venom (pool) 9 times on days 0, 14, 28, 71, 237, 289, 304, 473 and 487. For the first immunisation, the venom was emulsified with complete Freund's adjuvant (1st injection) and incomplete for boosters. Eggs were collected for 10 days after the 7th, 8th and 9th immunisation. To choose the optimal purification method, different protocols were evaluated: precipitation with ammonium sulphate (24 and 26% w/v), PEG-6000 (12% w/v) and caprylic acid (7% v/v). Thimerosal 0.01% (w/v) was added for preservation. The mean effective dose (ED50)

was assessed in NIH mice by mixing 3 mean lethal dose (LD50) of the venom with increasing volumes of IgY antivenom according to World Health Organization guidelines. The optimal LD50 was obtained by precipitation with ammonium sulphate instead of using PEG-8000 and caprylic acid. After 9 immunisations, 1 ml of IgY antivenom purified using PEG-8000 neutralised 158  $\mu$ g of venom. In addition, 1 ml of IgY antivenom purified by caprylic acid neutralised < 40  $\mu$ g of venom. However, 1 ml of IgY antivenom purified by ammonium sulphate neutralised 395  $\mu$ g of venom. In conclusion, immunisation of hens with sublethal doses of *C. d. terrificus* venom produced an antivenom with ED50 similar to those obtained in horses and could be an alternative production method. The IgY technology may enable the production of effective and affordable antivenoms.

### 174. 361 EPIDEMIOLOGY OF AA AMYLOIDOSIS COHORT STUDY. DIFFERENCES IN THE LATENCY PERIOD FROM THE DIAGNOSIS OF THE INFLAMMATORY CONDITION TO THE DIAGNOSIS OF AA AMYLOIDOSIS

María Adela Aguirre<sup>1,2</sup>, Elsa Mercedes Nucifora<sup>3</sup>, Marcelina Carretero<sup>4</sup>, María Soledad Sáez<sup>5</sup>, Patricia Beatriz Sorroche<sup>5</sup>, María Lourdes Posadas Martínez<sup>4,2</sup>

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**Introduction:** Amyloidosis is characterized by the deposition of positive Congo Red material. AA amyloidosis is associated with infectious, autoimmune, or idiopathic inflammation. The time from diagnosis of inflammation to diagnosis of amyloidosis is named latency. The importance of knowing the latency period, allows early suspicion of the entity and detection of risk factors for the development of amyloidosis. **Objective:** to estimate latency period from the diagnosis of the inflammatory condition to the diagnosis of AA amyloidosis in patients at Hospital Italiano de Buenos Aires from 01/01/2012 to 10/31/2019 and characterize these patients. **Methods:** A prospective cohort of adults with AA amyloidosis was designed in the Institutional Registry of Amyloidosis of Hospital Italiano de Buenos Aires. Enrollment was based on tissue AA confirmation. Information was collected on demographic characteristics, characteristics at diagnosis, characteristics of the underlying disease, treatment, and prognosis. **Results:** Idiopathic forms reached 56% ( $n=13$ ). The underlying diseases were autoimmune in 26% ( $n=6$ ) and infectious in 17% ( $n=4$ ). The median latency period was 20 years [interquartile range (IQR 1-32)], being 30 years (IQR 12-35) for autoimmune and 5 years (IQR 5-19) for infectious diseases. The main organic involvement was renal (87%). The treatment rate was 65%. During follow-up the overall mortality rate was 17% (confidence interval 95% 6-40%). **Conclusion:** The latency period was lower for infectious diseases than for autoimmune diseases in this cohort. Treatment of infections could prevent the development of kidney failure in this group of patients. Knowing the distribution of causes of AA amyloidosis and characteristics in our region is important and has a healthcare impact. As with rare diseases, the suspicion of amyloidosis is low. Dissemination of local data could help with early diagnosis.

### 175. 488 ZFP-36 PROTEINS IN MONOCYTE AND MACROPHAGE INFLAMMATORY SIGNALING AND DIFFERENTIAL TATION. MODELLING THEIR POST-TRANSLATIONAL MODIFICATIONS, INTRACELLULAR LOCATIONS, INTERACTOME, STRUCTURAL HETEROGENEITY AND HALF-LIFE

Cristian J. A. Asensio<sup>1,2,3</sup>, Simon Sarsfield<sup>1</sup>, Rob Wait<sup>1</sup>, Jane Crawford<sup>1,4,5</sup>

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ZFP36 and ZFP36L1 (L1) are RNA-binding nucleo-cytoplasmic

phospho-proteins with zinc-fingers, modulating the decay of AU-rich mRNAs such as those of some inflammatory cytokines. Their individual roles or redundancy in macrophages are unknown. Both are post-translationally modified with multi-site phosphorylation by many kinases and by other PTMs. The impact of all PTMs on their roles, location, mRNA binding, folding, modification code, half-life and interactions are understudied. We aimed to study them in THP1 and HeLa cell lines by subcellular fractionation, immunoprecipitation, immunoblotting, far-WB, dye binding, transfection, radiolabeling and 1D/2D gels, also using enzyme inhibitors and TLR ligands for cell treatments. We studied rZFP36 mutants by kinase assays and ZFP36 by informatic, interactome and MS analysis. As novel PTM, we considered the isomerization in proline-directed phospho-sites. By densitometry of their isoforms in gels, results suggested with statistical significance that the hyperphosphorylated forms of L1 and ZFP36 were cytoplasmic but insoluble, interacting with the cytoskeleton. Thus, both distribute in at least 6 locations: cytosol, cytoskeleton, mRNAs, stress-granules, P-bodies and nucleus. Besides, both cellular and rZFP36 isomerize. ZFP36 becomes a model for multi-site phosphorylation and isomerization. L1 levels were different in monocytic and macrophage states, suggesting a role in a differentiation switch but not in inflammation. ZFP36 was affected by inflammatory signaling but not by macrophage adherence or multination or ribotoxic stress. We visualize a complex rheostatic regulation in which ZFP36 is controlled by interactions with ions, proteins, mRNAs and proteasomes, behaving as a polyanion with electrostatic interactions and disordered regions that can isomerize. More studies are needed to understand their molecular heterogeneity and if they will become drug targets, to fine-tune their many activities without side effects

## MEDICINA REGENERATIVA Y NANOMEDICINA

### O1 COMUNICACIONES ORALES

FECHA Y HORA: 20/11/2024 11:30-12:30 H

LUGAR: AUDITORIO

COORDINADORES: MARIANO SCHUMAN,  
HEBE DURAN, RAMIRO QUINTA

#### 176. 030 DEVELOPMENT OF MIXED MICELLAR SYSTEMS AS IMMUNOSTIMULANT NANOPLATFORM FOR NOVEL SUBUNIT VACCINE FORMULATIONS

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The use of nanoparticulate systems as adjuvants has gained considerable strength, enhancing the efficacy and safety of subunit vaccines. Polymeric micelles (PMs) based on polyoxyethylene (PEO) and polyoxypropylene (PPO) tri-block copolymers represent a promising nanopatform for immunostimulant delivery. QS-21, a saponin fraction from *Quillaja saponaria*, is encapsulated within the AS01 adjuvant system in FDA-approved vaccines to boost immunogenicity and reduce hemotoxicity, though it remains a costly lipid-based platform. Our aim is to develop mixed PMs based on block copolymers (P123) and QS-21 as a novel immunostimulant nanopatform (P123/QS-21), characterize their physicochemical properties, evaluate *in vitro* hemotoxicity and *in vivo* immune response. The P123/QS-21 system was prepared by hydrating its components in PBS at 4°C overnight. Key assembly parameters, including hydrodynamic diameter ( $D_h$ ), polydispersity index (PDI), and critical micellar concentration (CMC), were determined using dynamic light scattering (DLS). Hemolytic activity was assessed, and the immune response was measured by antibody titers in blood serum and bronchoalveolar lavage (BAL), along with neutralizing antibodies in serum after in-

tramuscular immunization with the SARS-CoV-2 Spike recombinant protein (provided by NANOBIOTEC) formulated with P123/QS-21. The P123/QS-21 system demonstrated effective micellar assembly, with a  $D_h$  under 25 nm, PDI < 0.1, and a CMC value intermediate between those of P123 and free QS-21, indicating the formation of a new entity. This formulation showed reduced hemolytic activity compared to free QS-21, and achieved a two-fold increase in anti-Spike antibody titers in serum and a ten-fold increase in BAL. Notably, anti-Spike neutralizing activity was also observed. These findings represent a step forward in the development of novel and cost-effective nanoparticulate adjuvants for subunit vaccine formulations.

#### 177. 098 ENHANCED RADIOSENSITIZATION OF MELANOMA CELLS BY GOLD NANOPARTICLES AND POLYMERIC MICELLES COMBINED WITH DOXYCYCLINE

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Gold nanoparticles (AuNPs) were synthesized using polyoxyethylene (PEO) and polyoxypropylene (PPO) block copolymers (F127, F68, P85) through two methods: (i) direct formation in the presence of reducing copolymers, creating AuNPs-PMs complexes, and (ii) preformed polymeric micelles (PMs) and AuNPs (by Turkevich synthesis) resulting in hybrid AuNPs/PMs blends. Doxycycline (Doxy), a mitochondrial biogenesis inhibitor which acts as an active ligand, was adsorbed onto the surface of these nanostructures. All nanostructures were characterized by UV-Visible, DLS and TEM. Hydrodynamic diameters ( $D_h$ ) increased with higher copolymer molecular weights ( $M_w$ ) and concentrations (%w/v): AuNPs-PMs complexes ranged from 100-150 nm (0.5-5%) to 400-500 nm (10%); while AuNPs/PMs blends were slightly smaller, ranging from 20-60 nm (0.5-5%) to 500 nm (10%). P85 (lowest  $M_w$ ) displayed the smallest  $D_h$  (under 100 nm). All structures remained stable for 20 days. Copolymers prevented spontaneous AuNPs aggregation in presence of Doxy. Neither AuNPs (2.5-50 $\mu$ M), nor F127 and F68 PMs (0.1-1%) and their respective complexes and blends had a significantly impact on cell metabolic activity in A375 (radiosensitive) and Mel-J (radioreistant) melanoma cells. In contrast, free Doxy decreased melanoma cell viability below 75% at concentrations higher than 25 $\mu$ M, reaching 14-16% of viability at concentrations of ~1mM, while Doxy (25 $\mu$ M) combined complexes and blends displayed a mayor reduction in cell viability (<60%), for both A375 and Mel-J. Radiosensitization was initially studied by irradiating cells with gamma rays (2Gy, 137Cs), where cells pretreated with Doxy-combined complexes and blends showed a presumptive reduction in viability. Further clonogenic assays are underway to better understand this effect. The combination of Doxy with these nanostructures may offer a novel strategy to enhance radiotherapy efficacy in resistant melanoma.

#### 178. 111 CORNEAL ENDOTHELIAL DIFFERENTIATION OF HUMAN AMNIOTIC MESENCHYMAL STEM CELLS

Rodrigo Riedel<sup>1</sup>, Antonio Pérez-Pérez<sup>2</sup>, Julieta Gelardi<sup>3</sup>, Mariana Jaime<sup>4</sup>, Víctor Sánchez-Margalet<sup>2</sup>, Cecilia Varone<sup>1</sup>, Alejandro Berra<sup>5</sup>, Julieta Maymó<sup>1</sup>

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The human placenta amnion is a source of multipotent and pluripotent stem cells, including human amniotic mesenchymal stem cells (hAMSCs). hAMSCs possess immunomodulatory, anti-fibrotic, and anti-inflammatory properties, as well as high differentiation potential. They express embryonic and pluripotency markers, lack telomerase expression, and are non-tumorigenic, making hAMSCs a valuable and non-controversial tool for regenerative medicine. Corneal diseases, such as corneal endothelial dysfunction, are the fourth leading cause of blindness worldwide, and current treatment options have several obstacles. Recently, mesenchymal stem cells have garnered attention as a potential alternative source for corneal endothelial cells (CECs) due to their origin in the neural crest. This study aimed to differentiate hAMSCs into corneal endothelial-like cells. CEC differentiation was assayed by a specific CEC-induction medium. Western blot (WB), qRT-PCR, immunofluorescence (IF), and MTT assay were performed. First, a hAMSC isolation protocol using trypsin-collagenase digestion was successfully implemented. Following differentiation treatment, hAMSCs exhibited typical CEC morphology, characterized by hexagonal/polygonal-like cells. CEC-specific marker expression also was analyzed, revealing a significant induction in Zonula Occludens-1 (ZO-1), Na<sup>+</sup>/K<sup>+</sup>-ATPase, collagen VIII  $\alpha 1/\alpha 2$ , and paired-like homeodomain transcription factor 2 (PITX2) expression, measured by qRT-PCR and WB. In addition, a significant increase in the apical localization of ZO-1 and Na<sup>+</sup>/K<sup>+</sup>-ATPase was observed during CEC differentiation, evaluated by IF. Moreover, the CEC-induction medium enhanced cell viability for 16 days, measured by MTT assay. These findings suggest that hAMSCs can be successfully differentiated into corneal endothelial-like cells using a specific CEC-induction protocol, offering a promising cell source for regenerative medicine and potential clinical treatments for corneal endothelial diseases.

**179. 521 IDENTIFICATION OF BIOMARKERS BASED ON METAGENOMIC STUDIES OF THE GUT MICROBIOTA IN CHRONIC METABOLIC DISEASES**

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A study at Hospital de Clínicas "José de San Martín" aimed to evaluate the effects of physical activity and diet interventions on lifestyle and gut microbiota (GM) in individuals with obesity (OB), prediabetes (preDT), and type 2 diabetes (T2D), compared to a reference group (CNT). Materials and Methods: The study included 47 OB, 29 preDT, 13 T2D, and 23 CNT individuals. Fecal samples were analyzed for GM composition using metagenomic sequencing and bioinformatics tools. Clinical, biochemical, and anthropometric parameters and statistical analyses included correlations and comparisons were assessed with SPSS. Results: At baseline (T0), Firmicutes (Fir) and Bacteroidetes (Bac) were the predominant phyla. The T2D group had a lower abundance of Verrucomicrobia compared to CNT ( $p=0.072$ ). The OB, preD, and T2D groups had a higher Fir/Bac ratio, which was negatively correlated with weight ( $p=0.025$ ,  $r=-0.220$ ), BMI ( $p=0.05$ ,  $r=-0.190$ ), and inflammation (hsCRP) ( $p=0.036$ ,  $r=-0.211$ ). *Faecalibacterium* was less abundant in OB and preDT compared to CNT, with *Faecalibacterium prausnitzii* decreasing as metabolic conditions worsened ( $p=0.036$  and  $p<0.001$ , respectively). Lower species diversity was associated with higher hsCRP. After 6 months of lifestyle interventions (T6), OB showed significant reductions in waist circumference ( $p=0.001$ ) and HbA1c ( $p<0.001$ ). The preDT group had significant decreases in HbA1c ( $p<0.001$ ), weight ( $p<0.001$ ), waist circumference ( $p=0.001$ ), and BMI ( $p=0.003$ ). The T2D group saw declines in HbA1c ( $p=0.001$ ), triglycerides ( $p=0.032$ ), total cholesterol ( $p=0.021$ ), and hsCRP ( $p=0.012$ ). GM composition

and the Fir/Bac ratio remained unchanged. Conclusions: The study demonstrated that 6 months of lifestyle changes led to a GM composition in OB, preDT, and T2D groups that progressively resembled the CNT group, correlating with improvements in clinical, biochemical, and anthropometric parameters.

**180. 190 THE ANTIFIBROTIC POTENTIAL OF IMT504: MODULATION OF GLAST+ WNT1+ BONE MARROW STROMAL PROGENITORS AND HEPATIC MICROENVIRONMENT**

Borda Maximiliano<sup>1</sup>, Cantero María Jose<sup>1</sup>, Fiore Esteban Juan<sup>1,2</sup>, Sierra Romina<sup>1</sup>, Gómez-Bustillo Sofía<sup>3</sup>, Giardelli Gianlucca<sup>1</sup>, Lucia Cafaro<sup>1</sup>, Lucila Valentina Casella<sup>1</sup>, Mercedes Díaz Pedraza<sup>1</sup>, Camila Becerra<sup>1</sup>, Bayo-Fina Juan Miguel<sup>1,2</sup>, Schiavone Máximo<sup>1</sup>, Montaner Alejandro<sup>2,3</sup>, Mazzolini Guillermo Daniel<sup>1,2</sup>, Aquino Jorge Benjamín<sup>1,2</sup>

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**Objectives:** To evaluate the effects of IMT504 on fibrogenesis and the reversal of liver fibrosis in mice, as well as its impact on GLAST+Wnt1+ bone marrow stromal progenitors (BMSPs). **Methods:** Liver fibrosis in mice was induced by thioacetamide (TAA) administration and bile-duct ligation. Cre-loxP mice were used to identify GLAST<sup>+</sup> Wnt1<sup>+</sup> BMSPs and assess their contribution to liver cell populations. A combination of in vivo and in vitro assays, including flow cytometry, immunohistochemistry, and qPCR analyses, was conducted. **Results:** IMT504 significantly inhibited the progression of liver fibrogenesis and reversed established fibrosis. Early responses to IMT504 included the suppression of profibrogenic and proinflammatory markers, alongside an increase in hepatocyte proliferation. Additionally, IMT504 stimulated the proliferation and mobilization of GLAST<sup>+</sup> Wnt1<sup>+</sup> BMSPs through Wnt signaling, likely enhancing their contribution to endothelial- and hepatocyte-like progenitors. IMT504 had minimal effects on other BMSP subpopulations. Interestingly, both IMT504 and conditioned media from IMT504-pretreated GLAST<sup>+</sup> Wnt1<sup>+</sup> BMSPs shifted macrophages toward an anti-fibrotic phenotype, reduced hepatic stellate cells activation, and induced the expression of pro-regenerative markers in hepatocytes, aligning with the strong antifibrotic effects observed in vivo. **Conclusion:** IMT504 induces remodeling of the hepatic microenvironment through both direct and indirect mechanisms, acting on hepatic cell populations and modulating GLAST+ Wnt1+ BMSPs, respectively. These findings suggest that IMT504 is a promising candidate with potent antifibrotic properties.

**181. 202 SOLUPLUS® NANOMICELLES ASSOCIATED WITH BOVINE LACTOFERRIN: EFFECTS ON SHIGA TOXIN TYPE 2 SECRETION AND NEUTRALIZATION**

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Shiga toxin type 2 (Stx2) is the main virulence factor of Shiga tox-

in-producing *E. coli* (STEC) responsible for hemolytic uremic syndrome (HUS). Currently, no specific treatment for HUS is available, and antibiotics are contraindicated. We have previously shown that the association of Soluplus® nanomicelles (NM) with bovine lactoferrin (NM-bLf) significantly reduced STEC growth and bacterial adhesion on HCT-8 cells compared to bLf *in vitro*. In this study, we characterized NM-bLf and aimed to evaluate its effects on Stx2 secretion and neutralization. To better understand the association of NM and bLf, the NM-bLf was characterized by UV-Vis, fluorescence, and circular dichroism (CD) with different concentrations of NM (0.5–10 mg/ml). STEC (O157:H7) was cultured with NM 1%-bLf 0.1 mg/ml or bLf 0.1 mg/ml for 3h and 24h. Subsequently, Stx2 secretion was evaluated in the supernatants by ELISA. Additionally, the neutralization capacity of Stx2 was determined by co-incubating 1CD<sub>50</sub> of Stx2 with NM-bLf or bLf on Vero cells. The UV-Vis and fluorescence spectra indicated a moderate increase in bLf absorbance with NM 0.5 mg/ml, pointing to an improvement in protein solubility. The CD spectra in the far UV region showed an increase in the magnitude of the signal intensity (210 nm), probably due to a solubilizing effect on bLf by NM. Non-treated STEC secreted 51.9 ng/ml and 81.2 ng/ml of Stx2 on supernatant at 3h and 24h of culture. NM-bLf significantly inhibited Stx2 secretion by STEC after 3h and 24h of incubation compared with bLf (\*\*61% vs 20%; \*\*77% vs 45% respectively \*\*p<0.001). bLf did not show neutralizing effects directly on Stx2, neither NM-bLf showed this property *in vitro*. These findings indicate that coupling NM to bLf could effectively enhance some of bLf antimicrobial properties, including inhibition of Stx2 secretion, thus making it a possible emerging tool for addressing STEC infections and reducing the risk of HUS.

**182. 257 NANOSTRUCTURED LIPID CARRIERS FOR TRANSDERMAL DELIVERY OF CLOTRIMAZOLE IN CUTANEOUS AND SUBCUTANEOUS MYCOSES: FORMULATION, CHARACTERIZATION AND EX VIVO PENETRATION TESTS**

María F. Ladetto<sup>1,2</sup>, Claudia Salerno<sup>3</sup>, German A. Islan<sup>1</sup>, Guillermo R. Castro<sup>4</sup>, Romina J. Glisoni<sup>5</sup>, María L. Cuestas<sup>2</sup> <sup>1</sup>Laboratorio de Nanobiomateriales, Centro de Investigación y Desarrollo en Fermentaciones Industriales, Universidad Nacional de La Plata (CINDEFI UNLP-CONICET), La Plata, Buenos Aires, Argentina. <sup>2</sup>Universidad de Buenos Aires, Facultad de Medicina, Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPam UBA- CONICET), Buenos Aires, Argentina. <sup>3</sup>Universidad de Buenos Aires (UBA), Facultad de Farmacia y Bioquímica, Departamento de Tecnología Farmacéutica, Buenos Aires, Argentina. <sup>4</sup>Nanomedicine Research Unit (Nanomed), Federal University of ABC (UFABC), Santo André, SP, Brazil. <sup>5</sup>Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Nanobiotecnología (NANOBIOTEC UBA-CONICET), Buenos Aires, Argentina.

Nanostructured lipid carriers (NLC) were developed to control clotrimazole (CLT) release and enhance its transdermal delivery for treating cutaneous and subcutaneous mycoses. CLT, a widely used antifungal for topical treatments, presents challenges due to its lipophilicity, which limits solubility, absorption and bioavailability, whereas high-dose and prolonged treatments often lead to toxicity. To address these issues, CLT was incorporated into NLCs and further formulated into hydroxypropylmethylcellulose (HPMC) gels. Three NLC formulations were synthesized via emulsification/ultrasonication method, using cetyl-palmitate as solid lipid and poloxamer 188 as surfactant. Geraniol, eugenol, and oleic acid were selected as liquid lipids in order to enhance loading capacity, achieve therapeutic concentrations and improve therapeutic effectiveness. The resulting NLCs, with an average size of 130±15 nm and encapsulation efficiencies exceeding 95%, significantly improved the intrinsic solubility of CLT by 2,000 to 10,000 times. 2% HPMC was directly incorporated into the NLC suspensions to produce homogeneous gels. The antifungal efficacy of these formulations was confirmed through agar diffusion assays against 13 clinical isolates, showing at least a two-fold increase in activity compared to free CLT. *In vitro* release studies demonstrated controlled and sustained drug release,

fitting the Korsmeyer-Peppas model under sink conditions. *Ex vivo* studies using the Franz cell model with pig ear skin revealed that NLC formulations significantly enhanced CLT penetration with over 50% of the drug reaching the epidermis and, in the case of oleic acid-based NLCs, even the dermis, with no penetration to receptor fluid. These findings suggest that NLC-based gels represent a promising approach for the transdermal treatment of cutaneous and subcutaneous mycoses.

**P1 POSTERS**

FECHA Y HORA: 19/11/2024 16:00-17:00 H  
COORDINADORES: ROMORINI LEONARDO,  
SAFFIOTI NICOLÁS

**183. 017 CORNEAL REPAIR: THE ROLE OF GLAST+ PROGENITORS OF THE SCLEROCORNEAL LIMBUS AND THE EFFECT OF IMT504**

Álvarez Ordóñez Luis Fernando<sup>1</sup>, Borda Maximiliano<sup>1</sup>, Rodríguez Muedra María Eugenia, Camisa Joaquín, Montaner Alejandro<sup>2,3</sup>, Potilinski Constanza<sup>1,2</sup>, Gallo Juan Eduardo<sup>1,2</sup>, Aquino Jorge Benjamín<sup>1,2</sup>

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**Objectives:** To evaluate the behaviour of GLAST+ progenitors in the limbal epithelium and the effect of the oligodeoxynucleotide IMT504 after corneal injury. **Methods:** The cornea of the right eye was injured using alkali. Cre-loxP mice were used to identify GLAST+ cells in the anterior part of the eye and to assess their contribution to cornea cell populations. To evaluate the effect of IMT504 on corneal repair, CD1 mice were used. The ODN was subcutaneously injected 5 days after injury, and the animals were sacrificed 5 days later. The tissue was processed to produce whole mounts or cryosectioned and immunostained. **Results:** To analyze the distribution of cells expressing GLAST+, GLAST-CreERT2-Tom mice were injected with tamoxifen at postnatal day 2 (P2), the reporter gene was expressed in the progenitor layers of the anterior epithelium in the limbal region. Seven days post-injury, a significant number of Tom+ cells were observed invading the corneal epithelium, following a radial orientation pattern towards the center of the injury. These findings suggest that a subpopulation of limbal epithelial stem cells (LESCs) may express GLAST. IMT504 was found to inhibit the activation of the corneal stroma and fibrogenesis. Additionally, this ODN induced angiogenesis, which regressed at 30 days in most of the animals analyzed. **Conclusions:** Our hypothesis is that at P2, a significant subpopulation of quiescent LESCs expresses GLAST, which can be traced using GLAST-CreERT2-Tom mice. These animals could help, for the first time, analyze the kinetics of LESCs involvement in corneal repair and the effect of IMT504 on this process. IMT504 likely inhibits the activation of the corneal stroma and subsequent fibrogenesis, which could help prevent vision problems.

**184. 054 IMT504 ENHANCES LIVER REGENERATION AFTER PARTIAL HEPATECTOMY IN MICE**

Borda Maximiliano<sup>1</sup>, Lucia Cafaro<sup>1</sup>, Lucila Valentina Casella<sup>1</sup>, María José Cantero<sup>1</sup>, Mercedes Díaz Pedraza<sup>1</sup>, Camila Becerra<sup>1</sup>, Agustina Ábalo<sup>1</sup>, Montaner Alejandro<sup>2,3</sup>, Fiore Esteban Juan<sup>1,3</sup>, Aquino Jorge Benjamín<sup>1,3</sup>.

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**Objectives:** To evaluate the effects of the oligodeoxynucleotide (ODN) IMT504 on the liver regeneration and the role of GLAST<sup>+</sup>Wnt1<sup>+</sup> bone marrow stromal progenitors (BMSPs) in a mouse model of partial hepatectomy. **Methods:** GLAST-CreERT2-Tom or CD1 mice underwent partial hepatectomy and were treated with or without IMT504. Cre-loxP mice were used to identify GLAST<sup>+</sup>Wnt1<sup>+</sup> BMSPs and to examine their contribution to liver cells. A combination of *in vivo* and *in vitro* assays was applied, and flow cytometry, immunohistochemistry, and qPCR analyses were performed. **Results:** IMT504 treatment led to an increase in liver

size after hepatectomy compared to the control group, along with a higher mitotic index. This ODN induced the upregulation of markers associated with hepatoblast/hepatocyte proliferation (PCNA, HGF, Cyclin D1, Notch), hepatocyte function (IGF-1), DNA synthesis and repair (Gadd45a, Myb, Bcr2, Alb1), and liver progenitor cells (CD133, TWEAK1). Additionally, when incubated in conditioned media from hepatectomized liver, GLAST<sup>+</sup> Wnt1<sup>+</sup> BMSPs acquired features of hepatocytes (upregulation of Albumin, HNF-4a, AFP mRNA levels) and endothelial cells (upregulation of CD31, A1AT, vWF mRNA levels). Furthermore, IMT504 likely enhanced the mobilization and contribution of GLAST<sup>+</sup> Wnt1<sup>+</sup> BMSPs to the population of endothelial- and hepatocyte-like cells in the liver. **Conclusion:** IMT504 exhibits proregenerative effects, likely involving the recruitment of endogenous GLAST<sup>+</sup> Wnt1<sup>+</sup> BMSPs to the liver, and their contribution to liver cell populations.

#### 185. 395 ISOLATION AND CHARACTERIZATION OF ADIPOSE MESENCHYMAL STROMAL CELLS FOR ANTITUMORAL NANO-BASED CELL THERAPY

Kali Pellicer San Martín<sup>1</sup>, Eugenia Bühler<sup>2</sup>, Lucía Beaugé<sup>3</sup>, Paolo Lippi<sup>4</sup>, Luis Sommariva Odone<sup>4</sup>, Rodrigo E. Palacios<sup>3</sup>, N. Belén Rumie Vittar<sup>1,2</sup>, Luis E. Ibarra<sup>1,2</sup>

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Targeting therapeutic nanoparticles (NPs) to solid tumors, including those in the central nervous system (CNS), is a promising area of research aimed at enhancing diagnostic and therapeutic precision. Active NP targeting using cells could overpass various biological barriers to enhance NP transport and selectivity within tumors. One such strategy employs mesenchymal stromal cells (MSCs) as delivery vectors for actively targeted, photoactivatable polymeric nanoparticles to treat CNS tumors like glioblastoma (GBM). This approach leverages the natural recruitment of circulating immune and non-immune cells to tumors, enhancing light-mediated therapies' efficacy. MSCs were isolated from subcutaneous adipose tissue samples of human patients at the Centro de Traumatología y Artroscopia Privado. Both tissue explants and micronized fat obtained via mechanical disaggregation were cultured in various growth media. After ~20 days, successful isolation of MSCs was achieved, characterized by typical MSC morphology and adherence, spindle shape, and whirlpool patterns. Flow cytometry confirmed the identity of MSCs, with samples expressing CD44, CD73, and CD90 (≥ 95%) and lacking CD45 (≥ 95%). To evaluate their antitumor potential and capacity as NP vectors, we assessed GBM tumor cell proliferation when co-cultured with MSCs. None of the tested *in vitro* ratios (2:1, 5:1, 10:1, tumor-MSC cells) favored GBM cell proliferation. Notably, a 2:1 ratio showed an antiproliferative effect, warranting further studies to elucidate the underlying mechanisms. Additionally, we evaluated MSCs' potential as cellular vectors by assessing NP incorporation and cell viability post-loading with synthesized conjugated polymer NPs. NPC concentrations of up to 20 mg/L did not reduce MSC viability and were efficiently incorporated into the cells. Given the low immunogenicity, long circulation lifespans, ability to cross biological barriers, and poten

#### 186. 451 DEVELOPMENT OF NOVEL NANO-CAPSULES FOR LUNG-TARGETED DELIVERY OF PIRFENIDONE: FORMULATION, CHARACTERIZATION, AND IN VITRO EVALUATION

Micaela Juncal, Ezequiel Caputo, Eder Lilia Romero, Leticia Higa, María Julia Altube  
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Pirfenidone (Pfd) is an anti-fibrotic and anti-inflammatory drug used in the treatment of pulmonary fibrosis. Its oral administration has limitations due to rapid excretion ( $t_{1/2}$  of 2.4 h) and systemic adverse effects. The aim of this work is to develop antioxidant nanocapsules for efficient pulmonary delivery of Pfd. To this purpose, the encapsulation of Pfd in core/shell PLGA-PEG nanocapsules with a lipid interior of phosphatidylcholine, short-chain fatty acids, and the natural antioxidant bacterioruberin (Br) was optimized using the double emulsion technique. The physicochemical characteristics were studied by UV-vis spectroscopy, dynamic light scattering, and transmission electron microscopy. The nanoformulation, pNC-Br-Pfd, contained  $2470 \pm 174 \mu\text{g/mL}$  of final Pfd; presented an encapsulation efficiency of  $12 \pm 4\%$ , a Z-average of  $153 \pm 41 \text{ nm}$ , a polydispersity index of  $0.284 \pm 0.061$ , a Z potential of  $-45 \pm 6 \text{ mV}$  and spherical shape. Notably, these structural parameters were preserved following aerosolization using a vibrating mesh nebulizer. Moreover, pNC-Br-Pfd exhibited a biphasic release profile of Pfd, with  $57 \pm 3\%$  of Pfd released at 30 minutes and then a sustained increase over 48 hours. pNC-Br-Pfd showed cytocompatibility on THP-1 derived macrophages and MRC-5 fibroblasts for 24 h, up to  $20 \mu\text{g/mL}$  of Pfd and  $0.4 \mu\text{g/mL}$  of Br, measured by MTT, with no significant differences (n.s.) vs. control. On LPS-activated THP-1 macrophages, pNC-Br-Pfd inhibited IL-6 release by  $62 \pm 7\%$  ( $p < 0.001$ ) and free Pfd by  $27 \pm 3\%$  ( $p < 0.01$ ). Additionally, pNC-Br-Pfd inhibited reactive oxygen species ( $p < 0.0001$ ) to a greater extent than nanocapsules containing only Br ( $p < 0.1$ ) and free Pfd (n.s.) using the DCFDA probe. In summary, the pNC-Br-Pfd nanoformulation was cytocompatible and synergistically enhanced the activities of Br and Pfd, potentially amplifying their antioxidant and anti-inflammatory effects on inflammatory macrophages.

#### 187. 506 ISOLATION AND CHARACTERIZATION OF EXOSOMES DERIVED FROM THE HUMAN AMNIOTIC MEMBRANE CONDITIONED MEDIUM

Luciano A. Pérez<sup>1</sup>, Ricardo Malvicini<sup>2</sup>, Rodrigo Riedel<sup>1</sup>, Mabel Bonardi<sup>1</sup>, Mariana Jaime<sup>3</sup>, Diego Santa Cruz<sup>2</sup>, Cecilia Varone<sup>1</sup>, Natalia Pacienza<sup>2</sup> and Julieta Maymó<sup>1</sup>.

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The placental stem cells have been positioned as a central tool for the regenerative medicine. Their therapeutic potential to treat different diseases has been highly reported. There is plenty evidence about the anti-tumoral effects of the human amniotic membrane (hAM) stem cells, given by their antiproliferative, antiangiogenic and proapoptotic properties. In addition, extracellular vesicles, specially exosomes, have been recently described as important effectors of the anti-cancer hAM properties. Liver cancer is the fifth cause of cancer in the world, with a poor prognosis and survival. Alternative treatments to radio- or chemotherapy have been searched. We and other groups demonstrated the antitumoral effects of the amniotic membrane conditioned medium (AM-CM), but there is still a great lack of knowledge about the mechanisms involved. We have previously showed that the AM-CM inhibits hepatocarcinoma cells proliferation and promotes apoptosis. In this work, we aimed to isolate and characterize the exosomes present in the AM-CM and to analyse their ability to inhibit hepatocarcinoma cells survival. First, AM-CM exosomes were isolated by anion exchange chromatography and eluted with NaCl solution. The elution profile was characterized measuring protein and lipid concentration. CD63, a typical exosome marker, was determined by ELISA. Exosomes morphology was observed by transmission electron microscopy (TEM) and the quality and concentration of particles were determined by nanoparticles tracking analysis (NTA). Finally, antiproliferative exosomes effect was evaluated by MTT assay on HepG2 and Huh-7 cells. After chromatography elution, 8 fractions were obtained. Quantification of proteins and lipids showed a peak in fraction 4, consistent with the CD63 peak. After TEM analysis, AM-CM exosomes showed homo-



geneous spherical morphology and an average size between 80-100nm (n=3). Approximately,  $10^9$  particles of exosomes per ml were measured by NTA. MTT assay did not show changes in hepatocarcinoma cells viability after 24 h of exosomes treatment (0, 1-1 ug/ml). Our results would provide new tools for cancer treatment.

## P2 POSTERS

FECHA Y HORA: 20/11/2024 16:10-17:10 H

COORDINADORES: RIEDEL RODRIGO,  
BOLONTRADE MARCELA

### 188. 148 MICROTOMOGRAPHIC AND HISTOLOGICAL CHARACTERIZATION OF PERIAPICAL PROCESSES IN ADULT RATS WITH MATURE APICES. PRELIMINARY STUDY

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**INTRODUCTION:** Experimental endodontics is a very current aspect of scientific knowledge. There are studies on the evolution of periapical processes, but most describe immature apices of young Wistar rats. Since our studies focus on the evolution of chronic processes, the objective of this work was to characterize the microtomographic and histological aspects of apical processes with 7 and 10 days of evolution in adult Wistar rats. **MATERIALS AND METHODS:** Nine adult female Wistar rats (average weight = 179.2 g) were used and divided into 3 groups. The control group had no opening (n=3); the remaining 6 rats were anesthetized with ketamine/xylazine, the first lower molars were isolated, an opening was made, and the mesial canal was located, leaving the pulp chamber exposed to the oral cavity. Experimental groups were euthanized at 7 and 10 days post-opening (n=3 per group); their jaws were fixed in 10% buffered formalin for microtomographic and histological analysis. Data were expressed as mean (SD) and analyzed using one-way ANOVA (p<0.05, significant). **RESULTS:** In the microtomographs, tissue volume (TV) and bone volume (BV) parameters were measured in the periapical region and the apex of the mesial root. The BV percentage was 17.8 (0.3), 5.9 (0.9), and 6.7 (1.9) in the Control, 7-day, and 10-day groups, respectively. Significant differences (p<0.05) were observed between the control and the 7-day and 10-day groups; however, differences between the 7-day and 10-day groups were not significant. Histologically, teeth without an opening showed closed apices and an absence of vascular congestion. At 7 days, pulp hyperemia and inflammatory infiltrate were observed, and at 10 days, vascular congestion, periodontal inflammatory infiltrate, and root tissue resorption were noted. **CONCLUSION:** Exposure of the pulp tissue of adult rats to the oral environment for 7 and 10 days leads to the development of apical processes with different histological characteristics.

### 189. 182 REDOX-TRIGGERED SELF-ASSEMBLY OF HSA NANOPARTICLES

Shadia Matzkin<sup>1</sup>, Sofía Martínez<sup>2</sup>, Daniela Quinteros<sup>2</sup>, Ramón Peralta-Martínez<sup>3</sup>, Gonzalo de Prat-Gay<sup>3</sup>, Romina Julieta Glisoni<sup>1</sup>, Leonardo Gabriel Alonso<sup>1</sup>.

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Albumin-based nanoparticles have emerged as promising platforms for therapeutic applications due to their inherent advantages, including biocompatibility, biodegradability, non-immunogenicity, and safety, which make them ideal for clinical use. Among these, human serum albumin nanoparticles (HSA-NPs) are particularly notable for their potential to actively target the GP60 receptor, facilitating transcytosis across endothelial cells and enhancing therapeutic delivery. However, current synthesis methods often face challenges such as low reproducibility and reliance on potentially toxic chemical agents, which can limit their clinical applicability. In this study, we present a novel synthesis approach for HSA-NPs that addresses these limitations. Our method employs redox-triggered self-assembly under precisely controlled conditions, utilizing biologically compatible and non-toxic compounds. This approach yields nanoparticles with controlled sizes ranging from 90 to 130 nm by dynamic light scattering and well-defined secondary structures. By adjusting the reductant/thiol ratio, we successfully regulated the conformation of HSA within the nanoparticles, aiming to target different HSA receptors. We thoroughly evaluate the variables (e.g. degree of purification, dispersant medium, temperature, concentrations, among others) affecting nanoparticle properties, leading to homogeneous nanoparticles that exhibit optimal sizes, enhanced stability, and potential for targeted delivery. These features make our HSA-NPs highly promising candidates for drug delivery systems, particularly in the development of inhalable and intranasal therapies.

### 190. 188 ANALYSIS OF HSA-MIR-301A MODULATION IN HUMAN PLURIPOTENT STEM CELLS: IMPACT ON CELL CYCLE, VIABILITY, AND TARGET GENE EXPRESSION

Rodríguez Varela MS<sup>1</sup>, Vautier M<sup>1</sup>, Mucci S<sup>1</sup>, Chialva D<sup>1</sup>, Seveler GE<sup>1</sup>, Scassa ME<sup>1</sup>, Romorini L<sup>1</sup>

1. Laboratorios de Investigación Aplicada a Neurociencias - INEU (FLENI- CONICET)

Human embryonic and induced pluripotent stem cells (hPSCs) are endowed with an unusual cell cycle structure which consists of a short G1 phase. While E2F transcription factors (E2Fs) and microRNAs (miRNAs) are known to regulate this cycle, their interplay in hPSCs remains poorly understood. We had previously performed an RNA-seq analysis of small RNAs of human embryonic stem cells (hESCs) upon treatment with the pan E2Fs inhibitor HLM006474. From a differential expression analysis and by RT-qPCR validation, we identified a list of 7 miRNAs that includes miR-301a-3p as putative targets of canonical E2Fs in hESCs. Then, we aim to evaluate the role of miR-301a-3p on cell cycle, viability, and target gene expression in hPSCs, as miR-301a-3p was reported to be directly regulated by E2F1 and involved in the regulation of the above-mentioned processes in other biological models. Thus, we modulated the expression levels of miR-301a-3p by transfecting hPSCs with exogenous molecules (mimic/inhibitors). We observed that miR-301a over-expression or inhibition did not affect cell cycle distribution (measurement of DNA content by propidium iodide staining followed by flow cytometry analysis) or viability (determined by Trypan blue and propidium iodide staining). In addition, we analyzed by RT-qPCR the expression levels of putative miR-301a-3p direct or indirect target mRNAs. We determined that miR-301a-3p over-expression increased *OCT-4*, *E2F1* and *MBD2* mRNA expression levels in human induced pluripotent stem cells (hiPSCs) and miR-301a-3p inhibition decreased *CYCLIN A2* and *RUNX3* mRNA expression levels in hiPSCs and hESCs, respectively. Interestingly, we measured OCT-4 protein levels in hPSCs-transfected cells by flow cytometry analysis and observed that miR-301a over-expression decreased the percentage of OCT-4<sup>+</sup> cells. Our results suggest that miR-301a-3p is a miRNA that would be regulated by E2Fs and may participate in OCT-4 regulation.

### 191. 205 ENHANCED CYTOTOXICITY BY COMBINED MI-

### CROFLUIDIC-ASSISTED TADALAFIL- AND PACLITAXEL-LOADED POLYMERIC MICELLES IN GLIOBLASTOMA CELL LINES AND PATIENT-DERIVED TUMOR CELL CULTURES

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Nanomedicine is a promising platform for cancer chemotherapy, with polymeric micelles (PMs) being among the most studied carriers for hydrophobic drugs. Microfluidic technology, which allows precise fluid control at the microscale, is emerging as a method to optimize drug encapsulation in nanocarriers. In this study, we used microfluidics to encapsulate paclitaxel (PTX) and tadalafil (TADA), a phosphodiesterase 5 inhibitor, in PMs and assessed their combined effects on glioblastoma cell lines and tumor patient-derived cultures. PMs were created using methoxy-poly(ethylene glycol)-polycaprolactone as a polymer. The nanomedicines were characterized for size, polydispersity index (PDI), stability, drug concentration, and release profile. PTX and TADA content was quantified via reverse-phase HPLC, and therapeutic effectiveness was evaluated using MTT assays. Microfluidic-assisted PMs were compared with commercially available PTX formulations like Abraxane® and PMs prepared by conventional methods. The characterization revealed that microfluidic-assisted PMs had an average size of 100 nm with a narrow PDI. The in vitro release profile of PTX from PMs showed sustained, gradual drug release. In vitro assays demonstrated that PTX-loaded PMs produced by microfluidics exhibited significantly higher antitumor activity than those made by traditional methods, both in the U-251 glioblastoma cell line and a primary glioblastoma multiforme culture. Combined treatment with microfluidic-based PTX and TADA PMs resulted in significantly increased cytotoxicity at lower doses in both models compared to PTX PMs alone or Abraxane®. Overall, microfluidic-produced PMs resulted in a significant increase in cytotoxicity compared with traditional or commercially available PTX-loaded nanosystems, representing a promising alternative for developing new cancer therapies. Combining these nanomedicines may offer an effective strategy for treating glioblastoma multiforme.

### 192. 381 THREE-DIMENSIONAL MODEL OF STRIATAL STEM CELLS AS A METHOD OF STUDYING NEUROTROPHIC FACTORS IN REGENERATIVE MEDICINE

Cruz Gaitán Ana María (1)(2)(3), Carri Nestor (1), Lausada Natalia (1), Medori Mara (3), Spelzini Gonzalo (3), Leirós Gustavo (2), Ruíz Ignacio (2), Scicolone Gabriel (3).

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Neurotrophic factors (NFs) are crucial for neuronal development, proliferation, differentiation, survival, and nerve regeneration. This study evaluated the effects of NFs, specifically Noggin, FGF-9, and GDNF, on neurite growth (length and number) using a 3D model of neural spheroids (NS) derived from corpus striatum (CS) stem cells embedded in type I collagen gel. We also assessed NF-induced pro-

liferation in floating NS via MTT assay. Primary cells from the CS of E14 rat embryos were cultured under basal conditions. After 72 hours, a second passage was created by dissociating NS (clumped cells), followed by another 72 hours to allow new clump formation. NS were then embedded in collagen gel and treated with NFs for 24 and 48 hours. Expression of markers for proliferating cells (Ki67), progenitors (Nestin), neurons (TubIII, C-FOS), and glial cells (GFAP) was analyzed by immunocytochemistry. Another aliquot of NS was used to evaluate NF effects on proliferation. CS cell cultures clearly formed NS, main characteristic of neural stem cells (NSCs). In the 3D bioassay, trophic stimulation significantly increased the number and length of neurites compared to controls, with Noggin notably enhancing neurite outgrowth. After 48 hours, NS exposed to NFs showed a predominance of differentiation markers. FGF-9 stimulated the proliferation of floating NS. In conclusion, the 3D collagen gel assay effectively supports NSC differentiation, proliferation, and neurite outgrowth, as indicated by the immuno-profile of progenitor, neural, and glial cells. This assay also provides high reproducibility and resolution for tracking neural and glial outgrowth over a short culture period. tial for bioengineering, MSCs are promising NP delivery vectors.

### 193. 499 ENGINEERED EXTRACELLULAR VESICLES DERIVED FROM MESENCHYMAL STEM CELL CARRYING IGF-1 TO PROMOTE LIVER REGENERATION AFTER PARTIAL HEPATECTOMY

Mailín Casadei<sup>1</sup>; Ma. José Cantero<sup>1</sup>; Bárbara Bueloni<sup>1</sup>; Fátima Huamán Ormeño<sup>1</sup>; Lucía Lameroli<sup>1</sup>; Catalina Atorrastagasti<sup>1</sup>; Juan Bayo<sup>1</sup>; Guillermo Mazzolini<sup>1</sup>; Esteban Fiore<sup>1</sup>.

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**Introduction:** Liver transplantation is currently the only curative treatment for end-stage liver diseases. Impaired liver regeneration may cause liver failure after liver resection. Previously, we have demonstrated that the pro-regenerative effect on liver fibrosis of MSCs derived from umbilical cord (Human Umbilical Cord Perivascular cells, HUCPVCs), genetically modified to express the hepatoprotective factor IGF-1 (Insulin-like Growth Factor 1), is mediated by carrying IGF-1 on the Extracellular Vesicles (EV). **Aim:** To evaluate the effect on liver regeneration of EV carrying IGF-1 in mice model of partial hepatectomy (PH). **Material & Methods:** EV were isolated by ion exchange chromatography from supernatants of HUCPVC infected with adenoviruses codifying for IGF1 (EV-IGF1) or *green fluorescent protein* (EV-GFP). PH in C57BL6 mice were performed by surgical removal of right and left lobes (2/3 of total liver mass). After 1 hour of PH; EV-IGF1, EV-GFP or vehicle were administered by tail vein (1 dose, 45 ug/mice) and 3 days later animals were euthanized to liver sample collect. Liver Index (LI) were calculated as ratio of liver and body weight and Regeneration Index (RI) as % of recovered liver mass. Liver regeneration was evaluated by immunohistochemistry of PCNA+ proliferating cells and mRNA expression by qPCR. **Results:** In vivo treatment with EV-IGF1 led to a higher LI and RI compared to the controls groups. An increase in PCNA+ cells following EV-IGF1 administration indicated enhanced liver regeneration. Moreover, EV-IGF1 treatment upregulated markers associated with hepatocyte proliferation, such as PCNA, HGF, and Cyclin D1, as well as IGF-1, a marker of hepatocyte function. In contrast, the treatment with EV-IGF1 reduce the expression of pro-inflammatory genes such as IL-1 $\beta$ , TNF- $\alpha$  and iNOS. **Conclusion:** Our findings provide experimental evidence suggesting that EV-IGF1 could be a potential therapeutic agent for promoting liver regeneration following surgical injury.

### P3 POSTERS

FECHA Y HORA: 20/11/2024 16:10-17:10 H

COORDINADORES: VILLAREAL ALEJANDRO, OLEA DANIELA, BOUZA MARIA DEL ROSARIO

### 194. 088 FGF6, MIR302D AND MIR19B PROMOTE CARDIOMYOCYTE PROLIFERATION

Agustina Scharn<sup>1</sup>, María del Rosario Bauzá<sup>1</sup>, Alejandro Simonin<sup>2</sup>, Francisco Stefano Cimbaro<sup>1</sup>, Pilar Ferrer<sup>1</sup>, Alberto Crottogini<sup>1</sup>, Mariano Belach<sup>2</sup>, Fernanda Daniela Olea<sup>1</sup>, Paola Locatelli<sup>1</sup>.

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Cardiovascular disease is the leading cause of mortality worldwide, being myocardial infarction with significant cardiomyocyte (CM) loss one of its most severe consequences. Among the strategies to overcome this, is to stimulate cell cycle re-entry to promote cell proliferation. Our aim was to assess the *in vitro* effects of FGF6, miR302d and miR19b overexpression on cell proliferation and angiogenesis in neonatal rat CMs (NRCMs). Methods: NRCMs were transduced with a baculoviral vector encoding FGF6, miR302d and miR19b (Bv-FGF6-miR302d-miR19b) at MOI 200 and the effect of transgene overexpression was evaluated at 2 and 5 days post-transduction (PT). Cell proliferation was assessed by MTS assay, gene expression by RT-qPCR and immunocytochemistry. Angiogenesis was evaluated by tubulogenesis assay at the same time points. Results: Cyclin D1 and cyclin A2 expression were increased in CMs transduced with Bv-FGF6-miR302d-miR19b compared to Bv-Null at 2 days PT (Cyclin D1 fold increase:  $2.07 \pm 0.78$  vs  $1.03 \pm 0.29$ ,  $p < 0.05$ ; Cyclin A2:  $2.45 \pm 1.13$  vs  $1.14 \pm 0.6$ ,  $p < 0.05$ ) and 5 (Cyclin A2:  $3.71 \pm 1.38$  vs  $2.65 \pm 1.66$ ,  $p < 0.05$ ) days PT. Proliferation evaluation by MTS revealed increased cell division in cells incubated with supernatant of CM Bv-FGF6-miR302d-miR19b compared to CM Bv-Null at 2 (Cell proliferation:  $113.26 \pm 6.69$  vs CM Bv-Null:  $100 \pm 4.53\%$ ,  $p < 0.01$ ) and 5 (Bv-FGF6-miR302d-miR19b:  $109.97 \pm 2.29\%$  vs CM Bv-Null:  $100 \pm 11.69\%$ ,  $p < 0.05$ ) days PT. Immunocytochemistry showed an increase in CMs transduced with Bv-FGF6-miR302d-miR19b compared to Bv-Null at 2 days PT ( $46.26 \pm 13.17\%$  vs  $23.58 \pm 10.57\%$  de CM Ki67+,  $p < 0.01$ ) and 5 ( $34.71 \pm 13.68$  vs  $21.39 \pm 9.55\%$  CM Ki67+,  $p < 0.01$ ) days PT. Tubulogenic assay showed higher ring density in the Bv-FGF6-miR302d-miR19b vs Bv-Null at 2 and 5 days PT. Conclusion: FGF6, miR302d and miR19b overexpression in CMs induced cell proliferation, increased expression levels of cell proliferation genes and promoted angiogenesis *in vitro*.

**195. 204 THE LIVER OVEREXPRESSION OF BRECEPT, A NOVEL PAN-TGF- $\beta$  INHIBITOR, IMPROVES INSULIN SENSIBILITY IN A MASLD RAT MODEL**

Carolina Anahí Cámara<sup>1</sup>, Anabela La Colla<sup>1</sup>, Agustina Neto<sup>1</sup>, Tania Melina Rodríguez<sup>2,3</sup>, Stella Maris Echarte<sup>1</sup>, Ricardo Alfredo Dewey<sup>2,3,4,5</sup>, Andrea Chisari<sup>1</sup>

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common chronic liver disease, characterized by excessive accumulation of fat in the liver associated with metabolic dysfunction. Insulin resistance (IR) and hyperinsulinemia are central keys in the pathogenesis of MASLD. In a physiological state, insulin increases glucose intake and glycogen synthesis in hepatocytes. However, when the response to insulin is altered, hepatocytes shunt the excess glucose into lipogenic pathways, increasing liver steatosis. Nowadays, treatments for MASLD patients are limited. Recently, the TGF- $\beta$  pathway was linked to liver steatosis and IR. Brecept (Br), a pan-TGF- $\beta$  inhibitor, was developed by fusing T $\beta$ RII-SE, a novel soluble TGF- $\beta$  type II receptor splice variant, to the Fc portion of human IgG (T $\beta$ RII-SE/Fc). Thus, we aimed to study the effect of lentiviral-mediated liver overexpression of Br (Lv-Br) on insulin resistance in a rat model of MASLD induced by Western Diet (WD). We compared three groups: control, WD, and WD that received intrahepatic injection of the lentiviral vector encoding Br (WD+Lv-Br) at week 10. In week 21, animals were sacrificed. In serum samples, comparing

the WD+Lv-Br group with the WD group, we observed a strong tendency to reduce insulin resistance (decreased insulin concentration, HOMA-IR index, and increased QUICKI index). Moreover, in liver tissue stained with PAS, comparing the WD+Lv-Br group with the WD group, we observed a significant recovery of glycogen deposition in hepatocytes, which is closely related to a diminished IR. Finally, in liver tissue stained with H&E from the WD+Lv-Br group, compared with the WD group, we observed a remarkably decreased microvesicular steatosis in hepatocytes, which indicates an improvement in the ability of the liver to respond to insulin. Therefore, these results suggest that liver overexpression of Br exerts a beneficial effect on ameliorating IR in a MASLD model induced by WD in rats.

**196. 225 T $\beta$ RII-SE/Fc FUSION PROTEIN MODULATES LIVER INFLAMMATION AND LIPOAPOPTOSIS IN A RAT MODEL OF INDUCED LIVER FIBROSIS**

Anabela La Colla<sup>1</sup>, Carolina Anahí Cámara<sup>1</sup>, Tania Melina Rodríguez<sup>2,3</sup>, Stella Maris Echarte<sup>1</sup>, Ricardo Alfredo Dewey<sup>2,3,4,5</sup>, Andrea Nancy Chisari<sup>1</sup>

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<sup>4</sup>Centro de Medicina Traslacional (CEMET) Hospital de Alta Complejidad en Red "El Cruce" Nestor Carlos Kirchner, Florencio Varela.

<sup>5</sup>Rad Bio S.A.S.

Chronic liver diseases are characterized by an excessive wound-healing response that results in liver fibrosis, ultimately leading to organ failure. It is well established that transforming growth factor beta (TGF- $\beta$ ) promotes liver fibrosis. Thus, the development of agents with high potential for achieving a specific and long-lasting block of TGF- $\beta$  action is clinically relevant. We have recently described the presence in human cells of a new splicing variant of the TGF- $\beta$  type II receptor that renders a truncated mature protein of 57 amino acids known as T $\beta$ RII-SE. Previously, we showed that lentiviral-mediated overexpression of this truncated endogenous isoform of the human TGF- $\beta$  type II receptor Fc-tagged protein (Lv.T $\beta$ RII-SE/Fc) could regulate liver injury and fibrogenesis to exert its therapeutic effect against liver fibrosis. Therefore, the aim of this work was to deepen the mechanisms involved in the therapeutic effect of Lv.T $\beta$ RII-SE/Fc in a carbon tetrachloride (CCl<sub>4</sub>)-induced liver fibrosis rat model. Experimental groups were designed as follows: Control group received CCl<sub>4</sub> vehicle; CCl<sub>4</sub> group received CCl<sub>4</sub> for 10 weeks; Lv.T $\beta$ RII-SE/Fc + CCl<sub>4</sub> group received CCl<sub>4</sub> for 10 weeks and Lv.T $\beta$ RII-SE/Fc at week 4 (n=4-5). In accordance with previous results, we detected decreased hepatic Col1A1 mRNA expression in Lv.T $\beta$ RII-SE/Fc-treated animals when compared to the CCl<sub>4</sub> group. By RTqPCR, we observed diminished levels of TNF- $\alpha$ , IL-6, and TGF- $\beta$ 1 mRNAs in the Lv.T $\beta$ RII-SE/Fc + CCl<sub>4</sub> group compared to the CCl<sub>4</sub> group. Interestingly, we also observed that Lv.T $\beta$ RII-SE/Fc administration enhanced hepatic lipid accumulation, involving CD36, DGAT2, and SCD1 mRNA modulation. Accordingly, administration of Lv.T $\beta$ RII-SE/Fc also significantly diminished PUMA CCl<sub>4</sub>-induced mRNA expression level. These results suggest that lentiviral delivery of T $\beta$ RII-SE/Fc modulates liver inflammation and hepatocyte lipoapoptosis to exert its therapeutic effect against CCl<sub>4</sub>-induced liver fibrosis in rats.

**197. 297 DEVELOPMENT OF POLYMER 3D PRINTED SCAFFOLDS FOR BONE TISSUE REGENERATION**

Ignacio Ruiz<sup>1</sup>, Ana María Cruz Gaitán<sup>1</sup>, Jorge Aguilar<sup>2</sup>, Mariano Vasallo<sup>2</sup>, María Eugenia Balañá<sup>1</sup>, Gustavo J. Leirós<sup>1,2</sup>

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**Introduction:** The regenerative capacity of bone tissue is limited in extensive or pathological injuries. The transplantation of living bone tissue is a limited surgical procedure. Metallic or ceramic implants have limitations related to load, rejection, and high costs. Bioengineered bone substitutes emerge as a new therapeutic strategy. They must be osteoconductive/osteoinductive and require a 3D structure like the extracellular matrix of bone tissue. 3D printing allows the design and production of complex biomedical scaffolds with controlled microstructures using CT images of bone lesions. **Objectives:** This study aimed to evaluate design parameters affecting the mechanical and structural properties of polylactic acid (PLA) and polycaprolactone (PCL) constructs fabricated via 3D printing to serve as scaffolds for bone tissue substitutes. **Materials & Methods:** PLA and PCL scaffolds were fabricated using a fused deposition printer. Technical-grade filaments were used according to the manufacturer's recommended printing temperature. The design comprised a square prism, with square pore sizes ranging from 300-900  $\mu\text{m}$ . The printer nozzle was 0.1 mm in diameter, and the flow speed was varied between 0.1 mm/s and 0.2 mm/s. Porosity was determined by measuring void volume, and compression was evaluated using the standardized ASTM D695-15 method. **Results:** The porosity for PLA and PCL scaffolds range from 56-86% and 77-47% respectively and decrease with increasing flow speed. Both average compression moduli and ultimate compressive strength obtained in PLA and PCL was within the range of mandibular trabecular bone without cortical planes as referenced in the literature and increase with decreasing pore size. **Conclusions:** These results demonstrated that the porosity and compressive moduli obtained with the PCL or PLA scaffolds, designed in this study, were comparable to those of trabecular bone. Therefore, these scaffolds should be useful for fabricating cell-free bone substitutes.

**198. 300 INTEGRATION OF THREE-DIMENSIONAL CELL CULTURE TECHNIQUES WITH BIOMATERIALS FOR BONE REGENERATION DEVELOPMENTS**

Gastón Ignacio Angelini Marquiani <sup>1</sup>, Matías Eduardo Rizzo <sup>1</sup>, Angel Avila Ventre <sup>1</sup>, María Albertina Loureiro <sup>1</sup>, Jimena Dominguez <sup>1</sup>, Carolina Costa <sup>1</sup>, Monica Loresi <sup>1</sup>, Maximiliano D'adamo <sup>1</sup>, Matías Valenzuela Alvarez <sup>1</sup>, Marcela Fabiana Bolontrade <sup>1</sup>.

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Bone regeneration is complex and often fails to replicate original bone features. Mesenchymal stem cells and fibroblasts contribute by remodeling the extracellular matrix and supporting it calcification through cell recruitment or differentiation into specialized cells. Classic cell culture techniques face limitations in replicating the cells physiological niche accurately. To overcome this, 3D culture techniques like scaffold cellularization and spheroid production have been developed. In this study, we determined the properties of previously developed scaffolds, made from Platelet Rich Plasma (PRP) and polymerized Poly(lactic acid) (PLA). To assess the interaction between PRP and culture media, and thereby design a scaffold that can be effectively cellularized in dynamic exchange with the micro-environment, we analyzed the liquid penetration speed, finding that it remained at a constant speed at  $0.83 \pm 0.09 \text{ mm/h}$  during the first 6hs, after which it decreased until reaching a plateau, with a total penetration of 12mm after 24hs. Cell proliferation on PRP scaffolds was quantified by DAPI positive nuclear counting. This revealed a cell growth profile with a linear trend increasing daily by 75.3% ( $R^2$  0,906). Fluorescent vital staining with ethidium bromide and acridine orange was performed to evaluate live and dead cells in cultures grown with PRP and PLA, in addition to analyzing this distribution in spheroids. This allowed us to assess the 3D cellular architecture, showing a concentration of cells that enter apoptosis in the center with almost no presence of these in the superficial layer. Further, we assessed osteogenic differentiation within the scaffolds systems. The development of scaffolds incorporating preformed 3D cellular structures along with suspended cells could significantly improve the

functional capabilities of the scaffold. These results provide useful insights for designing cellularizable scaffolds suitable for advancing regenerative medicine applications.

**P4 POSTERS**

**FECHA Y HORA:** 21/11/2024 11:00-12:00 H

**COORDINADORES:** MAYMÓ JULIETA, VALENZUELA ALVAREZ MATIAS

**199. 362 DEVELOPMENT OF MAGNETITE/IRON OXIDE NANOPARTICLE SYSTEMS FOR APPLICATION IN COMBINED TREATMENTS WITH RADIOTHERAPY OF MELANOMA**

Cecilia Grissi<sup>1</sup>, Marina Perona<sup>2</sup>, Irene L. Ibañez<sup>1,6</sup>, Victoria A. Gomez<sup>3,5</sup>, Dolores Perez<sup>3,5</sup>, Mariela del Grosso<sup>1,5</sup>, Hebe Durán<sup>1,4,5</sup>

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<sup>5</sup>Instituto de Nanociencia y Nanotecnología (INN, CNEA-CO-NICET), Nodo Constituyentes, San Martín, Buenos Aires, Argentina.

A cancer stem cells (CSC) subpopulation in melanomas resistant to traditional therapies has been demonstrated. An increase in the expression of mitochondrial proteins has been described in CSCs. These multi-resistant melanoma cells were susceptible to inhibition of the mitochondrial respiratory chain in combination with other therapies, indicating that disruption of oxidative energy metabolism could be used for treating resistant melanomas. Mitochondrial targeting of therapeutic agents using nanoparticles (NPs) could be a way to overcome this issue. NPs can be modified for selective mitochondria targeting. In this sense, superparamagnetic iron oxide NPs (SPIONs) possesses unique properties that make them excellent radiosensitizing agents. The aim of this study was to functionalize SPIONs with triphenylphosphonium (SPIONs-TPP) for selective targeting to mitochondria and evaluate its radiosensitizing properties in melanoma cells. SPIONs were synthesized by the coprecipitation method, functionalized by linking TPP residue and characterized by Dynamic Light Scattering, Transmission Electron Microscopy, Fourier transform infrared spectroscopy, and Thermogravimetry. A375-G10 cells were incubated with different concentrations of SPIONs-TPP. Cell viability was measured by MTT method. Surviving fractions (SF) were obtained by clonogenic assay. SPIONs-TPP showed superparamagnetic behavior and low dispersion in shape and sizes. Basic groups of TPP anchored on the external surface of the coated magnetite were detected. No cytotoxicity was found in cells exposed up to 250  $\mu\text{g/ml}$  of NPs for 24 h and this concentration was used to irradiate cells with a <sup>137</sup>Cs gamma source (0-5 Gy). SF at 1 Gy was significantly reduced in MNPs-IR treated cells (0.077 and 0.03 for IR and SPIONs-TPP-IR treated cells, respectively,  $p < 0.001$ ). **Conclusions:** The method of preparation of modified SPIONs with TPP was effective. SPIONs-TPP shows radiosensitizing properties at 1 Gy in melanoma cells.

**200. 421 PAPAINE-BASED MICROPARTICLES FOR CHEMICAL TREATMENT OF DENTAL CARIES: FORMULATION AND CHARACTERIZATION**

Fiorella Ventura<sup>1</sup>, Nadia Chiaramoni<sup>2</sup>, Aldo Squassi<sup>1</sup>, Carlos F. Mendez<sup>3</sup>

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A chemical treatment model for dental caries has been proposed, based on the selective removal of necrotic and infected dentin using proteolytic enzymes. Objective: To develop a chemical caries removal system based on papain aggregates formulated as a suspension of defined-sized microparticles that can be dispensed in a controlled manner onto necrotic tissue, avoiding its action on dental pulp. Materials and Methods: Papain particles were synthesized by gamma radiation-induced crosslinking (10 kGy) of a solution (30 mg/ml) of pure enzyme in sodium phosphate buffer pH 7.0 containing 30% (v/v) ethanol. Particle size was determined by static light scattering using a He-Ne laser (633 nm) in a Mastersizer 2000E instrument. Proteolytic activity was analyzed by UV spectroscopy of tyrosine residues in a gelatin degradation assay in the presence and absence of cysteine, and by separation on polyacrylamide gels under denaturing conditions (SDS-PAGE). Results: 100% of the particles showed a size smaller than 20  $\mu\text{m}$ , with an average particle diameter of 0.37  $\mu\text{m}$ . Addition of cysteine to the particle suspension buffer resulted in enzyme activation with a significant increase ( $p < 0.05$ ) in proteolytic activity over time (0-60 min) at pH 7.0 compared to samples without papain addition, also evidenced by SDS-PAGE as a decrease in the 95 and 100 kDa bands of gelatin  $\alpha$ -chains and a concomitant increase in degradation products of lower molecular weight. Conclusion: Our results indicate that the proteolytic enzyme delivery system consists of an enzymatically active, defined-sized particulate material that may be useful for the chemical treatment of dental caries.

**201. 422 THREE DIMENSIONAL STUDY OF THE RECOVERY OF ALVEOLAR BONE DUE TO EXPERIMENTAL PERIODONTITIS. EFFECT OF THE LOCAL TREATMENT WITH LOW DOSES OF PTH 1-34**

Marina Soledad Bonanno<sup>1,2</sup>, Estefanía Magalí Zeni Coronel<sup>1,3</sup>, Diego Jorge Vazquez<sup>4</sup>, Gómez Ailin<sup>4</sup>, Mario Ricardo Davison<sup>5</sup>, Bozal Carola<sup>2</sup>, Susana Noemí Zeni<sup>1</sup>.

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The aim for this study was to evaluate the effect of a low dose of PTH 1-34 for periodontal tissue recovery in an experimental model of periodontitis by microCT technique. 12 adult female Wistar rats with ligature-induced periodontitis were divided into Lig and PTH groups ( $n=6$  each). 3 times per week during 21 days received saline solution and 0.2  $\mu\text{g}/\text{Kg}/\text{dose}$  of PTH 1-34 respectively. At the end, hemimandibles were extracted and scanned using a microCT system (Skyscan1272, Bruker). Volumetric alveolar bone parameter of bone volume fraction (BVF) was determined in the interradicular alveolar bone and in the complete alveolus. The microarchitecture parameters of trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular separation (Tb.Sp) were also determined. Results (mean $\pm$ SD for Lig vs PTH): In the interradicular alveolar zone, BVF did not reach statistical significance ( $35.1 \pm 2.2$  vs  $34.4 \pm 1.5$   $p=0.7$ ); while Tb.th and Tb.Sp showed a tendency to be lower in the Lig group ( $0.04 \pm 0.02$  vs  $0.05 \pm 0.01$   $p=0.09$  and  $0.1 \pm 0.03$  vs  $0.3 \pm 0.1$   $p=0.08$ , respectively). Conversely, Tb.N was significantly lower in the PTH group ( $9.4 \pm 1.7$  vs  $6.8 \pm 1.3$   $p=0.05$ ). Consequently, the microCT technique did not evidence recovery of the interradicular alveolar bone. When the complete alveolus was evaluated, PTH group showed significantly higher BVF value ( $23.0 \pm 0.6$  vs  $25.9 \pm 1.5$   $p=0.05$ ) but no significant differences in Tb.Th ( $0.06 \pm 0.03$  vs  $0.06 \pm 0.01$   $p=0.2$ ), Tb.N ( $3.9 \pm 0.5$  vs  $4.1 \pm 0.4$   $p=0.6$ ) and Tb.Sp ( $0.7 \pm 0.1$  vs  $0.6 \pm 0.08$   $p=0.5$ ) were observed. MicroCT showed that in the complete alveolus, the PTH group presented higher levels of BVF without differences in the other studied bone quality parameters. Under our experimental conditions, the intermittent administration of a low dose of PTH decreased the progression of periodontal disease without reaching a

complete recovery.

**202. 428 FUCOIDAN-COATED LIPOSOMES FOR PULMONARY DELIVERY OF AZITHROMYCIN AND CURCUMIN AS A NOVEL APPROACH TO TREAT COPD**

María Julia Altube<sup>1</sup>, Sofía Patané<sup>1</sup>, Horacio Jerez<sup>1</sup>, Ezequiel Latour<sup>2</sup>, Andrea Ponce<sup>3</sup>, Fernando Dellatorre<sup>2</sup>, Juan Manuel Figueroa<sup>4</sup>, Andrea Dugour<sup>1</sup>, Ana Paula Perez<sup>1</sup>

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Chronic obstructive pulmonary disease (COPD) is a lung condition primarily caused by an inflammatory response to cigarette smoke, with limited treatment options available. This study aimed to develop a novel formulation for COPD treatment, combining the macrolide antibiotic azithromycin (AZ) and the natural polyphenol curcumin (CUR) in cationic liposomes coated with fucoidans. Cationic liposomes containing AZ and CUR (LAC) were prepared by lipid film hydration and characterized using dynamic light scattering (DLS) and UV-vis spectroscopy. LAC coating with fucoidans derived from the algae *Myriogloea major* (LACFuc) was confirmed through DLS and Z potential measurement. The effects of LAC, LACFuc, and free AZ-CUR on THP-1 cells (human macrophages) were evaluated by assessing cell viability (MTT), intracellular ROS levels (DCFH-DA), and IL-8 release in LPS-stimulated cells (ELISA). Finally, IL-6 release was evaluated in a COPD model using Calu-3 cells (human lung epithelium) treated with a concentrated smoke extract (CSE). LAC exhibited a nanoscale size of  $333 \pm 65$  nm, low polydispersity ( $0.3 \pm 0.1$ ) and a positive Z potential of  $12 \pm 3$  mV. The concentrations of AZ and CUR were  $0.36 \pm 0.04$  and  $0.17 \pm 0.01$  mg/mL, respectively ( $n=7$ ), with an aqueous solubility increased 145-fold for AZ and 280-fold for CUR. After fucoidan coating, LACFuc showed an increased size ( $1137 \pm 201$  nm) and a shift to a negative Z potential ( $-33 \pm 2$  mV) compared to LAC. In LPS-stimulated THP1, at non-cytotoxic concentrations, LACFuc reduced ROS levels by 43  $\pm$  5% ( $p < 0.0001$ ) and IL-8 release by 1.7-fold ( $p < 0.0001$ ) compared to untreated control. In the COPD model, LACFuc reduced IL-6 release induced by CSE in Calu-3 cells ( $p < 0.0001$  vs. untreated CSE cells) to levels comparable to the unstimulated control. These findings show that LACFuc is a promising formulation for the treatment of lung inflammation and is worth further study to evaluate its therapeutic potential in COPD.

**203. 450 NEBULIZABLE NANOVESICLES WITH TACROLIMUS FOR TREATING PULMONARY FIBROSIS**

Ezequiel Nicolás Caputo, María José Morilla, María Julia Altube, Eder Lilia Romero

Centro de Investigación y Desarrollo en Nanomedicinas (CIDE), Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes

Idiopathic Pulmonary Fibrosis (IPF) is a chronic, progressive condition characterized by scarring and decreased lung function. Tacrolimus (Tac) is an immunosuppressant that has been studied for the treatment of IPF with positive results. However, its oral administration is limited due to numerous adverse effects. Inhalation of Tac would target the therapeutic site of interest more directly, but its low water solubility limits nebulization. Incorporating Tac into nebulizable nanovesicles could enhance pulmonary delivery efficiency and safety. In this study, nanovesicles targeting alveolar macrophages were prepared using archaeal lipids from *Halorubrum tebenquichense* (Arq-Tac) and compared with a conventional formulation of cholesterol and soybean phosphatidylcholine (Lipo-Tac). The formulations were prepared by the lipid film hydration method followed by son-

ication, extrusion, and filtration. Both formulations had nanoscale sizes as determined by dynamic light scattering: Arq-Tac  $253\pm 3$  nm, pdi  $0.46\pm 0.01$ , Z-potential  $-44\pm 3$  mV; and Lipo-Tac  $408\pm 23$  nm, pdi  $0.29\pm 0.07$ , Z-potential  $-4\pm 1$  mV. The drug loading capacity, measured in  $\mu\text{g}$  of Tac/mg of lipids, was  $77\pm 20$  for Arq-Tac and  $46\pm 33$  for Lipo-Tac. Additionally, both formulations maintained their structural stability during nebulization with a 90% recovery of lipids and no changes in size or pdi. Cytotoxicity was assessed on human THP-1 derived macrophages and human lung fibroblasts (MRC-5) using the MTT assay. No cytotoxicity was observed up to  $150 \mu\text{g/mL}$  of lipids and  $11.5 \mu\text{g/mL}$  of Tac after 24 hs of incubation. In LPS-activated THP-1 cells, both formulations were able to reduce IL-8 release, with  $45\pm 11\%$  for Arq-Tac ( $p < 0.01$ ) and  $58\pm 11\%$  for Lipo-Tac ( $p < 0.001$ ) compared to LPS-stimulated cells without treatment. These results suggest Arq-Tac nanovesicles offer higher Tac incorporation, better nebulization resistance, and effective IL-8 inhibition, making them strong candidates for further evaluation as antifibrotic agents.

#### 204. 536 ADVANCING MAGNETIC NANOTECHNOLOGY FOR FUTURE THERAPIES TARGETING INNER EAR CONDITIONS

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Hearing loss (HL) affects more than 5% of the global population, and projections suggest that up to 50% of young people could be affected in the coming years. While most HL cases are linked to local inflammation, treatment options remain limited due to the protective barriers of the inner ear. Intratympanic (ITT) administration is considered the most efficient drug delivery method. Despite this, the round window membrane (RWM) remains a significant barrier for most drugs. Our research seeks to overcome this challenge by using nanotechnology. Specifically, we propose the ITT injection of biocompatible magnetic nanoparticles (MNPs) loaded with Diclofenac (Dfc) (MNPs@FA.Dfc). These nanoparticles, which we previously developed for this purpose in our research group, will be guided through the RWM into the inner ear using an external magnetic field (EMF). In this study, we modeled the magnetic forces required to pull the MNPs through the RWM at both, short (2 mm) and long distances (2 cm) in a mouse's head. The amount of MNPs crossing the RWM strongly depends on the applied magnetic force and we found *ex vivo* that, at short distances (force on one MNP of about  $6 \times 10^{-18}$  N), 2-3% of the administered MNPs cross the RWM. Additionally, we initiated a concept trial of ITT injection using MNPs in a murine model, performed under anesthesia throughout the procedure. Auditory brain response testing confirmed that MNPs ITT injection and exposure to EMF for 1 hour did not elevate hearing thresholds, maintaining normal values after two months. These results suggest that the eardrum tissue was not compromised due to the injection and healed properly. These findings allow us to conclude that the MNPs can cross the RWM with the adequate design of the EMF, without affecting hearing. The amount of Dfc they can deliver within the inner ear still needs to be determined. The data presented are crucial for future *in vivo* trials using the developed administration system for therapeutic purposes.

#### 01 - COMUNICACIONES ORALES

FECHA Y HORA: 19/11/2024 11:00-12:00 H

LUGAR: AUDITORIO

COORDINADORES: REPETTO MARISA GABRIELA, BURGUEÑO ADRIANA

#### 205. 031 COMPARISON BETWEEN THE INTAKE OF A BREAD MADE WITH WHOLE OAT MALTED FLOUR AND A BREAD MADE WITH WHEAT FLOUR ON FUNCTIONAL PROPERTIES IN AN EXPERIMENTAL MODEL IN GROWING WISTAR RATS

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High consumption of white bread made with wheat flour has been correlated with the development of chronic diseases, hence the development of baked goods containing healthier ingredients. Whole oats can be malted, obtaining a flour with a greater amount of soluble low molecular weight fibers that, in addition to the beta-glucan content, improve the healthy properties to prepare functional foods. The aim of this study was to compare the effect of the intake of a bread made with whole oat malted flour (BOM) and a bread made with wheat flour (BW) on biomechanical bone parameters and volume stool during 60 days, in a rat model. A total of 16 newly weaned male Wistar rats (8/group) were fed with semisynthetic diets prepared with BOM and BW diet according to the American Institute of Nutrition Diet. Throughout the experience, feces were collected during the 60 days to determine the moisture content (%). At the end of the study rats were anesthetized and right femur was excised to measure the following biomechanical properties: Limit elastic load (Wy), Diaphyseal stiffness (Wy Dy) and Maximum fracture load (Wf max). BOM group presented a higher moisture content of the feces than BW group during the 60 days (%) ( $21.7.0\pm 1.2$  vs  $14.0\pm 1.5$ ,  $p < 0.0001$ ). The BOM group presented significantly higher biomechanical bone properties than BW group: Wy ( $120\pm 16$  vs  $84\pm 20$ ;  $p = 0.0434$ ), Wydy ( $216 \pm 49$  vs  $139. \pm 36$ ;  $p = 0.0125$ ) and Wfmax ( $147\pm 16$  vs  $125\pm 11$ ;  $p = 0.0293$ ). Conclusions: the high fiber content of BOM would be correlated with water retention and increased stool. On the other hand the intake of BOM, showed a prebiotic effect improving bone quality. These intestinal and bone benefits could lead us to affirm that bread made with malted oats could be considered a food with better functional properties than white bread and that it would be suitable for, carrying out a diet with healthier characteristics.

#### 206. 187 IMPACT OF DIETS ENRICHED WITH BEEF FAT ON THE METABOLISM OF C57BL/6J MICE

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C57BL6J mice are a widely used model for the evaluation of fat enriched diets. The objective of this work was to evaluate the effects of diets enriched with different percentage of beef fat on the metabolism of 12-week-old male C57BL6J mice. The mice were divided into 3 groups and fed for 16 weeks with: balanced feed (C; n=7), feed enriched with 7,5% beef fat (mB; n=7) feed enriched with 24% beef fat (B; n=7). The composition of the prepared feeds, body weight, intake, nutritional status and plasma biochemical parameters were evaluated. The food fed to group C had 6% of calories from fat; the group mB had 19% of calories from fat and the group B had 40% of calories from fat. The intake of group B was lower than groups C and mB during the 16-week treatment but no differences in body

weight were observed ( $p < 0.05$ , one-way anova). Regarding the nutritional status, it was observed that the caloric intake of group mB was higher than that of group C; total fat and saturated fat intakes differed among the 3 groups analyzed, being higher for group B; cholesterol intake was lower for the control group compared to the mB and B groups; no differences in feed efficiency were observed ( $p < 0.05$ , one-way anova). It was also observed that the liver weight/body weight index was higher for group C compared to group B, but no differences were observed in the total fat weight/body weight, testicle weight/body weight and epididymis weight/body weight indices ( $p < 0.05$ , one-way anova). Finally, in plasma, it was observed that group B presented higher total cholesterol, LDL-col and atherogenic index values than groups C and mB, and no differences were observed in glucose, HDL-col and triglycerides ( $p < 0.05$ , one-way anova). In the present study we observed that a diet enriched with beef fat influences total cholesterol, LDL-col and atherogenic index values independently of the percentage consumed and does not influence body weight.

**207. 200 ATTENUATION OF GLUCOLIPOTOXICITY ON BETA CELL FUNCTION AND PERIPHERAL TISSUE IN A DIET-INDUCED OBESITY MODEL: EFFECT OF COMPOUND A (CPDA)**

Miranda Sol Orellano<sup>1,2</sup>, Andrea Scelza-Figueroa<sup>1,2</sup>, Carolina Sétula<sup>1,2</sup>, Lucía Lameroli Mauriz<sup>2,3</sup>, Milagros Argañaras<sup>1</sup>, Pilar Montechi<sup>2</sup>, Catalina Atorrasagasti<sup>2,3</sup>, Marcelo Javier Perone<sup>1,2</sup>, Luz Andreone<sup>1,2</sup>.

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Type 2 diabetes (T2D) is an endocrine-metabolic disease characterized by hyperglycemia due to peripheral insulin resistance and the gradual loss of pancreatic  $\beta$ -cell function. The glucolipotoxic environment (GLTe) also negatively affects peripheral tissues aggravating the disease. We showed that compound A (CpdA), a non-steroidal dissociative GR-ligand, mitigates oxidative and ER stress in  $\beta$ -cells under the inflammatory environment of type 1 diabetes. Preliminary *in vitro* results indicated that CpdA also protects  $\beta$ -cell viability and function from in a GLTe. We aim to explore whether CpdA can attenuate the effects of GLTe on  $\beta$ -cells and peripheral tissues in a diet-induced obesity murine model. Eight-week-old male C57BL/6N mice were fed with either a standard chow diet (CD) or high-fat (20% w/w bovine fat) high-sugar (5% sucrose w/v in water) diet (Western diet, WD). From week 5 of diet mice were treated with CpdA (i.p. 2.5 $\mu$ g/g, 3 times a week) or vehicle. After 15 weeks, histological analyses of the pancreas, liver, and adipose tissue were performed by H&E staining and/or immunofluorescence. CpdA treatment in mice fed WD improved glucose tolerance (IpGTT) and increased insulin sensitivity (IpITT) vs. WD group ( $p < 0.05$ ). In WD group, histological analysis showed a higher proportion of large islets ( $p < 0.05$ ) and reduced insulin expression in  $\beta$ -cells ( $p < 0.05$ ) vs. CD mice; these effects were counteracted by CpdA treatment ( $p < 0.05$ ). Mice fed WD and treated with CpdA showed reduced hepatic steatosis and inflammation, resulting in a lower NAS score (MASLD progression) compared to WD mice ( $p < 0.05$ ). CpdA treatment diminished WD-induced local inflammation in adipose tissue (crown like structures,  $p < 0.05$ ). CpdA administration did not show apparent side effects neither affect body weight during treatment. We present evidence of the *in vivo* potential protective effect of CpdA, mitigating the harmful impact of GLTe on  $\beta$ -cell functionality and preserving peripheral tissues.

**208. 267 HEMIN TREATMENT ATTENUATES ENDOTOXEMIA ASSOCIATED WITH STEATOHEPATITIS INDUCED BY THE ADMINISTRATION OF A SUCROSE RICH DIET**

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Previously, we described the development of steatohepatitis associated with insulin resistance (IR) induced in rats by the administration of a sucrose-rich diet (SRD). Results from our laboratory demonstrate a hepatoprotective effect of hemin treatment in reducing the deleterious consequences of inflammation in the liver. However, whether these changes are associated with intestinal effects of hemin is still unknown. The aim of the present study was to determine the effects of hemin treatment on microbiota, intestinal barrier, and endotoxemia which are altered in SRD-rats. Male Wistar rats were fed a standard diet (C, n=6) or 30% sucrose in drinking water for 12 weeks (SRD, n=8). A subgroup of SRD animals received hemin (15 mg/kg/48h ip) during the last two weeks of dietary modification (SRD+H, n=8). Histological analysis of the ileum showed no epithelial changes due to hemin treatment as evaluated by H&E staining, nor in the count of Goblet cells as assessed by Alcian Blue staining. No effect was detected on the decreased expression of tight junction proteins ZO-1 and occludin induced by the diet. Consistently, bacterial DNA levels in the liver and blood remained elevated in both SRD subgroups. No differences were observed in the Firmicutes/Bacteroidetes ratio or in the short-chain fatty acid content in feces between SRD and SRD+H-treated animals. However, hemin treatment blocked the increase in circulating endotoxin levels observed in SRD-treated rats, ( $p < 0.05$  SRD+H vs. SRD) and the hepatic expression of LBP, an LPS binding protein, was higher in the SRD-group but, even higher in animals treated with hemin ( $p < 0.001$  SRD vs. C and  $p < 0.001$  SRD+H vs. C or SRD). In summary, our results indicate that hemin treatment does not attenuate diet-associated damage to the ileum but could lower circulating endotoxin levels by promoting hepatic clearance of LPS.

**209. 376 EFFECTS OF MACLEAYA CORDATA EXTRACT IN OXIDATIVE AND ANTI-INFLAMMATORY STATUS OF DAIRY CATTLE DURING THE TRANSITION PERIOD**

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The transition period (TP) in dairy cows is characterized by significant metabolic, endocrine, and inflammatory challenges. We aimed to evaluate the effects of extracts obtained from the perennial herb *Macleaya cordata* (MCE) on balance energy, systemic oxidative stress and anti-inflammatory status in cows during the TP. On day 24 prepartum, the supplemented group (MCG; n = 30) received an oral controlled-release bolus that releases a daily dose of 1.5 g MCE (Sangrovit®, Phytobiotics, Germany) over a 70-d period. The control group (n = 30) received placebo capsules. Individual milk production was recorded three times every 10 days. Body condition score was assessed at -24, 7 and 21 days relative to parturition, blood samples were extracted and beta-hydroxybutyrate acid (BHBA) was determined with test strips and an automatic analyzer. Blood was collected in tubes containing potassium fluoride/trisodium EDTA (Wiener Lab, Argentina) and tubes without anticoagulants. Serum was used to measure the concentrations of non-esterified fatty acids and glucose (GLU) using commercial kits (Randox Laboratories Ltd., UK and Wiener Lab, Argentina). Interleukin-1 (IL-1) and IL-6 concentrations were measured using an ELISA kit (Thermo Fisher Scientific, USA). Plasma was used to analyze malondialdehyde and 3-nitrotyrosine (3-NO<sub>2</sub>-Tyr) by LC-MS/MS. An interaction effect

was observed by milk yield and GLU concentration, where de MCG registered a greater production on day 30 and the highest GLU concentration on day 7 postpartum ( $P < 0.05$ ). In addition, 3-NO<sub>2</sub>-Tyr and IL-1 concentrations were lower in the MCG ( $P < 0.05$ ). No differences were observed in the other evaluated parameters ( $P > 0.05$ ). These results suggest that the MCE administered to dairy cows had positive effects on the antioxidant system, inflammatory state and milk yield during the TP.

## 210. 510 EFFECTS OF A HIGH CHLORIDE DIET ON BLOOD PRESSURE: ROLE OF CHLORIDE CHANNELS IN RENAL TARGET ORGAN DAMAGE

María J. Rudi<sup>1</sup>; Nicolás M. Kouyoumdzian<sup>1, 2</sup>; Melanie Kim<sup>1</sup>; Candela Dominguez<sup>1</sup>; Natalia L. Rukavina Mikusic<sup>1, 2</sup>; Hyun J. Lee<sup>1</sup>; Beatriz Perazzi<sup>3</sup>; Mónica Galleano<sup>4, 5</sup>; Marcelo R. Choi<sup>1, 2, 6</sup>.

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Excessive salt (NaCl) consumption leads to the development of arterial hypertension (AH) and target organ damage. The CIC-K1 and CIC-5 channels are essential regulators of the Cl<sup>-</sup> anion, but the contribution of this anion to the deleterious effects generated by salt remains unknown. The objective was to evaluate the effects of excessive Cl<sup>-</sup> consumption on the development of hypertension and the inflammatory and oxidative response in the kidney. Male Wistar rats (n=8/group) were divided into four groups and fed with different equimolar diets for 3 and 6 weeks: control (group C); NaCl 8% (group NaCl); high in Na<sup>+</sup>: Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>, 11.8% (group Na); high in Cl<sup>-</sup>: CaCl<sub>2</sub> 3.80%, KCl 3.06% and MgCl<sub>2</sub> 1.30% (group Cl). Systolic blood pressure were determined (SBP), and in the kidney: renal histology, excretory function parameters, oxidative stress (TBARS and GPx), inflammation and fibrosis markers (NF-κB and TGF-β), and protein expression of chloride channels CIC-K1 and CIC-5 and AT1 and AT2 receptors. Differences with a  $p$  value  $< 0.05$  were considered statistically significant (\*). An increase in SBP, GPx activity, and renal expression of p50-NFκB, TGF-β, and AT1R were observed in the NaCl and Cl<sup>-</sup> groups. AT2R expression was not affected in any of the groups. TBARS production increased in all experimental groups compared to C\*. The NaCl and Cl groups showed a higher expression of CIC-K1, while CIC-5 was reduced in the NaCl group compared to C\*. Histopathological analysis showed that animals in the NaCl and Cl groups showed more pronounced structural alterations characterized by tubular dilation compared to the C group. In conclusion, Cl<sup>-</sup> would be co-responsible, together with Na<sup>+</sup>, for triggering oxidative and inflammatory renal damage and increasing blood pressure. The CIC-K1 and CIC-5 channels would be involved in the deleterious effects of excessive chronic consumption of this anion.

### P1 - POSTERS

FECHA Y HORA: 19/11/2024 11:00-12:00 H

COORDINADORES: LEE HYUN JIN, ILLESCA PAOLA GUADALUPE

## 211. 026 ASSESSMENT OF CARDIOMETABOLIC RISK FACTORS IN CHILDREN WITH METABOLICALLY HEALTHY OBESITY

Belén Davico<sup>1</sup>, Amanda Yanez<sup>1</sup>, Laura Gaete<sup>2</sup>, Ezequiel Lozano Chiappe<sup>1</sup>, Leonardo Gomez Rosso<sup>1</sup>, María Soledad Saez<sup>3</sup>, Walter Tetzlaff<sup>1</sup>, María Gabriela Ballerini<sup>1</sup>, Augusto

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**Introduction:** Metabolically healthy obesity (MHO) does not exhibit traditional cardiometabolic risk factors, but data on lipoprotein levels and composition, activities of associated enzymes and lipid transfer proteins, such as lipoprotein associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) and paraoxonase 1 (PON1), and reverse cholesterol transport (RCT), constituted by cellular cholesterol efflux (CEE), and the activities of lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP), are scarce. We aim to compare these parameters in childhood MHO, childhood metabolically unhealthy obesity (MUO) and healthy controls. **Methods:** We included 21 children and adolescents with MHO, according to Damanhoury (2018), 15 with MUO and 19 controls (Age: 9 - 18 years). Anthropometric parameters and Tanner stage were registered. HOMA-IR was calculated. Glucose, insulin, lipid and high sensitivity C reactive protein (hsCRP) levels were measured by standardized methods. CEE and Lp-PLA<sub>2</sub>, LCAT and CETP activities were quantified by radiometric assays, PON1 activity by a colorimetric method and HDL antioxidant activity (AA) by a fluorometric technique. **Results:** The three groups had similar age, sex, Tanner stage, hsCRP levels, AA, CETP and PON activities. MHO exposed higher HDL-C levels than MUO and lower than controls ( $p < 0.01$ ). TG levels in MHO were lower than in MUO and similar to controls ( $p < 0.01$ ). Both MHO and MUO had higher HOMA-IR ( $p < 0.01$ ), Lp-PLA<sub>2</sub> activity ( $p < 0.05$ ) and LDL-C ( $p < 0.01$ ), apo B ( $p < 0.05$ ), and insulin ( $p < 0.01$ ) levels, and lower apo A-I ( $p < 0.01$ ) levels, LCAT activity ( $p < 0.05$ ), and CEE ( $p < 0.05$ ) than controls. **Conclusion:** Even without some traditional cardiometabolic risk factors, similar to pediatric MUO, childhood MHO shows altered glucose metabolism, vascular inflammation, a more atherogenic lipid profile, and impaired RCT. Preventive strategies should target not only MUO but also MHO early in life to reduce future cardiovascular risk.

## 212. 097 COMPARISON BETWEEN DIFFERENT VEGETABLE OILS AS SUPPLEMENTATION OF OMEGA 3 ON A DIET WITH SATURATED FATTY ACIDS. EFFECT ON SERUM FATTY ACID PROFILES OF GROWING RATS

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Omega 3 fatty acids are considered protectors of cardiovascular health, anti-inflammatory. A balanced diet is important to maintain optimal health status and prevent diseases. Objective: to analyze the effect of diet containing butter, as fat source, with and without the supplementation with canola oil, flax oil and chia oil as source of omega 3, on serum fatty acid profiles of growing rats. Weanling Wistar rats received for 10 days normocaloric diet and fat was provided by butter (B group). The other groups received the same diet supplemented with 24mg/day of canola oil (BC), flax oil (FB) and Chia oil (ChB). Control group(C) received diet AIN'93. Serum fatty acids profiles were determined by gas chromatography. Statistical analysis: ANOVA and Tukey as post hoc test. Results(%Area) were expressed as Mean±SD: OLEIC: C:9,34±1,22a; B:17,65±1,64bc; BC:20,07±3,21bc; BF:15,08±1,47b; BCh:20,07±2,94c. LINOLEIC: C:22,44±1,67c; B:8,19±1,12a; BC:10,57±1,00b; BF:10,07±0,66b; BCh:8,82±0,59ab. LINOLENIC: C:1,03±0,28c; B:0,35±0,10a; BC:0,93±0,14c; BF:0,51±0,09ab; BCh:0,73±0,23bc. EPA: C:1,15±0,19a; B:1,05±0,18a; BC:1,83±0,28b; BF:1,88±0,28b; BCh:2,74±0,45c. DHA: C:2,70±0,61a; B:2,02±0,34a; BC:3,59±0,51b; BF:3,75±0,57b; BCh:3,44±0,73b. Means with no letter (a,b,c) in common, were different ( $p < 0.05$ ). All experimental groups showed lower levels of linoleic acid and higher levels of oleic acid, with re-



spect to C. All supplemented groups presented higher levels of linoleic acid, EPA and DHA than M. In a short period of time, all oils used as a supplement were effective in increasing serum levels of omega 3 fatty acids with respect to a diet containing saturated fatty acid. Supplementation with canola oil, in a diet rich in saturated fatty acids, would be the most effective to increase linolenic acid levels in serum. This would be a consequence of the type of lipid received, being not only important the percentage of lipids but the composition of the fatty acids pattern.

**213. 136 PERCEPTION OF NUTRITIONAL STATUS AMONG THE STAFF OF THE FACULTY OF MEDICAL SCIENCES (FCM) AT THE NATIONAL UNIVERSITY OF ROSARIO (UNR)**

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Obesity is a chronic, multifactorial, and progressive disease that puts people's lives at risk. The perception of health, body image, and weight influences lifestyle choices, affecting overall health. Perceiving the risk of disease encourages preventive actions according to behavioral models. Therefore, we aimed to evaluate the concordance in the perception of nutritional status by comparing the measured Body Mass Index (BMI<sub>m</sub>) with the self-reported BMI (BMI<sub>s</sub>), calculated from self-reported weight and height, with the self-perception of nutritional status (BMI<sub>p</sub>), and with the use of 3 different pictograms (BMI1, 2, and 3 respectively) among the staff of FCM UNR. After signing informed consent, 283 people, including both teaching and non-teaching staff, completed a questionnaire and were then measured to calculate the BMI and categorize their nutritional status as underweight (B), normal (N), overweight (S), and obesity (O) (<20, 20-25, 25-30, >30kg/m<sup>2</sup>, respectively). The results were: BMI<sub>m</sub> (%): B: 4.1; N: 30.3; S: 34.5; O: 31.0. BMI<sub>s</sub> (%): B: 5.9; N: 29.0; S: 35.9; O: 29.3 Kappa=0.792 (substantial). BMI<sub>p</sub> (%): B: 2.8; N: 40.0; S: 51.0; O: 6.2 Kappa=0.406 (moderate). BMI1 (%): B: 1.4; N: 28.3; S: 43.8; O: 26.6 Kappa=0.441 (moderate). BMI2 (%): B: 5.5; N: 10.0; S: 45.9; O: 38.6 Kappa=0.349 (fair). BMI3 (%): B: 12.4; N: 36.9; S: 50.0; O: 0.7 Kappa=0.157 (poor). The lack of concordance reflects the low perception of nutritional status. It seems that obese individuals have difficulties in understanding or accepting the implications of obesity as a chronic disease, which is associated with multiple health risk factors.

**214. 149 A PRELIMINARY INSIGHT INTO EPICARDIAL ADIPOSE TISSUE SECRETOME EFFECT ON ENDOTHELIAL CELLS METALLOPROTEINASES ACTIVITY**

Gregorio Fariña<sup>1</sup>, Fernando Ferrerya<sup>1</sup>, Camila Spagnuolo<sup>1</sup>, Dajhana Silva Soria<sup>1</sup>, Valeria Zago<sup>1,2</sup>, Juan Patricio Nogueira<sup>3</sup>, Marcelo Damonte<sup>4</sup>, Gabriela Berg<sup>1,2</sup>, Magalí Barchuk<sup>1,2</sup>

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Cardiovascular diseases (CVD) are still the leading cause of death worldwide, despite the numerous preventive measures developed. Epicardial adipose tissue (EAT) is a visceral adipose tissue, which surrounds and directly communicates with the myocardium and coronary arteries, and is considered a risk factor for CVD. As an active organ, EAT can secrete several factors that can modulate its underlying structures metabolism, contributing to the atherogenic process and CVD. In this preliminary study, we aimed to evaluate metalloproteinases (MMP) 2 a 9 activities from endothelial cells exposed

to EAT secretomes from coronary patients. Methods: human artery endothelial cells (EA.hy926) were exposed to conditioned media of EAT from patients undergoing coronary by-pass graft (CAD, n=5) or valve replacement (noCAD, n=5), during 24 hours. Incubation of cells with DMEM was used as Control (C). After each treatment, MMPs activities in endothelial cells were assessed by gelatinolytic zymography. Metabolic profile of patients was evaluated in serum. Results: CAD patients presented a more deleterious metabolic profile, with increased insulin-resistance (IR) markers. MMP2 activity from endothelial cells was higher in NoCAD compared to C (0.30±0.01 vs 0.17±0.04 RU respectively, p=0.01), and in CAD compared to C (0.39±0.01 vs 0.17±0.04 RU respectively, p=0.0004) and to NoCAD (p=0.001). For MMP9, the same behavior was observed (C: 0.11±0.01; NoCAD: 0.24±0.01; CAD: 0.37±0.03 RU; p<sup>CvsNoCAD</sup>=0.002; p<sup>CvsCAD</sup>=0.005; p<sup>NoCADvsCAD</sup>=0.005). MMP9 activity was directly associated to IR markers, such as triglycerides/HDL-Cholesterol index (r=0.72, p=0.02). Conclusion: in IR, EAT deleterious secretome increases endothelial MMPs activities, which could contribute to endothelial dysfunction and to the atherogenic process, thereby increasing CVD risk.

**215. 301 REDUCTION IN CARDIOVASCULAR RISK AFTER BARIATRIC SURGERY IN MORBIDLY OBESE PATIENTS**

Ezequiel Lozano Chiappe<sup>1</sup>, Leonardo Gómez Rosso<sup>1</sup>, Maximiliano Martín<sup>1</sup>, Amanda Yáñez<sup>1</sup>, Belén Davico<sup>1</sup>, Augusto Bava<sup>2</sup>, María Soledad Sáez<sup>2</sup>, Cynthia Rodríguez<sup>2</sup>, Axel Beskow<sup>2</sup>, Susana Gutt<sup>2</sup>, Dong Woo<sup>2</sup>, Fernando Brites<sup>1</sup>.

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**Introduction:** Bariatric surgery (BS) is the most successful intervention for weight loss in patients with morbid obesity, offering benefits reducing cardiovascular disease (CVD) risk associated with dyslipidemia and inflammation. This study aimed to evaluate the effect of BS on lipid and inflammatory profiles, as well as on the activity of lipoprotein-related enzymes. **Materials and Methods:** Thirty-five patients with morbid obesity from the Italian Hospital of Buenos Aires were evaluated before, 6, and 12 months after BS. Weight, height, and body mass index (BMI) were recorded. Hemogram, high-sensitivity C-reactive protein (hsCRP), lipid profile, and plasma glucose levels were determined. Ratios of total cholesterol (TC)/HDL-C, triglycerides (TG)/HDL-C, TG and glucose (TyG), and neutrophils/lymphocytes (N/L) were calculated. Paraoxonase (PON) and arylesterase (ARE) activities of the PON1 enzyme, as well as lipoprotein-associated phospholipase A<sub>2</sub> activity, were measured by spectrophotometric assays, cholesteryl ester transfer protein (CETP) activity by a radiometric method, and HDL antioxidant activity by fluorometry. **Results:** BMI decreased significantly at 6 and 12 months after BS. Glucose, TG, and hs-CRP levels, as well as TG/HDL-C and TyG ratios decreased at 6 months and remained reduced at 12 months. TC/HDL-C and N/L ratios decreased at 12 months. Plasma HDL-C levels increased significantly at 6 and 12 months. Both PON1 activities decreased at 6 months but recovered the baseline values by 12 months. CETP activity was significantly reduced at 12 months. **Conclusions:** BS, primarily indicated for weight loss, also resulted in various benefits, including improvement in the lipid profile and the inflammatory state. Even if PON1 activities experienced an initial decrease, they recovered by 12 months. CETP activity also decreased after 12 months of BS, which agreed with the reduction in TG levels. Overall, these changes represent a significant reduction in CVD risk after BS.

**216. 481 RELATIONSHIP BETWEEN ADDED SUGAR, TOTAL SUGAR AND FAT INTAKE AND THE TRIGLYCERIDE-GLUCOSE INDEX AS A PREDICTOR OF CARDIOVASCULAR DISEASE**

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**Introduction:** The Triglyceride-Glucose Index (TyG) has been evaluated as a potential biomarker for insulin resistance and it has been associated with cardiovascular disease. However, its relationship with dietary intake has not yet been investigated. **Objective:** To evaluate the relationship between added sugar, total sugar, and fat intake with the TyG index as a predictor of cardiovascular disease. **Patients and Methods:** We conducted a cross-sectional study including adult volunteers, both teaching and non-teaching staff from the Universidad de Buenos Aires. A sociodemographic questionnaire was administered, and measurements of weight, height, and waist circumference were taken. Biochemical assessments were performed; the TyG index and the Atherosclerotic Cardiovascular Disease (ASCVD) lifetime risk were calculated. Dietary intake was assessed through four 24 hours dietary recalls. Statistical analysis was performed using SPSS software. **Results:** A total of 32 volunteers (40.0±11.0y), 78.1% (95%CI; 61.2-88.9%) female, were included. The prevalence of overweight was 53.3% (95%CI; 39.3-71.8%). The mean intake of total sugar, added sugar, and fat was 87.1 g/day, 66.6 g/day, and 86.1 g/day, respectively; 43.8% exceeded the WHO recommendation for added sugar consumption. 28.1% had total cholesterol greater than 200 mg/dL, and 18.8% had fasting glucose greater than 100 mg/dL. The average TyG index was 8.27 ± 0.37, and the average ASCVD risk was 27.5%. The TyG index was not associated with added sugar intake ( $p=0.49$ ), total sugar intake ( $p=0.52$ ), fat intake ( $p=0.49$ ), or ASCVD risk ( $p=0.44$ ); however, it was associated with total serum cholesterol ( $p=0.015$ ). **Conclusions:** Added sugar, total sugar and fat intake were not associated with the TyG index in the studied population. Dietary assessment should be performed in a larger number of volunteers to confirm these results.

#### 217. 482 COENZYMES Q9 AND Q10 AND MITOCHONDRIAL COMPLEXES IN HEART OF HIGH-FAT FED RATS SUPPLEMENTED WITH COENZYME Q10 (COQ10)

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We had previously shown that CoQ10 supplementation was effective increasing plasma [CoQ9] and [CoQ10], the two major ubiquinols in rats. The objective of this work was to study the effect of CoQ10 supplementation on the hearts of rats subjected to high-fat diet in terms of ubiquinol levels, oxidative stress and mitochondrial complexes. Male Wistar rats were divided into 4 groups: control diet (10% calories from fat) (C), control diet+50 mg CoQ10/kg body weight (BW)/day (CoQ), HF diet (60% of calories from fat) (HF) and HF diet+50 mg CoQ10/kg BW/day (HFCoQ) for 12 weeks. Heart

weight was statistically similar among the experimental groups. CoQ10 supplementation mitigated [Glutathione] decrease and TBARS increase that had been observed in HF groups. Heart homogenates [CoQ9] were 1.09±0.02, 1.50±0.04\*, 0.99±0.02\*, and 1.22±0.02\*<sup>#</sup>, and [CoQ10] = 0.20±0.01, 1.44±0.01\*, 0.24±0.01, and 0.64±0.01\*<sup>#</sup> (ug/mg protein) for C, CoQ, HF and HFCoQ respectively, \*vs C; <sup>#</sup>vs HF, <sup>Δ</sup>vs CoQ,  $p<0.05$ ). In mitochondria, the results were: [CoQ9] = 1.18±0.01, 1.48±0.04\*, 1.18±0.02, and 1.32±0.03\*, and [CoQ10] = 0.21±0.02, 1.33±0.03\*, 0.21±0.01, and 0.58±0.02\*<sup>#</sup> (ug/mg protein) for C, CoQ, HF and HFCoQ respectively, \*vs C; <sup>#</sup>vs HF,  $p<0.05$ ). The proportion reduced/oxidized forms in HF animals were lower than C, and CoQ10 supplementation led to an inversion in that proportion. Mitochondrial complexes CI-CII and CII-CIII activities were lower (5%, 18%) in HF animals respect to C ( $p<0.05$ ) and these activities were re-established in the HFCoQ. To conclude, CoQ10 supplementation of HF animals led to an increase in total [CoQ9] and [CoQ10] as well as in the proportion of the reduced forms, mitigating the mitochondrial complexes dysfunction and the oxidative stress conditions. Further experiments are necessary to understand the functional effects of the treatments and the mechanisms involved in the observed modifications.

#### 218. 500 DIETARY COMPONENTS ALTERED HEME PATHWAY REGULATION IN A MURINE MODEL OF ACUTE INTERMITTENT PORPHYRIA

María del Carmen Martínez<sup>1,2</sup>, Leda Oliveri<sup>1</sup>, Johanna Romina Zucconi<sup>1</sup>, Ana María Buzaleh<sup>1,2</sup>, Esther Noemí Gerez<sup>1</sup>

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Acute intermittent porphyria (AIP) is a hereditary disorder due to porphobilinogen deaminase (PBG-D) deficiency, characterized by neuroabdominal attacks. Secondarily, 5-aminolevulinic synthetase (ALA-S) is induced. Fasting is one of the triggering factors of acute attacks. To elucidate the mechanisms that lead to AIP onset and considering that a regulated diet can attenuate or prevent the attacks, the aim was to investigate how changes in the proportion of diet components affects the heme pathway regulation. C57BL/6 (WT) and PBG-D activity deficient mice (Pbgd<sup>+/-</sup>, PAI) of both sexes were used. Both WT and AIP mice were divided in 3 groups: 1 and 2 received standard diet (Purina 3) for 12 weeks; group 3 was fed with standard diet supplemented with 15% commercial olive oil during the same period; groups 2 and 3 were fasted 16 hours prior euthanasia. Serum glucose levels and lipid profile (high and low-density lipoproteins, total cholesterol and triglycerides) were measured. The effect on heme pathway was evaluated through activity and expression levels of regulatory enzyme ALA-S and PBG-D activity in liver. In both sexes, oil did not alter lipid profile compared to groups starved or not. Fasting caused a decrease in glucose levels in both AIP male (60%;  $p<0.05$ ) and WT male (40%;  $p<0.05$ ) respect to control animals; while in AIP females the drop was 80% ( $p<0.05$ ) without any variations in WT. ALA-S activity in AIP mice increased 150% ( $p<0.05$ ) in females and males due to fasting being higher when animals received olive diet: 300% ( $p<0.05$ ) in females and 150% ( $p<0.05$ ) in males. In WT mice, the increased activity was only observed in females. ALA-S protein expression was induced significantly in both fasted AIP groups regardless of sex, while in WT mice the increase was only produced in females. PBG-D activity was unchanged in all the studied groups. In conclusion, changes in dietary composition caused dysregulation of ALA-S1 expression in the AIP mouse model.

#### P2 - POSTERS

FECHA Y HORA: 19/11/2024 16:00-17:00 H

COORDINADORES: VIDUEIROS SILVINA, LABOUDETTE VERONICA, CASSANO DANIELA

#### 219. 018 EFFECT OF *Ligaria cuneifolia* INFUSION ("Argentine mistletoe") ON BOTH PLASMAIC CHOLESTEROL AND

### THE CELLULAR FACTORS THAT AFFECT ERYTHROCYTE DEFORMABILITY IN DYSLIPIDEMIC PATIENTS.

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*Ligaria cuneifolia* (Lc) is used to increase blood fluidity and decrease plasma cholesterol levels. Objectives: to analyze the effect of Lc infusion on plasma cholesterol levels (Cho) and cellular factors that affect erythrocyte deformability in patients with plasma cholesterol >200mg/dl. Methods: blood samples were collected for basal determinations (C) from 10 patients aged 50±15 years old, attending in the Cardiology Service at the Hospital Provincial of the Centenario. Patients received extract of Lc in tea bags to be taken for a month time. After 31 days, blood samples were again obtained (TLc). We assessed in plasma: Total Cho, HDLCho and LDLCho. In blood, cellular factors: Morphological Index (MI), according to Bessis, was calculated by the distinction of shapes using optical microscopy and the erythrocyte rigidity index (RI) was determined by filtration method, with nucleopore membranes. Hematological indices: Erythrocyte count was measured by a hemocytometer, hemoglobin by the cyanmethemoglobin method. Then, mean corpuscular volume and mean corpuscular hemoglobin concentrations were calculated. Statistical analysis was performed using Wilcoxon test. Results: Median and confidence interval (CI 95%). Cho: C: 230 (205–278) vs. TLc: 230 (200–251) ns; HDLCho: C: 59 (42–84) vs TLc: 63.5 (35–76) ns; LDLCho: C: 185 (140–239) vs. TLc: 169 (135–215) \*; MI: C: -2.1 (-1,3 – -3,3) TLc: -1,7 (-0,9–4,2) ns; RI C: 14.38 (7.94–20.97) TLc: 12.24 (7.07–19.09) \*. (\* p<0.05 vs C; ns: non significative vs. C). Hematological indices did not present changes. Conclusion: in the patients studied, treatment with Lc generated a significant decrease in LDLCho in blood levels, improving the erythrocyte deformability estimated by RI in dyslipidemic patients. In addition, considering that elevated values of plasma LDLCho are related to atherosclerosis developing, the importance of these results lies in considering Lc feasible to be used for the prevention of cardiovascular diseases.

### 220. 171 BIOMARKERS OF ENDOTHELIAL DYSFUNCTION AND CYTOKINE LEVELS IN HYPOTHYROIDISM: A SERIES OF META-ANALYSES

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Background: Hypothyroidism (HT) is associated with different comorbidities comprising increased arterial stiffness and decreased flow-mediated dilatation. The exact pathological mechanism of endothelial activation and dysfunction (ED) in HT remains unknown. We conducted a systematic review and meta-analyses to provide an overview of the pathogenesis of ED in HT. Research design and Methods: The literature search was done in February 2024 for studies analyzing traditional and novel circulating biomarkers of ED in patients with HT, including cytokines and chemokines. Random-effect models were used except when no heterogeneity was found. The protocol has been registered in the Prospective Register of Systematic Reviews (PROSPERO) under the identification number CRD42024540560. Results: Twenty-five different macromolecules and 66 studies were entered into analyses. HT was associated with increased levels of E-selectin, soluble intercellular adhesion mole-

cule-1, osteoprotegerin, and oxidized-LDL (p<0.02). Results were not conclusive for endothelin 1. Interleukin (IL)-6, IL-12 and CXCL10 were higher in HT (p<0.05). Subjects diagnosed with overt HT (OHT) may display a proinflammatory tendency with increased levels of IL-6 and interferon-γ, and decreased levels of TGF-β (p<0.05). Conclusions: The data presented and discussed here briefly highlights the association between HT and soluble biomarkers of ED. Inflammation is a plausible etiology for HT and associated cardiovascular risks. Inflammatory mediators released by activated T cells and macrophages may aggravate local tissue damage and systemic inflammation, which arouses more inflammation, forming a vicious circle leading to ED. Nevertheless, more studies are required to understand the underlying fundamental molecular mechanisms.

### 221. 224 ASTAXANTHIN ALLEVIATES EPIDIDYMAL ADIPOSE TISSUE OXIDATIVE STRESS IN ANIMALS FED A HIGH-SUCROSE DIET

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We recently showed that the administration of Astaxanthin (ASTX) -a carotenoid compound with strong antioxidant activity- was able to mitigate the increase in bodyweight gain and visceral adiposity by modulating adipose tissue lipogenesis and to ameliorate dyslipidemia in a diet-induced adiposity rodent model. In order to expand our previous findings, the main of the present research was to evaluate the effect of an ASTX-rich extract obtained from Paraná River basin decapod crustaceans (*Dilocarcinus pagei*) upon oxidative stress developed in the epididymal white adipose tissue (eWAT) of rats fed a high-sucrose diet. Male Wistar rats were fed for 90 days with 1 of 4 experimental diets: 1- Reference group (RD) received a standard commercial rodent diet, 2- High-sucrose diet (HSD) group received a HSD, 3- RD+ASTX group received a standard commercial rodent diet plus ASTX, 4- HSD+ASTX group received a HSD plus ASTX. The rats were given orally either ASTX (10 mg/kg body weight/day in sunflower oil) or only the vehicle. In eWAT were analyzed oxidative stress parameters: reactive oxygen species (ROS) levels, thiobarbituric acid reactive substances (TBARS), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) enzyme activities. Data were statistically analyzed by two-way ANOVA. Compared with HSD-fed rats, HSD+ASTX-fed animals exhibited lower levels of both ROS and TBARS (p<0.05). These changes were accompanied by a significant increase in GR activity (p<0.05), without modifications in CAT and GPx enzyme activities. No significant differences were observed between RD and RD+ASTX groups. In conclusion, the present results suggest that the carotenoid ASTX obtained from freshwater decapod crustaceans has beneficial effects against the prooxidant state developed in the eWAT of HSD-fed rats.

### 222. 252 EFFECT OF PREBIOTIC INULIN ON THE DEVELOPMENT OF OBESITY AND INSULIN RESISTANCE IN MICE FED A HIGH-FAT DIET: ROLE OF TNFR1 DISRUPTION

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Previously, we demonstrated that disruption of the TNFR1 signalling pathway increases liver inflammation and accelerates the progression of NAFLD to NASH in a high-fat diet (HFD) murine model. In this context, the intake of prebiotics such as inulin has emerged as a potential strategy for treating obesity and related metabolic disorders. The mechanisms underlying these effects remain largely unknown. The aim of this study was to evaluate the potential protective effect of inulin on the development of obesity and insulin resistance associated with the disruption of TNFR1 signalling in mice fed a HFD.

C57BL/6J wild-type (WT) and C57BL/6-Tnfrsf1atm1Imx/J knockout (TNFR1 KO) mice (n=4) were fed a 40% HFD for 16 weeks. Mice from both experimental groups were then treated with either a vehicle or inulin (0.5 g/kg body weight/day) administered by oral gavage three times a week for 4 weeks. Body weight gain was monitored throughout the treatment, showing a significant decrease in HFD-IN mice compared to HFD-VEH, while no significant differences were observed in KO mice (% weight change over the 4 weeks of inulin treatment: HFD-WT-VEH: 102.1±0.6; HFD-WT-IN: 94.4±1.0; HFD-KO-VEH: 99.3±0.6; HFD-KO-IN: 99.3±0.6). Plasma glucose levels were measured after a 12-hour fast (mg/dl): HFD-WT-VEH: 157.0±10.4; HFD-WT-IN: 132.7±6.8; HFD-KO-VEH: 116.0±9.0; HFD-KO-IN: 102.3±10\* (\*p<0.05 vs HFD-WT-IN). The oral glucose tolerance test revealed a reduction in the area under the curve in HFD-IN mice compared to HFD-VEH (p<0.052). However, no significant changes were between HFD-KO-IN and HFD-KO-VEH mice. To assess insulin signalling pathway activation, we evaluated the P-AKT/Total AKT ratio by Western Blot, finding a decrease in HFD-KO-IN (-43%; p<0.05) compared to HFD-WT-IN. Based on these results, we suggest that disrupting TNFR1 signalling impairs the protective effects of insulin treatment against the development of obesity and insulin resistance in mice fed a high-fat diet.

### 223. 366 ASSESSMENT OF TOTAL BODY WATER IN PREGNANCY USING BIOELECTRICAL IMPEDANCE

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Pregnancy is a physiological condition in which growth, total body water (TBW) and fat change. Knowledge about changes in body composition during this period is limited. This longitudinal study will evaluate TBW in each of the three trimesters of pregnancy using bioelectrical impedance (BIA) and at the third trimester using isotope dilution as reference method. The aim of the present work is to describe a pattern of BIA parameters at the first trimester to be evaluated along the pregnancy. The study was approved by Buenos Aires Province Ethics Committee. Up to this point, 7 primigravidae and 30 multigravidae from Posadas National Hospital and Maternidad Carlotto were enrolled in the study giving written consent to participate. Women came to the laboratory the morning after an overnight fast. Auto Reported prepregnancy body weight was registered. Height (H;m) and weight (W; kg) were measured in minimal clothes; weight gain (WG; kg) and body mass index were calculated (BMI=W/H<sup>2</sup>; kg/m<sup>2</sup>). To perform BIA, electrodes were placed in the hand and foot dorsal surface and a 632 uA alternating current was introduced. Measurements of Resistance (R, ohms) were made with multifrequency tetrapolar equipment (Bodystat Multiscan 5000) and TBW (L) was calculated as Lukaski prediction equation using the resistance index (H<sup>2</sup>/R). Considering TBW, body fat (BF, kg) was calculated as van Raaij equation. Results were expressed as media±SD (Percentile 5<sup>th</sup>-Percentile 95<sup>th</sup>). Gestational age was between 10,3 and 16,2 weeks and BMI was 27,3±5,9 (21,2-38). Resistance, TBW and FM were 556±80 (433-673), 32,4±5,4 (26-44,5) and 24,2±9,6 (12,1-44,2), respectively. Up to now, it has obtained the baseline of BIA parameters; measurements of R, TBW and BF will be assessed in the second and third trimester, searching for a valid indicator of changes in body composition associated with pregnancy outcomes.

### 224. 377 SUPPLEMENTATION WITH GRAPE POMACE EXTRACT MITIGATES HIGH FAT DIET-INDUCED ENDOTOXEMIA AND LIVER STEATOSIS IN MICE

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**Objective:** To investigate the therapeutic potential of grape pomace extract (GPE), rich in polyphenols, in mitigating obesity, insulin resistance, endotoxemia, and hepatic steatosis induced by a high-fat diet (HFD) in mice, as well as its effects on intestinal permeability using Caco-2 cells. **Materials and Methods:** Male C57BL/6 mice were fed a HFD or a standard diet for 13 weeks, with or without GPE supplementation (300 mg/kg/day). Body weight, glucose tolerance, lipid profile, and insulin resistance were measured. Hepatic steatosis, inflammation, endotoxemia, ALT levels, and plasma LPS were evaluated. Caco-2 permeability was assessed by transepithelial electrical resistance. Oxidative stress in Caco-2 cells was measured via DHE oxidation, and mRNA levels of NOX-1 and MLCK were assessed by RT-PCR. Levels of phosphorylated p65 (pP65) and ERK1/2 were analyzed. Statistical analyses were conducted using one-way ANOVA. **Results:** GPE mitigated HFD-induced obesity, insulin resistance and dyslipidemia. Additionally, GPE reduced liver weight, ALT levels, triglycerides content and steatosis. Also, lowered HFD-induced endotoxemia and activation of TLR4, NF-κB and the downstream expression of proteins involved in inflammation and oxidative stress (NOX4, 4-HNE, TNFα and F4-80). In Caco-2 cells, GPE inhibits TNFα-induced Caco-2 monolayer permeabilization, maintained tight junction (TJ) integrity, and fully prevented the increase in DHE oxidation and upregulation of pP65 protein levels, NOX-1 and MLCK mRNA expression, key regulators of TJ permeability. **Conclusion:** GPE supplementation mitigates HFD-induced obesity and the associated metabolic alterations including insulin resistance, liver inflammation and hepatic steatosis in part by modulating the integrity of the intestinal barrier and related endotoxemia. These results provide new evidence that supports the consumption of GPE, rich in bioactive compounds, to mitigate the adverse consequences of high fat consumption.

### 225. 486 ALCOHOL INDUCED MODIFICATIONS IN ADIPOSE TISSUE: A POTENTIAL RISK FACTOR FOR METABOLIC IMBALANCE?

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Adipose tissue (AT) has a remarkable ability to adjust its size and function in response to various internal and external signals, such as nutritional status and temperature. The regulatory circuits governing fuel storage and oxidation in white adipocytes and thermogenic in brown/beige adipocytes, are crucial for maintaining systemic energy homeostasis. However, when these pathways become dysregulated, it can lead to metabolic disorders and AT dysfunction, including obesity. This study aimed to investigate the effect of alcohol intake on the metabolic function of AT. Adult male mice (*Mus musculus*, CrlFcen) were exposed to 15% (v/v) ethanol in drinking water ad libitum for 12 days, followed by two days on water only. Males were euthanized and dissected to obtain AT from subcutaneous, gonadal, peri-retroperitoneal and brown adipose tissue (BAT) depots. Histological examination of the samples was performed after fixation and staining (H&E). AT lysates underwent Western blot analysis

for lipase expression (HSL, ATGL) and adipocyte markers (FABP4, CAV-1, UCP-1, Adiponectin). Alcohol consumption significantly increased ATGL and adiponectin expression in subcutaneous adipose tissue ( $p=0.05$ ), HSL expression in BAT ( $p=0.005$ ) and showed a tendency to increase HSL in retroperitoneal AT ( $p=0.07$ ). UCP1 expression levels did not show any difference between the groups in BAT depots. Histological analysis revealed a tendency to decrease the number of vacuoles in BAT and the vacuole area increased in gonadal tissue ( $p=0.02$ ) with treatment, suggesting triglyceride storage. We also evaluated the levels of glucose and other metabolites in serum to analyzed metabolic status of the mice. In sum, alcohol consumption induces significant alterations in adipose tissue metabolism and morphology. These results suggest that alcohol intake may influence fat storage and energy homeostasis, potentially altering the balance between white and brown adipose tissue, which could have implications for metabolic health.

### P3 - POSTERS

FECHA Y HORA: 20/11/2024 11:30-12:30 H

COORDINADORES: BARCHUK MAGALI, MACRI VANESA, LAVANDERA JIMENA

#### 226. 024 ABNORMAL LIPOPROTEIN METABOLISM IN SUBCLINICAL HYPOTHYROIDISM

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**Introduction:** The association between subclinical hypothyroidism (SH) and cardiovascular risk factors is controversial. Thus, evaluating novel biomarkers and key enzymes and proteins involved in lipoprotein metabolism and functionality is crucial. Among these, the main factors responsible for the antiatherogenic properties of HDL are the activities of lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), lipoprotein associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>), and the antioxidant activity of the enzyme paraoxonase (PON) 1. This study aims to evaluate the status of these parameters in adult female patients with SH. **Methods:** 13 adult women with SH and 10 healthy controls were included. Anthropometric data were registered. Levels of thyroid-stimulating hormone (TSH) and free thyroxine, lipid profile, and high-sensitivity C reactive protein (hsCRP) were measured using standardized methods. LCAT and CETP activities were determined employing developed radiometric methods. Lp-PLA<sub>2</sub> and PON1 activities were assessed using colorimetric methods and HDL antioxidant activity (AA) using an in-house fluorometric method. **Results:** Age, body mass index and hsCRP levels were similar in both groups. Patients with SH, in addition to higher TSH levels [6.86 (6.51–7.84) vs. 2.06 (1.71–2.75)  $\mu$ U/mL;  $p<0.01$ ], presented higher triglyceride levels ( $p<0.05$ ), lower HDL-cholesterol concentration ( $p<0.05$ ), and higher CETP activity ( $p<0.01$ ). LCAT, Lp-PLA<sub>2</sub>, and PON activities, and HDL AA were similar in both groups. Positive associations of CETP with TSH ( $r=0.54$ ;  $p<0.05$ ) and TG ( $r=0.43$ ;  $p<0.05$ ) levels were observed, as well as of HDL AA with PON ( $r=0.54$ ;  $p<0.05$ ) and HDL-cholesterol ( $r=0.46$ ;  $p<0.05$ ). **Conclusions:** Patients with SH exhibited a more atherogenic lipid profile, consistent with the increase in CETP activity, a key factor in lipoprotein remodeling. Our findings remark the importance of close monitoring of SH patients to identify the need for early intervention.

#### 227. 282 NATURAL ANTIOXIDANTS INHIBIT LIPID ACCUMULATION IN MURINE 3T3-L1 AND HUMAN ADIPOCYTE CELLS

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We previously demonstrated that antioxidant extract from *Ribes nigrum* (Cassis, Cs) inhibited neutral lipids and intracellular triglyceride (Tg) accumulation in 3T3-L1 murine adipocytes (mAD). We aim to evaluate the anti-adipogenic effect of new antioxidant extracts (EXT) in mAD and human adipocytes (hAD) obtained through differentiation of primary Adipose Stem/stromal Cells (hASC) in culture. EXT were obtained from leaves of *Bacharis articulata* (Carqueja, C), *Peumus boldus* (Boldo, B), and *Verbena bonaerensis* (Verbena, V) at 37 °C for 1h, and from Cs by enzymatic extraction at 42 °C for 1h. We differentiated both 3T3-L1 and hASC to mature adipocytes. Polyphenol content (Pph-mg/mL) and antioxidant capacity (AC-%) were assessed by Folin and DPPH assay, respectively. We evaluated Cs and EXT in mAD and hAD, and determined: Cytotoxicity by MTT assay; Neutral lipids content (Arbitrary units-AU) by OIL RED O staining (spectrophotometric and Image J analysis); Tg level (mg Tg/mg DNA). Results were compared with non-treated mAD (mADc) or hAD (hADc), considered as 100%. Pph: 9.03 (Cs); 0.06 (C); 2.00 (B); 0.03 (V); AC: 69% (Cs); 53% (C); 79% (B); 65% (V). EXT treatments weren't toxic for the cells. B and Cs were effective in neutral lipids decrease in mAD,  $p<0,05$  (100.00 $\pm$ 8.17 [mADc], 75.37 $\pm$ 3.33 [mAD+B], 91.01 $\pm$ 2.00 [mAD+Cs]). Only V had an effect in hAD  $p<0,01$  (100.00 $\pm$ 5.88 [hADc], 55.02 $\pm$ 4.36 [hAD+V]). EXT didn't reduce Tg levels in mAD (Cs did by 62%,  $p<0.05$ ), all EXT treatments reduced it in hAD, while B and Cs produced a significant decrease  $p<0,05$  (100.00 $\pm$ 16.96 [hADc], 33.49 $\pm$ 0.43 [hAD+B], 24.19 $\pm$ 2.46 [hAD+Cs]). C with the lowest AC and Pph had no significant effect in both models. Cs effect was similar in mAD and hAD reducing Tg content, and Cs also decreased neutral lipids in mAD. Since Cs had the highest Pph, we could suggest that polyphenols, through their AC, are relevant in mAD, while for the human model, other antioxidant components should play an important role.

#### 228. 304 TRANSCRIPTOMIC ANALYSIS OF PANCREATIC ISLETS IN A DIET-INDUCED MURINE MODEL OF METABOLIC SYNDROME

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Type 2 diabetes (T2D), associated with obesity and metabolic syndrome (MS), is characterized by abnormal glucose metabolism and affects the function and structure of pancreatic islets, as well as the survival and function of beta cells. This study aims to delve deeper into the molecular mechanisms behind MS-associated pancreatic islet dysfunction. Eight-week-old male C57BL/6N mice were fed either a standard chow diet (CD, n=5) or a Western diet (WD, n=10), supplemented with 20% w/w bovine fat and 50 g/L sucrose in water. Weight was monitored weekly, and glucose homeostasis was evaluated. After 20 weeks, pancreata were collected for histology, and pancreatic islets (n=3 per group) were isolated for RNA-seq analysis. Differentially expressed genes (DEGs) and biological processes were identified using gene ontology (GO) in RStudio, considering DEGs with  $p < 0.01$  and  $|\log_{2}FC| > 1$ . Weight gain in WD mice was

increased vs CD, with significant differences from week 4 ( $p < 0.05$ ). WD mice showed increased plasma cholesterol levels ( $p < 0.05$ ) and impaired glucose homeostasis (reduced glucose tolerance and increased insulin resistance). Pancreatic histological and immunofluorescence analysis corroborated alterations as already described for this model, eg. larger islets and diminished insulin expression. A total of 182 DEGs were identified between CD and WD groups, with 121 up-regulated and 61 down-regulated in WD mice. GO analysis showed that up-regulated genes were associated with pancreatic function, pro-inflammatory response, fat metabolism, and dietary adaptation, while down-regulated genes were related to cellular signaling, cytoskeleton organization, inflammatory response, and ion and glucose homeostasis. This study provides a detailed map of DEGs linked to MS-induced pancreatic islet dysfunction, laying the groundwork for identifying new biomarkers and therapeutic targets in T2D.

**229. 318 EXPLORATION OF POTENTIAL NEUROPROTECTIVE PROPERTIES OF PROTEINS FROM NON-CONVENTIONAL ORIGIN (FRESHWATER PRAWNS) IN A RODENT MODEL OF METABOLIC SYNDROME**

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Several studies, including from our group, demonstrated that Metabolic Syndrome (MetS) would be associated with the development of neurodegenerative disorders and cognitive decline. Therefore, the study of potential nutritional interventions that can prevent brain disorders that develop in this context are of main interest. The aim of this work was to evaluate the effect of a protein extract -rich in functional aminoacids- obtained from freshwater decapod crustaceans (*Macrobrachium borellii* prawn) upon the behavioral deficits and key molecules of brain energy metabolism in an experimental model of MetS. Male Wistar rats were fed for 90 days with 1 of 3 experimental diets: 1-Reference group (RD) received a standard commercial rodent diet, 2-High-sucrose diet (HSD) group received a HSD -containing casein as the protein source-, 3-HSD-CrP group received a HSD in which 50% of the protein source was replaced by a protein extract obtained from the freshwater prawn *M. borellii*. Amino acid composition of the diets was determined by HPLC. In these animals the Novel Object recognition (NORT) and T- maze memory tasks were performed in order to explore non-spatial and spatial memories, respectively. Protein mass levels of cerebral cortex hexokinase (HK) and AMP activated protein kinase (total AMPK and <sup>pThr172</sup>AMPK) were determined by western blot. Data were statistically analyzed by one-way ANOVA and Newman Keuls post-hoc. Partial substitution of the protein source was able to minimize the cognitive decline observed in HSD-fed animals, which was evidenced by a better performance of the HSD-CrP group in both NORT and T-Maze tasks. In addition, a significative increase ( $p < 0.05$ ) of HK and <sup>pThr172</sup>AMPK protein mass levels was observed in the cerebral cortex of HSD-CrP-fed animals. The results showed that *M. borellii* protein extract has beneficial effects upon the cognitive decline and disturbances of brain energy metabolism that develop in a MetS rodent model.

**230. 324 ADIPOCYTE HYPERTROPHY: SPEXIN AS A MODULATOR OF LIPID METABOLISM AND INFLAMMATION**

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Spexin (SPX) improves several metabolic parameters, adipose tissue (AT) hypertrophy and inflammation in obese rodent and fish

models. Obesity has been defined as the abnormal or excessive accumulation of AT. It is widely accepted that AT hypertrophy (higher size of cells) leads to insulin resistance, inflammation and metabolic alterations. Nowadays, many studies showed that depending on the fatty acid (FA) type consumed on diet, it may favor or not the development of these complications. Therefore, we aim to study the effect of SPX in lipid metabolism and inflammation in hypertrophic adipocytes in an in vitro model. The 3T3L1 cell line was differentiated into adipocytes and then treated with a saturated FA (lauric acid; LA; 250 and 500 $\mu$ M) for 48h. Samples were collected, and gene expression (GE) was assessed (ob, adipo, fas, hsl, il6, irs1). Additionally, functional analysis of lipolysis (glycerol content; basal and forskolin (FSK) induced) and lipogenesis (Triglycerides (TG) content) were quantified. One-way or Two-way ANOVA were used as statistical tests. For the latter, when interaction was significant post-test was applied. Treatment with higher dose of LA (500 $\mu$ M) resulted in a more evident hypertrophic model, showing inflammation, lipid metabolism and insulin signaling alterations. Briefly, il6 was increased ( $p < 0.05$ ) and adipo, fas and irs1 were decreased ( $p < 0.05$ ) while ob and hsl were not altered. TG content was elevated ( $p < 0.05$ ), and lipolysis was altered when stimulated with FSK ( $p < 0.01$ ). Then hypertrophic adipocytes were treated or not with SPX (1 and 10ng/ml) for 6h. Adipo and hsl GE were increased ( $p < 0.05$ ) and il6 was decreased ( $p < 0.05$ ). Lipogenesis was decreased after treatment ( $p < 0.01$ ) and lipolysis was increased only after FSK induction. Overall, SPX treatment induced an improvement mainly in lipid metabolism (GE and functional test) and inflammation (GE) in hypertrophic adipocytes, favoring a more functional metabolic profile.

**231. 332 ALTERATIONS IN IgG/ALBUMIN RATIO OF PLASMA PROTEIN AGGREGATES INDUCED BY HIGH INTENSITY INTERVAL TRAINING AND ITS IMPACT ON OXIDANT PRODUCTION**

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HIIT (High intensity interval training) involves explosive anaerobic exercise with brief recovery periods until exhaustion. A single bout of exercise enhances oxidative stress (OS), involved in long term muscle adaptation rather than impairing its function. In fact, the administration of antioxidants has been proposed to have detrimental effects on muscle growth. Objectives: To assess whether plasma protein aggregates promote the production of oxidants in PMN (polymorphonuclear cells) and the effect of HIIT on plasma proteins sensitivity to metal-induced aggregation. Materials and methods: Sprague-Dawley rats of 220g performed a HIIT exercise consisting of swimming bouts of 20 s with recovery periods of 10 s with a load of 9% body weight and a sedentary group remained as control. The glutathione (GSH) content was measured in erythrocytes after training by spectrophotometry. The plasma protein of sedentary and HIIT animals was aggregated with Fe(III) and the aggregation sensitivity was determined by optic density. The aggregated proteins were obtained in sedentary and HIIT groups and the potential proteins within the aggregates were outlined considering their molecular mass by SDS-PAGE. Results: A single HIIT session in rats decreases GSH in erythrocytes by 60% ( $p < 0.01$ ) indicating systemic OS. Fe(III) is a strong inducing agent of protein aggregation which was employed to test plasma protein sensitivity to metal aggregation. Notably, HIIT

does not significantly change the amount of aggregate despite OS generated by HIIT when compared to sedentary animals. However, Fe(III)-induced aggregates significantly increase in the IgG/Alb ratio when comparing sedentary and HIIT groups ( $p < 0.05$ ). No significant changes could be observed in other proteins. Thermally aggregated plasma proteins and IgG caused a significant decrease of GSH in PMN ( $p < 0.01$ ) *in vitro*. Conclusion: HIIT alters plasma protein sensitivity to aggregation which may contribute to OS by activation of PMNs.

**232. 334 TIME DEPENDENCE OF THE REVERSIBILITY OF Cu(II)-INDUCED PROTEIN AGGREGATES AND THEIR INTERACTION WITH POLYMORPHONUCLEAR CELLS**

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Cu(II) induces plasma protein (PP) aggregation and contributes to the formation of circulating protein aggregate (Agg). The reversibility of such Aggs may influence their clearance. Additionally, Cu(II) incorporates specific PP into the Agg which activate inflammatory pathways. Objectives: 1) To assess whether 1h or 24h incubation with Cu(II) renders IgG or PP Aggs irreversibly upon metal chelation. 2) To assess whether the Aggs obtained *in vitro* induce oxidative burst (OB) in polymorphonuclear cells (PMN). Materials and methods: The protein Aggs were obtained by incubation with Cu(II) for 1h or 24h. Agg solubility was tested against sodiumdodecylsulfate (SDS) and EDTA, followed by optic density. PMN from healthy individuals were incubated with the Aggs in order to determine their capacity to induce PMN OB following luminol oxidation. Thermally induced PP Agg was used as positive control. Results: 1h incubation Cu(II)-induced (Cu(II)-) IgG Aggs were fully dissolved by SDS ( $p < 0.001$ ) and EDTA ( $p < 0.001$ ). 1h incubation Cu(II)-PP Aggs were fully dissolved by SDS ( $p < 0.01$ ) and partially solubilized by EDTA ( $p < 0.05$ ). After 24h incubation Cu(II)-IgG Aggs were solubilized by SDS ( $p < 0.001$ ) but partially solubilized by EDTA ( $p < 0.05$ ). Notably, 24h incubation Cu(II)-PP Aggs were fully solubilized by SDS ( $p < 0.001$ ) but remained insoluble upon EDTA addition. PP incubation with Cu(II) leads to selective aggregation of IgG and other proteins. IgG Aggs activate PMN OB. Thermally PP Aggs initiated PMN OB. However, Cu(II)-PP Aggs obtained with 200  $\mu$ M Cu(II) which generates an IgG enriched Agg and 800  $\mu$ M Cu(II), which generates and Agg with relative protein content similar to plasma, did not induce OB. Conclusion: Cu(II)-protein Aggs become irreversibly Agg over time. The Aggs obtained *in vitro* are unable to induce OB. However, the *in vitro* production may differ from the physiological Agg formation and damage the Agg structure, impairing its interaction with PMN receptors.

**233. 480 IN VITRO AND IN VIVO EVALUATION OF A MAQUI BERRY (ARISTOTELIA CHILENSIS) EXTRACT AS INHIBITOR OF DIGESTIVE ENZYMES**

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Maqui berry administration has shown positive effects on fasting glucose and lipid levels, which may be related to the inhibition of digestive enzymes. This study aimed to evaluate the impact of an aqueous maqui berry extract (MBE) on  $\alpha$ -glycosidase and lipase activity, and to link this to the phenolic components of the MBE. MBE was prepared using a commercially available lyophilized maqui berry powder and the phenolic content was characterized using HPLC-MS/MS. Thirty phenolic compounds were identified with a total concentration of  $9.21 \pm 0.93$  mg/g<sub>extract</sub>, being delphinidin-O-diglucoside the one with the highest concentration. *In vitro*  $\alpha$ -glycosidase activity was determined using  $\alpha$ -glycosidase from *Saccharomyces cerevisiae* and from mice intestine homogenate. MBE showed an IC50 of  $0.011 \pm 0.001$  and  $2.69 \pm 0.72$  mg<sub>extract</sub>/mL, respectively (positive control: acarbose, IC50=  $0.109 \pm 0.011$  and  $0.077 \pm 0.019$  mg/mL). For *in vivo* assays, male Swiss mice were divided into 4 groups receiving by gavage: C (water + water), M (water + maltose 2 g/kg), A (acarbose 50 mg/kg + maltose 2g/kg), and MBE (MBE 160 mg<sub>extract</sub>/kg + maltose 2 g/kg). Glycemia was measured over 2 h, and areas under the curves (AUC) were calculated, with no significant differences between M and MBE. For lipase inhibition, *in vitro* assays were carried out using porcine pancreatic lipase. MBE showed an IC50 of  $1.24 \pm 0.19$  mg<sub>extract</sub>/mL (positive control: orlistat, IC50=  $(0.12 \pm 0.03) \times 10^{-3}$  mg/mL). For *in vivo* assays, mice were divided into 4 groups receiving by gavage: C (water + water), O (water + olive oil 5mL/kg), ORL (orlistat 25 mg/kg + olive oil 5mL/kg), and MBE (MBE 160mg<sub>extract</sub>/kg + olive oil 5m/kg). Triglyceridemia was measured over 4 h. AUCs showed no significant differences between O and MBE. In conclusion, MBE was capable to inhibiting the assayed digestive enzymes *in vitro*, but no effects were observed *in vivo*. These discrepancies could be related to the dose of MBE used *in vivo*; the possibility that the unmetabolized MBE phenolics are necessary to exert the inhibitory effects observed; and other factors to be further studied.

**P4 - POSTERS**

FECHA Y HORA: 20/11/2024 16:10-17:10 H

COORDINADORES: FERREIRA CORDONEDA MARIA DEL ROSARIO, WEISSTAUB ADRIANA

**234. 044 SHORT-TERM INTERMITTENT COLD THERAPY INCREASES THERMOGENIC CAPACITY AND REDUCES SUCROSE-INDUCED HEPATIC STEATOSIS**

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**Introduction:** Obesity and other metabolically related diseases associated with an excess of caloric intake can lead to non-alcoholic fatty liver disease (NAFLD). Its genesis is based on a multiple hit theory that argues that liver steatosis is one of the early events necessary to develop NAFLD. Thermogenesis is a catabolic process can significantly increase energy expenditure restoring the energy balance. Although there has been extensive research on brown adipose tissue (BAT), sustained activation that could be adapted to a clinical/therapeutic setting has proven to be challenging. Our lab has been developing a short-term intermittent cold protocol (IC) that can effectively activates BAT, increasing its oxidative capacity, as well as inducing the browning of white adipose tissue (WAT) therefore increasing the overall thermogenic capacity. **Aim:** To study the effect of IC on the activity of BAT and the browning capacity of WAT of C57bl6 mice treated with a sucrose-rich diet (SRD), and its effect on liver steatosis. **Materials & methods:** We subjected 8-week-old male C57bl 6 mice to increasing short term periods (5-15 min/day) at 4°C for a month IC, and we measured its effect on BAT, WAT, liver and serum parameters. On another set of experiments, we tested its therapeutic capacity by implementing the IC on 4-week treated SRD mice. **Results & Conclusions:** IC was able to induce a stronger

brown-like identity in BAT, increasing the oxidative capacity of the tissue, as well as, to induce browning of WAT. This was accompanied by a reduction in lipid droplet size. Four weeks of SRD treatment was able to increase lipid content in the liver shown by higher TAG levels versus Control (C) ( $0.23 \pm 0.01$  vs  $0.50 \pm 0.07$   $\mu\text{g}/\text{mg}$ ,  $p < 0.02$  vs C) and changes in tissue morphology and coloration. Furthermore, SRD-IC group show a restoration of tissue morphology as well as TAG content to control levels ( $0.23 \pm 0.01$   $\mu\text{g}/\text{mg}$  C vs  $0.20 \pm 0.01$   $\mu\text{g}/\text{mg}$ , ns) suggesting a potential role of IC on early stages of NAFLD.

### 235. 150 EPICARDIAL ADIPOSE TISSUE SECRETES METALLOPROTEINASES IN CORONARY ARTERY DISEASE AND THIS ACTIVITY IS MODULATED BY NITRO OLEIC ACID

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Epicardial adipose tissue (EAT) is a visceral AT surrounding the myocardium and coronary arteries, identified as an independent cardiovascular risk factor. Modified fatty acids are molecules that can provide protective effects or induce changes on tissue metabolism; among these, nitro-oleic acid (AONO2) is a compound with antioxidant and antiinflammatory properties. We have reported an increase in metalloproteinase (MMP) 2 and 9 activity in EAT from coronary patients, along with a pro-inflammatory profile. In this opportunity, our aim was to evaluate MMP2 and MMP9 activities in EAT secretomes, and the modulation exerted by AONO2 in tissue and secretory metalloproteinases activities. Methods: EAT and subcutaneous AT (SAT) biopsies from patients undergoing by-pass (CAD n=16) or valve replacement (NoCAD n=9) surgery were incubated in DMEM or DMEM+AONO2 10  $\mu\text{mol}/\text{L}$  during 6 hours. After each treatment, MMPs activities were evaluated in the EAT, SAT, and their secretomes by gelatinolytic zymography. Results: MMP9 activity was higher in EAT secretome from CAD than NoCAD ( $0.73 \pm 0.02$  vs  $0.64 \pm 0.03$  RU, respectively,  $p = 0.02$ ), as well as MMP2 (CAD:  $0.63 \pm 0.04$  vs NoCAD:  $0.46 \pm 0.01$  RU,  $p = 0.03$ ). AONO2 decreased MMP9 activity in EAT (DMEM:  $0.11 \pm 0.06$  vs AONO2:  $0.06 \pm 0.02$  RU,  $p = 0.0006$ ), SAT (DMEM:  $0.17 \pm 0.08$  vs AONO2:  $0.10 \pm 0.06$  RU,  $p = 0.0008$ ) and EAT secretome (DMEM:  $0.75 \pm 0.07$  vs AONO2:  $0.55 \pm 0.07$  RU,  $p < 0.0001$ ), and the same behavior was observed for MMP2 in EAT (DMEM:  $0.08 \pm 0.05$  vs AONO2:  $0.05 \pm 0.03$  RU,  $p = 0.02$ ) and its secretome (DMEM:  $0.60 \pm 0.10$  vs AONO2:  $0.45 \pm 0.10$  RU,  $p < 0.0001$ ). MMPs tissue activities were directly correlated with those in the secretomes, in both conditions (MMP9 DMEM:  $r = 0.6$ ,  $p = 0.05$ ; MMP9 AONO2:  $r = 0.7$ ,  $p = 0.02$ ; MMP2 DMEM:  $r = 0.8$ ,  $p = 0.01$ ; MMP2 AONO2:  $r = 0.7$ ,  $p = 0.05$ ). Conclusion: EAT secretes MMPs that could negatively impact in the endothelium and myocardium, and this could be attenuated by AONO2, potentially limiting inflammatory processes and deleterious effects in EAT underlying structures.

### 236. 264 COMPARATIVE EFFECTS OF OMEGA-3 FATTY ACIDS ON TRIGLYCERIDE-RICH LIPOPROTEINS: BEYOND TRIGLYCERIDES REDUCTION

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The activity of Lipoprotein Lipase (LPL) plays a pivotal role in triglyceride (TG) rich lipoprotein metabolism, with a paradoxical behavior in insulin-resistance (IR). In aorta, could explain vascular lipolysis and the generation of remnants (RLP). In atherosclerotic plaque, metalloproteinases (MMPs) play a role in its vulnerability. Omega-3 fatty acids (W3-FA) are well known for TG reduction, but their pleiotropic effects on cardiovascular risk are less understood. Our aim was to compare the effect of different pharmaceutical W3-FA formulations on RLP composition, as well as in LPL, MMP-2 and 9 activities in aortic and adipose tissue (AT) from IR rats. **Methods:** Male Wistar rats were fed a high sucrose diet (HSD, n=6), HSD + 4 g Icosapent Ethyl (HSD+IPE, n=6) or HSD + 4 g EPA/DHA (HSD+ED, n=6), for 12 weeks. In serum, lipid profile, glucose, and free FA were assessed. RLP were isolated by ultracentrifugation ( $d < 1,019$  g/mL) and characterized. Epididymal AT and thoracic aorta were isolated to assess LPL activity by radiometric assays, and MMP-2 and 9 activity by gelatinolytic zymography. **Results:** HSD group exhibited an atherogenic lipoprotein profile and elevated markers of insulin resistance. RLPs from the HSD group had higher TG content, which decreased more in HSD+IPE than HSD+ED. LPL activity in AT increased in HSD+IPE and HSD+ED, accompanied by a decrease in MMP-2 and 9 activity compared to HSD. MMP-9 activity showed a more significant reduction in HSD+IPE vs HSD+ED. In aorta, LPL activity decreased post-treatment, with more significant reduction in HSD+IPE. The effects on MMP activity in aorta mirrored those in adipose tissue. **Conclusion:** As expected, W3-FA treatment improved lipid profiles and RLP characteristics in insulin-resistant rats. However, IPE showed greater anti-inflammatory potential than EPA/DHA, warranting further research into the mechanisms involved.

### 237. 382 SEXUAL DIMORPHISM IN ADIPOSE TISSUE IMPROVEMENTS CAUSED BY SPEXIN IN OBESE MICE

Carolina Carla Garraza<sup>1</sup>, Sabrina Eliana Gambaro<sup>1</sup>, Patricia Fabiana Castro<sup>1</sup>, María Amanda Rey<sup>1</sup>, Andrés Giovambattista<sup>1</sup>, María Guillermina Zubiría<sup>1</sup>.

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Spexin (SPX) is a novel adipokine involved in several metabolic process, including body weight and caloric intake regulation, and adipose tissue (AT) functions. Previously, we showed beneficial metabolic effects in obese SPX-treated mice from both sexes under Fructose Rich Diet (FRD) intake. However, it remains unexplored the potential sex dimorphism in these effects. For this aim, two male and female mice (C57BL/6J) groups were studied: FRD mice (10 weeks of 20% w/v), and a similar group treated with SPX for the last 10 days of the protocol (ip. 29  $\mu\text{g}/\text{kg}/\text{day}$ ; FRD-SPX). Body weight and caloric intake were recorded every day. Glucose (GLU) and triglycerides (TG) plasma levels and liver TG content were quantified. AT depots (Inguinal (IAT), retroperitoneal (RPAT) and gonadal (parametrial (PAT) in females and epididymal (EAT) in males) were dissected and weighted. EAT and PAT were used for quantification of Ob, Adiponectin, PPAR $\gamma$ 2 and GALR2 by qPCR. Two-way ANOVA was used to determine factors (SPX and SEX) and interaction (SEXxSPX) effects. SPX treatment caused weight loss and decreased caloric intake ( $P_{\text{SPX}} < 0.05$ ), independently of mice sex. Plasma TG showed no changes, while plasma GLU showed decreased levels in female mice ( $P_{\text{SEX}} < 0.05$ ) and in SPX-treated mice from both sexes ( $P_{\text{SPX}} < 0.05$ ). Liver TG content was reduced by SPX treatment in mice from both sexes ( $P_{\text{SPX}} < 0.05$ ). Male mice showed increased masses of all AT depots ( $P_{\text{SEX}} < 0.05$ ), independently of SPX treatment, and SPX decreased visceral AT (EAT and PAT) in mice from



both sexes ( $P_{SPX} < 0.05$ ). Additionally, SPX treatment caused a beneficial lowering in mRNA expression of all AT functional markers (Ob, Adiponectin, PPAR $\gamma$ 2) and in GALR2, in a greater extent in obese female mice ( $P_{SEX \times SPX} < 0.05$ ). Overall, SPX caused a metabolic improvement in obese mice from both sexes, but a more marked beneficial effect in functional AT markers from female obese mice, indicating a potential sexual dimorphism in SPX effects. 1987

**238. 386 COENZYME Q10 (COQ10) SUPPLEMENTATION ON HIGH-FAT FED RATS: IMPACT ON WHITE ADIPOSE TISSUE**

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# Equally contribution

The aim of this work was to assess the effect of CoQ10 supplementation on adipose tissue of rats with high-fat diet. Male Wistar rats were divided into 4 groups: control diet (10% calories from fat) (C), control diet+50 mg CoQ10/kg body weight (BW)/day (CQ), HF diet (60% of calories from fat) (HF) and HF diet+50 mg CoQ10/kg BW/day (HFCQ) for 12 weeks. In this model, CoQ10 reduced HO-MA-IR, suggesting a beneficial effect on insulin resistance. Since adipose tissue is central to insulin sensitivity, this study focused on the epididymal white adipose tissue (EWAT). EWAT expansion was assessed by the EWAT/body weight ratio ( $1.4 \pm 0.1$ ,  $1.4 \pm 0.1$ ,  $2.2 \pm 0.2^*$  and  $1.6 \pm 0.2$ , for C, CQ, HF and HFCQ respectively, \*vs C,  $p < 0.05$ ) and by adipocyte area ( $680 \pm 60$ ,  $682 \pm 71$ ,  $1254 \pm 114^*$  and  $1054 \pm 102$   $\mu\text{m}^2$ , for C, CQ, HF and HFCQ respectively, \*vs C,  $p < 0.05$ ). Adipocyte hypertrophy is linked to oxidative stress and low nitric oxide (NO) bioavailability. NOX2 subunit expression was studied. The expression of catalytic subunit (gp91phox) did not show changes among groups, but the expression of the activator subunit (p47phox) was significantly lower in the HFCQ (-18%, HF vs HFCQ,  $p < 0.05$ ). The HF fed animals showed higher levels of endothelial nitric oxide synthase (eNOS) regardless CoQ10 supplementation, and eNOS phosphorylation at Ser1177 (an activator modification) did not differ significantly among the experimental groups. Redox status indexes, such as the thiobarbituric acid reactive substances and the expression of antioxidant enzymes, were modified by CoQ10 supplementation in control diet fed animals without effects in HF fed animals. These results suggest that CoQ10 supplementation could potentially reduce NOX2-dependent superoxide anion production without affecting NO production. Further experiments are necessary to evaluate other sources of reactive oxygen species and the inflammatory pathways, to establish the relevance of adipose tissue dysfunction in CoQ10-mediated effects in this model.

**239. 430 CHARACTERIZATION OF LEAP2 EXPRESSION ALONG THE SMALL INTESTINE UNDER DIFFERENT METABOLIC CONDITIONS**

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The liver-expressed antimicrobial peptide 2 (LEAP2) is an endogenous blocker of growth hormone secretagogue receptor (GHSR). LEAP2 acts as an GHSR antagonist, blocking ghrelin-evoked activation *in vitro* and reducing ghrelin-induced food intake, GH release and increase of glycemia. LEAP2 also modulates GHSR activity in a ghrelin-independent manner. Despite these actions suggest significant therapeutic potential, a limited number of studies have addressed its physiological relevance. In this context, our aim was to determine the levels and sites of LEAP2 expression throughout the small intestine, under normal metabolic conditions and fasting conditions, in male and female mice. C57BL/6 wild-type mice (females and males) were either kept in regular feeding (Control) or underwent a 48-hour fasting period (Fast). Following euthanasia, small intestines was extracted. Some of them were divided into four segments of approximately 10 cm each and used to create Swiss rolls. These rolls were sectioned, anti-LEAP2 immunohistochemistry was performed and cells expressing this peptide were counted. In parallel, other small intestines were used to assess levels of Leap2 mRNA. Also, plasma samples were collected to assess LEAP2 concentration. As expected, plasma LEAP2 concentration decreases in both fasted male and female mice. Also, we observed that cells immunoreactive for LEAP2 were present across all small intestine but enriched in duodenum and proximal portion of the jejunum. The levels of mRNA for Leap2 showed a similar pattern, as detected for the number of LEAP2 immunoreactive cells. Additionally, control animals exhibited a greater number of LEAP2-positive cells and mRNA levels for LEAP2 compared to fasted mice. No evident differences between male and female mice were detected. In conclusion, LEAP2 is mainly produced in proximal jejunum of the small intestine and the biosynthesis significantly reduced by fasting independently of sex and the portions of the small intestine.

**240. 458 EFFECT OF CANNABIS-DERIVED PRODUCTS ON OBESITY TREATMENT**

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Obesity constitutes a major risk factor for several diseases, such as type 2 diabetes, cardiovascular diseases, dyslipidemias, NAFLD, and cancer. Monosodium glutamate (MSG)-induced obesity is a model of visceral obesity. The animals develop a neuroendocrine-metabolic syndrome characterized by central hyper adiposity, resistance to insulin, reduced organ weight, etc. *Cannabis sativa*, has generated great expectations due to its therapeutic properties. Numerous studies suggest that the major bioactive components of the plant may be considered potential treatments for decreasing liver fat accumulation and other metabolic diseases. Newborn Wistar rats were divided into two groups: rats administered MSG (4 mg/g body weight) subcutaneously on days 2, 4, 6, 8, and 10 of life and rats that received 0.9% NaCl. These groups were divided into those treated with flower and root extract, and administered orally. On day 150 blood was drawn and the liver was dissected out. Body and biochemical parameters were measured. The body weight of the MSG rats was significantly greater than that observed in the control group ( $p < 0.05$ ). This increase was accompanied by the Lee Index (LI)  $> 0.3$  rise. We observe that both extracts, reverse these parameters ( $p < 0.05$ ). The addition of MSG modified carbohydrate metabolism as indicated by the TTG, with an increase in glucose observed in animals treated with MSG. This increase is reversed in

animals dosed with the different extracts ( $P < 0.05$ ). Oral administration of MSG induced dyslipidemia characterized by an increase in total cholesterol and triglyceride levels, which was reversed in the groups treated ( $P < 0.05$ ). We also observed a decrease in GOT and GPT ( $P < 0.05$ ). In conclusion, improvements in parameters associated with obesity were observed, demonstrating the effectiveness of the various extracts in preventing or reducing complications related to visceral obesity.

**241. 459 EXPERIMENTAL MODEL OF NUTRITIONAL OBESITY PROVIDES COMPELLING EVIDENCE FOR DAY-TO-DAY VARIATION IN OXIDATIVE STRESS BIOMARKERS AND THEIR POSSIBLE EFFECTS ON RAT LIVER**

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It is well known that the cause of obesity (OB) is multifactorial including genetic, environmental, and dietary factors, among which, high-calorie diets play a central role in the development of the disease. In adipose tissue, altered mitochondrial functionality contributes to impaired and subsequent lipid spillover to other tissues, e.g. the liver. Our objective was to evaluate some biomarkers of oxidative stress and histological parameters in our model of OB adult rat's liver. Male Wistar rats were weaned at 21 days of age and fed a normocaloric diet (NC) containing 366 kcal lipids/kg diet. Then, at 2 months of age, they were randomly separated and fed: one group, the NC diet (control group, CO) and the other, a high-fat diet (HFD, 1570.7 kcal margarine/kg diet, OB group), for the next 14 weeks. In the following 8 weeks two more CRCO and CROB groups were fed a chrono-diet. The animals were kept under 12 h-light:12 h-dark and 22–24°C conditions, with water and food ad libitum. All the experiments were performed following national and international guides for the care and use of laboratory animals and were approved by the CICUA (UNSL). Biomarkers such as TBARS, Catalase Activity (CAT), proteins, and Hidroxiprolina (HYP) levels were determined spectrophotometrically in liver homogenates. Statistical differences throughout the 24-h period were analyzed by one-way ANOVA. RESULTS: Our results showed that HYP and CAT levels were significantly lower in the CROB group compared to the CRNC group ( $p < 0.05$ ). A similar behavior presented the levels of MDA, but with lower values in the CROB group ( $p < 0.01$ ), compared to the CRNC group. CONCLUSION: we observed that HYP, TBARS and CAT levels were lower in the CROB group compared to the CRNC group. Therefore, it could be concluded that the consumption of protein-based chrono-diet could be related to the low concentration of biomarkers in the CROB group ( $p < 0.01$ ) compared to the CRNC group.

**P5 - POSTERS**

FECHA Y HORA: 21/11/2024 11:00-12:00 H

COORDINADORES: VALERIA ZAGO, MIKSZTOWICZ VERONICA

**242. 120 TRANSCRIPTOMIC ANALYSIS OF INSULIN-PRODUCING CELLS UNDER HORMESIS REVEALS MODULATION OF GENES ASSOCIATED WITH ENHANCED CELL FUNCTIONALITY AND SURVIVAL**

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rás<sup>1,2</sup>, Luz Andreone<sup>1,2</sup>, Marcelo Javier Perone<sup>1,2</sup>

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During the development of diabetes mellitus (T1D and T2D),  $\beta$ -cells suffer from endoplasmic reticulum and oxidative stress leading to, or exacerbating, inflammation. Hormesis is a phenomenon by which a harmful substance, administered in small doses, provides resistance to subsequent contacts with higher doses. Our previous studies demonstrate that hormesis mediated by physiological concentrations of IL-1 $\beta$  protects  $\beta$ -cells from dysfunction and death induced by proinflammatory cytokines. We aimed to elucidate the molecular mechanisms responsible for IL-1 $\beta$ -induced hormesis in rat insulinoma cells (INS-1E). INS-1E cells were cultured in RPMI with 10% FBS, conditioned with 10 pg/ml IL-1 $\beta$  for 72h (IL-1 $\beta$ <sup>low</sup>, IL-1 $\beta$  was renewed every 24h), and then challenged with a cytotoxic mix of cytokines (CYT; IL-1 $\beta$  100 pg/ml + IFN $\gamma$  5 ng/ml) for 16h. RNA was extracted, and libraries were prepared for RNA-seq analysis (Illumina). Differentially expressed genes (DEGs) between IL-1 $\beta$ <sup>low</sup>-treated cells and control cells were identified using false discovery rate (FDR) $< 0.01$  and  $|\log FC| > 0.6$ . Subsequent analyses used FDR $< 0.01$  and  $|\log FC| > 2$ . Biological processes related to DEGs were identified by gene ontology (GO) analyses of protein-coding genes. We found 125 and 878 DEGs between IL-1 $\beta$ <sup>low</sup>- and CYT-treated cells vs. control cells, and 353 DEGs between IL-1 $\beta$ <sup>low</sup>+CYT- vs. CYT-treated cells. As expected, GO analysis of CYT-challenged vs. control cells showed the activation of cytokine-mediated signaling pathways including the response to IL-1, type II interferon and the ERK1/2 cascade. GO analysis of IL-1 $\beta$ <sup>low</sup>+CYT- vs. CYT-treated cells identified the upregulation of cell-cell adhesion, insulin secretion and cell division, and the downregulation of cytokine-mediated signaling pathways. Transcriptomic analysis of IL-1 $\beta$ <sup>low</sup>-treated cells allowed us to identify genes and pathways that could play a major role in hormesis induction and  $\beta$ -cell protection against a cytotoxic mixture of proinflammatory cytokines.

**243. 189 EFFECT OF N-3 POLYUNSATURATED FATTY ACID SUPPLEMENTATION ON THE OXIDATIVE STRESS IN DAIRY COWS AT THE TRANSITION PERIOD**

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The transition period (21 days before calving until 21 days postpartum) represents a major adaptive challenge for the animal. During this period, a negative energy balance, oxidative stress, and a pro-inflammatory state can negatively influence the animal's health, production and reproduction. The present study evaluated in dairy cows, the effect of omega-3 polyunsaturated fatty acids (n-3 PUFAs) administration during the transition period on biomarkers of negative energy balance and oxidative stress, along with some antioxidant enzymes. The rations of the omega-3 group (O3G; n=18) were supplemented with calcium salts of fish and linseed oils: 0.4 kg dry matter (DM)/cow/day during 21 days prepartum and 0.65 kg DM/cow/day during 21 days postpartum. The control group (CG; n=18) received the same isoenergetic diet replacing the supplement with ground corn (0.8 and 1.30 kg DM/cow/day during prepartum and postpartum, respectively). Samples were obtained at - 21, 7 and 21 days relative to partum. Blood was obtained and immediately used to measure the concentration of beta-hydroxybutyrate acid (BHBA) by test strips and an automatic analyzer. Whole blood with heparin was used to determine the enzyme activities of superoxide

dismutase (SOD) and glutathione peroxidase (GPx), the concentration of serum non-esterified fatty acids (NEFAs) and total oxidative stress (TAS) using commercial kits. Plasma and liver samples (obtained through biopsies) were used to measure malondialdehyde (MDA) and 3-nitrotyrosine (3-NO<sub>2</sub>-Tyr) concentrations through LC-MS/Ms. NEFA and BHBA concentrations were higher in the O3G ( $p < 0.05$ ). The concentration of MDA in plasma showed an interaction effect *treatment x time*, being higher on day 21 postpartum in the O3G ( $p < 0.05$ ). No differences were observed in the other parameters evaluated ( $p > 0.05$ ). These results suggest that the animals of the O3G showed a higher lipomobilization without evidence of an alteration in oxidative stress biomarkers.

**244. 311 LEVELS OF BONE REMODELLING MARKERS AND THEIR RELATIONSHIP WITH CHANGES IN DENSITOMETRY ACCORDING TO AGE AND NUTRITIONAL STATUS. EXPERIMENTAL STUDY**

Estefania Magalí Zeni Coronel<sup>1</sup>, Marina Soledad Bonanno<sup>1</sup>, Mariana Seijo<sup>1</sup>, Susana Zeni<sup>1</sup>

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Bone is remodelled by the coordinated action of osteoblasts and osteoclasts. This remodelling is biochemically evaluated by the activity of these bone cells, specifically through the measurement of bone formation markers [osteocalcin (OCN)] and bone resorption markers [crosslaps (CTX)]. Bone remodelling follows a pattern similar to the growth velocity curve as a function of age. Objective: To evaluate longitudinally the variation in the levels of these markers of formation and resorption as a function of age and to relate these changes to those occurring in bone mass through densitometric analysis in the femur. Female Wistar rats (6 per group) were divided according to age into newborns (NB), weaning (W21), puberty (P45), young adults (YA90), and adulthood (A105). In each group CTX and OCN were assessed by ELISA and total femur bone mineral density (ftDMO) by DEXA (Hologic). Statistical analysis by ANOVA where different letters indicate significant differences with  $p < 0.05$ . Results (mean±SD) in the following order NB; W21; P45; YA90; and A105: CTX: 83.9±3.5C; 70.5±10.6BC; 52.5±7.4AB; 58.8±5.6AB; and 56.7±11.1AB; OCN: 2.49±0.19A; 3.06±0.05AB; 3.39±0.15ABC; 4.00±0.98BC; and 2.68±0.06; and ftBMD: not detectable; 91±12A; 176±13B; 254±8C; 268±5C. CTX decreased while OCN increased with age. The ftDMO accompanied in its increase the formation marker. Conclusions: To the best of our knowledge, these experimental results show for the first time longitudinally the variation that normally occurs in bone remodelling and its correlation with bone mass throughout the different stages of life.

**245. 455 IMPACT OF FUNCTIONAL LIPIDS ON THE REVERSION OF HEPATIC STEATOSIS IN ADULT RATS FED OBESOGENIC DIET**

Gisela Noemi Kuhn<sup>1</sup>, Candela del Rosario Wagner<sup>1</sup>, Carolina Daniela Gerstner<sup>1</sup>, Jimena Verónica Lavandera<sup>1,2</sup>, Claudio Adrián Bernal<sup>1,2</sup>

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Cafeteria (CAF) diet, leads to a deregulation of lipid metabolism which can progress to hepatic steatosis. Bioactive compounds: tocopherols (TP), phytosterols (PS) and conjugated linoleic acid (CLA) have antioxidant, antiobesogenic and triacylglycerol (TAG) modulating effects. Our objective was to evaluate the effect of the functional lipids (FL) combination on nutritional parameters and hepatic lipid accretion induced by a CAF diet. Male Wistar rats (327±13g) were fed 23 weeks in two periods (14 + 9 weeks). The first 14 weeks, animals consumed: Control (C) 4% soybean oil (n=12) and Cafeteria (CAF) 30% lipids (n=36), diets. Six animals from each group were sacrificed after 14 weeks. In the second period, 6 animals from each group: C (C/C) and CAF (CAF/CAF), continued with their respec-

tive diets, the rest of the CAF group changed to C (CAF/C), CLA 1% (CAF/CLA), TF+FE 0.5% each (CAF/TF+FE) or CLA+TF+FE (CAF/FL). Body weight (BW), body mass index (BMI), abdominal circumference (AC), serum and liver levels of TAG and cholesterol (CHO), C Reactive Protein (CRP) levels and liver fatty acids (FA) composition were measured. Hepatic histological sections (H&E) were analysed by software ImageJ. Student's t-test and One-Way ANOVA followed by Tukey's test ( $p < 0.05$ ) were used for statistical analysis. After 14 weeks, the BW (9%), AC (8%), BMI (29%), liver TAG (20%) and CRP (137%) were increased by CAF. After 23 weeks of treatments rats fed CAF/CAF vs C/C raised: BW (13%), AC (10%), BMI (24%) and CRP (266%). CAF/CLA and CAF/TF vs C/C decreased (30-34%) liver TAG and CHO and increased liver DHA (115 and 94%) and n-3 polyunsaturated fatty acids (76 and 59%) respectively. Hepatic histology showed a large increase of micro- and macro-vacuoles in CAF/CAF, whereas a significantly reduction of hepatic steatosis was observed in the CAF/LF group. Our data support the role of FL in the reversion of lipid accretion in liver caused by CAF diet.

**246. 519 POLYPHENOLS DERIVED FROM AGRICULTURAL CHERRY WASTE MITIGATE TNF ALPHA-INDUCED PERMEABILIZATION OF CACO-2 MONOLAYERS**

Aramburu Agustina<sup>1,2</sup>, Krasnov Matias<sup>1,3</sup>, Basanta M. Florencia<sup>2</sup>, Raffo Dolores<sup>4</sup>, Oteiza Patricia<sup>5</sup>, Erlejman Alejandra<sup>1</sup>

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<sup>5</sup>Nutrition Department, University of California Davis, CA, USA.

Discarded sweet cherries (*Prunus avium* L.) from harvest could serve as a valuable source of bioactive compounds, especially polyphenols, with a positive impact on intestinal barrier integrity. This study aimed to evaluate the anti-inflammatory effects of polyphenols extracted from discarded sweet cherries against TNF $\alpha$ -induced intestinal permeability. Polyphenols were extracted using Sep-Pak columns, yielding a Non-Digested Extract (ND Ext) rich mainly in Cyanidin-3-(6'-p-coumaroyl)glucoside (600 ± 42 mg/100 g powder) and smaller amounts of flavonoids and hydroxycinnamates. After simulating Dynamic Human Digestion (DHD), anthocyanins were recovered with a bioaccessibility of 72.8 ± 22.2% (n=3), and the Digested Extract (Dig Ext) was prepared. Caco-2 differentiated cells, a model of the intestinal epithelial barrier, were assessed for monolayer permeability post-TNF $\alpha$  treatment with or without polyphenol extracts by measuring Transepithelial Electrical Resistance (TEER). Caco-2 cells were pre-incubated with extracts (0.1-1 µg/mL) and then co-incubated with TNF $\alpha$  (10 ng/mL) for 6 hours. TNF $\alpha$  reduced TEER to 79.4 ± 5.1% versus control (100%). Cyanidins provided significant protection, with TEER reaching 93.2 ± 11.0% (n=3,  $p < 0.01$ ). ND Ext at 0.25 µg/mL preserved permeability with 99.9 ± 9.3% TEER, while Dig Ext at 1 µg/mL achieved 94.1 ± 18.2% TEER (n=3,  $p < 0.05$ ). In conclusion, sweet cherry polyphenols offer significant protection against TNF $\alpha$ -induced intestinal permeability, with effects maintained after digestion simulation. This suggests sweet cherries as a sustainable source of anti-inflammatory polyphenols, useful in developing novel antioxidant/anti-inflammatory agents, contributing to both health and sustainability.

**247. 531 EFFECTS OF HIGH-FAT AND HIGH-SALT DIETS ON MESENTERIC ADIPOSE TISSUE: ROLE OF THE NUTRIURETIC PEPTIDE SYSTEM**

Melanie Kim (1,2), Nicolás Kouyoumdzian (1,3), Silvana Cantú (1,2), Natalia Rukavina Mikusic (1,3), María Julieta Rudi (1), Candela Domínguez (1,2), Ana Puyó (1,2), Adriana Donoso (1,2), Marcelo Choi (1,3), Hyun Jin Lee (1,2).

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A growing evidence of the pathogenic potential of visceral adiposity that could link metabolic, kidney and cardiovascular diseases constitutes therapeutic targets to be taken into account. The role of the natriuretic peptide system and its receptors in mesenteric adipose tissue in the context of high-fat and high-salt diets (HFHS) is currently unknown. We studied the effects of diets on plasma levels of atrial natriuretic peptide (ANP), expression of NPR-A and NPR-C receptors in mesenteric adipose tissue and their relationship with systolic blood pressure (SBP) and visceral adiposity in male Wistar rats. Four groups were studied for 6 weeks: Control (C): balanced diet (0.4% NaCl); HF: 60% of calories from fat; HS: 8% NaCl overload in food; and HFHS. We determined: PAS; mesenteric adiposity index (AI); diuresis, creatinine clearance (CrCl), fractional excretion (EFNa) and urinary sodium (EUNa); plasma levels of ANP by ELISA and the expression of NPR-A and NPR-C by Western Blot. SBP increased in all three groups (mmHg, HF: 157±1; HS: 171±4; HFHS: 175±3 vs C: 123±1, p<0.01). In the HS and HFHS groups diuresis, EFNa and EUNa were higher than in the C and HF groups (p<0.05) with no changes in CrCl. Mesenteric AI (%) increased in HF: 0.76±0.01 vs C: 0.61±0.01, (p<0.01) while it decreased in the HS: 0.52±0.03 and HFHS: 0.58±0.01 vs. HF groups (p<0.01). Plasma levels of ANP (ng/ml) decreased in the HF: 0.25±0.02 and HFHS: 0.51±0.05 groups vs. C: 0.77±0.05, (p<0.01). The expression of NPR-A in adipose tissue was higher in HS, lower in HF vs. C (p<0.01), while HFHS presented a lower expression than C but higher than HF. On the other hand, the expression of NPR-C was higher in HF and lower in HS vs. C (p<0.01) and in HFHS it was higher than HS (p<0.05) and slightly higher than C. HF is associated with an increase in mesenteric adiposity, while HS decreases it. These effects could be due to a differential expression of NPR-A and NPR-C receptors at the level of adipose tissue.

## NEFROLOGÍA

### O1 - COMUNICACIONES ORALES

FECHA Y HORA: 20/11/2024 11:30-12:30 H

LUGAR: SALA DE CÁMARA

COORDINADORES: ANA MARIA PUYO, MARIA GABRIELA MARINA PRENDES

#### 248. 094 GELATINASES (MMP-2, MMP-9) AS RENAL BIOMARKERS IN DOMESTIC SPECIES

Luis Di Ciano\*, Belén Martínez Dell'Éra\*, Román Pareja, Carla Giampaoli, Emilio De Simone.

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The urinalysis is a non-invasive diagnostic tool for the detection of renal diseases among others. Urinalysis includes evaluation of physical characteristics, biochemical parameters, microscopic sediment, and enzyme estimation. Matrix metalloproteases (MMP-2 and MMP-9) have gelatinolytic activity and under normal conditions are filtered in the glomerulus and reabsorbed along the nephron. The use of MMPs as predictors of renal disease can be enhanced by characterizing their role in renal homeostasis. The aim of this work was to simultaneously evaluate the macro and microscopic characteristics, the examination of physical and chemical properties, the study of urinary sediment and the activity of MMP-2 and MMP-9 gelatinases in the urine of healthy equine (E), feline (F) and canine (C). The urinalysis was performed with reagent strips (Wiener Lab.). In the same urine samples, the gelatinolytic activity of MMP-2 and MMP-9 was evaluated by gelatine gel zymography. Variables were expressed as mean ± standard error of the mean (SEM). The urine of healthy felines and canines is clear in appearance, in contrast for that of equines which presented a cloudy and thickened appearance. The urinalysis showed that the urine pH of E (9 ±0.08) is more alkaline than those of F (6.67±0.67) and C (6±0.60 p<0.05) and

hyposthenuric (Urinary density, E: 1007 ±1.4 vs F: 1023±6.67 and C:1013±4.4 in g/ml, p<0.05). The activity of MMP-2 and MMP-9 in urine samples showed no differences. These results show that both metalloproteases are present in the urine of healthy animals and could participate in renal homeostasis. Changes in the activity of renal gelatinases could be associated to renal disease development, therefore, metalloproteases could be useful biomarkers of kidney injury.

#### 249. 105 EFFECTS OF SHIGA TOXIN 2 SUBLETHAL DOSE ON RENAL WATER HANDLE IN PREGNANT AND NON-PREGNANT RATS

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Shiga toxin-producing *Escherichia coli* causes hemolytic uremic syndrome with acute kidney injury. Previously, we showed that a sublethal dose of Shiga toxin 2 (Stx2) caused abortion in pregnant (P) rats, and significant less renal injury in P rats than in non-pregnant (NP) rats. Besides, it is known that during pregnancy increased nitric oxide production may lead to intrarenal vasodilation. This work aimed to study the effect of a sublethal dose of Stx2 on renal water handle in P and NP rats under different hydration conditions. Pregnant Sprague-Dawley rats, at day 8 of gestation, and NP rats, were intraperitoneally injected with 0.5ng Stx2/gbody weight (bw) (PS and NPS, respectively) or with eluent for control P (PC) and NP (NPC) rats. At 4 days post-injection, rats were placed in metabolic cages with water *ad libitum* (water<sub>ad</sub>) or water deprivation (water<sub>dep</sub>) for 18 hours. Blood and urine samples were collected to evaluate urinary flow (U), urine osmolality (Osm<sub>u</sub>), and transport of free water (Tc) reabsorbed in the collecting duct. Under water<sub>ad</sub>, NPS rats significantly increased U (16 ±1.1 ml/d×100gbw) and decreased Osm<sub>u</sub> (307 ±19.5 mOsmol/kg) and Tc, compared to NPC rats (U: 7 ±1.0 ml/d×100gbw; Osm<sub>u</sub>: 584 ±74.9 mOsmol/kg) (p<0.05). However, Stx2 did not modify these parameters in PS rats compared with PC rats. Under water<sub>dep</sub>, both PS and NPS rats significantly increased U (8 ±1.1 and 8 ±0.6 ml/d×100gbw, respectively), and decreased Osm<sub>u</sub> (583 ±91.2 and 553 ±31.8 mOsmol/kg, respectively) and Tc, compared to PC (U: 4 ±0.6 ml/d×100gbw, Osm<sub>u</sub>:1125 ±184.9 mOsmol/kg), and NPC rats (U: 3 ±0.4 ml/d×100gbw, Osm<sub>u</sub>:1202 ±126.8 mOsmol/kg), (p<0.05). In conclusion, under water<sub>dep</sub>, PS rats evidenced the renal water handle alterations caused by Stx2 sublethal dose, not shown under water<sub>ad</sub> conditions. We propose that the adaptive changes during pregnancy, such as systemic and intrarenal vasodilation, may prevent or mask Stx2 effects on renal water handle.

#### 250. 507 CLINICAL USEFULNESS OF WATER AND OSMOLAR EVALUATION IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD)

María Carola Baldoni<sup>1,2</sup>, María Lucía Rosenberg<sup>1,2</sup>, Natalia Elizabeth Riera<sup>1,2</sup>, Bruno Ezequiel Branca<sup>1,2</sup>, Jorge Eduardo Toledo<sup>1,2</sup>, Marina Claudia Khoury<sup>3</sup>, Roxana Peroni<sup>4,5</sup>, Elisabet Mónica Oddo<sup>1,2</sup>, Pablo Javier Azurmendi<sup>1,2</sup>.

1 Nefrología Experimental y Bioquímica Molecular, Instituto de Investigaciones Médicas Alfredo Lanari, Fac.de Med., U.B.A.; 2 IDIM, UBA-CONICET; 3 Docencia e Investigación, Instituto de Investigaciones Médicas Alfredo Lanari, Fac.de Med. U.B.A.; 4 Instituto de Investigaciones Farmacológicas (ININFA, UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. 5 Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires.

Electrolytes and water handling are pivotal in disease progression and treatment of ADPKD by ADH axis blockage (ADHb) with tolvaptan or high water intake. Urine osmolality could be a marker of the efficacy of ADHb. Measured urine Osm (m-uOsm) is a scarce

resource and it could be estimated by formula (c-uOsm). This study aimed to evaluate the water and electrolyte excretion in ADPKD patients and the c- and m-uOsm comparison. Daily urine (n=61) samples were collected from 2018 to 2024 from ADPKD patients naive for tolvaptan treatment (n=28) with  $78 \pm 4$  MDRD4-estimated GFR. m-Osm (in mOsm/Kg H<sub>2</sub>O) was determined by freezing point. Na<sup>+</sup> and K<sup>+</sup> were measured by indirect potentiometry and urea by urease kinetic method. c-uOsm (mOsm/L) was calculated by  $[\text{Na}^+ + \text{K}^+] (\text{mM}) \times 2 + \text{urea} (\text{mg/dl})/6$ . Daily free water (FWCL, L/d) and osmolar clearances (OCL, L/d) were calculated using a routine equation. The m- and c-uOsm were compared by the Bland-Altman plot. Diuresis, OCL, and FWCL were  $2.7 \pm 0.1$ ,  $2.8 \pm 0.1$ , and  $-0.1 \pm 0.1$ , respectively. Routine dietary recommendations (>2 L water and <2.4 g Na intake per day) were achieved only in 45 and 41% of the population. FWCL  $\geq 0$  was achieved at diuresis > 3. Patients with FWCL < or > 0 showed similar Na intake ( $3.7 \pm 0.5$  and  $3.0 \pm 0.2$  g/day, p=0.18) and plasma Osm ( $286 \pm 2$  and  $282 \pm 4$ , p=0.41) but different m-uOsm ( $231 \pm 26$  vs  $358 \pm 24$ , p=0.002). FWCL and diuresis multiple regression analysis showed that they depend on urea and Na excretion (Radj=0.58 and 0.34, p<0.005), respectively. The m-uOsm - c-uOsm was 11.7 (IC95%: -73.3 - 140.7), not different from 0 (p=0.99). This difference was higher in m-uOsm >300 than in  $\leq 300$  (p=0.039). Taking into account the high Na diet observed in our population, the water intake  $\geq 3$  L efficiently ADHb and then, decelerates disease progression. The c-uOsm showed concordance, but with high variability, with m-uOsm. The better performance in the c-uOsm <300 suggests their utility to monitoring the ADHb.

**251. 524 CHRONIC INHIBITION OF RENAL DOPAMINERGIC SYSTEM IN SPONTANEOUSLY HYPERTENSIVE RATS INCREASES BLOOD PRESSURE AND RENAL INFLAMMATION**

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Inhibition of peripheral dopa decarboxylase represents a pharmacological strategy for treatment of Parkinson's disease, but its effects on kidney function and blood pressure (BP) are unknown. Our objective was to evaluate if chronic administration of carbidopa (Cb) could lead to renal inflammation and increase in BP levels. Male Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) were divided into 4 groups (n=8/group): WKY and SHR (drinking water), WKY+Cb and SHR+Cb (Cb 25 mg/kg/day in drinking water) for 3 weeks. We determined: 24-hour fractional and urinary sodium excretion. Systolic blood pressure (SBP) was measured by tail-cuff method. Renal expression of the Na<sup>+</sup>, K<sup>+</sup>-ATPase pump (NKA), dopamine D1 and D2 receptors, Parkinson's protein 7 (PARK7) and NFκB (as anti-inflammatory and inflammatory markers) were determined by Western Blot. P<0.05 was considered significant (\* vs WKY; # vs SHR). SBP levels were modified only in the first week of treatment, with a greater increase observed in SHR+Cb vs SHR rats (mmHg:  $240 \pm 3\#$ ;  $210 \pm 10$ ). A significant reduction in fractional and urinary sodium excretion was accompanied by an increase in renal NKA expression in SHR and WKY rats (AU: WKY:  $1.00 \pm 0.08$ ; WKY+Cb:  $1.30 \pm 0.14^*$ ; SHR:  $1.30 \pm 0.23^*$ ; SHR+Cb:  $1.78 \pm 0.13^*\#$ ). In the WKY+Cb group there was a significant decrease in D1R expression (\*) and a non-significant increase in D2R. This was accompanied by a significant increase in PARK7 expression in SHR rats (\*) and a non-significant increase in NFκB expression in WKY+Cb (\*) and SHR+Cb rats (#). In conclusion, inhibition of renal dopamine synthesis with Cb alters tubular sodium handling, by increasing NKA activity and promoting greater sodium reabsorption. In the context of hypertension, these changes would be accompanied by a greater renal in-

flammatory response.

**P1 - POSTERS**

FECHA Y HORA: 20/11/2024 16:10-17:10 H

**COORDINADORES: ALBERTONI BORGHESE MARÍA FLORENCIA, ROSENBERG MARÍA LUCÍA**

**252. 142 POSSIBLE MECHANISMS INVOLVED IN HYPERTENSION PROGRAMMING IN RAT OFFSPRING BORN FROM MOTHERS WITH ADENINE-INDUCED CHRONIC KIDNEY DISEASE**

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Hsu et al demonstrated that maternal adenine-induced chronic kidney disease (CKD) programs hypertension in adult male rat offspring. We aimed to evaluate whether 21 day old rat offspring born from CKD Mothers (CKDm) presented alterations in renal development at structural and/or functional level. To achieve this goal, adult Sprague-Dawley (SD) female rats were treated for 3 weeks with Adenine 0.5% in standard rat chow powder (CKDm, n=4) or regular rat chow powder (Control mothers=Cm, n=4). Then, they were mated with control SD male rats. Offspring was divided into 4 groups: male and female born from Cm (MCm and FCm) or CKDm (MCKDm and FCKDm). After weaning (day 21), pups were weighed and sacrificed. Blood and urine samples were obtained to determine plasma and urinary creatinine and proteinuria. Kidneys were used to perform morphometric analysis in the external cortical (CA) and juxtamedullary area (JA); and to count the number of total glomeruli by acid maceration method. Tibias and femurs were measured using a caliper. Results expression: mean  $\pm$  SEM. Statistics: 2-way ANOVA. Offspring from CKDm had lower body weights (g) vs those from Cm [MCm:  $56.4 \pm 1.7$ ; MCKDm:  $53.1 \pm 1.9$ ; FCm:  $54.1 \pm 1.6$ ; FCKDm:  $51.5 \pm 1.6$ ; p<0.01] with sex differences (p<0.05). There were no changes in tibia or femur length, nor in plasma and urinary creatinine or proteinuria (n=6-8/group). We found a decreased number of glomeruli/mm<sup>2</sup> and total glomeruli in MCKDm vs MCm and in FCKDm vs FCm (p<0.05), with sex differences (p<0.01). We found an increased Total Glomerular Area (TGA) and Capillar Glomerular Area (CGA), both in CA and JA, in MCKDm vs MCm (p<0.01), and in FCKDm vs FCm (p<0.01). There were no changes in AGC/AGT% or Renal Filtration Surface Area in CA or JA. Our results suggest that, despite not finding changes in creatinemia or proteinuria, there are renal histomorphometric alterations in offspring born from CKDm, which may play a role, at least in part, in hypertension programming.

**253. 165 EFFECT OF FEMALE HORMONES SUPPLEMENTATION ON IMMUNE SYSTEM RESPONSE IN A RAT MODEL OF SALT-SENSITIVE HYPERTENSION**

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Previously we showed that ovariectomized (oVx) adult Wistar rats have a deranged renal sodium handling leading to salt-sensitive hypertension (HSS) upon a high sodium intake (HS). Also, we observed that changes occur in peripheral blood mononuclear cells (PBMC) of oVx HS rats, altering Na<sup>+</sup>K<sup>+</sup>ATPase (NKA) protein expression. The objective is to study the effect of hormonal supple-

mentation. We worked with female rats. At 60 days of life, half of the rats were oVx, and at 145 days intact female rats (IF) and oVx rats were divided into NS (0.24% NaCl) or HS (1% NaCl in drinking water) subgroups. Simultaneously, oVx rats were supplemented with 17  $\beta$  estradiol 3-benzoate (oVx NS E2 and oVx HS E2, 60  $\mu$ g/kg), progesterone (oVx NS P4 and oVx HS P4, 10 mg/kg) or vehicle in subcutaneous injections twice a week for three weeks. During the treatment, systolic blood pressure (SBP) was recorded. At the end of the experiment, blood samples were taken. The expression of NKA was studied in PBMC by Western Blot (WB) and the gene expression of NKA (Atp1a1) in PBMC was studied by quantitative polymerase chain reaction (qPCR). The elevated SBP (in mmHg) of the oVx HS group ( $135 \pm 2.3$  vs  $120 \pm 3.0$  IF HS) decreased with E2 ( $124 \pm 4.2$  oVx HS E2,  $p < 0.05$  vs. oVx HS) while it remained elevated with P4 ( $135 \pm 3.4$ ). WB studies in PBMC showed an overexpression of NKA in the oVx HS group that was reversed by the administration of both E2 and P4 ( $p < 0.05$  oVx HS vs. oVx HS E2, and oVx HS P4 groups). By qPCR, an increase in mRNA levels of NKA in oVx HS group was found ( $p < 0.05$ ). E2 reversed this increase. With P4, oVx NS had high gene expression while oVx HS decreased to non-quantifiable levels ( $Ct > 45$ ). We found a link between sexual hormone and NKA expression in PBMC. The increase in NKA activity in lymphocytes is related to cell proliferation, so the changes found show that the lack of hormonal regulation, especially E2, could determine the infiltration of immune cells in key organs described in hypertension.

**254. 218 IMPACT OF CONTRAST MEDIA ON THE PHARMACOKINETICS OF RADIOPHARMACEUTICALS DURING A BASIC RADIO RENOGAM**

Mariano Portillo, Fiorella Tesán, Diego Giaquinta Romero, Marcela Zubillaga, María Jimena Salgueiro.  
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Introduction: Drug-drug interactions are well-known to affect the kinetics, efficacy, and toxicity of conventional medications. In Nuclear Medicine (NM), it is common practice to use drugs to enhance diagnostic procedures, referred to as interventional NM. Objective: To assess the effect of iodine contrast media (ICM) on the results of a radionuclide basic renogram (RBR) in healthy control animals, providing insights into potential pharmacokinetic interactions. Materials and Methods: 9 healthy Sprague-Dawley rats (average weight 250 g) were used to perform RBR with  $^{99m}\text{Tc}$ -DTPA (37 MBq) under control conditions. Dynamic studies were acquired using a small-field-of-view gamma camera equipped with a high-resolution collimator and dedicated software for small animals. Images were taken at 1 frame/second during the first minute, followed by 1 frame/15 seconds for at least 30 minutes. After one week, the RBR was repeated one hour after intravenous administration of ICM (1 mg/kg body weight, Iopamidol, iodine 370 mg/mL). Results: RBR under control conditions displayed functional curves with the expected 3 phases: perfusion, function, and elimination. Average T<sub>max</sub> (2.3 min) and T<sub>50%</sub> (8 min) were within the normal range, as were the right and left relative renal functions (45-55%). After ICM administration, qualitative and quantitative changes affected the functional curves. Although all 3 phases were visualized, a prolonged T<sub>max</sub> (8 min) was recorded in some cases, and the elimination phase was significantly prolonged (T<sub>50%</sub> > 30 min). Despite these alterations, relative renal function remained within the normal range, and delayed elimination of  $^{99m}\text{Tc}$ -DTPA was observed. Conclusion: ICM interfered with normal renal function, as shown by RBR results. These findings highlight the need to consider the effects of ICM on renal function and to further explore its pharmacokinetic interactions with radiopharmaceuticals dependent on renal excretion.

**255. 219 CHANGES IN  $^{99m}\text{Tc}$ -DMSA BIODISTRIBUTION DUE TO INTERACTION WITH DOXORUBICIN AND IODINATED CONTRAST MEDIA**

Mariano Portillo, Fiorella Tesán, Marcela Zubillaga, María Jimena Salgueiro.  
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Objective: To study the alteration of the biodistribution of  $^{99m}\text{Tc}$ -DMSA after the administration of iodinated contrast media (ICM) in animals treated with doxorubicin (DOX). Materials and Methods: Six female Sprague Dawley rats were used. Baseline images were obtained after intravenous administration of 1 mCi of  $^{99m}\text{Tc}$ -DMSA (A). Three of them underwent  $^{99m}\text{Tc}$ -DMSA distribution imaging after the administration of 0.1 mg/kg of ICM (B). The other three were treated for two weeks with six doses of DOX (2 mg/kg) and underwent imaging after intravenous administration of 1 mCi of  $^{99m}\text{Tc}$ -DMSA at 24 (C) and 72 hours post-treatment (D). In the 72-hour images, 0.1 mg/kg of ICM was administered beforehand. Results: Qualitatively, the baseline images (A) showed the expected distribution pattern for this radiopharmaceutical. The images (B) displayed an altered distribution profile with a decrease in renal parenchyma uptake, an increase in hepatic parenchyma uptake, and visualization of large joints. In (C), the altered distribution persisted, with a marked increase in renal and hepatic uptake, as well as slight visualization of large joints. The images (D) exhibited a similar alteration pattern, with a predominant increase in large joints uptake and a slight increase in hepatic and renal parenchyma uptake. This was also reflected in the qualitative analysis. Conclusions: Our study demonstrates the alteration in the distribution of  $^{99m}\text{Tc}$ -DMSA in animals treated with doxorubicin, with and without contrast media, modifying the expected qualitative and quantitative imaging profile. In the context of hybrid imaging, it is important to establish the basis for the localization of probes used in diagnostic analysis and the understanding of the information obtained.

**256. 498 SEXUAL DIMORPHISM IN NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) RESPONSE TO ISCHEMIC ACUTE KIDNEY INJURY (IR-AKI)**

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NGAL increases in plasma and urine after IR-AKI. Our previous research indicates that females are renoprotected against unilateral ischemia-reperfusion (IR). Despite less damage, female rats have higher urinary NGAL (uNGAL) levels compared to males. NGAL is overexpressed in the ischemic kidney (IK) but the expression pattern varies by sex. We aimed to study plasma NGAL (pNGAL) and NGAL expression in the not injured contralateral kidney (CL) to understand the source of elevated uNGAL in females. Male (M) and female (F) Wistar rats (n=6 per group) underwent 40 minutes of unilateral renal ischemia followed by 1 day of reperfusion. Controls underwent sham operation. Renal NGAL was evaluated in IK and CL, both in the medulla (m) and cortex (c), using western blot and immunohistochemistry (IH). pNGAL was measured by ELISA. Statistical method: ANOVA post-hoc Tukey test. In both sexes NGAL was undetectable in controls and increased in IK. IR increased NGAL expression in CL (c: +100%\* in FCL vs FIK, +256%\* in MCL vs MIK; m: +316%\* in FCL vs FIK, +55% in MCL vs MIK). This increase was higher in male cortical tissue but higher in females in medullary tissue. No sex-related differences were observed when comparing between CL. Similar results to western blot were obtained by IH. pNGAL was higher in M compared to F submitted to IR (+24%\*). \* $p < 0.05$ . For the first time, we reported that unilateral IR induces NGAL expression in CL. This could imply a systemic renoprotective response to IR. Although our results cannot explain the increased uNGAL in females, they highlight CL's contribution to uNGAL, which does not reflect damage extent. This should be considered when evaluating NGAL as a urinary biomarker. We found that pNGAL response to IR varies by sex and correlates with renal damage, unlike uNGAL. Further studies are needed to explore NGAL's protective role and sexual dimorphism to improve its clinical application as biomarker.

**257. 515 RENAL PROTEIN MANAGEMENT IN A SUBLETHAL**

**MODEL OF HEMOLYTIC UREMIC SYNDROME IN RATS**

Mercedes Paciaroni (1), Sofia Vizcarra (1), Gabriela Figueiredo (1), Francisco Martella (1), Candela Kadar (1), Elsa Zotta (1,2), Federico Ochoa (1,2).

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In Argentina, HUS is the most frequent cause of acute renal failure in children. 30% may have renal sequelae with evolution to chronicity. The aim of our work was to study the renal protein management during the evolution to chronicity in an experimental sublethal model of HUS in rats. Sprague-Dawley rats (150-200 g, n= 5 per group) were injected intraperitoneally. The experimental group was inoculated with 0.25 mL of recombinant *E-coli* bacterial culture supernatant expressing Stx2 (sStx2) every 200 g of body weight (sublethal dose). The control group received the same volume of saline. Immunohistochemical studies were performed to detect FcRn, megalin, TGF  $\beta$ 1 and albumin after 1 week and 3 months post inoculation. Also at both times, two groups of animals were treated with enalapril (50 mg/Kd/d). Results: Immunohistochemistry showed at 1 week and 3 months an increase in FcRn and TGF  $\beta$ 1 tubular expressions ( $p < 0.001$  vs respect controls), although both presented a decrease when were treated with enalapril at 1 week ( $p < 0.001$ ). At 3 month the increase in FcRn expression was maintained during enalapril treatment ( $p < 0.001$ ) without alterations in TGF  $\beta$ 1 response. At glomerular level FcRn increased at 1 week and 3 months without enalapril effects ( $p < 0.001$ ). Megalin glomerular expression decreased at 1 week and 3 months, but it was increased with enalapril treatment at 3 months ( $p < 0.001$ ). Our results indicate that during the evolution to chronicity it could be developed a FcRn tubular adaptation mechanism that could be stimulated by enalapril, not observed in glomeruli. The TGF  $\beta$ 1 expression could be scape the regulation of enalapril at 3 months. Megalin glomerular expression could be related with the regulation by AARS at 3 months.

**258. 535 KIDNEY DAMAGE AFTER DISCONTINUING LITHIUM TREATMENT**

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Lithium (Li) remained the *gold standard* in the treatment of bipolar disorder. There is evidence that the prevalence of Chronic Kidney Disease is higher in patients treated with Li than in general population. The aim of this study was to evaluate whether kidney function and histopathological lesion improves after discontinuing Li treatment. Thirty *Wistar* rats were randomized into three groups: control group (CG) fed powered standard diet (PSD) for 4 months; and two experimental groups fed PSD supplemented with 60 mmol of Li/kg diet for 3 months (EG1 and EG2), and EG2 fed with PSD without Li for another month. Creatinine and Li concentrations were assessed at baseline, after 1, 3 and 4 months. Creatinine clearance was calculated by the standard formula. Kidneys were removed for histopathological analysis. The magnitude of the histopathological lesion was evaluated using a semiquantitative score (0: no evidence of significant alterations; 1: occasional dilation of cortical collecting

ducts and mild or absent hypertrophy; 2: occasional dilation with hypertrophy, no more than 3 consecutive foci; 3: marked dilation and hypertrophy in confluent foci). Lithemias were within therapeutic range used in humans in both EG. Creatinine levels showed no differences at baseline and after 1 month; were higher in both EG after 3 months ( $\chi^2=13.88$ ,  $p=0.001$ ) and were higher in the EG1 at the end of the experiment ( $\chi^2=12.57$ ,  $p=0.002$ ). Creatinine clearance showed no differences at baseline; was higher in both EG after 1 ( $\chi^2=8.54$ ,  $p=0.014$ ) and 3 months ( $\chi^2=12.57$ ,  $p=0.002$ ); and, at the end of the experiment, was higher in the CG, showing significant differences with EG1, ( $F=4.041$ ,  $p=0.036$ ; CG vs EG1:  $p=0.017$ ). There were no significant differences in histopathological lesion. Discontinuation of Li treatment appears to partially reverse renal dysfunction, although no significant difference was observed in renal histopathological damage.

**NEUROCIENCIAS****O1 COMUNICACIONES ORALES**

FECHA Y HORA: 19/11/2024 16:00-17:00 H

LUGAR: AUDITORIUM

COORDINADORES: ROSENSTEIN RUTH, RAMOS ALBERTO JAVIER

**259. 061 METHYLENE BLUE AS A NOVEL NEUROPROTECTIVE AGENT IN AN EXPERIMENTAL MODEL OF GLAUCOMA**

Ronan Nakamura<sup>1</sup>, Nicolás Sebastián Ciranna<sup>1</sup>, Juan Carlos Fernández<sup>1</sup>, Agustín Pedro Aranalde<sup>1</sup>, Ulises Ruiz<sup>1</sup>, Juan José López-Costa, Rafael Peláez<sup>2</sup>, Alfredo Martínez<sup>3</sup>, César Fabián Loidl<sup>1</sup>, Manuel Rey-Funes<sup>1</sup>.

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Introduction: Glaucoma is the leading cause of irreversible blindness worldwide. Even though several treatments exist to treat it, none of them addresses the main pathophysiological cause for blindness which is the neurodegenerative process in the retina. Given the evidence provided by our team on the neuroprotective role of methylene blue (MB) in other models of retinopathy, we propose MB as a candidate to reduce retinal damage in an episcleral vein cauterization (EVC) model of glaucoma. Materials & Methods: EVC surgery was performed in male *Wistar* rats ( $n=50$ ) unilaterally on two episcleral veins. Sham surgery was performed in the contralateral eye which served as control (CTL). Seven days post-surgery half of the animals started intraperitoneal MB treatment (2mg/kg) twice a day for 7 days. The other half received placebo. Oscillatory potentials (OP), scotopic (ERG) and pattern (PERG) electroretinograms were performed at day 7 and 15 post-surgery. At day 15 all animals were sacrificed. Eyes were enucleated and subjected to morphological studies with hematoxylin & eosin. Results: In EVC operated eyes ERG showed amplitude reduction of b-wave and OP alteration. In addition, PERG showed a significant reduction of N2-wave, and implicit time was significantly prolonged. Histological evaluation showed retinal ganglion cells (RGC) loss and reduction of inner retinal (IR) thickness. No significant differences were observed between CTL and CTL-MB groups. Our findings show that compared with EVC, EVC-MB had significant preservation of RGC and IR thickness. Electroretinography of EVC-MB showed significant waveform and amplitude preservation by MB. Both ERG b-wave and PERG N2-wave values account for inner retina neuroprotection and are consistent with our histological findings. Conclusion: MB is an effective neuroprotective drug in preserving retinal function and structure. This drug could therefore be used as a new therapeutic agent to prevent vision loss in glaucoma patients.

**260. 062 METHYLENE BLUE PREVENTS RETINAL LESIONS IN AN EXPERIMENTAL MODEL OF INTRAORBITAL OPTIC NERVE CRUSH (IONC)**

Nicolás Sebastián Ciranna<sup>1</sup>, Ronan Nakamura<sup>1</sup>, Juan Carlos Fernández<sup>1</sup>, Agustín Pedro Aranalde<sup>1</sup>, Ulises Ruiz<sup>1</sup>, Alejandra Paganelli<sup>1</sup>, Juan José López-Costa<sup>1</sup>, Rafael Peláez<sup>2</sup>, Alfredo Martínez<sup>3</sup>, Manuel Rey-Funes<sup>1</sup>, César Fabián Loidl<sup>1</sup>.  
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Introduction: Ocular and periorbital trauma may result in vision loss. In previous experiments of our team, hypothermia treatment in retinas subjected to IONC has proven to reduce morphological and molecular alterations. On the other hand, we have also showed that methylene blue (MB) reduces morphological and electrophysiological retinal distortion caused by perinatal asphyxia. In the light of these findings, we propose MB as a neuroprotective strategy. Materials & Methods: IONC surgery was applied to 45 days old Wistar rats. Sham surgery was performed in the contralateral eye which served as control. Intraperitoneal MB treatment (2mg/kg) was administered post-surgery and at 6, 12 and 24 hours. Oscillatory potentials (OP), scotopic (ERG) and pattern (PERG) electroretinography was performed 21 days post-surgery. After PERG evaluation, all animals were sacrificed. Eyes were enucleated for morphological studies with hematoxylin & eosin (H&E). Results: In IONC eyes, ERG showed a drastic amplitude reduction of the b-wave ( $p < 0.0001$ ), a-wave ( $p < 0.03$ ) and OP alteration ( $p < 0.0001$ ). PERG of IONC operated eyes showed a significant reduction of N2-wave ( $p < 0.0001$ ). Histological evaluation showed a large decrease in the number of retinal ganglion cells (RGC) ( $p < 0.0001$ ). No significant differences were observed between control (CTL) and CTL-MB groups. Our findings show that, compared with IONC group, IONC-MB had significant preservation of RGC. Electroretinography findings of IONC-MB showed significant waveform and amplitude preservation by MB. b-wave and N2-wave values, account for inner retina neuroprotection and are consistent with our histological findings. Conclusion: MB is an effective drug in preserving retinal histology and electrophysiological function after IONC induced damage. It could therefore be used as a neuroprotective strategy to preserve retinal integrity and prevent visual loss in ocular and periorbital trauma patients.

**261. 076 BLOOD AND BEHAVIORAL PROFILING IN A SCHIZOPHRENIA MOUSE MODEL: A PATH TO AN EARLY DIAGNOSIS AND NOVEL PHARMACOLOGICAL TREATMENT**

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Schizophrenia is a devastating disorder with still unknown etiology. Its diagnosis is based solely on observable signs and symptoms that emerges between late adolescence and early adulthood. People with schizophrenia show cognitive deficits, depression, social withdrawal, as well as metabolic and immunological abnormalities. However, the most remarkable symptoms are the positive ones, such as hallucinations and delusions. These symptoms are well treated with antipsychotics, but they do not improve cognitive, negative, immunological, or metabolic symptoms. In our lab, we generated a genetically modified mouse line that exhibits characteristics reminiscent to those described in schizophrenia patients. The main goal of our work is to understand the molecular bases underlying the onset of schizophrenia by studying this model and using it to search for specific markers for an early and objective diagnosis, as well as developing novel pharmacological treatments targeting unattended

symptoms. Adult mutant animals display molecular, cellular, physiological, and behavioral phenotypes similar to those described in patients. They also show glucose intolerance, imbalances in fatty acid levels, microglial reactivity, and a distinctive blood signature. We performed a peripheral blood characterization along the prodromic phase in search for mutant specific early biomarkers. A longitudinal study of the blood profile and glucose tolerance during the transition from adolescence to adulthood shows a characteristic pattern in the mutant group compared to the control group with a reduced neutrophil to lymphocyte ratio and progressive levels of glucose tolerance. Behavioral analysis reveals phenotypes associated with negative symptoms in the mutant group that emerge before adulthood. Our results show the potential of blood and behavioral analyses to stratify individuals who may be at risk of developing schizophrenia-associated phenotypes, with potential translational value.

**262. 262 CHARACTERIZING THE ASTROGLIAL RESPONSE IN A MODEL OF BRAIN EDEMA BY COLD INJURY**

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Astrocytes respond to Brain injury through a process of reactive astrogliosis involving transcriptomic, phenotypic and functional changes. Reactive astrogliosis is a progressive phenomenon and can achieve time-stable phenotypic changes. Brain edema, which is an increase in interstitial water content, is an early event after many different brain injuries and pathologies and might impact on astrocyte function. We hypothesize that transcriptomic changes underlying astrocyte reactive phenotypes in a context of brain edema are regulated by epigenetic mechanisms of histone posttranslational modifications. We here aimed to characterize astrocyte response in a model of cold injury, which was optimized by our group. We conducted all of our studies in adult female and male mice (C57/4-5 months) at 1 and/or 3 and 7 DPL (days post lesion). Using immunofluorescent co-labeling followed by epifluorescence and confocal microscopy we addressed immunoreactivity for astrocyte marker GFAP (glial fibrillary acidic protein), AQP4 (aquaporin 4) and C3 (complement 3). We also used immunomarkers to describe other reactive/responsive cells, such as NeuN (neuronal nuclear protein), IBA1 (microglial, ionized calcium-binding adaptor molecule 1) and NG2 (Nerve/glial antigen 2). Our results showed increased GFAP immunoreactivity at 1-7DPL and AQP4 immunoreactivity at 1DPL. We further observed an increase in C3 immunoreactivity with no clear colocalization to any cell type. NG2 showed subtle increased immunoreactivity at 1DPL. Immunoreactivity for microglia marker IBA1 increased at 3-7DPL while NeuN radically decreased at 1-7DPL. We conclude that the cold-injury model of brain edema promotes a progressive glial response and results suitable for addressing epigenetic changes in reactive astrocytes at different time points of reactive astrogliosis. It is of note that this model will allow addressing cellular response in a context of brain edema, a pathological condition of high clinical relevance.

**263. 328 AMYLOID BETA MODULATES ASTROCYTE ACTIVATION, GLUCOSE METABOLISM, AND INSULIN PATHWAYS: IMPLICATIONS FOR ALZHEIMER'S DISEASE**

Melisa Bentivegna<sup>1,2</sup>, Carlos Pomilio<sup>1,2</sup>, Amal Gregosa<sup>1,2</sup>, Nicolás González Pérez<sup>1,2</sup>, Daiana Vota<sup>2</sup>, Melina Bellotto<sup>1,2</sup>, Jessica Presa<sup>1,2</sup>, Flavia Saravia<sup>1,2</sup>, Juan Beauquis<sup>1,2</sup>

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Alzheimer's disease (AD) is the most common neurodegenerative disorder and the leading cause of dementia. Beyond amyloid beta (A $\beta$ ) and tau aggregation, inflammation and insulin resistance are also frequent in AD brains. Astrocytes are crucial in maintaining homeostasis and managing inflammatory responses. This study aimed



to 1) examine the metabolic and inflammatory status of PDAPP-J20 transgenic (TG) mice, an AD model, and 2) assess inflammatory activation, energy metabolism, and insulin signaling in astrocytes in vitro after A $\beta$  exposure. We hypothesized that A $\beta$ -treated astrocytes adopt a proinflammatory phenotype and lose homeostatic functions. Our findings revealed impaired insulin signaling (pAkt/Akt) in the hippocampus ( $p < 0.05$ ), along with reduced hippocampal insulin receptor (IR) levels ( $p < 0.05$ ). Immunolabeling for IR showed a decrease in GFAP+ astrocytes, which also displayed increased GFAP and S100b labeling ( $p < 0.05$ ), indicating proinflammatory reactivity. In vitro, A $\beta$  exposure led to increased NF $\kappa$ B nuclear translocation, elevated S100b ( $p < 0.0001$ ), and decreased EAAT2 expression ( $p < 0.0001$ ). Western blot and immunofluorescence analysis showed higher IR expression and increased membrane localization ( $p < 0.05$ ) in A $\beta$ -exposed astrocytes. However, insulin-prompted IR phosphorylation decreased and Akt phosphorylation remained unchanged after A $\beta$  exposure. A $\beta$ -exposed astrocytes also showed increased glucose uptake ( $p < 0.05$ ) and a trend towards higher lactate production and glycogen accumulation ( $p < 0.05$ ). Additionally, the number of mitochondria decreased, while superoxide production increased ( $p < 0.001$ ). Despite reduced lipid droplet density, their colocalization with mitochondria increased ( $p < 0.01$ ), suggesting a metabolic shift towards  $\beta$ -oxidation. These results suggest that hippocampal insulin resistance and glial reactivity are concurrent in experimental AD. A $\beta$ -exposed astrocytes adopt a proinflammatory phenotype with impaired insulin signaling and mitochondrial dysfunction, providing insight into AD pathophysiology.

**264. 359 SYNAPTIC MORPHOLOGY AND MITOCHONDRIAL ULTRASTRUCTURE ARE ALTERED IN THE PRIMARY VISUAL CORTEX IN GLAUCOMA**

Ailen G. Hvozda Arana<sup>1,2</sup>, Laura Caltana<sup>3</sup>, Julián Chao<sup>1</sup>, Claudia G. Reides<sup>1,2</sup>, S. Fabián Lerner<sup>1</sup>, Romina M. Lasagni Vitar<sup>1,2</sup>, Silvia Álvarez<sup>2,4</sup>, Pablo A. Evelson<sup>1,2</sup>, Sandra M. Ferreira<sup>1,2</sup>

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Glaucoma is the leading cause of irreversible blindness globally, impacting both the eye structures and brain regions associated with vision. Oxidative stress is crucial in the onset and advancement of the disease. The aim of this work was to assess the changes in mitochondrial ultrastructure and synaptic morphology in the primary visual cortex in a glaucoma model. Three-month-old Wistar rats underwent surgery where two episcleral veins in the left eye were cauterized: glaucoma group (G, n=4); the control group (n=4) received a sham procedure. Seven days post-surgery, the rats were euthanized, and the primary visual cortex was dissected. In the glaucoma group, the hemispheres were separated into ipsilateral (GI) and contralateral (GC) (CICUAL FFyB n° 3314). We assessed the mitochondrial and synaptic ultrastructure using transmission electron microscopy (TEM). Regarding mitochondrial ultrastructure transmission electron microscopy (TEM) images revealed slight clarification, swelling, and disruption of mitochondrial internal structures in both GC and GI compared to the control. On the other hand, although there was no significant difference in the number of synapses among groups, there was a decrease in the postsynaptic density thickness in GC ( $p < 0.05$ ). In addition, the synaptic curvature evaluated as the relation between synaptic length in the synaptic cleft and distance between the synaptic ends decreased in GC and GI compared to control ( $p < 0.001$ ). These findings indicate that glaucoma affects mitochondrial ultrastructure in the primary visual cortex as well as the synaptic morphology. Changes in postsynaptic density may reflect alterations in the density of postsynaptic receptors and the proteins involved in receptor-mediated signaling, leading to

impaired neuronal communication. Identifying the key drivers of mitochondrial dysfunction in glaucoma is essential for discovering new therapeutic targets to prevent disease progression.

**P1 POSTERS**

FECHA Y HORA: 19/11/2024 11:00-12:00 H

COORDINADORES: SILBERMAN MAGALI,  
SILVEYRA MARIA XIMENA

**265. 006 MODULATION OF SIRT6 ACTIVITY AND/OR EXPRESSION LEVELS AS A THERAPEUTIC STRATEGY FOR GLUCOSE INDUCED VISUAL IMPAIRMENT**

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Hyperglycemia increases the risk of developing eye disorders and vision impairment. In this context, although multifactorial etiology of Diabetic Retinopathy (DR) have been extensively studied, vision recovery has been limited. Anti-VEGF agents are currently used as one of the strategies to treat both early and advanced stages of proliferative DR. SIRT6 is a histone deacetylase that acts as a glucose homeostasis regulator, neuroprotector, Hif1 $\alpha$  co-repressor (and therefore VEGF modulator), genome stability promotor by DNA damage prevention. We have described a unique role of SIRT6 in the retinal physiology and retinal degeneration and VEGF upregulation was found in models of SIRT6 deficiency. The multiplicity of functions played by SIRT6 makes it a suitable candidate as a therapeutic target for DR treatment that would allow to tackle both the neurodegenerative and the vascular aspects of the visual deficit associated with the disease. We aimed to assess the therapeutic effect of SIRT6 modulation in vitro by using the novel allosteric activator MDL800 and by regulating SIRT6 expression. We evaluated different concentrations and time points as well as cell viability, morphology and proliferation in the glial cell line MIO-M1 exposed to low or high glucose. High glucose exposure induced an increase of the acH3K9 levels (SIRT6 substrate) and MDL-800 induced a decrease of this modification which is consistent with SIRT6 activation. MDL-800 had no effect over cell morphology, viability, proliferation and SIRT6 levels. Of note, a moderate reduction of VEGF levels were found both in MDL-800 treated cells and in SIRT6 deficient cells in which the protein was re-expressed. Genetic regulation of SIRT6 expression, alone or combined with pharmacological modulation of its activity, would represent a suitable approach as a therapeutic strategy for high glucose induced retinal cell impairment.

**266. 025 CEREBRAL MITOCHONDRIAL FUNCTION OF WEANED ANIMALS AFTER ACUTE HYPOBARIC HYPOXIA**

Analia Czerniczyniec<sup>1</sup>, Antonella M. Romero Fernandez<sup>2</sup>, Analia Karadayian<sup>3</sup>, Melisa Etchegoyen<sup>3</sup>, Marisa Moriando M<sup>4</sup>, Silvia Lores-Arnaiz<sup>3</sup>, Lidia E. Costa<sup>4</sup>, Pablo H. La Padula<sup>4</sup>.

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During the gestational stage, organisms are physiologically adapted to hypoxia (*Mount Everest in utero*,  $pO_2$  equivalent to 8849 m). This work evaluates the persistence of memory and the mechanism of this beneficial adaptive effect in weaned animals. Considering that the brain is highly sensitive to hypoxia damage, we evaluated the impact of acute hypoxia on the mitochondrial function of cerebral cortex and hippocampus of weaned rats. Male Sprague Dawley rats (21 days old just weaned) were subjected to a simulated altitude of 4,400 m (58.7 kPa=440 mmHg) in a hypobaric chamber for 48h (48HH). Same number of sibling rats remained as controls (101.3 kPa=760 mmHg). Oxygen consumption, mitochondrial membrane potential, nitric oxide levels (NO), and hydrogen peroxide ( $H_2O_2$ ) production were measured in isolated mitochondria from both tissues. After 48HH, both brain regions preserved tissue integrity evaluated by histopathological studies. In hippocampal mitochondria, no significant changes were observed in oxygen consumption, NO levels, and  $H_2O_2$  production. However, mitochondrial membrane potential increased by 28% ( $p<0,05$ ). In cerebral cortex, while oxygen consumption and mitochondrial membrane potential were preserved, NO levels decreased (37%;  $p<0,05$ ) and  $H_2O_2$  production increased (53%;  $p<0,05$ ). The observed changes in NO levels and in mitochondrial membrane potential suggest that these factors could play a crucial role in maintaining mitochondrial function under hypoxic conditions. The hippocampal mitochondria, possibly due to the tissue's involvement in spatial and memory functions needed for the recently weaned organism, would be more restrictive to oxidative production than cortical mitochondria. We suggest that the observed mechanisms displayed could shed light on the preservation of memory following gestational hypoxia, highlighting a potential adaptive benefit carried into postnatal life.

**267. 046 CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY IN WOMEN WITH BREAST CANCER: A RETRO-PROSPECTIVE COHORT STUDY**

Melina Arana Amantini<sup>1,3</sup>, Guillermo Córdoba<sup>2,3</sup>, Yoanna Vanni<sup>2,3</sup>, Mónica Varela<sup>2,3</sup>, Claudio Paletta<sup>2,3</sup>, Delia Soriano<sup>1,3</sup>, Florencia Coronel<sup>1,3</sup>.

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Chemotherapy-induced peripheral neuropathy (CIPN) is one of the main adverse effects of antitumor treatment. The aim of our study was to examine the prevalence and clinical characteristics of CIPN in breast cancer patients treated with paclitaxel. A total of 45 patients with early-stage breast cancer treated at Austral University Hospital in the last 3 years were evaluated. Patient-reported CIPN-related symptoms were assessed via the international EORTC QLQ-CIPN16 questionnaire. Additional variables analyzed included age, weight, body mass index, tumor type, chemotherapy regimen, symptoms at the time of treatment, and persistence of symptoms. The study population included women aged 25-71 years. The predominant tumor phenotype was ER/PR-positive, and the most frequently used chemotherapy regimen was adjuvant anthracycline-based therapy. Upon completing the questionnaire, 82% of the patients reported experiencing at least one symptom during treatment with paclitaxel. The prevalence of clinical CIPN was 62%, defined by the presence of at least one symptom with moderate or severe intensity. In contrast, the retrospective analysis of clinical records indicated a prevalence of 15%. No differences were detected between patients with and without CIPN when evaluating anthropometric, tumor-related and treatment-related variables. Half of the patients with CIPN presented both sensory and motor symptoms,

while 29% and 18% presented exclusively motor and sensory symptoms, respectively. At the time of the interview, 46% of patients still experienced at least one symptom. Notably, all these patients had experienced more severe disturbances during chemotherapy. The results indicate a high prevalence of CIPN-related symptoms during and after paclitaxel administration in our population. Clinical under-reporting and persistent symptoms emphasize the need to incorporate questionnaires into clinical practice to better detect symptoms and guide interventions for patient's well-being.

**268. 099 RESVERATROL PROTECTS FETAL BRAINS FROM MATERNAL IMMUNE ACTIVATION BY MODULATING NRG/ERBB AXIS**

Ana Julia Ticchi, Andrea De Laurentis, Fernando Correa CONICET, Laboratorio de Neuroinmunología, CEFyBO, Facultad de Medicina, UBA

Resveratrol (Rsv) has been shown to exert neuroprotective effects in murine models of neuroinflammation. Maternal immune activation (MIA) exerts a negative impact on fetal brain development and it has been associated to neuropsychiatric disorders in the adulthood. Several reports suggest a role for the NRG1/ErbB pathway, which can influence neuroinflammatory processes, highlighting the potential protecting role of resveratrol against MIA-induced neurodevelopmental disruption. **Objectives:** To study the possible neuroprotective effects of Rsv in the fetal brain in a model of MIA induced by bacterial lipopolysaccharide (LPS) and to characterize the NRG/ErbB axis involved in the modulation of the microglial response to these stimuli. **Material and Methods:** Rsv was administered to Balb/c mice on gestational day 15, followed by LPS or vehicle. Fetal brains were then collected to assess COX-2, iNOS, IL-6, and NRG/ErbB axis expression. Additionally, BV2 microglial cells were treated with LPS, NRG1, and/or resveratrol to analyze the expression of these mediators. **Results:** LPS-triggered MIA significantly increased the fetal brain expression of COX-2, iNOS, and IL-6 ( $p<0.05$ ). However, Rsv administration effectively prevented this inflammatory response. Analysis of the NRG1/ErbB signaling pathway revealed that MIA reduced mRNA levels of NRG1 ( $p<0.05$ ) in fetal brains, effect that was prevented by Rsv. Interestingly, Rsv tended to decrease mRNA levels of ErbB2, but this inhibitory effect was not observed when Rsv and LPS were co-administered. NRG1 did not alter LPS-induced iNOS or COX-2 in BV2 cells but reduced LPS- enhanced IL-6 levels. We confirmed the expression of NRG1 and its receptors in murine microglial cell line BV2. **Conclusion:** Rsv administration counteracts LPS-induced expression of pro-inflammatory mediators as well as the changes of the NRG1/ErbB axis in fetal brains exposed to MIA. Conversely, NRG1 only reduced the LPS-induced expression of IL6 in BV2.

**269. 178 ASTROCYTES AND IMT504 ROLE IN THE REMYELINATION PROCESS**

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Many central nervous system diseases involve demyelination, a pathological process in which myelin is lost from around axons. Remyelination, the regeneration of myelin sheaths following demyelination, is crucial for restoring axonal functionality. Although successful remyelination generally relies on the proliferation and differentiation of oligodendrocyte progenitor cells, other cell types also play critical roles in the regeneration process. For instance, recent studies have highlighted both beneficial and detrimental roles of astrocytes in remyelination. IMT504 is a non-CpG oligodeoxynucleotide comprising 24 nucleotides and characterized by two specific PYNNTTTTGT sequences. We have recently demonstrated the beneficial effects of IMT504 on the regulation of microglial activation and oligodendrogenesis. In this study, our goal is to investigate the effects of IMT504 on astrocytes from the subventricular zone and

corpus callosum (CC) in a rat demyelination model induced by cuprizone (CPZ). We administered subcutaneously IMT504 every day for five days before CPZ withdrawal. Brain samples were then analyzed 0 (T0), 3 (T3), and 7 (T7) days after CPZ withdrawal. Additionally, we examined the direct effects of IMT504 on astrocyte cell cultures. Our immunohistochemical results revealed no significant changes in GFAP+ cells per area. However, Western blot analyses in the CC showed an increase in GFAP protein levels at T0 and a decrease at T3 and T7 in demyelinated animals injected with IMT504 as compared to demyelinated rats injected with saline solution. No significant changes were observed in BrdU assays on cell proliferation in astrocyte cultures treated with IMT504, whereas differential mRNA expression of A1/A2-associated phenotype genes was detected in the presence of IMT504. Overall, these results show a direct effect of IMT504 which changes astrocytes' activation patterns.

**270. 179 OLIGODEOXYNUCLEOTIDES AS EMERGING DRUGS FOR DEMYELINATING DISEASES: ROLE OF IMT504 IN MICROGLIAL CELL ACTIVATION AND OLIGODENDROCYTE PROGENITOR CELL PROLIFERATION**

Alexis Silva Silva<sup>1,2</sup>, Patricia Mathieu<sup>1,2</sup>, and Ana M. Adamo<sup>1,2</sup>.  
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Demyelination consists in myelin loss from around axons, while remyelination restores myelin and resolves functional deficits. Multiple sclerosis is a high-incidence inflammatory demyelinating disease in which remyelination frequently fails. IMT504 is a non-CpG oligodeoxynucleotide consisting of 24 nucleotides and characterized by 2 specific PyNTTTTGT sequences. Given the regenerative and immunomodulatory properties of IMT504 and our previous results showing its beneficial effects on neuroinflammation and remyelination in an animal demyelination model, this work aims to study IMT504 role in microglial and oligodendrocyte progenitor cell (OPC) contribution to remyelination. Primary cultures of microglia and OPCs were obtained from cerebral cortical tissue of 1- to 2-day-old rats. Microglia were cultured with 1% or 10% FBS, treated with IMT504 or saline solution for 24 h, and subsequently incubated for 1 h with fluorescent latex beads for phagocytosis assays. Microglia were also harvested for Western blot analyses on the signaling pathways involved in activation and RT-qPCR analyses of microglial cytokine differential expression. OPCs were treated with or without IMT504 in the presence of BrdU, cultured for 24 h, and fixed for immunocytochemistry. Results showed that IMT504 increased microglial phagocytic capacity in the presence of 1% or 10% FBS as compared to control cells. Also, IMT504 increased microglial proliferation, in agreement with signaling pathway activation. Furthermore, IMT504 increased the levels of TNF $\alpha$  and IL-10 mRNA and reduced those of IFN $\gamma$  and IFN $\beta$  mRNA. Finally, results showed that IMT504 reduced OPC proliferation and induced their differentiation. These findings support potentially beneficial properties of IMT504 which may aid therapy development for demyelinating diseases.

**P2 POSTERS**

FECHA Y HORA: 20/11/2024 11:30-12:30 H

COORDINADORES: KARADAYIAN ANALIA,  
CARUSO CARLA MARIANA

**271. 335 STUDY OF THE FETAL OXYTOCINERGIC SYSTEM IN A MURINE MODEL OF MATERNAL IMMUNE ACTIVATION**

Zafarian Jazmín, Ticchi Julia, Murillo Murillo Marisol, Canela Delàs Yilena, Farina Mariana, Correa Fernando, De Laurentis Andrea

Previous results from our lab show that LPS-elicited inflammation resulted in an increased secretion of oxytocin (OXT) in rats. It has been shown that OXT may modulate neuronal and glial cell activity, serving as a neuroprotectant and exerting anti-inflammatory effects on microglia. Additionally, we have also studied the anti-inflamma-

tory role of the endocannabinoid system, both through its direct pro-homeostatic actions and by activating the oxytocinergic system. The objective was to assess the impact of maternal immune activation (MIA) on mouse fetal brain oxytocinergic system. Given that MIA activates resident glial cells in the fetus, components of the oxytocinergic and endocannabinoid systems were analyzed, along with the effects of OXT and anandamide (AEA) on BV2 microglial cell line subjected to lipopolysaccharide (LPS). LPS administration to Balb/c mice on day 15 of pregnancy (MIA) increased the expression (Western blot) of OXT receptor ( $p < 0.05$ ) in placenta and fetal brain from these mothers compared to control. Brain CB1 and CB2 cannabinoid receptors expression were increased ( $p < 0.05$ ). FAAH and NAPE-PLD enzymes expression were also analyzed. BV2 cell cultures stimulated with LPS (100 ng/ml) also increased the OXT receptor protein expression ( $p < 0.05$ ). OXT did not modify basal or LPS-induced levels of the receptor. OXT reduced ( $p < 0.05$ ) TNF- $\alpha$  and IL-6 levels (ELISA), while increased IL-10 on control and LPS treated cells. LPS also increased CB1 and CB2 expression in BV2 ( $p < 0.05$ ). Anandamide (AEA,  $10^{-6}$ M) prevented the increase in TNF- $\alpha$  and IL-6 levels, via CB2-mediated mechanism. Notably, the addition of AEA and AM630 reversed ( $p < 0.05$ ) LPS-induced protein expression of OXT, CB1 and CB2 receptors. In conclusion, MIA enhances the activation of oxytocinergic and endocannabinoid systems in fetal brain as well as in microglial cells. OXT and AEA exert anti-inflammatory effects on these cells but it remains unclear whether they act through the same or separate pathways.

**272. 405 THE NEUROACTIVE STEROIDS ESTRADIOL AND PROGESTERONE PRODUCE A NEUROMODULATORY EFFECT ON KETAMINE-INDUCED HYPERLOCOMOTION IN MALE ORCHIECTOMIZED RATS**

Natalia Belén González<sup>1</sup>, Esther Vargas<sup>2</sup>, Paloma Ramírez<sup>3</sup>, Giuliana Germani<sup>2</sup>, Ricardo Cabrera<sup>1,2,3</sup>.

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Ketamine is a dissociative anesthetic widely used as a drug of abuse worldwide. It is characterized by inducing psychotic symptoms in humans, mainly sensoriperceptive phenomena such as hallucinations and delusional thinking disorders. In animal models, ketamine induces psychotomimetic effects that include sensoriperceptive and cognitive alterations generating, among others, increased locomotor activity (LA). This process is known as sensitization and involves variations in the mesocorticolimbic system's dopaminergic, glutamatergic, and GABAergic neurotransmission. Sex steroids, on the other hand, exert a key neuromodulatory role in the differential response to different substances of abuse. This work aimed to study the neuroadaptive phenomena stimulated by ketamine in a psychosis-type experimental model in orchietomized male rats (ORX), combined with s.c administration of Estradiol ( $E_2$ ) and Progesterone ( $P_4$ ). Adult male rats of the Sprague-Dawley strain, orchietomized and non-orchietomies, were used. The experimental groups were: 1. No ORX: Control (C), Ketamine (25mg/kg)(i.p) (K), Control +  $E_2$  (4mg/kg) +  $P_4$  (0.1mg/kg)(CEP), Ketamine +  $E_2$  +  $P_4$  (KEP) 2. ORX: Control (CO), Ketamine (KO), Control +  $E_2$  +  $P_4$  (CÉPO), ketamine +  $E_2$  +  $P_4$  (KEPO). Animals were evaluated in the open field following a stimulus dose of ketamine, using Ethowatcher to quantify meters/10 minutes. Data were expressed as mean+SEM and analyzed by ANOVA 2 and, Bonferroni. We observed a significant increase in LA in the CO group to the C group ( $P < 0.0001$ ). Likewise, we observed a significant increase in LA in the KO group to the K group ( $P < 0.0001$ ). The administration of  $E_2$  and  $P_4$  in CEP control animals induced a discrete increase in LA in the C group ( $P < 0.05$ ). However, in ketamine-sensitized animals,  $E_2$  and  $P_4$  supplementation significantly increased LA in the K group ( $P < 0.0001$ ). No significant differences were observed between the CO and COEP groups. However, steroid supplementation significantly increased LA in the KEPO group to the KO group ( $P < 0.0001$ ). We conclude

that the decrease in testosterone concentration induces a potentiation in ketamine sensitization. E<sub>2</sub> and P<sub>4</sub> supplementation increases this potentiation, indicating a notorious interaction by genomic and non-genomic mechanisms proposing positive neuroadaptive plastic phenomena on ketamine-induced modifications.

**273. 424 ASSESSMENT OF MUSCLE ACTIVITY IN HEMI-PARKINSONIAN RATS BY NONINVASIVE CUTANEOUS ELECTROMYOGRAPHY**

Paloma Ramírez<sup>3</sup>, Natalia González<sup>1</sup>, Julieta Mellimaci<sup>2</sup>, Senena Fragalá<sup>2</sup>, Ricardo Cabrera<sup>1,2,3</sup>

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In neurodegenerative(nd) diseases, cell death generates irreversible changes in different brain functions. In Parkinson's disease, patient presents irreversible motor disorders, up to CNS dysfunctions. Electromyography(EMG) allows the study of the muscular electrical signal through electrodes. Classical EMG enables the analysis of muscle function with invasive techniques, not allowing the free movement of the individual. Cutaneous(ct) EMG solves these drawbacks while preserving the safety and efficiency of the measurements. This work aimed to evaluate, through ct EMG measurements, the possible neuroprotective(np) effects of progesterone(P4) and estradiol(E2) on the EMG modifications associated with the nd phenomenon induced by 6-OHDA in adult male Sprague-Dawley rats. The experimental groups were: Control(C), Hemiparkinsonian(HP), C+E2+P4(CEP), HP+E2+P4(HPEP), C+Tamoxifen(T)+E2+P4(CTEP), HP+T+E2+P4(HPTPEP). Data was expressed as mean±SEM and analyzed by ANOVA 1, Bonferroni, and/or T-test. We observed a significant decrease in myoelectric activity(ME) in the HP group to the C group(p<0.0001). When applying E2 and P4, we observed a significant increase in ME in the CEP group versus the C group(p<0.0001). Similarly, the HPEP group presented a substantial rise in ME to the HP group(p<0.0001). However, it did not significantly compensate for the ME with respect to the CEP group(p<0.0001). When blocking E2 receptors with T, we observed a significant decrease in ME in the CTEP group to the CEP group(p<0.0001). The same was found in the HPTPEP group versus the HPEP group(p<0.0001). We conclude that treatment with E2 and P4 has no effect, which could be explained by a genomic modulatory effect of E2 on the nigrostriatal DA system and its interaction with GABAergic and Ach, among others, to which we would add a non-genomic rapid impact measured by P4 on the same systems. In addition, ct electromyography allowed us to monitor the np evolution over time as a therapeutic prognosis.

**274. 425 MITOCHONDRIAL AND AUTOPHAGIC ALTERATIONS IN SGSH-DEFICIENT NEURONAL MODELS OF MUCOPOLYSACCHARIDOSIS TYPE III**

Valentina Saud<sup>1</sup>, María Colonna<sup>1</sup>, Roxana Gorojod<sup>1</sup>, Marcos Gabriel Francia<sup>3</sup>, Alejandra Sonia Guberman<sup>3</sup>, Mónica Lidia Kotler<sup>1</sup>, Carlos Javier Pomilio<sup>2</sup>, Soledad Porte Alcon<sup>1</sup>.

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Mucopolysaccharidosis type III (MPSIII), is a rare lysosomal storage disorder that primarily leads to neurodegeneration starting in early childhood. MPSIIIA is caused by mutations in the gene responsible for N-sulfoglucosamine sulfohydrolase (SGSH), an enzyme involved in the degradation of heparan sulfate within lysosomes. Our laboratory has created SGSH-deficient HT22 neuronal cell lines, which we have labeled as 12, 15, and 124. This study aims to explore these

models to better understand the effects of SGSH deficiency on mitochondrial integrity and autophagy. Preliminary data indicated the accumulation of glycosaminoglycans in MPSIIIA cells, particularly in cell line 124 (p<0.05) as evidenced by DMMB staining. Our studies demonstrated an increased proportion of cells with fragmented mitochondria in MPSIIIA lines, and consistent with these findings, MitoTracker staining has shown a reduction in the mitochondrial aspect ratio (p<0.001 in cell lines 12, 15, and 124 vs wild-type). Then, we evaluated mitochondrial dynamics by measuring the expression levels of the fission protein DRP1 in cytosolic fractions by Western Blot (WB), and found a non-significant decrease in MPSIIIA lines. Further investigation is required to determine its presence in the mitochondrial fraction. Moreover, we analyzed LC3/MitoTracker colocalization in immunofluorescence assays and observed an increase in the M1 colocalization index in cell line 124 (p<0.0001), suggesting that mitochondria may be enclosed within autophagosomes in this model. Additionally, autophagic flux was quantified by adding the lysosomal inhibitor bafilomycin (LC3-II turnover measured in WB), revealing an increase in cell line 12 compared to the control (p<0.01). These results advance our MPSIIIA model characterization, revealing key mitochondrial and autophagic alterations, which could contribute to future therapeutic strategies. We plan to expand on these results to further inform these data.

**275. 444 LEARNING AND MEMORY ARE DIFFERENTIALLY AFFECTED BY SEX IN THE MCGILL-R-THY1-APP RAT MODEL OF ALZHEIMER'S-LIKE BRAIN AMYLOIDOSIS**

Martín Habif, Natalia Coletti, Mauro Exequiel Alfaro, María Victoria Oberholzer, María Belén González, Diana Alicia Jerusalinsky

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Over the last decade, many studies have shown a sex difference in neuropathology and cognition in mouse models of Alzheimer's disease (AD). The McGill-R-Thy1-hAPP (Tg) transgenic rat model of AD harbors the human amyloid precursor protein gene with two familial (Swedish/Indiana) AD mutations, which promote amyloid-β (Aβ) accumulation. We recently reported deficits in Long Term Memory (LTM) formation and social interaction in 4- and 6-month-old hemizygous (Tg+/-) males, whereas 4-month-old females appeared to learn and remember, just like their wild-type (wt) littermates. To further characterize cognitive aspects in Tg rats to discriminate age-related deficits from those that might be due to amyloidosis, we evaluated 12-month-old animals. Both male and female wt and Tg rats similarly explored an open field (OF), habituating to the environment. In the inhibitory avoidance (IA) to a mild-foot-shock task, wt and Tg females formed LTM, while only wt females displayed persistence at 14 days; wt males showed LTM without persistence, and Tg males did not evidence LTM. The wt rats of both sexes showed Short-Term Memory (STM) and LTM for object recognition (NOR), whereas Tg animals showed only STM. In contrast to wt males, wt females were able to discriminate a novel object location (NOL), but Tg rats (both sexes) were unable to complete the task. Our results indicate sexual dimorphism in the learning and establishment of associative memories in middle-aged rats, especially in hippocampus-dependent tasks. They also show dimorphism in the Tg model, especially in aversive and spatial associative memory, and significant impairment in spatial memory. Thus, this highlights the importance of including sex as a variable when interpreting cognitive behavior data. We also suggest that similar changes might be present in preclinical stages of AD, and thus their early detection should be improved, as they might go unnoticed given the neuronal and cognitive reserve of humans.

**276. 503 GLUN2A KNOCK DOWN INDUCES CHANGES IN GLUCOSE CONSUMPTION IN NEURONS**

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Synaptic plasticity refers to long lasting changes in synapses and involves changes in neuron and astrocyte morphology and also, in the expression of cytoskeleton and synaptic proteins, including NMDA receptors (NMDAR). These glutamate receptors are heterotetramers composed by two obligatory (GluN1) and two regulatory subunits, being GluN2A and GluN2B the most expressed in cognitive related brain structures. In the last years, *grin2A* mutations (which codify for GluN2A protein) were associated with complex phenotypes that led to neurodevelopmental disorders, including the occurrence of seizures and in some cases, decreased GluN2A levels. In parallel, the development of seizures was associated to alterations in astrocyte metabolism. In order to better understand the role of GluN2A reduced expression in synaptic plasticity, metabolism, and behavior, we induced a GluN2A knock-down (GluN2A-KD) in two models: mature hippocampal neuronal cultures and the CA1 hippocampal region of young adult Wistar rats. *In vivo*, the GluN2A KD animals showed increased seizure susceptibility after PTZ injection. Furthermore, *in vitro*, the GluN2A-KD neurons showed higher glutamate sensibility and a more immature morphology. Since most brain energy is used to sustain synaptic activity and seizure outcome was related with alterations in metabolism; in this work we analyze how GluN2A-KD would be associated to changes in metabolism measuring astrocytic glycogen content and GYS1 expression. Preliminary results suggest that GluN2A down regulation alter both glutamate responsiveness and also glycogen metabolism, which would facilitate seizure outcome that is a hallmark in patients carrying *grin2A* mutations.

### P3 POSTERS

FECHA Y HORA: 20/11/2024 16:10-17:10 H  
COORDINADORES: BELFORTE JUAN,  
VALDEZ SUSANA

#### 277. 153 ANTIOXIDANT AND NEUROPROTECTIVE IN VITRO EFFECT OF FULL-SPECTRUM EXTRACTS DERIVED FROM THE FEMALE INFLORESCENCES OF *CANNABIS SATIVA* L

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Oxidative stress is the principal cause of neurodegenerative diseases and metabolic syndromes, with the nervous system being particularly susceptible due to its high oxygen demand and limited antioxidant defenses. In recent years, researchers worldwide have concentrated on exploring the diverse biological effects of compounds isolated from *Cannabis sativa* L. (*C. sativa* L.), including their potent antioxidant properties. The aim was to evaluate the antioxidant profile and neuroprotective potential of four full-spectrum extracts derived from the female inflorescences of *C. sativa* L., thereby covering all four chemotypes. We determined the total phenolic content of the extracts by the Folin-Ciocalteu method and expressed it in µg equivalents of chlorogenic acid (CGA) per gram of dry flower weight: QI (BW) 21.92 ± 4.27, QII (D) 20.99 ± 4.53, QIII (P) 35.70 ± 4.23, and QIV (R) 18.20 ± 2.05. Furthermore, several *in vitro* antioxidant capacity assays were performed (DPPH, reducing power assay, and HO<sup>•</sup> scavenging). To evaluate neuroprotective activity, subtoxic concentrations (0.25, 0.50, and 1.0 µg of cannabinoids per mg of resin) from two extracts (QIII and QIV) were tested *in vitro* on glutamate-damaged HT-22 neuronal cells. First, we demonstrated that pre-treatment with these extracts increased cell viability and protected against apoptosis induced by glutamate toxicity. Then, we showed that slight differences in the composition of full-spectrum extracts can influence and modulate mitochondrial functions, thereby altering diverse biological processes. These results suggest that *C. sativa* L. full-spectrum extracts exhibit effective neuroprotective properties *in vitro*, indicating their potential as sources of compounds with beneficial impacts on human health, particularly with neurodegenerative disease.

#### 278. 262 CHARACTERIZING THE ASTROGLIAL RESPONSE IN A MODEL OF BRAIN EDEMA BY COLD INJURY

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Astrocytes respond to Brain injury through a process of reactive astrogliosis involving transcriptomic, phenotypic and functional changes. Reactive astrogliosis is a progressive phenomenon and can achieve time-stable phenotypic changes. Brain edema, which is an increase in interstitial water content, is an early event after many different brain injuries and pathologies and might impact on astrocyte function. We hypothesize that transcriptomic changes underlying astrocyte reactive phenotypes in a context of brain edema are regulated by epigenetic mechanisms of histone posttranslational modifications. We here aimed to characterize astrocyte response in a model of cold injury, which was optimized by our group. We conducted all of our studies in adult female and male mice (C57/4-5 months) at 1 and/or 3 and 7 DPL (days post lesion). Using immunofluorescent co-labeling followed by epifluorescence and confocal microscopy we addressed immunoreactivity for astrocyte marker GFAP (glial fibrillary acidic protein), AQP4 (aquaporin 4) and C3 (complement 3). We also used immunomarkers to describe other reactive/responsive cells, such as NeuN (neuronal nuclear protein), IBA1 (microglial, ionized calcium-binding adaptor molecule 1) and NG2 (Nerve/glial antigen 2). Our results showed increased GFAP immunoreactivity at 1-7DPL and AQP4 immunoreactivity at 1DPL. We further observed an increase in C3 immunoreactivity with no clear colocalization to any cell type. NG2 showed subtle increased immunoreactivity at 1DPL. Immunoreactivity for microglia marker IBA1 increased at 3-7DPL while NeuN radically decreased at 1-7DPL. We conclude that the cold-injury model of brain edema promotes a progressive glial response and results suitable for addressing epigenetic changes in reactive astrocytes at different time points of reactive astrogliosis. It is of note that this model will allow addressing cellular response in a context of brain edema, a pathological condition of high clinical relevance.

#### 279. 358 FUNCTIONAL VALIDATION OF THE PSEN1 R358P AND PSEN1 T119I VARIANTS IN ALZHEIMER'S DISEASE: AN IN VITRO STUDY

Diego García Chialva<sup>1</sup>, Diego Cifarelli<sup>1</sup>, Luciana Isaja<sup>1</sup>, Manuela Apecetche<sup>1</sup>, Laura Martínez Ojeda<sup>2</sup>, Tatiana Itzcovich<sup>2</sup>, Patricio Chrem-Mendez<sup>3</sup>, Gustavo Sevlever<sup>1</sup>, María Scassa<sup>1</sup>, Ezequiel Surace<sup>2</sup>, Leonardo Romorini<sup>1</sup>

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Alzheimer's disease (AD) is a neurodegenerative disorder and the leading cause of dementia worldwide. It is characterized by progressive neuronal degeneration and the accumulation of beta-amyloid plaques (Aβ) and neurofibrillary tangles (NFT) in the brain. AD manifests in sporadic AD (sAD) and familial AD (fAD). fAD is associated with inherited genetic mutations affecting amyloid precursor protein (APP) processing, involving genes such as *APP*, *PSEN1*, and *PSEN2*. The identification of two novel *PSEN1* variants, p.T119I and p.R358P in early-onset AD patients at FLENI provided a unique opportunity to study their possible implications in fAD. Notably, the patient harboring the *PSEN1* R358P variant also carried a novel *SORL1* variant (Gly1536Asp). Noteworthy, genetic variants in *SORL1* are now considered a major AD risk factor. To evaluate the role of these two novel *PSEN1* variants in APP processing, we developed a cellular model using *PSEN1* Knock-Out (KO) HEK293T cells created through CRISPR/Cas9 technology. We

assessed the  $A\beta_{42}/A\beta_{40}$  ratio (AD biomarker) in the supernatant of *PSEN1* KO-cells transfected with expression vectors coding for *APP* and either *wild-type PSEN1*, the novel *PSEN1* variants or *PSEN1* A246E (a known pathogenic mutation). We observed a significant ( $p < 0.05$ ) increase in the  $A\beta_{42}/A\beta_{40}$  ratio in HEK293T cells transfected with *PSEN1* A246E or *PSEN1* R358P plasmids and a slight trend towards an increase in cells transfected with *PSEN1* T119I vector. In the case of *PSEN1* R358P-transfected cells, the increase in the  $A\beta_{42}/A\beta_{40}$  ratio observed was primarily due to the decrease in  $A\beta_{40}$  levels in the supernatant. These findings suggest a potential pathogenic role for the *PSEN1* R358P variant in fAD, independent of the co-occurring SORL1 mutation.

## 280. 394 GLIAL REACTIVITY IS AN EARLY EVENT IN zQ175 HUNTINGTON'S DISEASE MICE

Diego Rivas Castillo, María Friser Frederiksen, Julieta Saba, Federico López Couselo, Lila Carniglia, Mercedes Lasaga, Daniela Durand, Carla Caruso.

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Huntington's Disease (HD) is a neurodegenerative disorder characterized by the degeneration of striatal neurons, leading to motor and cognitive symptoms. Neuroinflammation is involved in neurodegeneration and is recognized by increased GFAP-positive astrocytes and Iba1-positive microglia. In this study, we analyzed glial reactivity in zQ175 knock-in (HD) and wild-type (WT) mice at 4, 8, or 12 months (M) of age or primary cultured striatal microglia and astrocytes from WT and HD mice by examining GFAP and Iba1 expression in coronal brain sections by immunohistochemistry. Additionally, we assessed HMGB1 expression, a nuclear protein that, when released, triggers an inflammatory response, and its receptors TLR2 and TLR4. **Results:** Cortical GFAP expression did not differ at any age in female or male WT and HD mice. Striatal GFAP expression is increased in female HD mice 4 and 8-month-old (M) ( $p < 0.05$ ) but not at 12M. Male HD mice show increased striatal GFAP expression at 8M ( $p < 0.05$ ). Iba1 expression was significantly higher in 8 and 12M male HD mice striatum and cortex ( $p < 0.05$ ). Striatal HMGB1 expression was elevated in female HD mice at 4M and 8M ( $p < 0.05$ ) whereas in male HD mice showed HMGB1 increased at 8M ( $p < 0.05$ ). In the cortex of HD mice, HMGB1 was lower in 8M female and male and 12M male HD mice ( $p < 0.05$ ). Striatal cultured astrocytes from HD mice exhibited increased nuclear HMGB1 levels ( $p < 0.05$ ). TLR2 expression did not differ between WT and HD striatal astrocytes while TLR4 expression was not detected. Striatal HD microglia showed increased HMGB1 and TLR4 expression ( $p < 0.05$ ) but similar TLR2 levels than striatal WT microglia. **Conclusion:** HD mice exhibit neuroinflammation earlier in the striatum of female than male HD mice. Moreover, astrocytes and microglia from HD pups already show increased levels of HMGB1 suggesting that neuroinflammation is an early event and may contribute to HD pathogenesis.

## 281. 448 DEVELOPMENT OF AN ULTRASENSITIVE ELISA FOR THE DETECTION OF AMYLOID B OLIGOMERS (ABOS): IMPLICATIONS FOR EARLY DIAGNOSIS OF ALZHEIMER'S DISEASE (AD)

Mauro Exequiel Alfaro<sup>1</sup>, María Victoria Oberholzer<sup>1</sup>, Martín Habif<sup>1</sup>, María Raquel de Campos<sup>2</sup>, María Belén González<sup>1</sup>, Vanina Grippo<sup>3</sup>, Ismael Calandri<sup>4</sup>, Adriano Sebollela<sup>2</sup>, Ricardo Allegri<sup>4</sup>, Diana Alicia Jerusalinsky<sup>1</sup>

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Alzheimer's disease (AD) pathology primarily affects the temporal and frontal lobes, impairing cognitive functions and leading to dementia. Soluble A $\beta$  oligomers (A $\beta$ O), derived from the Amyloid

Precursor Protein (APP) through proteolysis, play a crucial role in AD pathogenesis. A $\beta$ O are associated with synaptic alterations and early memory deficits. We developed a technique to detect A $\beta$ O in cerebrospinal fluid (CSF), enabling efficient assessment and study of potential links to cognitive impairment. In our study, we utilized the transgenic rat model McGill-R-Thy1-APP (Tg $^{+/-}$ ), which expresses human APP with familial AD mutations (Swe/Ind). We employed a single-chain Fv (scFv) antibody fragment, NUsc1, derived from the sequence of the specific anti-A $\beta$ O monoclonal antibody NU1, to identify A $\beta$ O. This scFv antibody fragment, consisting of the hypervariable portions of the light and heavy variable chains of NU1 linked by an artificial linker-peptide, lacks an Fc region, making it less immunogenic and much smaller (55 kDa) than the average IgG (150 kDa). NUsc1 has demonstrated high affinity for A $\beta$ O. Significantly elevated levels of A $\beta$ O were observed in the CSF of Tg $^{+/-}$  rats compared to wild-type littermates, confirming the efficacy of the ultrasensitive ELISA for the quantitative assessment of A $\beta$ O. Our next step was to investigate whether these molecules' levels correlate with cognitive impairments. The correlation between increased A $\beta$ O levels and the progression of cognitive impairments suggests that this method could provide a precise technique for early diagnosis, potentially enhancing the efficacy of available therapies by enabling interventions before significant neurodegeneration occurs. We plan to apply this ELISA to human CSF samples from AD patients obtained from FLENI. Implementing this method with human samples could significantly advance early diagnosis, opening new avenues for molecular diagnostics and the development of more targeted therapeutic strategies.

## 282. 456 EARLY-LIFE FLUOXETINE EXPOSURE: EVALUATION OF CRITICAL WINDOWS FOR RAT JUVENILE SOCIAL PLAY AND NON-PLAY BEHAVIORS

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Social interaction deficits are a key feature of some neurodevelopmental disorders; however, little is known about their neurobiology. Interestingly, the serotonin pathway has been highlighted to regulate emotional behaviours in rodents. We have shown that early-life exposure to fluoxetine, a serotonin selective uptake inhibitor, can impair social interaction later in life. Nevertheless, it hasn't been determined whether there is a specific time window for such disruption. Here, we administered fluoxetine (sc.: 10 mg/kg) or saline to male Wistar rats during one of three putative critical windows: the postnatal (PN;P4-11), pre-weaning (PreWean;P12-19), or weaning (Wean;P20-27) periods, and studied (P30-40) in an unfamiliar group-matched pair paradigm, social play and non-play behaviors. PN group had longer pinning bouts, with no change in the number or latency to the first one. PreWean- pinning number, latency and duration were similar to control. Weaning group showed fewer pouncing and pinning events. Regarding non-play behaviors, PN group showed increased time doing mounting and longer crawling and following events. PreWean had shorter crawling bouts and Wean had no effect on these behaviors. On social recognition, PreWean increased the number of peer-sniffing events and Wean increased the latency to the first one. In relation to behaviors directed to the environment, Wean increased the latency to the first rearing. Brains from Wean group were fixed and the synaptic marker Synaptophysin (SYN) evaluated in the hippocampus by immunohistochemistry. There was no effect of Fluoxetine on CA3 SYN-immunostaining, suggesting other brain areas could be required for the observed social impairment. Our results demonstrate that Fluoxetine exposure around weaning time is particularly deleterious for juvenile social play behavior. These findings indicate a crucial role of serotonin in the maturation of brain systems that modulate social interaction.

FECHA Y HORA: 21/11/2024 11:00-12:00 H  
 COORDINADORES: BEAUQUIS JUAN, BAEZ  
 VERONICA, GALIGNIANA MARIO

**283. 084 EFFECTS OF GLUN2A KNOCKDOWN ON BEHAVIOR AND NEURONAL SIGNALING IN IN VIVO AND IN VITRO MODELS**

Fernanda Mariana Silva<sup>1</sup>, María Florencia Acutain<sup>1</sup>, Luisina Castellari<sup>1</sup>, Abril Muñoz<sup>1</sup>, Verónica Baez<sup>1,2</sup>.

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N-methyl D-Aspartate receptors (NMDARs) are glutamate-gated ion channels that play a role in synaptic plasticity, memory, learning and neurodevelopment. One of the most expressed subtypes in the adult brain is GluN2A-NMDAR. The correct spatiotemporal expression of the GluN2A subunit is essential for maturation, stabilization and refinement of synaptic connections. Recently, mutations in *grin2A* (the gene that codifies for GluN2A) were related to intellectual disability and epileptic syndromes. Also, it was shown that *grin2A* variants led to a decrease in GluN2A levels. For this reason, we aimed to evaluate the effects of reduced GluN2A expression *in vitro*, in primary cultured hippocampal neurons; and *in vivo*, in Wistar rats. To this end, young adult Wistar rats were intrahippocampal injected with an adeno-associated vector containing a shRNA against GluN2A (AAV-sh2A) or a "scramble" sequence (AAV-shsc). At 14 days post-injection (DPIs), a battery of behavioral tasks were performed, in order to evaluate spatial memories and social behavior. Obtained results *in vivo* showed that the decrease in GluN2A expression (GluN2A-KD rats) led to differences in both spatial and social behavior. On the other hand, mature hippocampal cultured neurons of 14 days *in vitro* (DIVs), were transfected with the AAV-sh2A or the AAV-shsc. The cultures were incubated for 6 DIVs and then used to perform immunofluorescence assays against pCAMKII- $\alpha$  and pERK1/2, two effectors of GluN2A-NMDAR. Northworthing, in the *in vitro* model, GluN2A-KD neurons showed an increase in pERK levels, in both soma and nucleus, and also a decrease in the pCAMKII $\alpha$  total level. These findings suggest that lowering GluN2A expression induced behavior deficits that could be attributed to an impairment in the intracellular signaling of GluN2A-NMDAR.

**284. 214 GAIT CHANGES OF PATIENTS WITH PARKINSON'S DISEASE APPLYING RHYTHMIC AUDITORY STIMULATION AT THE COMMUNITY ENVIRONMENT**

Conrado Borgatello<sup>2</sup>, Hernán G. Latini<sup>1</sup>, Rodrigo Juárez<sup>1</sup>, María Eugenia Ferri<sup>1</sup>, Ignacio Primo<sup>1</sup>, Manuel Rodríguez<sup>1</sup>, Silvana B. Rosso<sup>2</sup>

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**Introduction:** External Rhythmic Auditory Stimulation (RAS) in rehabilitation programs has shown benefit in improve spatiotemporal gait variables in patients with Parkinson's disease (PD) in controlled environments, but the impact on community walking has been little researched. **Objective:** To determine the effects of RAS on spatiotemporal gait variables in patients with PD in the community environment (CE). **Method:** 27 patients, 1-3 Hoehn & Yahr stages (H&Ys) were evaluated. 10-Meter Walk Test (10MWT) variables such velocity, step numbers, stride length, and cadence were measured at the gym (baseline) with RAS and at the CE without and with RAS. RAS was applied by a metronome dosed at baseline cadence. The data were analyzed by ANOVA (mean  $\pm$  SD). Cohen's *f* was used to estimate the effect size. **Results:** The use of the RAS at the CE increased gait velocity (1.189 m/sec  $\pm$  0.35) compared to walking at the CE without the RAS (1.06 m/sec  $\pm$  0.31, \*\* *p* < 0.01; *f* = 0.157). There was a decrease in step number (16.48  $\pm$  4.023 vs. 17.63  $\pm$  3.702; \* *p* < 0.05; *f* = 0.129) and an increase in stride length (1.277  $\pm$  0.2792 vs. 1.180  $\pm$  0.2329; \*\* *p* < 0.01; *f* = 0.158) between the experimental conditions of walking at the CE with and without RAS.

For cadence, the results showed no significant differences between the conditions. Regarding H&Ys, we found a significant decrease in step numbers only in patients H&Ys  $\geq$  3 with RAS compared with patients who did not use the RAS at the CE (17.93  $\pm$  4.061 vs. 19.2  $\pm$  3.61, \* *p* < 0.05; *f* = 0.146). Analyzing stride length, we found significant differences in patients with the RAS and without the RAS at the CE in H&Ys  $\leq$  2 (1.417  $\pm$  0.2646 vs. 1.313  $\pm$  0.2207; \* *p* < 0.05; *f* = 0.173), and H&Ys  $\geq$  3 (1.165  $\pm$  0.2441 vs. 1.074  $\pm$  0.1876; \* *p* < 0.05; *f* = 0.177). **Conclusions:** RAS may be considered a useful tool to improve gait spatiotemporal variables in patients with PD at the CE, mainly in more advanced H&Ys.

**285. 228 MORPHOMETRIC ANALYSIS OF HYPOTHALAMIC TANCYTES AND THEIR POSSIBLE STRUCTURAL PLASTICITY IN RESPONSE TO METABOLIC-RELATED HORMONES**

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**Introduction:** hypothalamic tanycytes are polarized ependymogial cells that line the base of the third ventricle and project through various hypothalamic nuclei and the median eminence. They facilitate the bidirectional exchange of metabolic and hormonal cues between the bloodstream and the hypothalamus and participate in intercellular communication within hypothalamic neuronal circuits that regulate energy homeostasis. In this context, literature suggests that tanycytic morphology may change depending on the energy balance as part of a physiological response that adapts to the metabolic state of the organism. **Objectives:** here, we investigated the structural plasticity of these cells in response to some hormones related to energy metabolism in a primary culture of rat hypothalamic tanycytes. **Methods:** we incubated 7-day cultured tanycytes for 48 hours with different concentrations of dexamethasone, insulin or T4. We then quantified the length of individual cells and the area of their somas and processes. Data were compared using Kruskal Wallis test followed by Dunn's post hoc test. **Results:** We found that dexamethasone decreased cell length similarly across the tested range (K-W *P*<0,0001, *P*<0,0001) without changing the area of somas (K-W *P*=0.030, *P*>0.05), but decreasing the area of processes (K-W *P*<0,0001, *P*<0.05). A low concentration of insulin decreased cell length (K-W *P*<0,0001, *P*<0.0001) and affected both the area of somas (K-W *P*<0,0001, *P*<0.0001) and processes (K-W *P*<0,0001, *P*<0.0001), while a high concentration of this hormone had no effect on cell morphology. T4 did not significantly affect the cell length or area of somas or process of cultured tanycytes. **Conclusion:** This evidence shows that glucocorticoids and insulin modify the morphology of tanycytes *in vitro*. Next, we will investigate whether these hormonal stimuli affect the morphology of tanycytes and their interaction with vessels or diverse hypothalamic cell types in *in vivo* conditions.

**286. 457 UNRAVELING THE ROLE OF ASTROCYTES IN AN EXPERIMENTAL MODEL OF NEURODEVELOPMENTAL SYNAPTOPATHY**

María Belén Gomez<sup>1</sup>, Giuliana Colonna Soldavini<sup>1</sup>, Martin Gabriel Codagnone<sup>1,2</sup>, Analía Gabriela Reinés<sup>1,2</sup>

<sup>1</sup>Instituto de Biología Celular y Neurociencia "Prof. de Robertis" (IBCN, UBA-CONICET), <sup>2</sup>Cátedra de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires

Synaptic remodeling involves a delicate balance between synaptic formation and elimination, regulated by communication among microglia, astrocytes, and neurons. Alterations in any of these cell types may lead to synaptopathies that ultimately underlie behavioral changes. To study the cellular and molecular basis of neurodevelopmental synaptopathies, we used a rat model induced by prenatal administration of valproic acid (VPA, 450 mg/kg i.p., E10.5). We previously showed that cortical neurons from VPA animals are intrinsically modified and that microglia are adapted to the neuronal

environment; however, the role of astrocytes and their crosstalk with neurons and microglia remain unclear. We aimed to characterize the morphological profile of astrocytes isolated from VPA animals (reactive fibrillar and non-reactive non-fibrillar according to GFAP immunostaining), assess their response to synaptic terminals (ST) by measuring synaptophysin (SYN) immunostaining, and study the effect of soluble microglial factors on this response. Cortical astrocytes isolated from control and VPA male Wistar rat pups were cultured, exposed to microglia-conditioned medium (MCM) and/or ST. Astrocytes isolated from VPA animals exhibited increased basal reactivity compared to those from control animals. Exposure to ST enhanced reactivity in control astrocytes but failed to further increase it in astrocytes isolated from VPA rats. Simultaneously, astrocytes from VPA animals showed higher SYN staining compared with those from controls. In astrocytes from control animals, MCM increased their reactivity, which remained the same after ST addition. In astrocytes from VPA animals, MCM did not modify their basal reactivity, which increased with the further addition of ST. These results indicate that astrocytes from VPA animals differ from controls and suggest an increased phagocytic capacity under basal conditions and in response to microglial signals, contrasting with in vivo VPA cortical synaptic pattern.

**287. 526 EFFECTS OF DIFFERENT TYPES OF ENVIRONMENTAL ENRICHMENT IN BEHAVIOR AND ADULT HIPPOCAMPAL NEUROGENESIS**

Daniela R Montagna<sup>1</sup>, Rocío B Foltran<sup>2</sup>, Karen M. Stefani<sup>1</sup>, Agustina Resasco<sup>3</sup>, Silvina L Diaz<sup>1</sup>.

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Environmental enrichment (EE) is the ensemble of elements in housing to facilitate natural motivated behaviors. Complex EE increases adult hippocampal neurogenesis and improves animal welfare in rodents. However, not much is known about the effect of simple EE. The aim of this work was to analyze the effect of simple and complex EE on adult hippocampal neurogenesis and the affective state of mice. 9-week-old Swiss mice of both sexes were allocated into standard, complex EE (CE) and two simple EE (tube or board house) housing conditions for 8 weeks. Also, C57 mice were subjected to the same protocol and data obtained are being analyzed. Observations in the home cage, Splash, Novelty Suppressed Feeding (NSFT), Urine Sniffing (UST) tests and the Object Pattern Separation (OPS) were performed. The cell division marker BrdU was administered 4 weeks before mice were sacrificed and brain tissue was sliced for immunofluorescence assays. CE induced a decrease in anxiety-like behaviors in the NSFT in both sexes, but no significant changes in the other behavioral tests. Concerning the observations in cage, CE induced a significant increase in activity in general ( $p = 0.02$ ), only in female mice, as well as a significant increase in agonistic behavior in females. EE induced a significant decrease in stereotypical behavior ( $p = 0.05$ ) in females, both by CE and BH. Only CE induced an increase in survival of newborn cells. Our results show that these types of simple EE do not induce massive changes in several behaviors that are commonly used in the field of neuroscience, neither in survival rates of newborn neurons in the hippocampus. However, the reduction in stereotyped behavior is indicative of improved animal welfare, suggesting that tube or board house could be added in mice cage to improve their welfare.

**ONCOLOGÍA**

**O1 COMUNICACIONES ORALES**

FECHA Y HORA: 19/11 13:00-14:00 H

LUGAR: SALA DE CÁMARA

COORDINADORES: LACUNZA EZEQUIEL, GABRI

**MARIANO ROLANDO, MAZZAIRA GISELA**

**288. 070 MOLECULAR SUBTYPING OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA FROM A MULTICENTRIC ARGENTINIAN STUDY REVEALS RNA-SEQ SUPERIORITY FOR CLASSIFICATION AND PROGNOSTIC IMPLICATIONS OF CYTOTOXIC CELL ABUNDANCE**

María Sol Ruiz<sup>1,2</sup>, Daniel Avendaño<sup>1,2</sup>, Ignacio Gomez Mercado<sup>1,2</sup>, María Laura Lacreu<sup>1,2</sup>, Ezequiel Sosa<sup>1,2</sup>, Lucila Viappiani<sup>1,2</sup>, Mercedes Abbate<sup>1,2</sup>, María Cecilia Riccheri<sup>3</sup>, Elba Vazquez<sup>1,2</sup>, Geraldine Gueron<sup>1,2</sup>, Javier Cotignola<sup>1,2</sup>

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B-cell Acute Lymphoblastic Leukemia (B-ALL) is the most common pediatric cancer, with over 20 identified molecular subtypes enabling risk-adapted therapy and novel treatment strategies. This study aimed to determine the molecular subtypes of B-ALL and explore their association with clinical outcome in the Argentinian pediatric population. We analyzed bone marrow samples from newly diagnosed patients enrolled in the ALLIC-GATLA 2010 clinical protocol using transcriptome sequencing and a range of bioinformatic tools to identify fusion transcripts, single nucleotide variants, gene expression profiles, and to estimate immune microenvironment composition. ALLSorts tool successfully classified 30 out of 32 samples, with a high concordance rate (17/18) compared to current subtyping methods (karyotyping, RT-PCR). We identified 9 different subtypes, being hyperdiploid, DUX4 and *ETV6:RUNX1* the most frequent in our cohort. Gene expression analysis provided additional subtype-specific evidence, including *CRLF2* (Ph-like) and *HDAC* overexpression (*MEF2D*), *CD33/CD10* (*ZNF384*) and *DUX4/CD371/ERG* (*DUX4*) dysregulation. Molecular subtypes were significantly associated with relapse incidence and positive Minimal Residual Disease measured on day 15 of treatment ( $p < 0.05$ ). We also identified a subset of patients with a higher abundance of cytotoxic cells, associated with an increased risk of relapse/death ( $HR = 4.5$ ;  $p = 0.05$ ). Analysis of an independent high-risk cohort (TARGET B-ALL phase II), confirmed that a higher cytolytic score correlated with a greater relapse risk ( $HR = 2.66$ ,  $p = 0.001$ ). RNA-seq demonstrated superior molecular classification capability, successfully classifying over 90% of patients, compared to approximately 35% with conventional methods. The integration of multiple bioinformatic tools was essential for enhancing sensitivity. Importantly, the abundance of cytotoxic cells was independent of molecular subtype and associated with a worse prognosis.

**289. 138 DECODING DISEASE HETEROGENEITY THROUGH OSTEOPOINTIN REGULATION IN PROSTATE CANCER BONE METASTASIS**

Pablo Sanchis<sup>1,2,3</sup>, Agustina Sabater<sup>1,2,3</sup>, Julia Lechuga<sup>1,2</sup>, Mora Gatti<sup>1,2,3</sup>, Juan Bizzotto<sup>1,2,3</sup>, Estefania Labanca<sup>4</sup>, Pia Vallacco<sup>2</sup>, Geraldine Gueron<sup>1,2</sup>, Nicolas Anselmino<sup>4\*</sup>.

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Prostate cancer (PCa) exhibits significant heterogeneity, shaped by both genomic alterations and the tumor microenvironment. Osteo-



pontin (*SPP1/OPN*) has been associated with disease severity in a subset of PCa patients with bone metastasis (BM), making it essential to understand the molecular mechanisms driving *SPP1* expression for better disease management. Here we conducted a comprehensive transcriptomics analysis using publicly available datasets (GSE74685, SU2C-PCF, and Westbrooke et al.) to compare PCa across different metastatic sites and treatment conditions. A distinct subgroup of BM patients was identified, characterized by elevated *SPP1* levels and an activated Protein Kinase A (PKA) pathway. To further investigate this link, we employed an indirect co-culture system to mimic the interaction between PCa cells (PC3; AR-negative) and osteoblast precursors (MC3T3) *in vitro*. By integrating transcriptomic (RT-qPCR and RNA-seq) and secretomic (ESI-MS/MS) data, we dissected the critical elements involved in the PCa-bone cell crosstalk. Our findings revealed that bone-derived factors such as Col1a1 and Fn1 significantly induced *SPP1* expression in PCa cells via PKA activation ( $P < 0.05$ ), underscoring its pivotal role at the PCa-bone interface. Additionally, experimental and clinical data suggest that the androgen receptor (AR) modulates the PKA/*SPP1* axis. Notably, longitudinal patient samples analyses revealed increased *SPP1* expression in a subset of patients following enzalutamide treatment (an AR signaling inhibitor), alongside increased PKA activity ( $P < 0.05$ ). In summary, our study identifies PKA as a key regulator of *SPP1/OPN* expression in PCa within the context of BM, highlighting *SPP1/OPN* as a potential biomarker for tumors with active PKA signaling and offering new insights into the heterogeneity of treatment responses in PCa patients.

**290. 194 CANCER IMMUNOTHERAPY USING REPLICATIVE ADENOVIRUSES EXPRESSING BITES AND IMMUNOLOGICAL CHECKPOINT INHIBITORS**

Mauricio Vargas Lopez, Felipe Nuñez, Osvaldo L. Podhajcer, Maria Veronica Lopez.

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Immunotherapy has revolutionized cancer treatment, achieving notable success by combining drugs with complementary mechanisms of action. Among these, immunological checkpoint inhibitors (ChPIs, such as PD-L1/PD-1) and Bispecific T-cell engagers (BiTEs) are prominent. BiTEs activate T cells by binding them to tumor cells. Oncolytic adenoviruses (OAVs) are particularly effective, transforming “cold” tumors into “hot” ones with active immune responses. They achieve this through two mechanisms: directly attacking malignant cells and inducing a secondary anti-tumor immune response by releasing neoantigens via MCI. The project's goal was to “arm” an OAV with immunomodulatory genes (a BiTE and a ChPI). The adenovirus developed carries a selected tumor specific promoter (TSP) driving the activity of the adenovirus E1A gene, a BiTE targeting the EGF receptor and an anti-PD-L1. This novel OAV was tested *in vitro* in ovarian cancer cell lines, examining the expression and biological activity of the BiTE and the anti-PD-L1, and evaluating its efficacy in a murine model of peritoneal human ovarian cancer dissemination. Our OAV was able to induce the expression of both the BiTE and the ChPI, was lytic *in vitro* on all ovarian cancer lines and exhibited high *in vivo* efficacy. In conclusion, we developed an OAV capable of producing a BiTE and an anti-PD-L1 intratumorally. This multi-drug approach is a step forward for immunotherapeutic drugs combination, as the infected cells produce the OAV, the BiTE and the ChPI locally and simultaneously, reducing systemic toxicities associated with combining multiple drugs.

**291. 220 ADIPOSE MICROENVIRONMENT IN BREAST CANCER: MOLECULAR SUBTYPE-DEPENDENT EFFECT**

Priscila Pagnotta<sup>1,2</sup>, María Lujan Crosbie<sup>3</sup>, Natalia Santiso<sup>3</sup>, Anabela Ursino<sup>3</sup>, Celeste Frascaroli<sup>3</sup>, Alicia Amato<sup>3</sup>, Rubén Drezman<sup>4</sup>, Juan Carlos Calvo<sup>1</sup> and Judith Toneatto<sup>1</sup>

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Breast cancer (BC) is the most common and deadliest cancer among women of all ages, classified into molecular subtypes including triple negative (TN) and hormone receptor positive (HR+). The tumor microenvironment, mainly adipose tissue, plays a crucial role in regulating disease progression. The aim was to compare the role of soluble factors released by the TN and HR+ breast adipose tumor microenvironments in disease progression. Conditioned media (CM) from adipose explants adjacent to HR+ and TN tumors ( $n = 29$ ; MCT) were used to treat HR+ (MCF7 and T47D) and TN (MDA-MB-231) cell lines, respectively, and contrasted with CM from female donors ( $n = 21$ ; MCN). CM induced changes in tumorigenic processes: MCN and MCT decreased proliferation in the TN model without altering migration capacity compared to T47D line. Additionally, MCT decreased cell adhesion in the TN line compared to the T47D line, while it increased the migration in the T47D line. In the HR+ model, incubation with MCT led to an increased protein expression of Nanog and a tendency to reduce Klf4. These results were in line with elevated levels of CAV-1, MMP9 and Vim, which are associated with a worse prognosis. In contrast, MCN did not show a clear pattern and exhibited differences between the MCF7 and T47D lines, highlighting the importance of using multiple models within the same molecular subtype. On the other hand, MCT induced a decrease in the protein expression of Nanog, while the MCN decreased CAV-1 and MMP9 levels in the TN model. We conclude that the effect of soluble factors released by the adipose microenvironment depends on the molecular subtype of BC. In the HR+ subtype, these factors contribute to a pro-tumorigenic profile while they exhibit an anti-tumorigenic effect in TN subtypes, similar to the role of breast stroma in a healthy context. Studying these differences enhances the understanding of BC's particularities and expands the potential for complementary current clinical options.

**292. 289 KANSL2 REGULATES RRNA BIOGENESIS IN GLIOBLASTOMA CELLS**

Nicolás Budnik<sup>1</sup>, María Belén Piñero<sup>1</sup>, Yilong Zhou<sup>2</sup>, Lucía Canedo<sup>1</sup>, Zdenek Andrusik<sup>3</sup>, Joaquín Espinosa<sup>3</sup>, Ken Kobayashi<sup>4</sup>, María Shvedunova<sup>2</sup>, Meike Wiese<sup>2</sup>, Asifa Akhtar<sup>2</sup>, Carolina Perez Castro<sup>1</sup>.

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*4. Laboratorio de Agrobiotecnología, Instituto de Biodiversidad y Biología Experimental Aplicada (IBBEA-CONICET-UBA) -FBMC - FECEN- Universidad de Buenos Aires.*

KANSL2 is a subunit of the non-specific lethal (NSL) chromatin-modifying complex involved in epigenetic programming. KANSL2 regulates cell plasticity and tumorigenesis in glioblastoma multiforme (GBM), which is characterized by high heterogeneity and low survival rate of patients. Previously, our group demonstrated that KANSL2 plays a critical role in cellular plasticity and the aggressiveness of GBM. To study the transcriptional network and target genes of KANSL2 in GBM, we analyzed the expression of KANSL2 in GBM samples from RNA-seq data, confirming an upregulated expression of KANSL2 mRNA in tumors, and a positive correlation with the stemness index score. By performing gene enrichment analyses, we uncovered that higher expression of KANSL2 was strongly associated with the term “Ribosome”. Two independent shRNAs against KANSL2 in U87 GBM cells reduced the expression of 45s and 28s rRNA precursors, POLR1E and MOF, the enzymatic subunit of the NSL complex (Anova  $p < 0.05$ ). In contrast, KANSL2-RFP overexpression caused opposite effects (t-test  $p < 0.05$ ). RNA-seq analysis of 3D cultures of KANSL2-KD derived from patient-derived

GBM cells revealed a significant downregulation of ribosomal biogenesis-related genes. Flow cell cytometry analysis using propidium iodide staining showed a late G1 phase arrest of KANSL2-KD U87 cells (Anova  $p < 0.05$ ), confirming that cell proliferation depends on the presence of KANSL2. We conclude that KANSL2 regulates GBM cell plasticity, ribosomal biogenesis, and cell proliferation.

### 293. 432 CASEIN-KINASE II INHIBITOR CIGB-300 EXHIBITS ANTITUMOR ACTIVITY IN PANCREATIC DUCTAL ADENOCARCINOMA MODELS

Florencia Gonzalez Moran<sup>1,2</sup>, Carla S. Capobianco<sup>1</sup>, Luisina Solernó<sup>1,2,3</sup>, Candela Llavona<sup>1,2</sup>, Daniela L. Papademetrio<sup>4,5</sup>, Silvio Perea<sup>6</sup>, Juan Iovanna<sup>2,7</sup>, Juan Garona<sup>1,2,3</sup>, Daniel F. Alonso<sup>1,2,3</sup>, Hernán G. Farina<sup>1,3</sup>, María F. Gottardo<sup>1,2,3</sup>

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Pancreatic ductal adenocarcinoma (PDAC), has a 5-year survival rate of less than 5% due to late diagnosis and poor treatment response. CK2 is a serine-threonine kinase that is usually deregulated in malignant cells and is deeply involved in proliferation and apoptosis control. CIGB-300 is a peptidic inhibitor of CK2 that impairs CK2 enzymatic activity by a substrate- and enzyme-binding mechanism. The aim of the present preclinical study was to investigate the antitumor effects of CIGB-300 in PDAC. Our results showed that CIGB-300 reduced the viability of PANC-1 and PANC02 cell lines, obtaining  $IC_{50}$  values of  $\approx 300 \mu M$  and  $\approx 250 \mu M$  respectively. CIGB-300 also inhibited colony-formation and clonogenic growth in PANC02 cells ( $p < 0.05$ , ANOVA). In addition, we observed that CIGB-300 exerts a pro-apoptotic action (TUNEL assay,  $p < 0.05$ ,  $\chi^2$ ). *In vivo*, syngeneic mice C57BL6 were injected s.c. with PANC02 cells and, after 30 days of cell inoculation, an incomplete surgical resection model was carried out. Mice were treated after surgery with vehicle (control) or CIGB-300 (10 mg/kg i.p., daily 5 doses/week) for two weeks. CIGB-300 treatment significantly reduced residual tumor growth ( $p < 0.05$ , T test) and increased mice survival ( $p < 0.05$ , Longrank test). Treatment with CIGB-300 was also assessed on PANC02-tumor-bearing mice in addition to gemcitabine (25 mg/kg i.p. 3 doses/week, every-other-day). Combined therapy significantly inhibited tumor progression, enhancing the anti-PDAC effects of gemcitabine-based chemotherapy ( $p < 0.05$ , ANOVA). These results identified CIGB-300 as a promising co-adjuvant agent for the therapeutic management of PDAC.

### 294. 460 OVERCOMING PLACLITAXEL CHEMORESISTANCE IN TRIPLE NEGATIVE BREAST CANCER WITH HISTAMINE H<sub>3</sub> RECEPTOR ANTAGONISTS

Ignacio A Ospital 1, Melisa B Nicoud 1, Paolo Lauretta 1, Agueda Velazco 1, Rocio Martinez Vivot 1, João P S Fernandes 2, Vanina A Medina 1

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Triple-negative breast cancer (TNBC), which accounts for 10-15% of newly diagnosed breast cancer cases, is the most aggressive sub-

type with a particularly poor prognosis. Although Paclitaxel (PTX) is a standard first-line therapy for TNBC, its use is limited by poor solubility, significant side effects, and the development of chemoresistance. We have previously identified the presence of histamine H3 receptor (H3R) in TNBC samples, which was associated with poor prognosis. Notably, LINS01022 H3R antagonists have shown therapeutic potential by inhibiting cell proliferation, inducing apoptosis, and reducing tumor size in 4T1 TNBC tumor-bearing mice. This study aimed to evaluate the antitumor effects of LINS01022 and its potential to enhance PTX therapy in 4T1 TNBC cells and their PTX-resistant counterparts (4T1 R). LINS01022 inhibited the clonogenic proliferation in 4T1 R cells in a dose-dependent manner ( $IC_{50} = 3.2 \mu M$ ). Furthermore, LINS01022 potentiated PTX-induced reduction in cell proliferation and viability in both 4T1 R and parental 4T1 cells ( $P < 0.01$ ). Cell apoptosis was evaluated by Annexin V and TUNEL. The results showed that LINS01022 significantly increased apoptosis in the chemoresistant 4T1 R model similar to its effect in 4T1 cells. Additionally, LINS01022 enhanced the apoptotic effect of PTX in these assays. Chemoresistance in tumor cells is often driven by increased efflux pump activity and expression. The impact of LINS01022 on efflux modulation was assessed using the rhodamine 123 accumulation assay. At a concentration of 25  $\mu M$ , LINS01022 significantly inhibited pump activity in 4T1 R cells, increasing intracellular fluorescence by up to 500% compared to control ( $P < 0.05$ ), demonstrating its potential to overcome drug resistance. In conclusion, LINS01022 is a promising therapeutic candidate for TNBC, with potential to reverse chemoresistance and potentiate the antitumor effects of PTX.

### 295. 505 OVERCOMING BETACATENIN-MEDIATED IMMUNOTHERAPY RESISTANCE IN HCC: THE THERAPEUTIC POTENTIAL OF SMYD2 INHIBITION

Barbara Bueloni<sup>1</sup>, Mariel Fusco<sup>2</sup>, María Jose Cantero<sup>1</sup>, Nicolas Marcolini<sup>1</sup>, Lucia Lameroli<sup>1</sup>, Mailin Casadei<sup>1</sup>, Catalina Atorrasagasti<sup>1</sup>, Mariana Malvicini<sup>2</sup>, Esteban Fiore<sup>1</sup>, Guillermo Mazzolini<sup>1,3</sup>, Juan Bayo<sup>1</sup>

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**Introduction:** Hepatocellular carcinoma (HCC) is the second most lethal cancer worldwide. The first-line treatment for advanced HCC is the combination of immune checkpoint inhibitors (ICIs) and anti-angiogenic agents. However, the molecular class characterized by  $\beta$ -catenin mutations is associated with "immune exclusion" in HCC, and therefore might be immunoresistant. The methyltransferase SMYD2 exhibits abnormal expression in various tumors, including HCC. In addition, SMYD2 facilitates the activation of non-epigenetic substrates commonly mutated in HCC, such as  $\beta$ -catenin. Our aim was to explore if SMYD2 inhibition could reverse the resistance to ICIs therapy of  $\beta$ -catenin mutated HCC. **Methods:** Human HCC RNA-Seq datasets were used to study SMYD2 correlation with oncogenic and immunosuppressive pathways. The PM299L HCC cell line (hyperactive  $\beta$ -catenin) was used to study the effect of SMYD2 inhibition on Wnt pathway activation. RNA-Seq analysis was employed to study transcriptome changes in HCC cells upon SMYD2 inhibition. *In vivo* effect of SMYD2 inhibition and its combination with anti-PD1 antibody was evaluated in the orthotopic PM299L murine HCC model. Immune response was studied by flow cytometry. Inflammatory profile of J774 macrophages was assessed by qPCR. **Results:** SMYD2 expression negatively correlates with immune and apoptosis-related genes. RNA-Seq analysis revealed that SMYD2 inhibition downregulates genes related with cell cycle and Wnt pathway. Notably, LLY507 and AZ505 (SMYD2 chemical inhibitors) strongly hinder tumor growth *in vivo*. SMYD2 inhibition shifts J774 macrophages towards a pro-inflammatory profile and downregulates the immunosuppressive secretion profile of PM299L cells. AZ505 antitumor therapy synergizes with anti-PD-1 and in-

creases CD8<sup>+</sup> CD107<sup>+</sup> activated T cells. **Conclusions:** Inhibition of SMYD2 reverse immunosuppressive transcriptional programs in HCC, emerging as a promising therapeutic target for HCC in combination with ICLs.

## O2 COMUNICACIONES ORALES

FECHA Y HORA: 21/11 11:00-12:00 H

LUGAR: AUDITORIUM

COORDINADORES: LAMB CAROLINE,  
RAMIREZ DARIO

### 296. 004 ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY (ADCC) INDUCED BY ACTIVE IMMUNOTHERAPY WITH RACOTUMOMAB IN PEDIATRIC PATIENTS WITH HIGH-RISK NEUROBLASTOMA

Valeria I. Segatori<sup>1,2</sup>, Nicolás Pinto<sup>3</sup>, Guido Felizzia<sup>4</sup>, Victoria Sobrero<sup>3</sup>, Mariana Varela<sup>3</sup>, David Verón<sup>3</sup>, Cynthia A. Gulino<sup>1</sup>, Silvia Eande-Eberle<sup>4</sup>, Diego Fernández<sup>4</sup>, Marcelo Guthmann<sup>5</sup>, Marcelo Tinelli<sup>5</sup>, Eduardo Spitzer<sup>5</sup>, Ignacio Demarco<sup>5</sup>, Walter Cacciavillano<sup>4</sup>, Guillermo L. Chantada<sup>2,3</sup>, Daniel F. Alonso<sup>1,2</sup>

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5) Laboratorio Elea-Phoenix

6) mAbxience

Racotumomab is an anti-idiotypic mouse monoclonal antibody that targets N-glycosylated glycoconjugates like NeuGcGM3 ganglioside, a tumor neoantigen expressed in many human cancers. After a successful Phase II/III trial, racotumomab has been approved in Latin American countries as maintenance therapy for adults with advanced non-small cell lung cancer. A subsequent Phase I trial in pediatric patients with refractory malignancies demonstrated the safety of repeated racotumomab vaccination in children and evidence of induction of immune response. ADCC is a crucial mechanism by which host antibodies target tumor cells expressing specific antigens, triggering cell-mediated destruction. Here, we evaluated the induction of ADCC in high-risk neuroblastoma patients included in a Phase II trial with racotumomab (ANMAT Disp. 3997/16; NCT02998983). The trial accrued 39 patients of median age 68 months (range 33-156) who achieved complete or partial remission or had non-progressive disease after standard therapy. Patients received racotumomab plus alum (0.4 mg/dose, intradermal) in 5 biweekly doses followed by 10 monthly doses to complete one year of treatment. We evaluated Fc-mediated ADCC using the Promega Reporter Bioassay based on engineered effector cells expressing the high affinity Fc gamma IIIa receptor and luciferase. Serum samples from 38 patients were available for evaluation during the study period. Most patients (37/38, 97%) developed human anti-mouse antibodies (HAMA) and treatment elicited a specific IgM and/or IgG response to NeuGcGM3 in 16 patients (16/38, 42%). Eight patients (8/38, 21%) showed a significant increase ( $p < 0.05$ ) of ADCC response against NeuGcGM3-expressing X63 target cells at 3 and/or 6 months after starting treatment. Our data confirm for the first time the induction of ADCC following racotumomab immunotherapy in certain pediatric patients with high-risk neuroblastoma. Clinical significance of findings remains to be established.

### 297. 135 EXPLORING THE ROLE OF MOLECULAR TARGETS IN THE TRANSITION FROM *IN SITU* TO INVASIVE BREAST CANCER

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Ductal carcinoma in situ (DCIS) is an early form of breast cancer (BC) where epithelial neoplastic cells are confined within mammary ducts. While DCIS can progress to infiltrating carcinomas (IDC), predicting when or which cases will advance remains challenging. In a murine model, intraductal inoculation of MCF10DCIS.com cells lead to spontaneous progression from DCIS to IDC, with MT1-MMP, a membrane metalloprotease, playing a key role. MT1-MMP is overexpressed at the invasion front, defining two cell populations: MT1-MMP<sup>high</sup> and MT1-MMP<sup>low</sup>. Transcriptome analyses of these populations, compared with high-grade human DCIS, revealing SPARC and CLCA2, as key actors potentially involved in early BC progression. In a previous study, immunofluorescence analysis of invasive tumors from intraductal injection models revealed increased SPARC and CLCA2 expression in peripheral areas and at the stromal interface, similar to MT1-MMP. Now we compare SPARC and CLCA2 expression in patient frozen samples of BC (n=22) using RT-qPCR and immunohistochemistry (IHC), based on expected mRNA-protein correlation. While RT-qPCR showed high SPARC levels ( $p < 0.05$  vs control, Mann-Whitney U test), IHC revealed heterogeneous expression between stromal and neoplastic regions. For CLCA2, which lacks stromal expression, both techniques were consistent. Interestingly, inverse expression between CLCA2 and SPARC in neoplastic tissue was observed ( $p = 0.0046$ , paired t-test) with high neoplastic CLCA2 observed when SPARC is mainly stromal. In conclusion, our comparison of RT-qPCR and IHC for SPARC and CLCA2 suggests that these methods may capture different aspects of gene expression. Different compartments might need to be considered. The relationship between SPARC and CLCA2 underscores their complex interplay in BC progression, and further analysis might be required to better understand their functions.

### 298. 195 OVEREXPRESSION OF BRACHYURY (BRACHY) AND INSULIN-LIKE GROWTH FACTOR RECEPTOR (IGF1R) IN THYROID PAPILLARY CARCINOMA (TPC) CELLS: DIFFERENT PHENOTYPES AND ASSOCIATION WITH PEDIATRIC THYROID NODULAR PATHOLOGY

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In pediatrics, thyroid tumor stratification is difficult to assess. In human carcinomas Brachy has been identified as a regulator of Epithelial-mesenchymal transition. IGF1R play important roles in neoplastic growth. No information about Brachy and IGF1R expression in pediatric thyroid nodular disease is available. **Aim:** To evaluate Brachy & IGF1R expression in pediatric thyroid nodular samples and to study Brachy & IGF1R overexpression effect in vitro & in vivo. **Methods:** Immunostaining for Brachy & IGF1R was performed in pediatric Thyroid Papillary Carcinomas (TPCa), Follicular Adenomas (FA) or Benign Thyroid Nodular disease (BTN). TPCa cells overexpressing Brachy or IGF1R were used for Phalloidin staining, viability & apoptosis assays, wounding assays, gene (qPCR) & protein (WB) expression. Cells were injected in female nude mice (1e<sup>8</sup>cells/flank) **Results:** 50 samples were analyzed, 17 from BTN. Only TPCa and FA showed positive staining for Brachy (15/24TPCa;5/9FA) and IGF1R (11/24TPCa;4/9FA). In TPCa, positivity for IGF1R was only detected when Brachy was present. All patients with Brachy+/IGF1R-

immunostaining belonged to Intermediate/High-risk group and all but one, persisted with Indeterminate/Incomplete response 2 years post-surgery. In vitro, Brachy overexpression increased proliferation ( $p < 0.05$  vs TPC) and cell migration ( $p < 0.05$  vs TPC), decreased e-cadherin and increased vimentin & fibronectin expression. The opposite profile was found in IGF1R overexpressing clones. In vivo, by d35 100% of tumors from Brachy cells had volumes above 60mm<sup>3</sup>, while those from TPC or IGF1R clones were smaller ( $p < 0.001$  ANOVA). **Conclusion:** In TPCa Brachy+IGF1R- staining was associated with initial higher risk and indeterminate/incomplete response at 2 years. In vitro Brachy overexpression led to a mesenchymal like phenotype and favored tumor growth. Conversely, IGF1R expression favored epithelial features. Results suggest potential opposite roles for Brachy and IGF1R Thyroid tumor biology.

### 299. 226 MAPPING THE HO-1 INTERACTOME AS A KEY TO UNDERSTANDING NEUROENDOCRINE PROSTATE CANCER

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**Abstract:** Heme-oxygenase 1 (HO-1), the rate-limiting enzyme for heme degradation, has been implicated with a non-canonical antitumor role in prostate cancer (PCa). This study explores the hypothesis that HO-1 exerts its antitumor effects through its protein interactome, aiming to identify HO-1's nuclear interactors and their potential association with specific PCa phenotypes. PCa cells were treated with hemin (80  $\mu$ M, 24 h), a specific pharmacological inducer of HO-1, followed by HO-1 immunoprecipitation and LC-ESI MS/MS analysis, leading to the identification of 42 HO-1 interactors. To explore the relevance of these interactors in PCa, we analyzed RNA-seq data from the MDA-PCa-PDX series (PCa Patient-Derived Xenografts Program, MD Anderson Cancer Center), which reflects the heterogeneity of PCa. Unsupervised clustering based on the expression of HO-1 interactors revealed that samples with high expression of these genes were predominantly associated with neuroendocrine prostate cancer (NEPC). Further bioinformatics analysis using the Beltran et al. dataset, comprising transcriptomic and clinical data from 44 NEPC patients, demonstrated that the expression of HO-1 interactors distinctly clustered a subgroup of NEPC samples characterized by elevated levels of *DIRAS2*, *ILF3*, *NUMA1*, *SRSF6*, *DDX17*, *CCDC175*, *GPATCH1*, *DDX5*, *SFPQ*, *SAFB*, *BCLAF1*, *DDX27*, *SRSF7*, *RBMX*, and *SRSF3*. Principal Component Analysis further linked these 15 genes with a specific NEPC subtype, large cell neuroendocrine carcinoma. Notably, *ILF3*, *DDX17*, *BCLAF1*, and *SAFB* were detected in the nuclear fraction of PCa cells, and ChIP Atlas analysis suggested that these proteins are DNA-binding factors with regulomes significantly associated with neurological disorders. These findings propose that HO-1 may play a critical role in NEPC through its interactors, potentially serving as valuable markers for NEPC subtypes.

### 300. 275 UNVEILING THE ROLE OF KSHV-INFECTED HUMAN MESENCHYMAL STEM CELLS IN KAPOSI'S SARCOMA INITIATION

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Kaposi's sarcoma (KS) is an AIDS-defining cancer and a significant global health challenge caused by Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8). The most aggressive form of KS is found in HIV-1-infected individuals (acquired immunodeficiency syndrome-associated KS, AIDS-KS), where it is generalized and disseminated, localizing in the skin and visceral organs, including the gastrointestinal tract and lungs. KS may derive from KSHV-infected human Mesenchymal Stem Cells (hMSCs) that migrate to sites characterized by inflammation and angiogenesis, promoting the initiation of KS. **Methods:** We carried out KSHV infection of hMSCs and then subjected them to MSC conventional media or KS-like pro-angiogenic culture conditions for different time points. We performed RNA-sequencing and pathway analysis of the Differentially Expressed Genes (DEGs) between these different environmental conditions. **Results:** by analyzing the RNA sequences (Log FC>|1|; FDR<0.05) of KSHV-infected primary hMSCs, we have identified specific cell subpopulations, mechanisms, and conditions involved in the initial stages of KSHV-induced transformation and reprogramming of hMSCs into KS progenitor cells. Under pro-angiogenic environmental conditions, KSHV can reprogram hMSCs to exhibit gene expression profiles more similar to KS tumors, activating cell cycle progression, cytokine signaling pathways, endothelial differentiation, and upregulating KSHV oncogenes indicating the involvement of KSHV infection in inducing the Mesenchymal-to-Endothelial (MEndT) transition of hMSCs. **Conclusion:** This finding underscores the significance of pro-angiogenic conditions in facilitating KSHV-induced proliferation and reprogramming of hMSCs towards MEndT including a gene expression profile closer to that of KS tumors providing further evidence of these cell subpopulations as precursors of KS cells that thrive in a pro-angiogenic environment

### 301. 313 UNRAVELING THE IMPACT OF CARBON MONOXIDE-RELEASING MOLECULES ON PROSTATE CANCER CELL BEHAVIOR

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Carbon monoxide (CO) is a physiological gasotransmitter known for its anti-inflammatory and antioxidant properties, playing crucial roles in various cellular processes. Carbon Monoxide-Releasing Molecules (CO-RMs) have been developed to safely deliver CO to cells, showing promising therapeutic potential across multiple diseases. This study aimed to assess the effects of three water-soluble CO-RMs—CORM-3, CORM-401, and CORM-A1—on key hallmarks of prostate cancer (PCa). Using two distinct PCa cell lines, PC3 and MDA PCa 2b, we evaluated cell viability across a range of CO-RM concentrations (25-150  $\mu$ M; 6 h) to identify doses that do not compromise cell viability for further analyses. Confocal microscopy with the DCFH-DA probe revealed that CO-RM treatment significantly reduced reactive oxygen species (ROS) levels in both cell lines ( $p < 0.05$ ). Furthermore, CO-RMs enhanced cell adhesion ( $p < 0.05$ ) and inhibited cell migration, particularly with CORM-3 ( $p < 0.05$ ). Further, mitochondrial integrity and biogenesis were significantly altered as shown by MitoGreen and TMRE staining. Metabolically, PC3 cells treated with CORM-401 exhibited significant reductions in ATP content, LDH activity, and LDH levels ( $p < 0.05$ ), while the opposite effects were observed in MDA PCa 2b cells, indicating that CO-RM effects are influenced by cell-specific characteristics. On the molecular level, CO-RMs repressed markers of oxidative stress (*SOD-2*), metabolism (*LDH*), proliferation (*Ki67*), and angiogenesis (*VEGF*) in PC3 cells ( $p < 0.05$ ), as assessed by RT-qPCR. This study demonstrates that CO-RMs effectively modulate key biological processes in PCa cells, highlighting their potential as therapeutic agents for PCa.

#### P1 POSTERS

FECHA Y HORA: 19/11 11:00-12:00 H

COORDINADORES: CASAS ADRIANA,  
PETERS GISELLE

#### 302. 107 SYNERGISTIC PROAPOPTOTIC AND ANTIMIGRATORY EFFECT OF THE COMBINATION OF 2-NITROFLAVONE AND GEFITINIB IN BREAST CANCER CELLS

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Gefitinib, a drug used for the treatment of cancer which inhibits epidermal growth factor receptor (EGFR), has recently been proposed for the treatment of certain types of breast cancer. 2-nitroflavone (2'NF) is a synthetic flavone obtained in our institute which was demonstrated to have antitumoral effects and to impair EGFR signaling. Since EGFR is associated with carcinogenesis and is implicated in the mechanism of action of some flavonoids, a combinatory therapy between 2'NF and gefitinib is proposed. We have recently described a synergistic antiproliferative effect of the combination of 2'NF and gefitinib in human breast cancer cells. The objective of the present work was to evaluate the proapoptotic and antimigratory effects of this combinatory treatment. For that purpose, MDA-MB-231 (triple negative) and MCF-7 (luminal A) breast cancer cells were treated with 2'NF, gefitinib or a combination of both. Acridine orange/ethidium bromide staining examined under a fluorescent microscope was used to evaluate apoptosis in both cell lines. Cell migration was assessed in MDA-MB-231 cells by the scratch assay. Results were analyzed by ANOVA. Treatment with 2'NF or gefitinib at two concentrations (5 and 10  $\mu$ M) induced apoptosis in both cell lines. The effects of equimolar combinations of the drugs at both concentrations were significantly higher than the effect of each drug alone ( $n=7$ ,  $p < 0.05$ ). For scratch assays, the drugs were used at low concentrations (1 and 2  $\mu$ M) that proved not to affect cell proliferation but only cell migration. The migration of cells treated with a combination of 2'NF and gefitinib was lower than that of cells treated with the drugs alone ( $n=3$ ,  $p < 0.05$ ). In conclusion, the combination of 2'NF with gefitinib demonstrated to have a synergistic proapoptotic

and antimigratory effect on breast cancer cells, in addition to the previously described antiproliferative effect, supporting its potential use as new therapy for breast cancer.

#### 303. 115 SOLUBLE GUANYLYL CYCLASE ALPHA1 SUBUNIT PROMOTES HUMAN ENDOMETRIAL AND CERVICAL TUMOR GROWTH

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Cervical and endometrial cancers rank fourth and sixth, respectively, among the most common cancers in women worldwide. Various dysregulated proteins contribute to their development, one of which is the nitric oxide receptor enzyme, soluble guanylyl cyclase (sGC), primarily composed of the sGC $\alpha$ 1 and sGC $\beta$ 1 subunits. We previously demonstrated that sGC $\alpha$ 1 knock-down reduces proliferation, survival, and migration in endometrial and cervical carcinoma cell lines (ECC-1 and HeLa, respectively). To further investigate these findings, this study aimed to examine the effect of sGC $\alpha$ 1 overexpression on tumor development, both in vitro and in vivo. In vitro results showed that sGC $\alpha$ 1 overexpression promoted cell cycle progression and reduced cell death in both cell lines, increasing the S phase in ECC-1 cells ( $p < 0.05$ ) and the G2/M phase in HeLa cells (flow cytometry,  $p < 0.001$ ). Biochemical analyses revealed that sGC $\alpha$ 1 overexpression elevated the levels of p-AKT (T308) ( $p < 0.05$  or lower), PCNA ( $p < 0.05$ ), and VEGF ( $p < 0.05$ ) in both HeLa and ECC-1 cells (vs. respective controls, western blot). Furthermore, cells overexpressing sGC $\alpha$ 1 were more resistant to cytotoxic stimuli, including serum deprivation or treatment with 10 nM vinorelbine or 10 nM docetaxel (MTT cell viability assay,  $p < 0.05$  or lower vs. respective control cells). Finally, tumor growth was assessed in mouse xenograft models, where HeLa and ECC-1 cells overexpressing sGC $\alpha$ 1 exhibited increased tumor growth, as evidenced by larger tumor volume ( $p < 0.001$ ) and weight ( $p < 0.001$  vs. respective control cells). These findings underscore the significant role of sGC $\alpha$ 1 in the tumor biology of both endometrial and cervical carcinomas, suggesting that it could be a potential therapeutic target for these cancers.

#### 304. 131 CHARACTERIZATION OF MOLECULAR ALTERATIONS AND CLINICAL CORRELATIONS IN PEDIATRIC MEDULLOBLASTOMA

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Medulloblastoma (MDB) is the most common embryonal brain tumor (~20%). MDB are both classified into histological subtypes: classic (CL), desmoplastic/nodular (DN), extensive nodularity (MBEN) and large cell/anaplastic (LC/A) and molecular groups: WNT-activated (WNT), SHH-activated and TP53-wildtype (SHH TP53-), SHH-activated and TP53-mutant (SHH TP53+) and non-WNT/non-SHH group (noWNT/noSHH). The latter defined by molecular alterations and each with distinctive prognosis. Our aim was to characterize molecular alterations in MDB and correlate them with clinical data and outcome. IHC, FISH, Sanger sequencing and NanoString were performed on 84 MDB biopsies. Initial clinical risk (ICR) was de-

finied by age, tumor's surgical resection and metastasis. Median age was 5 years (0.1-16 years) and male:female was 1.4:1. 55 cases (65.5%) were categorized as high and 29 (34.5%) as standard ICR. The LC/A subtype was predominant in 36 cases (42.9%) followed by CL in 29 (34.5%), DN in 14 (16.7%), MBEN in 4 (4.8%). The predominant molecular group was noWNT/noSHH in 51 cases (60.7%) followed by SHH TP53- in 13 (15.5%), SHH TP53+ in 10 (11.9%) and WNT in 9 (10.7%). Genetic alterations in TP53 was the most frequent in 24 cases (27.38%) followed by NMYC in 18 (21.4%), GAB1 in 16 (19.1%), Iso17 in 11 (13.1%), CMYC in 8 (9.5%), and Chr6 monosomy and CTNNB1 in 4 (4.8%) each. Tumors with high ICR presented a decreased OS ( $P=0.04$ ) but, no significant differences were found among independent molecular alterations and OS. No significant differences were found in OS among histological subtypes. LC/A subtype was predominant in the noWNT/noSHH group ( $P<0.0001$ ). Identifying molecular alterations in MDB is crucial in the present context of molecular medicine, where patients are stratified into risk groups and tumors are treated according to the molecular alteration detected instead of the tumor type, particularly in the developing child.

**305. 238 ASSESSMENT OF THE IMMUNOLOGICAL MIMICRY OF GD2 TUMOR-ASSOCIATED GANGLIOSIDE PEPTIDOMIMETICS FOR THEIR USE IN THE DEVELOPMENT OF ACTIVE IMMUNOTHERAPIES**

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Differential expression in transformed cells and involvement in tumor progression make tumor-associated glycans (TACAs) good antigens for the development of active immunotherapies. However, TACAs are poorly immunogenic since they are unable to trigger a proper immune response when administered alone. To enhance their immunogenicity, peptides obtained from phage display libraries that mimic their structure, called peptidomimetics, can be used alone or fused to antigen presentation platforms. Among TACAs, the ganglioside GD2 is significantly overexpressed in neuroblastomas, correlating with tumor progression and poor outcomes, making it a validated therapeutic target. The aim of this work was to evaluate whether two peptidomimetics of the ganglioside GD2, named GDc and GDe, exhibit immunological mimicry against the TACA, to further conjugate them to a presentation platform based on Virus-Like Particles (VLPs) derived from the Z protein of the Junín virus. Following confirmation that at least one of the peptides elicits dendritic cell stimulation *in vitro*, we employed molecular biology techniques to conjugate the GDc and GDe sequences to Z, thereby generating expression vectors. These vectors were subsequently utilized to immunize mice according to a biweekly administration protocol. The administration of both peptidomimetics generated humoral immune response in a fraction of the animals, evaluated by ELISA against purified GD2. Additionally, a 40% enhancement in splenocyte proliferation was observed in immunized compared to control mice ( $p<0.01$ ), characterized by a higher proportion of CD8+ T lymphocytes (16% for GDc and 20% for GDe,  $p<0.05$ ). These findings provide robust proof-of-concept evidence demonstrating the immunological mimicry of GDc and GDe peptides. This foundational research establishes the groundwork for developing a promising immunotherapeutic strategy that targets the GD2 ganglioside through its conjugation to a novel antigen presentation platform.

**306. 251 ALL-TRANS RETINOIC ACID (ATRA) AND CISPLATIN (CDDP) COMBINED TREATMENT IMPAIRS TUMOR GROWTH AND METASTASIS DEVELOPMENT IN AN EXPERIMENTAL IN-VIVO LUNG CANCER MODEL**

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Lung cancer remains a significant public health challenge and is still one of the leading causes of cancer-related death in Argentina, and Cisplatin (CDDP) is the gold standard treatment for this disease. However, its effectiveness is often diminished due to the development of resistance, a process associated with the presence of cancer stem cells (CSCs). The Retinoic Acid System has been implicated in CSC maintenance and expansion, making it a promising therapeutic target. The aim of this study is to evaluate the efficacy of combining ATRA with CDDP on tumor progression and metastasis dissemination of LP07 cells, a lung cancer model that spontaneously arose in BALB/c mice. To assess the impact of the combined treatment on CSC metastatic potential, mice were intravenously inoculated with 7,500 LP07 oncosphere-derived cells. Treatment began 48h before cells inoculation by subcutaneously inserting a slow-release ATRA pellet (10 mg/mice) and the intraperitoneal administration of CDDP (2 mg/kg, 3 times/week). Twenty-one days later, mice were euthanized, and lung metastases were quantified. In a separate experiment, 200,000 LP07 cells were inoculated subcutaneously. Upon tumor detection, treatments were initiated (ATRA as before, CDDP 2 mg/kg, 2 times/week), and tumor volume was recorded twice a week. After three weeks- treatment, mice were euthanized and lung metastases were counted. The combined treatment resulted in a significant reduction in both macro and micro lung metastases compared to the control group in the first protocol. In the second assay, a notable decrease in tumor volume and lung metastases was observed exclusively in mice receiving the combination therapy. Notably, CDDP monotherapy had no significant impact on LP07 tumor dissemination. Our findings suggest that ATRA and CDDP combination effectively impair tumor progression and metastasis spread, suggesting that ATRA could enhance the CDDP efficacy, particularly in CDDP-resistant lung tumors.

**307. 364 SOLUBLE TNF $\alpha$  BLOCKADE ENHANCES TRASTUZUMAB-DERUXTECAN ANTITUMOR EFFECT IN HER2-POSITIVE BREAST CANCER**

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Breast cancer is the most common and the leading cause of cancer deaths in women. Trastuzumab deruxtecan (T-DXd), an antibody-drug conjugate, inhibits topoisomerase I and has a bystander effect in HER2+ breast cancer cells. Previously, we demonstrated that soluble TNF (sTNF) induces MUC4 expression, which hides the trastuzumab binding site on HER2. Blocking sTNF with INB03 (DN) sensitizes cells to trastuzumab. Our objective was to evaluate sTNF blockade on T-DXd treatment in HER2+ breast cancer preclinical models. We treated the HER2+MUC4+ JIMT-1 cell line with IgG, DN, T-DXd or the combination to study signaling pathways by western blot. We assessed proliferation using Incucyte or Countess II. Nude mice bearing JIMT-1 tumors were treated with 5 mg/kg IgG or T-DXd 5, 2.5 or 1.25 mg/kg i.v. on days 0, 7 and 14; 10 mg/kg DN i.p. twice/week or the combination (n=6-8) and tumor volume was measured. T-DXd at 2.5 and 5  $\mu$ g/ml inhibited cell proliferation in JIMT-1 cells and DN addition deepened the effect. The *in vivo* dose-response curves showed tumor growth inhibition of 83% (T-DXd 5 mg/kg), 61% (T-DXd 2.5 mg/kg) and 37% (T-DXd 1.25 mg/kg) vs. IgG ( $P<0.0001$ , 0.0001 and 0.05, respectively). Adding DN enhanced this inhibition, increasing it to 98% (5 mg/kg), 81% (2.5 mg/kg) and 73% (1.25 mg/kg)  $P<0.0001$  vs. IgG. T-DXd 1.25 mg/kg+DN achieved an antitumor effect similar to 5 mg/kg T-DXd alone. We observed an increase in cGAS and ISG15 expression when treated with T-DXd, alone or with DN *in vivo* and *in vitro*. Moreover, p16 was increased under T-DXd treatment measured by western blot. There are several reports of the adverse effects of T-DXd, which lead to treatment discontinuation. In this work, we demonstrated that adding DN could allow for a dose reduction of T-DXd without compromising

its antitumor effect in preclinical models. Moreover, treatment with T-DXd induces the activation of cGAS and interferon-related proteins that could activate the senescence program.

**308. 476 IMPACT OF ACYL-COA SYNTHETASE 4 INHIBITOR IN EPITHELIAL OVARIAN CANCER**

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Acyl-CoA synthetase 4 (ACSL4) is an enzyme that plays a significant role in arachidonic acid metabolism. It has been involved in pathological processes, including prostate, liver, and breast cancer. We have previously developed and characterized an inhibitor of ACSL4, PRGL493. We aimed to examine the effect of the ACSL4 inhibitor in epithelial ovarian cancer (EOC). Ovarian cancer is the third most prevalent gynecological malignancy and the leading cause of mortality within this category. Our previous research showed significantly higher ACSL4 levels in EOC patient samples relative to normal tissue. Western blot analysis revealed elevated levels of ACSL4 in the EOC lines A2780, OV-90, and SKOV-3 compared to non-tumorigenic HOSE cells. The PRGL493 inhibitor significantly reduced cell proliferation in EOC cells. Here we investigated the inhibitor's impact on cell migration, ABC transporters activity and tumor sphere formation. PRGL493-treated SKOV-3 cells showed significantly slower wound closure after 20 hours compared to controls ( $p < 0.05$ ). Efflux assays showed higher accumulation of doxorubicin and Hoechst in PRGL493-treated cells compared to controls, indicating lower transporter's activity. PRGL493 also impaired tumor sphere formation in SKOV-3 cells. We then tested the combination of PRGL493 with chemotherapeutic drugs on SKOV-3 cells. After 96 hours of treatment with suboptimal doses of carboplatin (1 mg/ml), paclitaxel (0.15 nM), and doxorubicin (0.027  $\mu$ M), alone or with PRGL493 (10  $\mu$ M), cell viability was assessed via MTT assay. The combination of the drugs with PRGL493 enhanced the inhibition of cell proliferation. The results show that ACSL4 inhibition has an impact in cell migration, ABC transporter activity and sensitizes SKOV-3 cells to chemotherapeutic treatment.

**309. 483 THYMOSIN B4 EXPRESSION IN BREAST CANCER AND ITS EPIDEMIOLOGICAL ASSOCIATION WITH HEALTH HABITS**

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Thymosin  $\beta$ 4 has been hypothesized to be a tumor promoter as its expression frequently increases during disease progression and is associated with poor prognosis in several tumor types; however, this has not been clearly demonstrated for breast tumors. We proposed to analyze the protein and mRNA expression of Thymosin  $\beta$ 4 (T $\beta$ 4 and TMSB4X, respectively) in human breast biopsies from oncological patients at HZEOL and its association with relevant clinico-pathological parameters as well as with relevant health habits such as alcohol intake, smoking, etc. For this purpose, epidemiological surveys were carried out in patients diagnosed with breast cancer, mRNA was extracted from the tumor tissue samples of each patient, determining the expression of TMSB4X by RT-PCR

and the expression of T $\beta$ 4 at protein level by immunohistochemistry. The association between Thymosin  $\beta$ 4 expression with relevant clinico-pathological parameters (histological type and stage, lymph node status, histopathological classification, family history, previous oncological disease, etc) and different health habits was studied.

The results show that tumor biopsy samples present low levels of T $\beta$ 4 expression. Increased T $\beta$ 4 has been correlated with the development of metastasis and poor prognosis and would regulate the expression of genes critical for angiogenesis and cell migration; although its functional role in tumor progression remains poorly understood. However, in this study we have not found a positive correlation between T $\beta$ 4 expression and tumor invasiveness, measured as a function of tumor stage. Of the health habits analyzed, statistical analysis showed a significant correlation between T $\beta$ 4 expression with smoking, both at the mRNA and protein level. This highlights the need for primary prevention health campaigns focused on the impact of health habits as risk factors for different diseases, including, as in our case, breast cancer.

**P2 POSTERS**

FECHA Y HORA: 19/11 11:00-12:00 H

COORDINADORES: URTREGER ALEJANDRO, LAURA TODARO

**310. 065 EFFECT OF RENAL TUMORS ON THE MORPHOLOGY AND FUNCTIONALITY OF SURROUNDING ADIPOSE TISSUE**

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In addition to the genetic and epigenetic changes that occur in epithelial cells, tumor progression also depends on the bidirectional dialogue between epithelial tumor cells and the adjacent tumor microenvironment (TM). In addition to tumor cells, the TM contains a plethora of non-tumor cells, with adipocytes being the major component, particularly in clear cell renal cell carcinoma (RCC). Adipose tissue (AT) is an endocrine and immunoregulatory organ that contributes to human physiology. In this work, we evaluated the effect of renal tumors on the surrounding adipose tissue (AT), seeking to elucidate the mechanisms by which the presence of the tumor modifies the environment. To this end, fragments of normal human perirenal TA (hRAN) were incubated for 48 h with conditioned media (CMs) from renal tumors. Then, in the treated TA, changes in the expression of: 1) perilipin A (marker of mature adipocytes); 2) UCP1, PPAR $\gamma$ , PGC1 $\alpha$ , c/EBP $\alpha$  and TBX1 (brown/beige TA markers); 3) leptin, adiponectin (adipocytokines); 4) MCT1, MCT4 and GLUT 1 (lactate and glucose transporters, respectively); 5) ER $\alpha$ , ER $\beta$ , AR and PgR (estrogen, androgen and progesterone receptors, respectively). We found: a) an increase (trend) in the expression of browning and differentiation markers, b) a significant increase in the expression of leptin ( $p < 0.05$ ), MCT1 ( $p < 0.05$ ), and ER $\alpha$  in hRAN incubated with tumor MCs vs. ctrl-MCs. Furthermore, microscopic observation of histological sections of hRAN treated with tumor MCs and stained with H&E showed browning foci in a large number of areas of the TA (multilocular polygonal cells). We conclude that the perirenal adipose tissue undergoes a process of adaptation to the changes generated by the surrounding tumor, which in turn favors tumor development.

**311. 139 UNLOCKING METABOLIC VULNERABILITIES IN PANCREATIC CANCER BY TARGETING PROTEIN KINASE A**

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Pancreatic cancer (PC) remains one of the most lethal malignancies, with a 5-year survival rate of just 13%. PC tumors are characterized by a highly fibrotic stroma, composed predominantly of collagen fibers. Our previous findings demonstrated that a collagen-rich environment activates Protein Kinase A (PKA) signaling in prostate cancer, leading to metabolic rewiring in tumor cells. Given the pivotal role of a fibrotic stroma in both PKA activation and PC progression, this study aimed at elucidating the role of PKA in the metabolic phenotype of PC. PANC-1 cells, a PC cell line, were treated with the potent PKA inhibitor H89 across a concentration gradient from 1  $\mu$ M to 80  $\mu$ M. Concentrations above 20  $\mu$ M significantly impaired cell viability ( $P < 0.05$ ), prompting us to focus on 2.5 and 10  $\mu$ M H89 to assess metabolic effects without inducing cell death. At the cellular level, PKA inhibition led to a significant decrease in ATP levels, increased lipid accumulation (Bodipy 493/503 staining), and higher levels of lipid peroxidation (Bodipy 665/676 probe;  $P < 0.05$ ). Additionally, blocking PKA activity resulted in an increased mitochondrial membrane potential, as indicated by JC-1 staining. At the molecular level, we observed significant upregulation of *HIF1A1* and *VDR*, alongside dysregulation of *EHHADH* and *ALDH1L2*, suggesting enhanced lipid biosynthesis in H89-treated cells. Furthermore, the metabolic transporters *SLC16A1* and *GLUT1* were markedly upregulated upon PKA inhibition. Collectively, these findings highlight PKA as a crucial regulator of the metabolic phenotype in PC and underscore the profound lipid metabolic reprogramming induced by PKA inhibition, offering novel insights into potential therapeutic targets for this deadly disease.

### 312. 140 ANDROGEN RECEPTOR NEGATIVELY REGULATES THE PKA/OSTEOPONTIN AXIS IN PROSTATE CANCER: IMPLICATIONS FOR AR-TARGETING THERAPIES

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Osteopontin (*SPP1*) is a secreted protein associated with a poor prognosis in prostate cancer (PCa), and it is notably upregulated in a subset of PCa bone metastasis. We have previously identified that type I collagen and fibronectin activate protein kinase A (PKA) which subsequently regulates *SPP1* expression in PCa cells. Considering that the androgen receptor (AR) is a crucial transcription factor in PCa pathogenesis and the main target of current PCa therapies, this study aims to elucidate the role of the AR in the PKA/*SPP1* signaling axis. Analysis of the SU2C-PCF dataset, comprising transcriptomics data from 444 metastatic PCa patients, revealed that high AR scores correlate with reduced *SPP1* mRNA expression ( $P < 0.05$ ), suggesting an inverse relationship between AR and *SPP1*. Consistent with this, the AR-positive C42B PCa cell line exhibited no detectable *SPP1* expression (RT-qPCR) compared to the AR-null PC3 cells, supporting the hypothesis of AR-mediated regulation of *SPP1*. Further, PC3 cells treated with the PKA activator forskolin (FK; 10  $\mu$ M for 24 h) significantly increased *SPP1* mRNA levels (fold change: 2.5;  $P < 0.05$ ). To directly examine the interaction between

AR and the PKA/*SPP1* pathway, we transfected AR-negative PC3 cells with a human AR expression vector. AR expression was confirmed by RT-qPCR and Western blot, and AR activity was validated using the PSA-Luc reporter plasmid following dihydrotestosterone (DHT) treatment (10 nM). Although, FK-induced *SPP1* expression was unaffected in AR-transfected PC3 cells, DHT treatment significantly reversed FK-mediated *SPP1* upregulation, demonstrating the critical role of AR activity in this pathway. These findings demonstrate that AR exerts a negative regulatory effect on the PKA/*SPP1* axis, underscoring a potential role of this axis during treatments with AR inhibitors.

### 313. 151 LIPOIC ACID REDUCES MIGRATION AND INVASION IN HEPATOCELLULAR CARCINOMA CELLS BY INHIBITING EPITHELIAL-MESENCHYMAL TRANSITION VIA AN AMPK-P53 PATHWAY

Anabela C. Ferretti<sup>1</sup>, Florencia Hidalgo<sup>1</sup>, Carla Borini Etichetti<sup>2</sup>, Alejandro Pariani<sup>1</sup>, Tomás Rivabella Maknis<sup>1</sup>, Javier Bussi<sup>3</sup>, Felipe Zecchinati<sup>1</sup>, María C. Larocca<sup>1</sup>, Cristián Favre<sup>1</sup>

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**Introduction:** We previously demonstrated that lipoic acid (ALA), an AMPK activator with antitumor effects in various cell types, inhibits migration in hepatocellular carcinoma (HCC) cell lines. This effect was absent in the Hep3B cell line (TP53 null). **Objectives:** We hypothesized that this selective action was linked to the AMPK target p53 and we aimed to study its role in ALA mechanism. **Methods:** To explore this hypothesis, we detected pAMPK and p53 levels in control and AMPK silenced HepG2/C3A cells. We also developed a p53-silenced cell line (shTP53) from these cells and assessed migration, invasion, and mRNA levels of E-cadherin (CDH1), vimentin (VIM), and SNAIL (SNAI1) after ALA (0.5 mM) treatment.

In addition, we conducted a bioinformatics analysis of the expression of these and other epithelial-mesenchymal transition (EMT) markers in HCC patients from the TCGA database, evaluating potential changes with TP53 status (WT versus MUT) and their impact on survival. **Results:** ALA tripled p53 levels in parallel with those of pAMPK, which was suppressed by AMPK silencing. In control cells, ALA reduced migration by 60%, but only by 30% in shTP53 cells. ALA reduced invasion by 50% in isogenic cells ( $p < 0.05$ ) but this effect was blocked in shTP53 cells. ALA significantly increased mRNA levels of CDH1 and decreased those of VIM, but only in control cells. In patient analyses, we observed significant increases in expression compared to healthy tissue ( $\log_2FC > 1$ ) for VIM, MMP2, SNAI2, MMP9, and TWIST1. CDH1 was the only marker whose relative expression depended on TP53 status: its distribution showed more preserved levels in TP53 WT patients ( $p < 0.05$ ). MMP9 was the only gene whose levels impacted survival based on TP53 status ( $p < 0.02$ ). **Conclusion:** ALA emerged as a potent anti-migratory agent in HCC cells through an AMPK-p53 pathway, inhibiting EMT. E-cadherin appears to play a central role in this process, further supported by its association with TP53 in patients.

### 314. 157 STUDY OF NON-TARGETED EFFECTS OF RADIATION IN AN ANAPLASIC THYROID CELL LINE

Romina Oglio<sup>1</sup>, Jennifer Miranda<sup>1</sup>, Carla Rodriguez<sup>1</sup>, Marina Carpano<sup>1</sup>, Mariana Casal<sup>3</sup>, Federico Gomez<sup>3</sup>, Guillermo Juvenal<sup>1,2</sup>, Marina Perona<sup>1,2</sup>, Lisa Thomasz<sup>1,2</sup>.

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Anaplastic thyroid carcinoma (ATC) is an extremely aggressive thyroid malignancy. ATC has a median survival of 5 months regardless aggressive treatments that involve surgery, radiation and chemotherapy. Radiation-induced bystander effect is the phenomena occurring in non-irradiated cells as a result of signal transmission from an irradiated cell. In turn, irradiated cells could benefits from



the feedback signals sent from the bystander non-irradiated cells promoting tumor resistance. However, the mechanism regulating the bystander signaling that could promote the mitigation of the radiobiological effects in ATC are not yet well understood. Our goal is to explore possible factors involved in tumor resistance in ATC. Methods: ATC cells (8505C) were irradiated with 0, 2 and 5 Gy. After 72 hours, conditioned medium (CM) of irradiated 8505C was obtained. Proliferation (MTT, cell counting), migration (wound healing), intracellular ROS (DCHFDA), gene expression (qPCR) and p-NF- $\kappa$ B (immunocytochemistry) were analyzed. Results: Radiation increased ROS production (1.5 fold;  $p < 0.01$ ) was correlated with augmented mRNA levels of NOX4(2.8 fold) and NOX5(2.5 fold). We observed an increase mRNA levels of TNF $\alpha$ (2.8 fold), TGF $\beta$ 1(2 fold) and COX2(3.5 fold), while Gal3 was decreased (0.5 fold;  $p < 0.01$ ) in irradiated 8505C cells. In non-irradiated cells, CM induced ROS production by a 1,35 fold( $p < 0.05$ ) and enhanced cell migration (15%,  $p < 0.05$ ). We found that CM from 5 Gy irradiated cells, significantly increased mRNA of COX2, TNF $\alpha$ , INF $\beta$ , TGF $\beta$ 1,  $\beta$ -catenin and Gal3 and decreased NRF2 and Gal1. Pretreatment with TGF $\beta$ 1 inhibitor (10  $\mu$ M SB431542) restored NRF2 and Gal1 expression. Moreover, the p-NF- $\kappa$ B expression in the cell nuclei of bystander non-irradiated 8505C was also increased significantly (1.5 fold,  $p < 0.01$ ). Conclusion: Irradiated cells and bystander non-irradiated 8505C could secrete signals to promote tumor resistance.

### 315. 167 EXPRESSION AND SUBCELLULAR LOCALIZATION OF P300 AND ITS RELATION TO PROTEINS INVOLVED IN BREAST CANCER PROGRESSION

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Breast cancer (BC) is a heterogeneous disease that includes different molecular and clinical subtypes. Triple negative BC (TNBC) is a molecular subtype of BC that is known for having a poor prognosis and limited therapeutic options. Therefore, it is necessary to investigate potential tumor markers and therapeutic targets for this pathology. Previously, we have demonstrated that inhibition of p300 has an antitumoral effect in BC and TNBC cell lines, and decreases tumor progression in the animal models. In addition, we show that the presence of p300 in the cytoplasm correlates with increased survival of patients suggesting that its nuclear localization is necessary for the pro-tumoral effects. Therefore, we proposed to investigate the expression and localization of p300 and its association with proteins involved in tumor progression of BC and TNBC by immunohistochemistry. In human BC biopsies, we observed a positive association between p300 expression and Elf5,  $\beta$ -catenin, GEF-H1 and HO-1 protein ( $p < 0.05$ ). In human TNBC biopsies, higher p300 levels were associated with decreased nuclear  $\beta$ -catenin and increased membrane  $\beta$ -catenin expression ( $p < 0.05$ ); furthermore, higher cytoplasmic p300 levels correlated with higher E-cadherin expression ( $p < 0.05$ ). In a murine syngeneic model of LM3 cells we detected an increase in E-cadherin and Elf5 expression and a decreased in cytoplasmic  $\beta$ -catenin expression in tumors of animal injected with p300 genetically-silenced cells compared to the control group ( $p < 0.05$ ). In a murine xenograft model of MDA-MB-231 cells, we detected an increase in membrane  $\beta$ -catenin and E-cadherin expression in the tumors of animals injected with VV59 (a specific pharmacological inhibitor of p300) compared to the control group ( $p < 0.05$ ). These results show a positive association between p300 expression and proteins involved in tumor progression in BC. They also demonstrate an antitumor effect for inhibition and cytoplasmic translocation of p300 in TNBC.

### 316. 237 AQUAPORIN-8 (AQP8) KNOCKDOWN POTENCIATES MITOCHONDRIAL REACTIVE OXYGEN SPECIES (ROS)

### ANTIPROLIFERATIVE EFFECTS IN HEPATOCELLULAR CARCINOMA (HCC)

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**Introduction:** AQP8, classified as a peroxiporin, has been linked to cancer progression in recent studies. Although it is established that H<sub>2</sub>O<sub>2</sub> transport is a crucial signaling driver for cancer, the different outcomes between tissues remain unclear. **Aim:** to study the role of AQP8 in HCC and the AQP8 modulation in vitro. **Methods:** In silico analysis of HCC patients were performed from The Cancer Genome Atlas database. The gene expression and the survival rate were assessed using Kaplan–Meier survival analysis. The aquaporin-8 gene-knockdown was made by using a short hairpin RNA (shRNA)-expressing adenovirus vector (Adshaqp8). HepG2 cells were exposed to Adshaqp8 or an empty vector (SCR) at a multiplicity of infection of 50. After 72 h of culture, MTT and ROS production studies were performed. **Results:** Studies revealed a significantly lower survival rate in patients with high AQP8 expression compared to those with low AQP8 expression ( $p = 0,008$ ). Comparison of paired normal and tumor tissues showed a significantly higher expression of AQP8 in tumor tissues ( $p = 0,0069$ ). AQP9 expressed in the plasmatic membrane demonstrated the opposite effect with a higher survival rate in high AQP9 expression patients ( $p = 0,033$ ) and a lower expression in tumor tissue ( $p = 0.0004$ ). HepG2 cells exposed to Adshaqp8 showed 82% decrease in mitochondrial AQP8 vs Control ( $p = 0,002$ ). Exposure to Adshaqp8 or SCR show no toxicity, as measured by LDH release into the media. MTT studies exhibit a significant decrease in the IC50 in AQP8-knockdown cells treated with a mitochondrial pro-oxidant (Control: 15,72  $\mu$ M, Adshaqp8: 10,53  $\mu$ M;  $p = 0,049$ ). ROS generation studies indicated a tendency toward mitochondrial ROS accumulation in AQP8-knockdown cells compared to control. **Conclusion:** Our data suggest that AQP8 may play a role in mitochondrial H<sub>2</sub>O<sub>2</sub> signaling in HCC cells. This finding could have potential therapeutic implications for chemotherapeutic compounds that affect mitochondrial metabolism.

### P3 POSTERS

FECHA Y HORA: 19/11 16:00-17:00 H

COORDINADORES: ALVAREZ MARÍA DE LUJAN, CEBALLOS MARIA PAULA

### 317. 022 DIFFERENTIAL EFFECTS OF HUMAN PERIPROSTATIC ADIPOSE TISSUE-DERIVED VESICLES ON PROSTATE CANCER MICROENVIRONMENT BASED ON TUMOUR AGGRESSIVENESS

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Prostate cancer (PCa) is a prevalent and complex malignancy influenced by its surrounding microenvironment, particularly periprostatic adipose tissue (PPAT). This study investigates the influence of extracellular vesicles (EVs) secreted by human PPAT on PCa progression. EVs were isolated from PPAT samples derived from low-risk (ISUP  $\leq$ II) and high-risk (ISUP  $>$ II) PCa patient's and tested on androgen-sensitive PCa cell lines (22Rv1), differentiated macrophages from THP-1, and HUVEC cells. EVs from non-tumorous extraperitoneal perivesical adipose tissue (PVAT) were used as controls. EV stimuli concentration was standardized at 2.5  $\mu$ g/ml. The effects of these EVs on PCa cell proliferation, and migration were assessed, along with macrophage polarization effects, by measuring M1 and M2 mRNA markers (CD80, CD86, CD206, CD163, CD68) and inflammatory genes (IL-10, IL-1 $\beta$ , MCP-1, TNF- $\alpha$ , iNOS). Tube formation assays were performed in HUVEC cells to evaluate angiogenesis effect. Results demonstrated that PPAT-derived EVs impacted PCa behaviour differently based on tumour grade. Low-risk PPAT-derived EVs enhanced cell proliferation in 22Rv1 cells more than high-risk EVs. In migration studies, high-risk PPAT-derived EVs significantly promoted cell migration more than low-risk EVs. When analysing the macrophage polarization effect of the secreted EVs, we observed that low-risk PPAT-derived EVs increased mRNA levels of the M2 marker (CD206), while high-risk PPAT-derived EVs significantly increased the M1 marker (CD86), raising the M1/M2 ratio. Additionally, low-risk PPAT-derived EVs induced inflammation by upregulating IL-1 $\beta$  and TNF- $\alpha$  mRNA levels. Angiogenesis assays revealed that high-risk PPAT-derived EVs significantly induced neovascularization, leading to more nodes, junctions, meshes, and segments. These findings highlight the role of PPAT-derived EVs in modulating the PCa microenvironment and suggest their potential as therapeutic targets or diagnostic biomarkers.

### 318. 113 THE N4 ARYL SUBSTITUTED THIOSEMICARBAZONE T2 REDUCES INVASIVENESS OF TRIPLE NEGATIVE BREAST CANCER CELLS IN A MOUSE INTRADUCTAL INOCULATION MODEL

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Breast cancer is the leading cause of cancer death in women worldwide while triple negative breast cancer (TNBC) is its most aggressive subtype. Our group has been studying the action of the N4 aryl substituted thiosemicarbazone T2 on TNBC which has shown reduction of growth, invasiveness and metastatic ability on 4T1 tumors in subcutaneously inoculated mice. In this work, we assessed the effect of T2 treatment on the 4T1 cell mouse intraductal inoculation model. Cells were inoculated through the fourth mammary gland nipple of female BALB/c mice and treatment with T2 25 mg/kg was initiated 48h after inoculation (a.i.) for a total of 5 intraperitoneal doses administered every other day. Two weeks a.i., mice were euthanized and their mammary glands were removed for wholemount followed by paraffin embedding and sectioning. Through hematoxylin-eosin staining, mammary gland ducts were classified according to the presence of 4T1 cells as empty, lumen with *in situ* cell growth or with invasive cell growth beyond the duct walls. Glands from mice treated with T2 had a lower percentage of ducts with invasive cell growth (13 $\pm$ 1% vs 20 $\pm$ 3% in control group,  $p < 0.05$ ). We assessed the proliferation marker PCNA and the EMT associated transcription factor  $\beta$ -catenin through immunofluorescence. There was a reduction in proliferating cells on slides from T2 treated mice (27 $\pm$ 6% vs 43 $\pm$ 4% in control group,  $p < 0.05$ ).  $\beta$ -catenin was found to have differential distribution depending on duct status. Cells within the ducts showed membranous localization of  $\beta$ -catenin while cells invading beyond the ductal wall had a diffuse mark. For both conditions, T2 treatment resulted in lower levels of  $\beta$ -catenin expression (36 $\pm$ 6AU

vs 60 $\pm$ 1AU,  $p < 0.01$  for *in situ* cells and 20 $\pm$ 4AU vs 44 $\pm$ 8AU,  $p < 0.05$  for invading cells). These results show that T2 treatment reduces both cell proliferation and invasive capacity of intraductally inoculated 4T1 cells and this effect is concomitant with  $\beta$ -catenin modulation.

### 319. 168 IN VITRO STUDIES OF THE EFFECTS OF DIFFERENT MODALITIES OF ONCOLOGICAL RADIATION ON MONO-LAYER AND ONCOSPHERES OF ANAPLASTIC THYROID CANCER

Susana Nieves<sup>1</sup>, Aldana Schey<sup>3</sup>, Antonella Pastini<sup>1,3</sup>, Estanislao Pavlik<sup>1,3,4</sup>, Martina Buschittari<sup>1,3,4</sup>, Marina Carpano<sup>1</sup>, Luciano Rossich<sup>1</sup>, Paula Ramos<sup>1</sup>, Paula Curotto<sup>1</sup>, Mariana Casal<sup>1,3</sup>, Pablo Menéndez<sup>3</sup>, Cristina Zarlenga<sup>1</sup>, Alejandro Urteger<sup>2,3</sup>, María Alejandra Dagrosa<sup>1,2</sup>

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**Background:** Anaplastic thyroid cancer (ATC) and poorly differentiated thyroid cancers, though representing less than 10% of all thyroid neoplasms, are highly resistant to conventional therapies. External ionizing radiation, including X-rays and boron neutron capture therapy (BNCT), are often employed as a complementary treatment. The cancer stem cells (CSCs) are a subpopulation known for their high tumorigenic potential and resistance to chemo- and radiotherapy, playing a crucial role in maintaining thyroid tumor growth. The histone demethylase KDM1A is implicated in cancer progression, stem cell pluripotency, and multidrug resistance. **Objective:** This study aims to evaluate the impact of various radiation therapies on CSCs by analyzing their capacity to form oncospheres and the expression of KDM1A, a key factor in CSC maintenance and radioresistance. **Materials and methods:** Proliferating human ATC cells (8505C) were divided into the following groups: 1) Control (untreated); 2) X-rays; 3) NCT (neutron thermal irradiation); and 4) BNCT (irradiated with thermal neutrons and boron-10). Cells were irradiated with doses ranging from 1 to 5 Gy. The following assays were performed: Clonogenic assay, micronucleus formation and KDMA1 gene expression (RT-qPCR) were studied. To evaluate the ability to form oncospheres, cells were seeded in low-adhesion surfaces in serum-free culture media supplemented with B27 and EGF. **Results:** The BNCT treatment resulted in a significant decrease in cell survival ( $p < 0.05$ ) and an increase in micronucleus formation per binucleated cell. The number of oncospheres larger than 70 $\mu$ m significantly decreased over time, while their average diameter increased from day 3 to day 6 in the X-ray, NCT, and BNCT groups. KDMA1 expression showed a slight, non-significant decrease across all irradiated groups. **Conclusions:** Despite radiotherapy, a population of CSCs persists, which may be associated with KDM1A expression.

### 320. 169 BORON-ENRICHED TYROSINE KINASE INHIBITORS (TKIs) FOR THE TREATMENT OF CANCER BY COMBINING TARGETED THERAPY AND BORON NEUTRON CAPTURE THERAPY (BNCT)

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**Background:** Previously we have shown that boron neutron capture therapy (BNCT) based on the <sup>10</sup>B (n, <sup>4</sup>He) <sup>7</sup>Li nuclear reaction could be a radiotherapy technique applied to the treatment of malignant solid tumors such as cutaneous melanoma (CM) and advanced thyroid cancer (ATC). Looking for strategies to improve the effectiveness of BNCT, new boron agents as lapatinib and erlotinib, which are currently used in target therapy, were joined to organometallic carboranes containing boron (B). Both drugs are inhibitors of cellular enzymes tyrosine kinases (TKIs) related to tumor proliferation and progression. **Objective:** The aim of these studies was to evaluate the use of erlotinib and lapatinib enriched with boron for the treatment of CM and ATC. **Materials and Methods:** Proliferating human melanoma (Mel J) and undifferentiated thyroid cancer (8505C) cells were incubated with borated erlotinib (C27) or lapatinib (C10, C14) at concentrations lower than their IC50 (3-12 µM) for 1 and 24 hours. Boron content was measured by ICP-AES spectroscopy and the cellular microdistribution studied by neutron autoradiography. After, cells were incubated with 10 ppm of <sup>10</sup>B and irradiated in the RA 3 Facility (Flux = 5 · 10<sup>9</sup> n/cm<sup>2</sup> sec). The cell survival through colorimetric assay 3-(4, 5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide and cell proliferation by immunocytochemistry (Ki67 antibody) were measured. **Results:** Mel J cells showed greater cellular uptake of the C27 and instead 8505 cells showed a significantly higher uptake of both, C10 and C14. Autoradiography showed internalization of the bimodal compounds. Irradiation studies demonstrated a significant decrease in cell survival and a lower number of Ki67 positive cells indicating a decrease in cell division for both TKIs. **Conclusions:** Both TKIs could be used for the treatment of CM and ATC by targeted therapy and BNCT respectively.

**321. 199 FIRST REPORTED IMMUNOHISTOCHEMICAL STUDIES AND q-PCR EXPRESSION OF LAT1 AND PEPT1 TRANSPORTERS IN THE HAMSTER CHEEK POUCH ORAL CANCER MODEL**

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Boron Neutron Capture Therapy (BNCT) is a tumor selective particle radiotherapy that combines preferential tumor uptake of <sup>10</sup>B compounds and neutron irradiation. BNCT mediated by BPA (borono-phenylalanine) has been studied in many clinical trials. We have studied BPA biodistribution and BPA/BNCT in the hamster cheek pouch oral cancer model. In this model the tumor is surrounded by precancerous tissue that gives rise to additional tumors and allows for the study of mucositis commonly seen in Head and Neck cancer

patients (HNC), which can be a dose limiting toxicity. We observed therapeutic boron concentrations in the tumor vs the precancerous and normal tissue. BPA/BNCT showed high tumor responses but severe mucositis in the precancerous surrounding tissue. LAT1 is mainly responsible for BPA uptake and is upregulated in HNC. In addition to BPA, we tested some novel boronated dipeptides in the same model, these can be taken up by PEPT1 as well as LAT1. In this study, we evaluated for the first time in the hamster cheek pouch oral cancer model expression of LAT1 and PEPT1 in tumors, precancerous and normal tissue. Tumor-bearing hamsters were divided into [no BPA] or [BPA injected, 300 mg/kg] groups. 3 h later, tumor bearing and contralateral normal pouches were excised for LAT1 and PEPT1 immunohistochemistry (IHC) and LAT1 expression by q-PCR (Rpl13a as housekeeping gene). PEPT1 IHC was negative in tumor, precancerous and normal pouch. Instead, similar to HNC patients, LAT1 IHC and q-PCR results showed that tumor cells are strongly LAT1 positive, with q-PCR showing increased LAT1 gene expression with BPA treatment (p<0.05). LAT1 IHC in the basal epithelium of precancerous and normal tissue in BPA treated and non-treated animals were also positive, explaining the accumulation of BPA and consequent mucositis in precancerous tissue following BNCT. This study provides additional evidence that the hamster oral cancer model is suitable for evaluating boron agents and BNCT.

**322. 209 EXPRESSION OF CD146, PDGF-Rβ, CD3, AND CD8 IN SPORADIC COLORECTAL CANCER AND ITS CORRELATION WITH TUMOR CHARACTERISTICS AND DIABETES**

Florencia Adriana Lohmann<sup>1</sup>, Martín Isac Specterman Zabalá<sup>1</sup>, Julieta Natalia Soarez<sup>1</sup>, Maximiliano Dádamo<sup>1</sup>, Monica Alejandra Loresi<sup>1</sup>, María de las Nieves Diaz<sup>2</sup>, Walter Hernan Pavicic<sup>1</sup>, Marcela Fabiana Bolontrade<sup>1</sup>, Marcelo Raul Risk<sup>1</sup>, Juan Pablo Santino<sup>2</sup>, Carlos Alberto Vaccaro<sup>1,2</sup>, Tamara Alejandra Piñero<sup>1</sup>.

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Colorectal cancer (CRC) is one of the most prevalent malignant neoplasms globally. According to the World Health Organization, CRC is the second leading cause of cancer-related deaths in Argentina. Most CRC cases are sporadic, caused by somatic mutations and epigenetic changes linked to modifiable factors like diet, sedentary lifestyle. The tumor microenvironment includes immune cells like CD3+ and CD8+ T lymphocytes and angiogenesis-associated cells expressing CD146 and PDGF-Rβ. This study focused on evaluating the expression of CD146, PDGF-Rβ, CD3, and CD8 in tumor tissues from patients with sporadic CRC, assessing the influence of diabetes and tumor characteristics on these expressions, and comparing logistic regression and Random Forest (RF) models for patient status prediction. Immunohistochemical analysis was performed on samples from patients at Hospital Italiano de Buenos Aires. Tumor area segmentation utilized convolutional neural networks, with biomarker quantification conducted using Python tools. Combining clinical-epidemiological data with statistical and artificial intelligence analyses, patient outcomes were evaluated over 12 months. Significant associations were found between biomarker expression and tumor site, with higher expression in the left colon. Non-diabetic patients showed elevated CD3 and CD8 levels, while diabetic patients had higher CD146 and PDGF-Rβ. Moderate correlations were observed between CD146 and PDGF-Rβ (R=0.34), and CD3 and CD8 (R=0.42). Logistic regression showed better accuracy and recall balance, whereas RF was more precise in classifying disease-free patients. The current study is focused on this specific sample group, and future work will involve expanding the cohort to include diverse populations and exploring new AI tools to further validate and enhance the findings.

**323. 315 DESIGN AND FEASIBILITY OF A RAPID METHOD TO ESTIMATE THE PROPORTION OF LIVE BACILLI AND**

### THE HOMOGENEITY OF AN IMMUNOTHERAPEUTIC BACILLE CALMETTE-GUÉRIN BY FLOW CYTOMETRY

Vanessa Seery<sup>1</sup>, Tania Lovera<sup>2</sup>, Adriana Cangelosi<sup>1</sup>, Sofía Arribillaga<sup>2</sup>, Patricia Geoghegan<sup>1</sup>, Claudia Argüelles<sup>2</sup>.

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Bacille Calmette-Guérin (BCG) is a live attenuated strain of *Mycobacterium bovis*, used as an immunotherapeutic (BCGi) in the treatment of superficial bladder cancer. At the INPB-ANLIS, it is produced in static cultures according to the guidelines of the World Health Organization (WHO). Among other quality parameters, the product should contain a high number of live cells (viability) and be made up mainly of single bacilli. Currently, the method recommended by the WHO to test viability is the counting of colony-forming units, which is time-consuming. Homogeneity is evaluated by microscopy with the degree of clumping of a BCGi smear. Unlike these methods, flow cytometry (FACS) can simultaneously detect live cells and cellular aggregates, which could facilitate the control of BCGi batches. We aimed to develop a method for the estimation of bacterial viability and homogeneity in BCGi by FACS. Viability staining of BCGi was tested using carboxyfluorescein succinimidyl ester (CFSE) at 1, 5, and 25  $\mu\text{M}$  incubated at different times, and fluorescein diacetate (FDA) at 10, 25, and 100  $\mu\text{g}/\text{ml}$  incubated at different times. A fresh batch of BCGi was compared with 3 consecutive expired batches. Samples were analyzed using a BD FACSLytic flow cytometer. Comparing the stains, FDA provided a better definition of the live bacterial population than CFSE. Additionally, using 10  $\mu\text{g}/\text{ml}$  of FDA for 15 minutes resulted in the best definition of live cells, and an inverse correlation between viability and the time since the preparation of the batches ( $p=0.17$ , Spearman correlation). Finally, the homogeneity between the samples could be assessed using an FSC-W vs FSC-A plot with a logarithmic scale. Singlets were more than 80% of the events in all samples. This study demonstrated that FACS provides effective tools for detecting live mycobacteria in BCGi and for assessing its homogeneity. It showed potential improvements that could be implemented for quality control of BCGi produced at INPB-ANLIS.

#### P4 POSTERS

FECHA Y HORA: 19/11 16:00-17:00 H  
COORDINADORES: RUIZ MARÍA LAURA,  
VILLASVERDE MARCELA SOLANGE,  
CALLERO MARIANA

### 324. 156 GLYCOSYLATED 4-METHYLEMBELLIFERONE EXHIBITS ANTITUMOR EFFECT IN EXPERIMENTAL COLORECTAL CANCER

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Colorectal cancer (CRC) remains as a second cause in cancer-related deaths. Also, rapidly shifts to diagnosis at a younger age in a more advanced stage. Drug glycosylation enhances pharmacokinetic properties, improves bioavailability, and reduces toxicity, making it a promising alternative for cancer treatments. We have previously reported that coumarin 4-methylumbelliferone (4Mu) can affect tumor cell growth and modulate the tumor microenvironment

leading to an antitumor response across various cancer types. We enzymatically synthesized a glycosylated derivative of 4Mu, named 4MuR. This study aimed to assess the anti-tumor properties of 4MuR in experimental colorectal cancer models. Our *in vitro* results showed that the glycosylated drug significantly affects cell viability. CRC murine cell line CT26 cells exhibited an  $\text{IC}_{50}$  value of  $688.7 \pm 6 \mu\text{M}$  for 4Mu and  $309.6 \pm 6 \mu\text{M}$  for 4MuR. Notably, 4MuR induced cellular death at a concentration of 0.1  $\mu\text{M}$ , whereas 4Mu-treated cells showed resistance. Further analysis through apoptotic assays revealed that 4MuR induced apoptosis in CT26 cells more effectively than 4Mu ( $p<0.05$ ). At a higher concentration of 500  $\mu\text{M}$ , 81.2 % of 4MuR-treated cells were apoptotic compared to 10.7 % in 4Mu-treated cells. To assess the *in vivo* anti-tumor efficacy of 4MuR, BALB/c mice ( $n=20$ , two independent experiments) were subcutaneously inoculated with CT26 cells. Treatments started on day 9 post-inoculation (with tumor volumes of  $\sim 60 \text{ mm}^3$ ) via oral administration (200 mg/kg). Remarkably, 4MuR significantly reduced tumor growth compared to control and 4Mu-treated mice ( $p<0.01$ ). Moreover, histological analysis of tumor tissue revealed necrotic areas and mononuclear cell infiltrates in the 4MuR-treated animals with no damage in other organs. In summary, our findings suggest that 4MuR holds potential as a therapeutic agent for CRC treatment.

### 325. 221 EFFECT OF TYPE-I INTERFERONS (IFNs) ON PANCREATIC TUMOR CELL MIGRATION IN RESPONSE TO GEMCITABINE

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive, fast-growing type of cancer. PDAC has an extremely poor prognosis because of its insensitivity to current therapy. The invasiveness of PDAC is due to the epithelial-mesenchymal transition (EMT), a process induced by different molecules that are modulated, including matrix metalloproteinases (MMPs). Gemcitabine (Gem) is the most frequently agent used for PDAC treatment. Type-I IFNs have shown to possess antitumoral properties. We investigated the role of Gem,  $\text{IFN}\alpha$  and  $\text{IFN}\beta$  in pancreatic tumor cells migration. We studied cell migration by wound-healing assays. MIAPaCa-2 and PANC-1 cells were seeded in 96-wells plate until confluence. Monolayer was cut with a p200 tip, and cells were incubated with treatments of 1 mg/ml Gem and 1000 UI/ml IFNs. We took photographs at 0, 24 and 48 h at the same wound area. The organization and location of actine filaments in cells were studied with a Phalloidin-Alexa 546 staining after 24 h of Gem and IFNs treatments. The activity of MMPs was evaluated by gelatin zymography. The MMPs were collected from supernatants of both cell lines upon 24 h of Gem and IFNs treatments. The activity of MMP2 and MMP9 was calculated as the percentage of densitometry values of each band. Results show that the treatments with  $\text{IFN}\beta$ , alone and with Gem, have a greater tendency to decrease cell migration. Furthermore, the zymography results show that the activity of MMPs decreased with IFNs and Gem treatments. MIAPaCa-2 cells show that the activity of MMPs decreased in a 5% with  $\text{IFN}\beta$  treatment, but the combination with Gem reverses that decline ( $p<0.05$ ). PANC-1 cells show the combination of IFNs plus Gem does not reverse it. To conclude, our results suggest that the combination of  $\text{IFN}\beta$  and Gem is an effective treatment for inhib-

iting cell migration. Therefore, IFN $\beta$  combined with Gem could be a potential alternative therapy for PDAC.

**326. 258 SMALL CD3+CD81+ EXTRACELLULAR VESICLES RELEASED BY STARVATION PROMOTE AUTOPHAGY IN A PARACRINE FASHION THROUGH THE FOXO3A PATHWAY IN PANCREATIC CANCER CELLS**

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Pancreatic ductal adenocarcinoma is an extremely aggressive cancer without an effective treatment and scarce life expectancy. Pancreatic tumors are usually to be in a highly desmoplastic and hypovascularized microenvironment. Then it is important to understand the ability of pancreatic cancer cells to resist the habitual oxygen and nutrient limiting condition. Consequently, autophagy is an important cell response program to deal with these adverse conditions. Moreover, extracellular vesicles (EVs) are gaining exponential relevance in intercellular communications and the coordinated behavior of cell populations. With the objective of improving our knowledge of pancreatic cancer cells response to poor nutrient settings we submitted Panc-1 cell cultures to starvation. We found that under starvation EVs-associated tetraspanins, CD9, CD63 and CD81, mobilize towards domains-like structures into the plasma membrane of pancreatic cancer cells. Accordingly, a specific increase of small EVs (sEVs) is observed in the starved cells. Interestingly, the sEVs from starved cells, but not those from cells at basal condition, strongly induce the autophagy pathway even in cultures with optimal nutritional media. Furthermore, this ability to induce autophagy is specific to CD63/CD81 double positive sEVs and are effective even in the non-tumoral pancreatic cell line hTER-HPNE. This sEVs-mediated autophagy seems to be intermediated, at least in part, by the FOXO3a pathway. Worth to note, although EVs release is back to basal level after 1 h of recovery post-starvation, those EVs possess a residual effector capacity of autophagy induction suggesting a quantity/quality decoupling of secreted vesicles. Our results suggest that pancreatic cancer cells under poor nutritional environments release specific sEVs which in turn activate the FOXO3a pathway and autophagy flux in recipient cells.

**327. 259 B. LACTIS INL1 IMPROVES MACROPHAGE IMMUNE FUNCTION AND MODULATES EVENTS ASSOCIATED WITH COLORECTAL CANCER CELL AGGRESSIVENESS**

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The human milk-derived strain *Bifidobacterium animalis* subsp. *lactis* INL1 (*B. lactis* INL1) (agreement UNS-UNL N°REC-1092496-2) displays properties of a novel probiotic. Previously we showed that in colorectal cancer (CRC) cells, certain probiotics modulate gene expression associated with macrophage activity and that *B. lactis* INL1 improves macrophage physiology under lipopolysaccharide (LPS) action. Herein we investigate if *B. lactis* INL1 is able to modulate: 1- the macrophage activity in an inflammatory context and 2- the interaction between macrophages and CRC cells (which are under a pro-tumor factor action). We observed that pretreatment with 5% of cell-free supernatant (CFS) from *B. lactis* INL1 for 3 h (condition that not affect cell viability) enhances the migratory and phagocytic capacity of RAW264.7 macrophages promoted by LPS exposure for 24 h. Previously we showed that parathyroid hormone-related peptide (PTHrP) is a pro-tumor factor that favors HCT116 cells aggressiveness via E-cadherin deregulation. Also, our *in silico* analysis revealed that the macrophage inhibitory cytokine GDF15 is overexpressed in HCT116 cells. In view of these findings, we next proceeded to evaluate the expression of both markers. Western blot analysis revealed that the pre-treatment of HCT116 cells with CFS from *B. lactis* INL1 followed by PTHrP exposition for 24 h reverses the changes in E-cadherin and GDF15 expression induced by PTHrP. Finally, we evaluated whether the conditioned medium from HCT116 cells pre-treated with *B. lactis* INL1 CFS (MCT-T) and exposed to PTHrP affects macrophage function. Using a viability assay, we observed that MCT-T enhanced macrophage viability compared to conditioned medium from cells under PTHrP action but untreated with probiotic. These results suggest that expression and secretion of GDF15 from tumor cells may mediate the suppressive effects of tumor conditioned medium on immune cells, and that *B. lactis* INL1 could modulate this process.

**328. 260 METRONOMIC CHEMOTHERAPY (MCT) WITH CYCLOPHOSPHAMIDE (CY) AND LOSARTAN (LOS): EFFECT ON MACROPHAGES POPULATIONS IN THE TUMOR MICROENVIRONMENT (TME)**

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Macrophages play an essential role in innate and adaptive immunity. MCT consists in the chronic administration of low doses of chemotherapeutic drugs, without prolonged drug-free periods. The administration of metronomic CY+LOS in mice bearing the triple negative M-234p mammary adenocarcinoma has shown therapeutic efficacy with low to null toxicity, inhibition of tumor growth and increase of survival rate with decrease in mitosis and Ki-67<sup>+</sup> tumor cells, increase in eosinophils and T CD8<sup>+</sup> cells numbers in TME. Also, the extracellular matrix showed less HIF-1 $\alpha$ <sup>+</sup>,  $\alpha$ SMA<sup>+</sup> cells and collagen deposit. Our aim was to determine the effect of MCT with CY+LOS on the M1, M2 and total macrophages (M $\emptyset$ ) cells populations in the TME of M-234p tumor bearing mice. Female BALB/c mice were challenged s.c. with M-234p (day 0) and on day 7 they were divided in experimental groups: **C**: Control, with no treatment; **CY**: Treated with CY 25mg/kg/day; **LOS**: Treated with LOS 150mg/kg/day; **CY+LOS**: Treated as **CY** and **LOS**. We analyzed tumor samples before treatment (days 3, 5 and 7) and in experimental groups (days 7, 14 and 21). We observed that **CY** ( $P < 0.001$ ) and **CY+LOS** ( $P < 0.0001$ ) decreased tumor growth respect to **C** group, from day 14 of treatment on. M2 cells ( $P < 0.01$ ) and M $\emptyset$  ( $P < 0.05$ ) showed an increase between days 3 and 7 and no changes were observed in the M1 population, which was lower than M2 ( $P < 0.0001$ ) during pretreatment days. In the **C** group, M2 continued to increase with respect to M1 cells on days 7, 14 and 21 ( $P < 0.0001$ ). In contrast, **CY+LOS** exhibited an opposite behavior compared to **C** group, where the M1 increased respect to the M2 cells: days 7 ( $P < 0.01$ ), 14 and 21 ( $P < 0.0001$ ). We conclude that the metronomic **CY+LOS** treatment caused: 1) inhibition of M234-p tumor growth; 2) reduction of M2 macrophages; 3) increase in M1 cells; 4) the polarization of macrophages towards antitumor M1

cells, combined with the previously analyzed modifications in the TME, would contribute to its therapeutic efficacy.

### 329. 320 EXPRESSION OF IMMUNE CHECKPOINT HUMAN LEUKOCYTE ANTIGEN G IN TUMORAL CELL LINES CULTURED IN HYPOXIC MICROENVIRONMENT

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The immune checkpoint HLA-G is a non-classical MHC class I molecule that acts as local immunosuppressor. HLA-G has restricted tissue expression in physiological conditions. However, its expression can be ectopically induced in several tumors. The quick growth of tumor cells creates a hypoxic environment where low oxygen levels lead to the accumulation of hypoxia inducible factors (HIF), which modulate gene expression of angiogenesis, cell proliferation and migration. In this context, HIF could positively modulate HLA-G expression in tumor cells. The aim of this work is to evaluate the hypoxia effect on HLA-G expression in two tumor cell lines, named JEG-3 and T47D. Both lines were incubated under chemical (with deferrioxamine 200µM, DFX) and physiological (with 1% O<sub>2</sub> incubator) hypoxia conditions. HIF and HLA-G expression were measured by western blot (WB), flow cytometry and RT-qPCR. A preliminary chromatin immunoprecipitation assay (ChIP) was done. First, hypoxia culture conditions were tested by measuring HIF-1α and -2α by WB. Both factors showed a peak expression at short incubation times (6h), in chemical and physiological hypoxia conditions. Only with DFX incubation, HIF-1α levels increased gradually in a time-dependent manner. Then, HLA-G expression was analyzed. JEG-3 is a choriocarcinoma cell line that naturally expresses HLA-G and hypoxia incubation caused an increase in mRNA levels and an up-trend at the protein level but these were not significant. In contrast, the breast tumor cell line T47D does not express HLA-G and when was cultured under hypoxia showed levels of mRNA and started to express HLA-G significantly. ChIP assay was performed only on JEG-3 and preliminary results showed that HIF-1α and -2α can interact with HLA-G promoter sequence. In conclusion, chemical and physiological hypoxia upregulated HLA-G expression levels in both tumor cell lines, showing a more notable effect in T47D than in JEG-3. Thus, HIFs could act as positive modulators.

### 330. 333 EVALUATION OF SEX DIFFERENCES IN THE PREVALENCE AND TIMING OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY AMONG COLORECTAL CANCER PATIENTS TREATED WITH OXALIPLATIN

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Oxaliplatin is a first-line chemotherapeutic agent used in the treatment of colorectal carcinoma. Its main adverse effect is neurotoxic damage, which primarily affects peripheral nerves, resulting in a condition characterized by sensory, motor, and autonomic symptoms. The most frequent and severe symptom is neuropathic pain. To date, there are no strategies to prevent and/or reverse chemotherapy-induced neuropathy and pain (CIPN). Whether the prevalence and/or severity of this condition vary by sex remains unknown. In this study, we examined a cohort of colorectal cancer (CRC) patients (CIE #P23-016) receiving oxaliplatin treatment at Hospital Universitario Austral in 2019 and 2021. After reviewing the clinical records, we identified a total of 56 patients who met these criteria. Our study included 56 patients, comprising 30 men (54%) and 26 women (46%), aged between 25 and 84 years. While 46% of the patients received treatment in the adjuvant setting, 54% of them

received oxaliplatin as first line treatment. Treatment regimens included FOLFOX in 45% and XELOX in 55% of cases. Notably, 41 patients (73%) developed CIPN, with women being significantly more likely to develop CIPN compared to men (p=0.01). In fact, 88% of female patients manifested CIPN-related symptoms, while 60% of male patients did. Moreover, 21 patients (51%) developed CIPN during treatment and 19% post-treatment. Most male patients (55%) manifested CIPN-related symptoms both during and after chemotherapy, while most female patients (65%) manifested symptoms only during the period of administration. Our findings reveal a significant sex-related difference in CIPN prevalence, with women being more affected than men. The association between sex and CIPN underscores the need for sex-specific management and treatment strategies. Female patients may require closer monitoring and potentially more aggressive interventions to mitigate CIPN during and after chemotherapy.

## P5 POSTERS

FECHA Y HORA: 20/11/2024 11:30-12:30 H

COORDINADORES: SAHORES ANA, COLO GEORGINA, CABILLA JIMENA PAULA

### 331. 035 DNA DAMAGE: EFFECT OF GENE THERAPY IN COMBINATION WITH METFORMIN ON GLIOBLASTOMA CELLS

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Cells have complex mechanisms that monitor DNA integrity and activate repair mechanisms when there are deficiencies or errors during replication. A potential result of the damage is a permanent alteration in DNA structure that can ultimately promote cell death. The aim of our work was to demonstrate DNA damage as part of the cytotoxic mechanisms displayed by cytosine deaminase::uracil phosphoribosyl transferase/5-fluorocytosine (CDU/5-FC) suicide gene system and hIFNβ gene on U251, a human GBM cell line, alone or in combination with metformin (3mM) and the presence of proteins associated with repair mechanisms. We found that both gene therapies in combination with metformin increased DNA damage after 48 h of lipofection (Comet assay; p<0.05). By immunofluorescence assays, we evaluated the presence of histone YH2AX and observed a significant increase with both gene therapies combination with metformin. Since mammalian cells can repair DNA DSBs mainly by homologous recombination (HR, pATM) and non-homologous end joining (NHEJ, Ku80). We investigated this mechanism after 78 h of lipofection and we observed an increase in Ku80 protein in cells lipofected with suicide gene. MET potentiated its effect. In parallel, the combination of MET with the 5FU chemotherapy (the active product of CDU/5FC) reproduced these results. Finally, we evaluated the presence of pATM. We observed a significant increase in cells lipofected with suicide gene (CDU/5FC) and treated with MET. Similar results were obtained in those cells lipofected with hIFNβ gene. These results indicate that both gene therapies enhance DNA damage, increasing their efficacy as a potential treatment for GBM, which is even greater in combination with MET.

### 332. 038 FECAL MICROORGANISMS FROM CRC PATIENTS PROMOTES THE INCREASE OF CANCER STEM CELL PROPERTIES BY DIRECT ACTION IN COLORECTAL CANCER CELLS

Juliana Lourdes Bernacchia<sup>1</sup>, Alejandra Graciela Palma<sup>1</sup>, Francisco Damián Rosa<sup>1</sup>, María Florencia Quintanilla<sup>1</sup>, Juan

Nicolas Arán Bedia<sup>1</sup>, Ana Ludueña<sup>2</sup>, Adriana Noemí De Paulis<sup>3</sup>, Eugenia Bertona<sup>3</sup>, Natalia Mangieri<sup>3</sup>, Melisa Fernández<sup>3</sup>, Adrián Sambresqui<sup>4</sup>, Oscar Laudanno<sup>4</sup>, Mónica Vázquez-Levin<sup>5</sup>, María Fernanda Rubio<sup>1</sup>, Mónica Alejandra Costas<sup>1</sup>.

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We have previously shown there are signals and pathways that could be induced directly by microorganisms from human colorectal cancer (CRC) fecal samples in epithelial colorectal cells, independent of the immune system, like TEM signals, that could probably contribute to CRC development. Moreover, we have found that relative abundance of some bacteria shows differences, according to healthy or disease conditions and induce biological effects by direct action over CRC cells. In this work we investigated the direct effect of fecal CRC samples in colorectal cancer cells, over cancer stem cell (CSC) properties and identification of the most abundant bacteria in lysates of the infected monolayers. We performed experiments of infection of the human colorectal cancer HCT116 cell line with microorganisms from fecal samples from healthy (control) or CRC patients at a multiplicity of infection (MOI) 5 from the IDIM. We found that infection with CRC samples induced the increase in number (131.2%) and size of tumorspheres respect to those from healthy donors. Moreover, immunofluorescence assays of infected monolayers shown an increased expression of the CSC marker CD133 (26.4%), that is consistent with the increased nuclear translocation of the nuclear receptor coactivator RAC3 and NF- $\kappa$ B ( $p < 0.05$ ), whose association with CSC we have previously described. Using microbiological assays from lysates of infected cells, we identified 98% of the colonies were *Enterococcus faecium* for cells infected with CRC samples, while 99% were *Escherichia coli* for those infected with healthy donors. Bioinformatic analysis from Cancer Biportal of TCGA human colon adenocarcinoma samples shown that high content of *E. faecium* was related to mutations of some oncogenes like APC, TP53, TTN, KRAS, PIK3CA. In addition, a higher expression of RAC3 was associated to a higher content of *Enterococcus* ( $p < 0.05$ ). Direct action of microorganisms from CRC fecal samples contributes to increase the CSC properties.

### 333. 350 ASSOCIATION BETWEEN NUCLEAR MRP4/ABCC4 EXPRESSION PATTERNS AND DIFFERENTIATION LEVELS IN PANCREATIC CARCINOMA CELLS

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Multidrug resistance-associated protein 4 (MRP4/ABCC4) is an ATP-binding cassette (ABC) transporter predominantly localized in the plasma membrane, where it exports substrates such as cAMP, PGE2, and various chemotherapeutic agents. Its overexpression has been associated with increased cell proliferation in several cancers, including pancreatic ductal adenocarcinoma (PDAC). Previously, we reported the nuclear expression of MRP4 in PDAC tumors and carcinoma cell lines. This study aims to investigate whether the presence of MRP4 in the nuclei is associated with the degree of differentiation in PDAC cell lines, specifically in poorly differentiated (PANC1), moderately differentiated (BXPC3), and well-differentiated (HPAF-II) cells. Western blot (WB) assays were performed on en-

riched nuclear, total membrane, and cytosolic fractions from these cell lines. Additionally, immunofluorescence staining for MRP4, GAPDH, and DAPI was performed on both intact cells and isolated nuclear fractions using confocal microscopy. The WB results indicate that MRP4 is expressed in the nucleus of PANC1, BXPC3, and HPAF-II cells. Furthermore, the nuclear amount of MRP4, determined by colocalization with DAPI in individual cells, correlates with the overall expression levels of MRP4. However, confocal analysis of isolated nuclear fractions revealed distinct expression patterns: a diffuse or "cloudy" appearance in poorly differentiated cells, in contrast to a more perinuclear distribution in better-differentiated cells. These findings not only confirm the presence of MRP4 in the nuclei of PDAC cells but also suggest that its expression pattern is associated with the degree of differentiation in pancreatic cancer cells. Although the nuclear role of MRP4 remains unclear, we speculate that its perinuclear localization in differentiated cells could implicate a role in the transport of molecules, such as cAMP, across the nuclear membrane.

### 334. 351 EVALUATION OF BORON NEUTRON CAPTURE THERAPY (BNCT) IN MELANOMA AND LUNG CARCINOMA CELLS WITH A NOVEL BORONATED COMPOUND BASED ON BORONDIPYRROMETHENEDIFLUORIDE

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Introduction: BNCT is a radiotherapeutic modality based on the selective incorporation of boron to cancer cells and the nuclear reaction  $^{10}\text{B}(n,\alpha)^7\text{Li}$ . The aim of this study was to evaluate BNCT in human melanoma (A375) and lung cancer (A549) cells with a novel boronated compound (BODIPY-BSH). This compound was obtained by conjugating sodium borocaptate (BSH) to a borondipyrromethenedifluoride (BODIPY), which presents an intrinsic fluorescence/BNCT dual functionality. Methods: Cytotoxicity was determined by MTT assay. Cell incorporation of BODIPY-BSH was detected by fluorescence microscopy and intracellular boron concentration quantified by atomic emission spectrometry (ICP-AES). BNCT *in vitro* experiments were performed by irradiating cells with thermal neutrons from the RA-3 facility (CAE-CNEA) with doses 0-5 Gy. The following experimental groups were used for both A375 and A549 cells: BNCT: cells incubated with BODIPY-BSH 100  $\mu\text{M}$  for 2 h and irradiated; NT: cells irradiated with neutrons without BODIPY-BSH; C: Control cells without treatment and CB: control cells incubated with BODIPY-BSH for 2 h. Survival curves were obtained by clonogenic assay and fitted to the linear-quadratic model. Results: No cytotoxicity was found for both cell lines treated with BODIPY-BSH up to 100  $\mu\text{M}$ . Cytoplasmic incorporation of BODIPY-BSH was observed by fluorescence microscopy. Intracellular boron concentration determined by ICP-AES demonstrated a maximum at 2-4 hours of incubation. BNCT demonstrated a significant decrease of survival vs. NT. Results of survival fraction at 1 Gy were: for A375:  $0.75 \pm 0.06$  (NT) y  $0.47 \pm 0.02$  (BNCT) and for A549:  $0.51 \pm 0.09$  (NT) y  $0.28 \pm 0.01$  (BNCT). Conclusions: BODIPY-BSH is a promising agent for BNCT. Further evaluation of BNCT *in vivo* will be performed. The versatility of BODIPYs will allow the incorporation of other motifs into

the basic structure to generate multifunctional theranostic probes and active targeting to cancer cells.

### 335. 360 RAT EXPERIMENTAL MODEL FOR A RADIOLOGICAL SAFE EVALUATION OF RADIOACTIVE MICROSPHERES DEVELOPED IN CNEA

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Transarterial Radioembolization (TARE) involves a selective intra-arterial injection of microspheres (MS) into the hepatic tumor, causing local ischemia and irradiation. The National Atomic Energy Commission (CNEA) develops an yttrium-90 aluminosilicate (YAS) MS, which are currently in the preclinical research phase. For the animal model choice, it should be considered radiological risks for the operator. Before using radioactive MS, the procedure must be tested with non-radioactive MS to ensure radiological safety. Objective: to determine an experimental model suitable for the biological evaluation of radioactive MS. Materials and methods. Non-radioactive radiopaque YAS-89 MS of 25-50  $\mu\text{m}$  were used. I) vehiculation and specific activity (SA). MS were tested through a 2.8F Cook catheter and needles from 27 to 31G. Theoretical values of MS mass (mg) to be injected in rats were calculated from a range of SA (Activity/MS). II) model selection: Wistar anesthetized rats of 250 g were used, injected with 20 mg of MS in a) intra-arterial in right or left kidney, b) intra-hepatic as a bolus in an accessible liver lobe. MS distribution was verified with mammograph and TAC. III) strategy for safety injection of MS and surgery. Develop of a protective screen. Results. I) MS were easily transported through catheter and needles up to 30G. For an injection of 37MBq, 0.9-4.48 mg MS are needed for a range of SA of 2500 to 500 Bq/MS II) a) MS were detected completely filling the arterial network. The model was discarded because MS are not selectively confined. b) MS stay confined at the injection site. The right median liver lobe was chosen for its accessibility for surgery III) external radiation exposure is reduced with a 3-side 1 cm acrylic shield adapted for long laparoscopic forceps. Conclusions: MS intrahepatic injection in the right median lobe is the most suitable model, using a 1cm thick acrylic shield and long forceps to ensure radiological protection.

### 336. 462 TARGETING HISTAMINE H<sub>3</sub> RECEPTOR: A NOVEL APPROACH FOR LUNG CANCER THERAPY

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Lung cancer is the leading cause of cancer-related deaths worldwide, with non-small cell lung carcinoma (NSCLC) being the most common type. Despite therapeutic improvements, survival rates remain low. Evidence has shown the pivotal role of histamine in cancer biology. Elevated histamine H<sub>3</sub> receptor (H<sub>3</sub>R) levels were detected in NSCLC samples, correlating with poorer survival. The aim of this work was to evaluate the antitumoral properties of H<sub>3</sub>R antagonists: LINS01022, a novel piperazine compound, and Pitolisant, a first-in-class FDA-approved compound for the treatment of narcolepsy-related daytime sleepiness. Cell viability, clonogenic proliferation, cell counting, cell apoptosis (Annexin-V and TUNEL) and migration (wound-healing assay and transwell system) were assessed in human A549 adenocarcinoma and H596 adenosquamous carcinoma cells. Treatment with LINS01022 and Pitolisant produced a significant concentration-dependent inhibition of cell proliferation in A549 (IC<sub>50</sub> = 2.7 and 4.5  $\mu\text{M}$ , respectively) and H596 cells (IC<sub>50</sub> = 8.1 and 20.5  $\mu\text{M}$ , respectively). Both H<sub>3</sub>R antagonists reduced cell migration, while increasing cell apoptosis (P<0.01). *In vivo* models were developed by subcutaneous inoculation of H596 cells in nude mice. Treatment with H<sub>3</sub>R antagonists (10 mg/kg per day during 2 weeks, i.p.) produced a significant inhibition of exponential tumor growth, reducing tumor weight with respect to controls, and exhibiting a safe toxicological profile. We conclude that selective H<sub>3</sub>R antagonists offer therapeutic potentials for lung cancer treatment.

#### P6 POSTERS

FECHA Y HORA: 20/11/2024 11:30-12:30 H

COORDINADORES: LODILLINSKY CATALINA, MALVICINI MARIANA, RICO MARÍA JOSÉ

### 337. 073 ONCOLOGICAL APPLICATIONS OF CELLULOSE-DERIVED MATERIALS. STUDY OF NEW LEVOGLUCOSE-NONE DERIVATIVES ON BREAST CANCER PROGRESSION

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Breast cancer remains the leading cause of cancer-related deaths among women worldwide. Pyrolysis of soybean hulls has yielded a bio-oil rich in levoglucosenone, which exhibits significant antiproliferative activity against breast cancer cells. This compound can be used to synthesize a variety of derivatives with potentially enhanced antitumor efficacy. Notably, we have demonstrated that the derivatives: FC-IV-58A, FC-IV-59A, and BPG-III-37D induce cell death at lower IC<sub>50</sub> values compared to the original compound (4.76±2.1 $\mu\text{M}$ , 3.99±1.3 $\mu\text{M}$ , and 6.55±2.3 $\mu\text{M}$  vs. 12.23±0.5 $\mu\text{M}$ , respectively, in LM3 cells). In the present study, we evaluated the effects of these three derivatives on cell adhesiveness, metalloprotease secretion, and tumor growth. The treatment with FC-IV-59A and FC-IV-58A for 24 h significantly reduced the adhesive capacity of LM3 cells (p<0.05 vs. control, ANOVA test). In contrast, while treatment with BPG-III-37D reduced adhesion up to 50%, this difference did not reach statistical significance. Zymography revealed the presence of MMP-9 in the conditioned media. The treatment of LM3 cells with the leading compound or with FC-IV-58A and BPG-III-37D did not alter MMP-9 enzymatic activity. However, FC-IV-59A treatment resulted in a notable increase in MMP-9 secretion. This increase may be attributed to elevated cellular content released following a pronounced cell death induction. Finally, *in vivo* assays employing BALB/c mice showed that systemic administration of the compounds at doses of 4 mg/kg led to an important reduction of tumor size after 17 days. This effect



was more pronounced with compound FC-IV-59A, which exhibited the lowest IC50 values in vitro. Although further research is needed, our findings suggest that the new derivatives showed significant effects in preclinical models, both in vitro and in vivo, leading us to propose drug combination trials, which may offer enhanced efficacy compared to single-agent treatments.

**338. 201 [177Lu]Lu-PSMA 617 A POTENTIAL THERAPY FOR TRIPLE-NEGATIVE BREAST CANCER**

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**Introduction:** 177Lu-labeled prostate-specific membrane antigen (PSMA) radioligand therapy is a new treatment option for metastatic castration-resistant prostate cancer (mCRPC). PSMA is overexpressed in prostate cancer, but it is also expressed in some breast cancer tumor cells and in endothelial cells during angiogenesis. Triple negative breast cancer (TNBC) is a highly aggressive breast cancer associated with a poor prognosis and the absence of progesterone and estrogen receptors and a low HER2 expression severely limit the therapeutic possibilities. The aim of this study was to evaluate the application of [177Lu]Lu-DOTA-PSMA-617 in a biological model of triple negative breast cancer. **Method:** Female BALB/c mice between 20-25 g were used as recipients of xenografts 4T1 triple negative breast cancer cells. Biodistribution (BD) studies and therapeutic efficacy were performed. The animals were injected with 2.2 MBq of [177Lu]Lu-DOTA-PSMA-617 of specific activity 0.5 mCi/ $\mu$ g of ligand, through the tail vein. The animals were sacrificed, the organs of interest were removed, weighed and then their activity was measured with a NaI well counter. The therapeutic efficacy of [177Lu]Lu-PSMA was analyzed after intravenous administration of a single dose of [177Lu]Lu-PSMA (60 MBq) and tumor progression was monitored. **Results:** [177Lu]Lu-PSMA-617 was obtained with specific activity greater than 0.5 mCi/ $\mu$ g (19.27MBq/nmol) with RP between 95-99% (n=5). The BD studies showed a tumor/blood ratio of  $3.4 \pm 1.7$  and a tumor/kidney ratio of  $0.05 \pm 0.03$  (240 min post injection). The therapeutic studies shows significantly smaller tumor volumes in [177Lu]Lu-PSMA-617 treated animals compared to the control group. [177Lu]Lu-PSMA-617 inhibited tumor growth by 90 % (p<0.001) 10 days after treatment and 60 % (p>0.01) 16 days of treatment. **Conclusion:** This study shows a potential application of [177Lu]Lu-PSMA-617 in TNBC.

**339. 375 POTENTIAL EMPLOYMENT OF H1 ANTIHISTAMINES AS A NOVEL STRATEGY TO IMPROVE THE EFFECTIVENESS OF RADIOTHERAPY IN BREAST CANCER**

Nora Mohamad<sup>1</sup>, Tamara Galarza<sup>1</sup>, Rosa Bergoc<sup>3</sup>, Ernesto Crescenti<sup>3</sup>, Juan Perazzo<sup>3</sup>, Alicia Klecha<sup>1,2</sup>, Gabriela Martin<sup>1,2</sup>

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Radiotherapy is used after breast cancer (BC) surgery to kill remaining tumor cells. Radio-resistance can increase the risk of recurrence or metastasis. Recent research suggests that H1 antihistamines (H1R-A) may improve survival rates in BC patients and are effective in treating experimental tumors. Herein, we studied the potential use of H1R-A as a new approach that could enhance the response to radiotherapy. A bioinformatic analysis of the TCGA database (BC patients) using the R2 and cBioPortal platforms revealed that tumors with lower mRNA HRH1 levels showed a trend towards better responses for all treatments, significant in TNBC and HER+ subtypes (p=0.045). Radio-responsive tumors had lower HRH1 levels

(p=0.018) and 6 genes were differentially expressed between radio-responsive and resistant tumors (p<0.01) with one, PRKCG, related to DNA repair and linked to HRH1 signaling pathways. 5  $\mu$ M H1R-A loratadine (L) or 2Gy  $\gamma$ -irradiation halved clonogenicity (p<0.05) in TNBC MDA-MB-231 cells. Combined treatment drastically reduced colony formation vs each single treatment (p<0.01). 2Gy-induced increase in mammospheres formation, indicating an enrichment in CSC, was prevented by H1R-A (p<0.05). Female nude mice were transplanted with 2Gy-irradiated or not MDA-MB-231 tumors and received or not L 2.5 mg/kg/day p.o. for 30 days (2Gy-C-2GyL-L). Tumor growth rate was similar in C and 2Gy, lower in L and the lowest in 2GyL (p<0.05). C were poorly differentiated adenocarcinomas, L lowered such undifferentiation grade with atypical gland formation. 2Gy increased it but 2GyL reduced it vs 2Gy. L and 2GyL tumors disclosed similar % of necrotic areas to C and 2Gy respectively, though they were smaller. 2Gy tumors had the highest fibrosis and vascularization levels. All C mice exhibited marked splenomegaly but 30% of L mice and only 15% of 2Gy and 2GyL presented it (p<0.001). Research on H1R-A holds promise in creating new combination therapies to improve the efficacy of radiotherapy

**340. 398 MOLECULAR MECHANISM OF THE ANTITUMORAL EFFECT OF ALOYSIA POLYSTACHYA ON COLORECTAL CANCER CELLS**

Alejandra Graciela Palma<sup>1</sup>, Juliana Bernacchia<sup>1</sup>, Paola Di Leo Lira<sup>2</sup>, Valeria Moscatelli<sup>2</sup>, Francisco Damián Rosa<sup>1</sup>, María Florencia Quintanilla<sup>1</sup>, María Fernanda Rubio<sup>1</sup>, Daiana Retta<sup>2</sup>, Mónica Alejandra Costas<sup>1</sup>

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Several studies have demonstrated the benefit of natural products in the treatment of many diseases, and we found in a previous work that the extract of the plant *Aloysia polystachya* (AP) has a cytotoxic effect on human colorectal cancer (CRC) cells, which could be through the combination of hydrophilic and hydrophobic components. Here we investigated the possible components of AP and molecular mechanisms involved in its cytotoxic action on CRC. We analyzed the chemical composition of AP essential oil by chromatography, since we have previously observed its cytotoxic effect on human CRC HCT116 cells. We found that two of the most abundant components were thujone and carvone (10.3% and 38.5% respect to total components, respectively). Next, we decided to investigate the cytotoxicity over HCT116 cells of these AP components as well as the flavonoids catechin and quercetin that we have previously described as components of AP extract. We found that all these components induced cell death (% cytotoxicity: thujone 60%, carvone 100%, quercetin 27%, catechin 20%) at concentrations similar to that found with AP (1:8 dilution). The cell death induced by flavonoids was significantly inhibited (quercetin 0%, catechin 0%) when the coactivator RAC3 (antiapoptotic) was downregulated. In view of these results and the knowledge that quercetin is a ligand of the Aryl Hydrocarbon Receptor (AHR), overexpressed in CRC, we investigated if RAC3 is involved in AP effect through AHR, which induces pro-apoptotic signals. We observed an increment in AHR activity by reporter assay in the presence of AP and when RAC3 expression is increased, suggesting that AP stimulates AHR pathway and RAC3 is an AHR coactivator (p<0.001, Tukey test). From these results we conclude that AP cytotoxic effect over CRC cells could involve at least thujone and carvone, whose signaling remains to be investigated, and the activation of AHR by flavonoids or other components where RAC3 could be an AHR coactivator.

**341. 402 MOLECULAR MECHANISM LINKING MAGE-A9 EXPRESSION TO POOR PROGNOSIS IN HEAD AND NECK CANCER**

Melisa Suberbordes<sup>(1)</sup>, Valeria Torralba Agu<sup>(1)</sup>, Micaela Escalada<sup>(1)</sup>, Candela Vidal<sup>(1)</sup>, Ana Raimondi<sup>(2)</sup>, Martin Monte<sup>(1)</sup>.

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MAGE-A9 belongs to MAGE type I genes (Melanoma Antigen Genes) and it is therefore expressed in germ cells and silenced in normal somatic tissues with an abnormal re-expression in tumor tissues of different origins. MAGE-A9 expression is strongly associated with poor prognosis, including head and neck cancer. However, little is known about the molecular mechanism governing this output. Previously, we showed for the first time that MAGE-A9 inhibits the transcriptional activity of the oncosuppressor p53 without altering its protein levels and probably through HDAC2 and/ or TRIM28 interactions. To better understand the mechanisms underlying MAGE-A9 action, we explored the MAGE-A9 interactome obtained from two different human cell lines. Analysis of significant and top ranked interactors confirmed in both cell lines, suggest that MAGE-A9 interactors could be associated with DNA repair and damage response, Wnt/ $\beta$ -Catenin signaling, and the epithelial-mesenchymal transition process. Selected interactors were MAGE-A9 specific and did not interact with other MAGE-I proteins. To verify whether MAGE-A9 could be involved in these processes, we first generated a biological model consisting of human head and neck cancer cell lines (HN12) genetically modified to express doxycycline-inducible MAGE-A9 protein. We started studying the Wnt/ $\beta$ -Catenin signaling. We observed that MAGE-A9 expression correlates with enhanced transcriptional activity of  $\beta$ -Catenin by assessing its transcriptional targets cMyc, N-Cadherin and VEGF mRNA through RT-qPCR. In addition, the expression of MAGE-A9 resulted in increased  $\beta$ -Catenin protein levels as observed in co-expression experiments. Finally, doxycycline-induced MAGE-A9 in HN12 cells caused an increase in cell migration detected by wound-healing assay. We observed that wound closure was doubled in MAGE-A9-expressing cells compared to the control. These results suggest that MAGE-A9 could be involved in the regulation of Wnt/ $\beta$ -Catenin signaling as part of a specific oncogenic function, and it could explain, at least in part, MAGE-A9 association to poor prognosis in head and neck cancer. Research on MAGE-A9 expression in head and neck cancer is an ongoing project in our laboratory and is aimed to continue working on this hypothesis.

**342. 418 OPTIMISING METHODS FOR HUMAN THYROID PRIMARY CELL CULTURE ISOLATION**

Valentina Arenal<sup>1</sup>, Clara Madsen<sup>1</sup>, Lucía Fernández Chávez<sup>1</sup>, Exequiel Gonzalo Alonso<sup>1</sup>, Pamela Pichel<sup>2</sup>, Sergio Recio<sup>2</sup>, María Eugenia Fermento<sup>1</sup>, Eliana Noelia Alonso<sup>1</sup>, María Julia Ferronato<sup>1</sup>, María Marta Facchinetti<sup>1</sup>, Alejandro Carlos Curino<sup>1</sup> and Georgina Pamela Coló<sup>1</sup>.

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According to the GLOBOCAN 2020 database on cancer incidence and mortality, thyroid cancer (TC) has the ninth-highest cancer incidence worldwide. Basic research contributes to developing diagnostic and treatment alternatives. Cell models play a crucial role *in vitro* study of the thyroid. Permanent thyroid cell lines widely used in laboratory research typically originate from tumors. However, there is a need to compare these tumor cells with cells originating from normal thyroid tissue. Primary cell lines are invaluable for exploring cancer biology and investigating novel treatments. This study aims to optimize an isolation protocol of human primary thyroid cells from histologically non-tumor, tumor, and metastasis tissues. Primary cultures are laborious to obtain and then difficult to maintain in culture. We tried five different methods for isolating primary cells that combine mechanical disaggregation and enzymatic digestion. These methods use distinct enzymatic compositions, incubation times, and mechanical approaches, including centrifugation. We have arrived at a simple, rapid, and effective method to culture cells from thyroid biopsies for subsequent studies. Our previous results have revealed that GEF-H1 plays a pro-tumor role in different tumoral cell lines. We investigate the expression of GEF-H1, which belongs to

the RhoA-GTPase activator family, in primary cells. Using immunofluorescence staining, we observed significantly higher GEF-H1 protein expression in cytokeratin-19 positive thyroid carcinoma cells compared to normal thyroid cells ( $p < 0.0001$ ). Our results propose a cheap and easy isolation thyroid primary cells protocol and suggest that GEF-H1 could be used as a potential tumor biomarker in TC.

**P7 POSTERS**

FECHA Y HORA: 20/11/2024 11:30-12:30 H

COORDINADORES: CARDAMA GEORGINA,  
YANEFF AGUSTÍN

**343. 023 MECHANISMS OF TNF-INDUCED LIPOLYSIS IN ADIPOCYTES AND THE INHIBITORY ROLE OF NCOA3**

Francisco Damián Rosa, Sebastián Gutierrez, Juliana Bernacchia, María Florencia Quintanilla, Alejandra Graciela Palma, Mónica Alejandra Costas, María Fernanda Rubio  
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In previous research, we observed higher levels of TNF in the mammary tumour microenvironment of patients compared to that of benign lesions. Although it is well known that TNF induces lipolysis in 3T3-L1 cell line differentiated to adipocytes (3T3ad), the underlying signalling mechanisms remain unclear. The aim of this study is to elucidate the pathway through which TNF or T47D breast cancer cells conditioned medium (CM) activates lipolysis in adipocytes. To this end, 3T3ad were incubated with TNF or CM, in the presence of sulfasalazine (S), an NF- $\kappa$ B inhibitor, or SP600125 (SP), a JNK inhibitor. Only combined treatment was able to reverse the increased in glycerol secretion induced by TNF or CM measured by colorimetric assay (S+SP+CM:  $0.8 \pm 0.2$ , S+SP+TNF:  $1.1 \pm 0.2$ , S+SP:  $1.4 \pm 0.3$ , B:  $1.0 \pm 0.1$ ). Given that NCoA3 is a coactivator of NF- $\kappa$ B, we transfected 3T3 cells with a plasmid expressing a short hairpin RNA targeting NCoA3 (sh) to assess its role in lipolysis. Interestingly, reducing its expression enhanced TNF- or CM-induced glycerol secretion (shB:  $4.1 \pm 0.1$ , shTNF:  $5.6 \pm 0.1$ , shCM:  $8.9 \pm 0.1$ , wtB:  $1.0 \pm 0.2$ ;  $p < 0.05$  respect to wtB; shTNF or shCM  $p < 0.05$  respect to shB). To confirm these findings, we transfected 3T3 cells with plasmids overexpressing a full length (FL)- or a nuclear localisation-deficient-NCoA3 ( $\Delta$ ns). NCoA3 overexpression completely inhibited TNF-induced glycerol secretion (FL TNF:  $1.3 \pm 0.4$ ,  $\Delta$ nsTNF:  $1.3 \pm 0.3$ , wtB:  $1.0 \pm 0.3$ ; TNF:  $2 \pm 0.1$   $p < 0.05$  respect to B), whereas it only partially inhibited CM-induced secretion (FL CM:  $2.2 \pm 0.4$ ,  $\Delta$ nsCM:  $3.1 \pm 0.3$ , CM:  $3.7 \pm 0.1$ , B:  $1.0 \pm 0.3$ ;  $p < 0.05$  respect to B; FL CM  $p < 0.05$  respect to CM). In conclusion, our study demonstrates that simultaneous inhibition of NF- $\kappa$ B and JNK pathway is necessary to prevent TNF- or CM-induced lipolysis. In addition, NCoA3 plays an inhibitory role in lipolysis, blocking the TNF-induced pathway and partially inhibiting the pathway activated by CM, therefore the activation pathway is not identical to that of TNF.

**344. 191 EFFECTS OF A YERBA MATE EXTRACT ON SPONTANEOUS DISSEMINATION AND COLONIZATION OF LEWIS LUNG CARCINOMA CELLS IN SYNGENEIC C57BL/6 MICE**

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Yerba mate (*Ilex paraguariensis*) is a plant widely distributed throughout South America. Over the past decade, our group has characterized a Yerba Mate extract (YMe) and demonstrated its antitumor properties in breast and colon cancer using *in vitro* and *in vivo* models. Based on these findings, we further investigated the effects of the YMe in lung cancer, which currently ranks among

the cancers with the highest incidence and mortality rates in both men and women. *In vitro* evaluation resulted in a negative modulation of YMe on cell proliferation, adhesion and migration, which are central cellular events linked to tumor growth and progression. Considering the results observed *in vitro*, the aim of this study was to evaluate the effects of YMe on spontaneous dissemination and tumor cells colonization of Lewis lung carcinoma. For this purpose, YMe or maltodextrin (vehicle of the extract formulation) were administered to female C57BL/6 mice during four weeks prior to tumor cells inoculation and the treatment was extended until the end of the experiment. The animals were injected subcutaneously in their right flank with  $3 \times 10^5$  LL/2 cells. Routine measurements of the mice body weight and tumor size were performed. No statistical differences were found in the volume of primary tumors between the experimental groups. Notably, the incidence, defined as the number of mice with superficial pulmonary nodules relative to the total tumor-bearing animals, was significantly reduced ( $p < 0.001$ ) in the YMe-treated group (20%) compared with the vehicle group (80%). In addition, a significant decrease in both the total amount and size of pulmonary nodules per mouse was observed. These results were confirmed by histopathological evaluation. In conclusion, the findings of this study indicate that YMe effectively inhibits pulmonary dissemination and colonization of lung cancer cells. Future studies will focus on elucidating the underlying mechanisms responsible for these observed effects.

#### 345. 254 EFFECT OF THE RUNX2 INHIBITOR CADD522 IN HUMAN BREAST CANCER MODELS

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FGFR inhibitors have been used to treat endocrine resistant breast cancer. However, many patients remain refractory to these treatments. We have demonstrated previously an interactive loop between FGFR2 and RUNX2 and that RUNX2 overexpression generates resistance to both endocrine and FGFR inhibitors treatments. Thus, we are interested in evaluating the possibility of using RUNX2 inhibitors to treat endocrine resistant tumors. We have already shown that the RUNX2 inhibitor CADD522 (CAD) reduced the proliferation of T47D cells with a constitutively activated FGFR2 (FGFR2CA) or overexpressing RUNX2 *in vitro*. The specific aim of this study was to investigate the effect of CAD *in vivo* using the T47D breast cancer models. T47D-Control (C) and RUNX2-overexpressing (RUNX2) cells were sc injected into NSG mice. Once tumors reached a size of 20-30 mm<sup>2</sup>, CAD (10 mg/kg) was administered intraperitoneally (3 times/week for 3 weeks). CAD significantly inhibited tumor growth in T47D-Control tumors ( $p < 0.0001$  C vs CAD), CAD-treated control tumors showed a reduction in tumor weight ( $p < 0.01$  Control vs CAD) and increased stromal remodeling compared to untreated controls. In contrast, RUNX2-overexpressing tumors were resistant to CAD therapy and all mice bearing these tumors developed lung metastasis. Our findings suggest that T47D-RUNX2 tumors may require higher concentrations of CAD for their inhibition, highlighting the important role of RUNX2 in tumor growth regulation. We propose that the T47D-R2CA model, which exhibits constitutively active FGFR2 and physiologically elevated RUNX2 levels, may be more suitable for further CAD efficacy studies. Finally, our results highlight the potential of CAD as a complementary treatment to standard hormone therapy in luminal breast cancer. High RUNX2 expression could serve as a predictive factor for therapy outcomes and may be associated with a more aggressive breast cancer phenotype.

#### 346. 305 NATIONAL PRE-CLINICAL IMAGING LABORATORY (LANAIP-CNEA): A NON-INVASIVE TECHNOLOGY FOR BIOMEDICAL RESEARCH IN LINE WITH THE 3R'S PRINCIPLES IN ANIMAL EXPERIMENTATION

Andrea Monti Hughes<sup>1,2</sup>, Yago Dores Dores<sup>1</sup>, Lucila Rogulich<sup>1</sup>, Agustina M. Portu<sup>1,2</sup>, Verónica A. Trivillin<sup>1,2</sup>, Clarisa Lopez Bularte<sup>1</sup>; Gustavo A. Santa Cruz<sup>1</sup>, Emiliano C.C. Pozzi<sup>1</sup>

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Imaging technologies are an important tool in biomedical research. They allow a non-invasive *in vivo* study of biochemical and biological processes, anatomical, physiological and functional parameters (blood flow, tissue permeability, etc.), molecular and cellular processes in real time. Some of the pathologies that get the most of this technology are cancer, cardiovascular, neurodegenerative, musculoskeletal diseases. From an ethical point of view, in line with the "3Rs" ethical principles in animal experimentation, this technology provides more information from a smaller number of animals, increasing the statistical power of the data and reducing the level of experimental variation. The National Pre-Clinical Imaging Laboratory (LANAIP) was created within the framework of the Research and Development activities in human health that are carried out by the National Atomic Energy Commission (CNEA) and with the aim of enhancing knowledge and applications that ultimately will contribute to nuclear medicine and radiotherapy. Its mission is to support the development of scientific protocols in cancer and human diseases in general, by providing high-resolution preclinical images to national laboratories. The LANAIP is equipped with a multimodal ALBIRA Si platform (Bruker), with the potential to combine PET (Positron Emission Tomography), SPECT (Single Photon Emission Computed Tomography) and CT (Computed Tomography). The first images obtained from <sup>18</sup>F-DG PET/CT in the Hamster cheek pouch oral cancer model, <sup>18</sup>F-DG PET/CT Diffuse lung metastases model in BDIX rats and <sup>99m</sup>Tc-MDP SPECT/CT in Balb/c mice were obtained this year. All animals were anesthetized with isoflurane and recovered completely without negative effects. With these results, Albira imaging equipment has been successfully installed, moving the project to an operative step. The licensing procedures have been recently approved by the Nuclear Regulatory Authority (ARN).

#### 347. 337 METRONOMIC CHEMOTHERAPY (MCT) WITH CYCLOPHOSPHAMIDE (CY) + LOSARTAN (LOS) ACHIEVES BETTER THERAPEUTIC EFFECT AND LESS TOXICITY THAN MAXIMUM TOLERATED DOSE (MTD) WITH CY IN M-234p TRIPLE NEGATIVE MAMMARY ADENOCARCINOMA BEARING MICE

Soto, Eva<sup>1</sup>; López, María del Pilar<sup>1</sup>; Masson, Agnes<sup>1</sup>; Rozados, Viviana R.<sup>1,2</sup>; Scharovsky O. Graciela<sup>1,2</sup>; María José Rico<sup>1,2</sup>; Leandro E. Mainetti<sup>1,2</sup>

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Triple-negative breast cancer (TNBC), characterized by the absence of estrogen (ER) and progesterone (PR) receptors, and low expression of human epidermal growth factor receptor 2 (HER2), exhibits high aggressiveness, risk of metastasis, and mortality. Conventional cancer therapy has reached a plateau of therapeutic efficacy, prompting the development of new strategies. Metronomic chemotherapy (MCT) proposes that low doses of chemotherapeutic drugs, administered chronically, reduce tumor growth, resistance, and drug-induced toxicity. MTD, though effective in tumor reduction, often results in increased tumor resistance and significant drug toxicity. We aimed to evaluate the effectiveness of MCT with CY+LOS compared to conventional chemotherapy MTD with CY, in tumor growth, metastases, white blood cell count (WBC), and survival. With this purpose, BALB/c mice were inoculated s.c. with M-234p (n=40). When the tumor was palpable, the experimental groups were assembled: CONTROL (no treatment), CY (25mg/kg/day), LOS (200mg/kg/day), CY+LOS (combined), and MTD (150 mg/kg/day on day 0 and 100mg/kg/day on days 2 and 4, followed by a resting period of 14 days, till new cycle). MCT was administered in the drinking water while MTD was given i.p. Tumor growth on day 27, differed significantly from CONTROL, with CY+LOS (P=0.0351) and MTD (P=0.0097). Median survival was CY+LOS (25 days), CONTROL (16 days), and MTD (12 days). Normal WBC count ranges from 2,000 to 10,000 WBC/mm<sup>3</sup>. At euthanasia, CY+LOS maintained WBC under normal parameters (median;

range=5,163;1,150-12,333), whereas **MTD** had a decreased WBC (median; range=700;600-13,700). No metastases were observed in **MCT** groups, while lung metastases were present in 37.5% of the animals in the **MTD** group. In conclusion, compared to MTD, metronomic with **CY+LOS**: 1) maintains WBC within normal limits, reducing the toxicity associated with conventional chemotherapy; 2) decreases metastasis development; 3) extends survival time.

### 348. 489 BASAL CHEMORESISTANCE OF STROMAL CELLS AND THEIR ROLE IN OSTEOSARCOMA PROGRESSION

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Osteosarcoma (OS) is the most common malignant bone tumor, with a stagnant five-year survival of 15-30% for patients with lung metastases. Metastases drastically reduce survival and complicate treatment, underscoring the need for improved therapies. Doxorubicin (Dox) is used in OS chemotherapy. Our previous results showed that the fibroblast secretome modulates the response to Dox in metastatic (LM7) and non-metastatic (SAOS2) OS cells, enhancing drug sensitivity. This prompted us to investigate the role of non-cancer stromal cells in chemotherapy, focusing on the basal chemoresistance of fibroblasts, as part of a comprehensive screening of the bidirectional communication between stromal and tumor cells. We determined the IC50 of fibroblasts against Dox. Fibroblasts treated with Dox for 2, 24 and 48 hs showed no significant changes in viability ( $4,52 \times 10^{-6}$  mM  $\pm$   $2,6 \times 10^{-6}$  mM,  $6,10 \times 10^{-6}$  mM  $\pm$   $3,6 \times 10^{-6}$  mM,  $7,06 \times 10^{-7}$  mM  $\pm$   $5,3 \times 10^{-7}$  mM, respectively). The higher IC50 values were observed at 2 and 24 hs, with minimal values at 48 hs. The early values show a trend that could be linked to a dynamic response of fibroblasts to Dox, possibly related to adaptive mechanisms of cell resistance. The IC50 value at 48 hs suggests a trend towards increased sensitivity to Dox over time. However, the maximum IC50 values for fibroblasts remain lower than those for OS tumor cells, indicating that stromal cells have a lower basal chemoresistance compared to tumor cells, which have IC50 values that are two orders of magnitude higher). Interestingly, when tumor cells were challenged to Dox while being conditioned by fibroblasts, the IC50 decreased by 5,24 times and 3,96 times in non-metastatic and metastatic OS cells, respectively. This would be indicative of a mechanism involving horizontal transference of biological properties from the stromal cell compartment to the tumor cells. These results contribute to understanding the role of stromal cells in cancer niches and to improve therapy design.

### 349. 497 NATURAL NAPHTHOQUINONES AND SESQUITERPENE LACTONES: EFFECT ON PANCREATIC DUCTAL ADENOCARCINOMA CELLS

Nadia T. Mirakian<sup>2</sup>; Viviana Pulido<sup>1</sup>, Lucy E. Bonilla<sup>1</sup>, Maximiliano Díaz<sup>1</sup>, Adriana Carlucci<sup>5,6</sup>; Valeria P. Sülzen<sup>2,3</sup>; Daniela L. Papademetrio<sup>1,4</sup>.

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Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease with an increasing global incidence, ranking in Argentina the 6<sup>th</sup> position. It remains the most difficult cancer to deal with. Treat-

ment with gemcitabine increases the average survival of patients by just 6.7 months. Achillin, a sesquiterpene lactone, and lapachol and  $\beta$ -lapachone, two natural naphthoquinones, have shown anti-inflammatory and antitumor properties. The aim of this study was to evaluate the cytotoxic effect of the three compounds in two PDAC cell lines, PANC-1 and MIAPaCa-2. The effect of the compounds was evaluated by assessing the metabolic activity by XTT assay, the cell proliferation by BrdU incorporation assay and the cell death by Hoechst 33342/propidium iodide staining, after 72 hours of treatment. Lapachol was able to induce a percentage of death of  $41.88 \pm 18.97$  on PANC-1 cells at a dose of  $396,4 \mu\text{M}$  ( $p < 0.001$ ), and  $23.90 \pm 2.85$  on MIAPaCa-2 cells at a dose of  $138,8 \mu\text{M}$  ( $p < 0.001$ ). In both cell lines, the inhibition of proliferation and the decrease of metabolic activity reached 80%, in a dose-dependent manner ( $p < 0.001$ ). Achillin induced complete inhibition of metabolic activity and cell proliferation at a dose of  $1500 \mu\text{M}$ , achieving 100% of cell death by treatment with  $2000 \mu\text{M}$  ( $p < 0.001$ ).  $\beta$ -lapachone also induced a complete inhibition of cell proliferation and a decrease in metabolic activity but at lower doses, reaching 100% of cell death at  $8 \mu\text{M}$ . However, the response appears to be dose independent, exhibiting all-or-none behavior. Our results show that achillin and  $\beta$ -lapachone present greater effect than lapachol on PDAC cells. However,  $\beta$ -lapachone would have a narrow range of therapeutic index. In conclusion, achillin and lapachol could be considered candidates for further studies in the search for new treatments for clinical use.

## P8 POSTERS

FECHA Y HORA: 20/11/2024 16:10-17:10 H

COORDINADORES: MENACHO MARQUEZ MAURICIO ARIEL

### 350. 010 GLIOBLASTOMA CELL MIGRATION IS BLOCKED BY THE COMBINATION OF GLUCOSE METABOLISM INHIBITORS AND METFORMIN

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Glioblastoma (GBM), the most common primary malignant brain tumor in adults, is a highly vascular and infiltrative tumor. GBM cell migration has been related to an exacerbated activity of the VEGF/HIF1 $\alpha$  axis. In addition, GBM is a highly metabolic neoplasia. The aim of the present work was to explore the antimigratory effects of metformin (MET, antidiabetic drug, OXPHOS inhibitor), 2-deoxyglucose (2DG, HK inhibitor) and 6-aminonicotinamide (6AN, G6PDH inhibitor) on U251 cell line. Cells were seeded over coverslips; upon confluency a scratch was made using a tip. Cells were treated with 1 mM 2DG, 5 mM MET and 25  $\mu\text{M}$  6AN or a combination of them. The rate of wound closure was monitored before 24 h under a microscope. Images were analyzed by the Image J software. The combination of MET/6AN significantly decreased GBM migration ( $p < 0.05$  vs control). We also evaluated the migratory capacity of 3D cultures. GBM spheroids were developed by the hanging drop method, grown for 2 days and reseeded over ordinary (adherent) wells. After 24 h of treatments, the outgrowth was calculated as the migratory area. From 3D structures, both combinatory approaches MET/2DG and MET/6AN decreased migratory capacity ( $p < 0.05$ , vs control). To better understand the mechanism involved in these results we investigated the VEGF/HIF1 $\alpha$  axis by Elisa (48 h, supernatants) and WB (24 h, whole cell lysates) respectively. After treatment, the combination of MET/6AN significantly decreased the presence of VEGF ( $p < 0.05$ , respect to control) and HIF1 $\alpha$  protein of GBM

cells ( $p < 0.05$ , vs control). We hypothesize that these effects could also impair angiogenesis, thus reinforcing its anti GBM potential. In summary, the combination of glucose metabolic inhibitors and MET impairs the migration of GBM cells probably by affecting the VEGF/HIF1 $\alpha$  pathway. Altogether, the results reported here support further studies to investigate the potential use of this metabolic modulation approach in a clinical setting.

**351. 033 A NOVEL PENICILLIN DERIVATIVE INHIBITS THE NF- $\kappa$ B PATHWAY IN DABRAFENIB-SENSITIVE AND RESISTANT MELANOMA CELLS**

Sofia Bajicoff<sup>1</sup>, Mercedes Debernardi<sup>2</sup>, Florencia Cayro<sup>2</sup>, Camila Chocan<sup>1</sup>, Marcela Villaverde<sup>3</sup>, Carina M.L. Delpiccolo<sup>4</sup>, Ernesto G. Mata<sup>4</sup>, Viviana C. Blank<sup>1</sup>, Leonor P. Roguin<sup>1</sup>

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We have previously shown that downregulation of  $\beta$ -catenin levels in melanoma cells is one of the mechanisms involved in the antitumor activity of the penicillin derivative TAP7f. In this work, we decided to explore whether TAP7f modulates NF- $\kappa$ B pathway in melanoma cells sensitive (A375S) and resistant (A375R) to the BRAF inhibitor dabrafenib. After incubating cells with a 20  $\mu$ M concentration of TAP7f for different times, Western blot assays revealed that the penicillin derivative increased I $\kappa$ B expression levels in A375S ( $1.38 \pm 0.15$ ,  $p < 0.001$ ) and A375R ( $1.33 \pm 0.18$ ,  $p < 0.001$ ) cells after 30 min of incubation. Consequently, a significant reduction of I $\kappa$ B phosphorylation was observed in both cell lines after 30 min (A375S:  $0.37 \pm 0.16$ ,  $p < 0.001$ ; A375R:  $0.76 \pm 0.14$ ,  $p < 0.01$ ). We next studied the effect of TAP7f on the phosphorylation of the p65 subunit of NF- $\kappa$ B, which is known to be related to the activation of the pathway. We found that TAP7f decreased p-p65 levels after 15 and 30 min of incubation in both cell lines ( $p < 0.001$ ). We also demonstrated that TAP7f, after 24 h of treatment, reduced the expression levels of downstream proteins regulated by NF- $\kappa$ B, such as Bcl-2 and Bcl-xL, in A375S and A375R cells. Furthermore, qPCR assays showed that TAP7f decreased mRNA levels of Bcl-2, IL6, IL1 and TNF alpha, all NF- $\kappa$ B target genes, in the melanoma cells studied. Additionally, we found that Bay 11-7082, an NF- $\kappa$ B inhibitor, inhibited cell migration in a similar manner to TAP7f, supporting the involvement of the NF- $\kappa$ B pathway in the migration process. Taken together, our results revealed that TAP7f, in addition to regulating Wnt/ $\beta$  catenin pathway, also inhibits the activation of NF- $\kappa$ B, which plays a pro-tumorigenic role in several malignant tumours. Based on these findings, the penicillin derivative TAP7f could be considered a promising candidate for the treatment of melanoma cells, regardless of their resistance level to BRAF inhibitors.

**352. 133 BMP7 PROMOTES CISPLATIN CHEMORESISTANCE OF CERVICAL CANCER CELLS THROUGH THE PI3K/AKT SIGNALING PATHWAY**

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Chemotherapy with cisplatin is one of the standard treatments for patients with advanced or recurrent cervical cancer (CC). However, chemoresistance can develop, compromising its efficacy in clinical practice. We previously observed an indirect role of bone morphogenetic protein 7 (BMP7) in cisplatin chemoresistance and in processes associated with cell plasticity in CC by acting on endothelial cells.

In this work, the objective was to investigate a direct role of BMP7 in chemoresistance to cisplatin in CC and the molecular mechanisms involved. First, we evaluated the viability of both control HeLa cells and HeLa cells overexpressing BMP7, obtained via CRISPR/dCas9 technology, when exposed to different concentrations of cisplatin using the neutral red assay. Compared to control cells, BMP7-overexpressing HeLa cells exhibited higher viability under increasing concentrations of cisplatin and a higher half-maximal inhibitory concentration (IC50) of cisplatin. These results were confirmed by trypan blue dye exclusion testing. Then, we investigated the impact of this cytokine on markers involved in apoptosis and cell cycle regulation modulated by cisplatin using Western Blot analysis. We found that overexpression of BMP7 reverses the PARP cleavage, the decrease in cyclin D1 expression, and the Akt phosphorylation induced by cisplatin in CC cells. An increase in Akt phosphorylation levels was further observed in BMP7-overexpressing cells. Finally, we employed a trypan blue assay to determine the effect of LY294002, a phosphatidylinositol-3-kinase (PI3K) inhibitor, which is a key enzyme involved in the activation of Akt, on the cell viability of CC cells. LY294002 reversed the increase in cell viability induced by BMP7 under cisplatin treatment. Taken together, these results provide evidence that BMP7 plays a significant role in cisplatin resistance in cervical cancer cells by influencing the PI3K/Akt pathway.

**353. 164 DIFFERENCES IN THE DNA DAMAGE RESPONSE OF ANAPLASTIC THYROID CANCER CELLS TREATED BY DIFFERENT IONIZING RADIATION THERAPIES**

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**Background:** Anaplastic thyroid carcinoma (ATC) is an aggressive malignancy with poor response to conventional treatments. Disease recurrence and radioresistance to various radiotherapeutic modalities including low- and high-linear energy transmission (LET), X-rays and boron neutron capture therapy (BNCT), have been described. Different signaling pathways are involved in the response to radiation-induced DNA damage (RDD), such as the TGF $\beta$ /Smad or nuclear Sirtuins/NAD<sup>+</sup>, which maintain genomic stability. **Objective:** This study aims to compare the RDD induced by different ionizing radiation therapies and explore the underlying mechanisms of radioresistance in ATC cells. **Materials and methods:** Human ATC cells (8505C) were divided into the four groups: 1) Control (untreated); 2) X-rays; 3) NCT (neutron thermal irradiation); and 4) BNCT (irradiated with thermal neutrons and boron-10). Cells were irradiated in the RA-3 nuclear reactor (neutron flux =  $5.10^9$  n/cm<sup>2</sup>/sec) or in an X-ray accelerator, with physical doses ranging from 1 to 5 Gy. The following studies were performed: clonogenic assay, micronucleus formation in binucleated cells, gene expression of DDR-related proteins (RT-qPCR), and COX-2 protein expression (immunocytochemistry). **Results:** BNCT showed a decrease in cell survival at 1 and 3 Gy ( $p < 0.001$ ) and an increase in micronucleus formation at 3 Gy. At 2 h post-irradiation, BNCT induced higher mRNA levels of Atr ( $p < 0.05$ ), Tgf- $\beta$  ( $p < 0.001$ ), rTgf- $\beta$ 1 ( $p < 0.001$ ), and Sirt1 ( $p < 0.01$ ) compared to X-rays. Conversely, X-ray treatment significantly increased the expression of Atm ( $p < 0.01$ ) and Smad 2 ( $p < 0.05$ ) relative to BNCT and NCT. Additionally, BNCT also increased Cox-2 gene expression ( $p < 0.001$ ), correlated with higher protein levels at 48 h. **Conclusion:** The DDR and radioresistance mechanisms are different for each radiotherapy highlighting the potential for combining ionizing radiation with targeted inhibitory drugs to enhance treatment efficacy.

**354. 256 THE TREATMENT WITH A HER-INHIBITOR DECREASES STEMNESS IN TRIPLE-NEGATIVE BREAST CANCER CELL LINES AND INDUCES GROWTH INHIBITION IN DIFFERENT TUMOR MODELS**

Diego Javier Brites Neira<sup>1</sup>, Andrés Bechis<sup>1</sup>, Lizeth Aixa Ariza Bareño<sup>1</sup>, Aldana Magaly Schey<sup>1</sup>, Luciana Cañonero<sup>1</sup>, Alejandro Jorge Urteger<sup>1</sup>, Laura Beatriz Todaro<sup>1</sup>.

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The human epidermal growth factor receptor (HER) family comprises tyrosine kinase receptors that play critical roles in breast and gastric cancer development. Lapatinib is a dual tyrosine kinase inhibitor (TKI) that targets HER1 and HER2 by binding to the ATP-binding site of their intracellular domains. Currently, Lapatinib is used in combination with capecitabine in advanced HER2-positive breast cancer that progressed following standard treatments that include anthracyclines, taxanes and trastuzumab, as well as in postmenopausal patients where hormonal therapy is indicated. Previously, we have identified that HER2 is expressed in the cancer stem cell (CSC) subpopulation of several triple-negative and HER2-negative breast cancer cell lines. In this study we proposed: 1) evaluate the effect of HER inhibition in various human and murine tumor models and in their CSC population, and 2) elucidate the mechanisms underlying Lapatinib's effects. Our findings revealed that Lapatinib increased cytotoxicity, leading to significant oncospheres growth inhibition in HS578T and LM38LP, triple-negative breast cancer cell lines. Furthermore, this treatment reduced cell viability in multiple breast cancer models, including human (HS578T) and murine (4T1, LM38-LP) triple-negative and HER2-positive (BT-474) cell lines. Similar effects were observed in human hepatocellular carcinoma (HUH7) and prostate cancer (PC3) cell lines. Finally, we compared the effect of Lapatinib with those of Trastuzumab (monoclonal antibody against HER2) and Cetuximab (monoclonal antibody against EGFR) in several breast cancer cell lines mentioned above. While Lapatinib and Cetuximab both significantly reduced cell viability, Trastuzumab showed no effect. Our findings, along with previous results, highlight the potential of Lapatinib repositioning as a therapeutic option, beyond HER2-positive breast cancer.

**355. 325 BORON NEUTRON CAPTURE THERAPY + OLIGO-FUCOIDAN SEAWEED EXTRACT: RADIOTOXICITY AND TUMOR CONTROL STUDIES IN AN *IN VIVO* EXPERIMENTAL ORAL CANCER MODEL**

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Boron Neutron Capture Therapy (BNCT) is a tumor selective particle radiotherapy that combines selective tumor uptake of <sup>10</sup>B compounds and neutron irradiation. In the hamster cheek pouch oral cancer model, we previously demonstrated the therapeutic effect of BNCT mediated by boronophenylalanine (BPA). To improve tu-

mor control and reduce radiotoxicity, the aim of this study was to combine Oligo-Fucoidan with BNCT. Oligo-Fucoidan is a sulfated polysaccharide isolated from *Laminaria japonica*, with anticancer and inflammatory properties. Hamsters were cancerized with DMBA in mineral oil (0.5%) twice a week during 12 weeks. Animals with tumors were subjected to (GROUP 1) BPA/BNCT at 2.6 Gy precancerous tissue (n= 7 animals), (GROUP 2) BPA/BNCT+Oligo-Fucoidan 2.6 Gy (n= 4 animals), (GROUP 3) BPA/BNCT 1.8 Gy (n= 3 animals), (GROUP 4) BPA/BNCT+Oligo-Fucoidan 1.8 Gy (n= 5 animals). Topical application of Oligo-Fucoidan (200 mg/kg) was performed during 14 days before BNCT and 14 days after BNCT. The clinical status of the animals, tumor control and radiotoxicity in terms of mucositis in precancerous tissue were evaluated at 7, 10, 14, 21 and 28 days after BNCT. In the 2.6 Gy experiment, no radioprotective effects of Oligo-Fucoidan were observed, as 86% (GROUP 1) and 100% (GROUP 2) of the animals exhibited severe mucositis after BNCT. Both groups exhibited similar and high percentages of tumor overall responses (83% and 79% respectively). Based on previous studies in which we demonstrated that Oligo-Fucoidan increased significantly tumor absolute boron uptake, in this study, we decided to lower the absorbed dose to precancerous tissue (1.8 Gy) in order to reduce radiotoxicity. We observed that Oligo-Fucoidan tended to reduce the percentage of animals with severe mucositis from 67% to 20%. Overall tumor response in both groups is currently under evaluation. Oligo-Fucoidan could be a potential radioprotector when combined with BPA/BNCT for the treatment of oral cancer.

**P9 POSTERS**

FECHA Y HORA: 20/11/2024 16:10-17:10 H

COORDINADORES: FISZMAN GABRIEL

**356. 009 THE ROLE OF FERROPTOSIS IN RADIATION-INDUCED CELL DEATH BY VALPROIC ACID ON ANAPLASTIC THYROID CANCER**

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Anaplastic thyroid cancer (ATC) is one of the most aggressive malignancies in humans. Novel strategies to control it, like hypofractionated radiotherapy or radiotherapy combined with chemotherapy, are therefore necessary. Valproic acid (VA) is a clinically available anti-convulsant and histone deacetylase inhibitor. Furthermore, VA radiosensitizes different cancer cell lines, including thyroid ones. This study aimed to evaluate the effect of VA on cellular radiosensitivity and the participation of ferroptosis in this process in ATC cell lines. **Methods:** KTC-2 and C643 cell lines were incubated with VA (1 mM) and cell viability was measured by MTT method. Radiosensitization was determined by clonogenic assay. Ferroptosis was determined by intracellular reactive oxygen species (ROS) levels measured by the fluorescent dye 2',7',-dichlorofluorescein-diacetate (DCFH-DA); BODIPY 581/591 C-11 was used to detect membrane lipid peroxides. Ferroptosis marker PTGS2 expression was evaluated by qPCR. Finally, clonogenic assay with ferroptosis inhibitor Ferrostatin-1 (Ferr-1) was performed in KTC-2 cells. **Results:** VA incubation decreased cell survival in radioresistant KTC-2 cells (p<0.001 for 2 Gy and p<0.05 for 3,5 Gy vs. control). ROS levels increased in both irradiated cell lines, with and without VA (p<0.05). VA also increased lipid peroxides values compared to irradiated only cells at 24 hours (3.5 Gy, p<0.001) and 48 hours (2 Gy, p<0.05) post-irradiation in KTC-2 cells. PTGS2 gene expression was up-regulated in irradiated cells at 24 hours in both cell lines (p<0.05). Clonogenic assay with Ferr-1 showed recovery of cell survival in cells irradiated alone at a dose of 1 Gy (p<0.01), and 2 Gy in cells treated with VA and irradiated (p<0.01). **Conclusion:** Gamma radiation could induce ferropto-

sis in ATC cells. Also, VA radiosensitized KTC-2 cells and ferroptosis could be one mechanism involved in this process.

**357. 129 CHARACTERIZATION OF SPHERICAL AND VIRUS-LIKE MESOPOROUS SILICA NANOPARTICLES MODIFIED WITH A SILICON(IV) PHTHALOCYANINE FOR PHOTODYNAMIC THERAPY**

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Phthalocyanines are well-known as effective photosensitizers for photodynamic therapy. In this work, we evaluated the phototoxic properties of the lipophilic tetra-*tert*-butyl silicon(IV) phthalocyanine dihydroxide (SiPc) covalently bound to synthesized spherical and virus-like mesoporous silica nanoparticles (SSN and VMSN) in murine colon carcinoma CT26 cells. The hydrodynamic diameters of both nanoparticles, determined by dynamic light scattering, were similar (SSN=194±43 nm; VMSN=219±31 nm). In order to identify the optimal photosensitizer-vehicle formulation, nanoparticles were modified with different surface concentrations of SiPc, ranging from 5 to 50 µmol/g. Although aggregation of SiPc bound to the surface of SSN was detected from 18 µmol/g, the monomeric state was preserved with VMSN, even at the highest concentration. However, the stability of the modified nanoparticles suspensions decreased at SiPc concentrations above 30 µmol/g. When CT26 cells were incubated with both SiPc-SSN and SiPc-VMSN complexes, no effect on cell viability was evident in the dark, while a significant decrease was shown after irradiation with 0.68 Jcm<sup>-2</sup>. The phototoxic action was independent of the SiPc surface concentration, but the amount of nanoparticles required decreased as the SiPc density increased. On the other hand, nanoparticle morphology affected SiPc phototoxicity, since IC<sub>50</sub> value of 2.8±0.3 µM obtained with SSN was improved to 0.4±0.1 µM when the photosensitizer was bound to VMSN at the same density (18 µmol/g). In addition, the cellular uptake of SiPc conjugated with VMSN was 7.5-fold higher compared to SSN, as revealed by fluorescence detection. In conclusion, these results demonstrated that while both types of silica nanoparticles were efficient vehicles to obtain a potent silicon(IV) photosensitizer, SiPc phototoxic action and cellular uptake in CT26 cells were significantly enhanced with VMSN, being the optimal modification density of 18 µmol/g.

**358. 134 ROLE OF BMP7 IN VASCULOGENIC MIMICRY FORMATION IN CERVICAL CANCER**

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Vasculogenic mimicry (VM) is a process in which aggressive cancer cells mimic endothelial cells to form blood vessel-like structures, allowing tumors to obtain nutrients and grow independently of tumor angiogenesis. This characteristic is associated with poor prognosis in various cancers, including cervical cancer (CC). We previously observed an indirect role of bone morphogenetic protein 7 (BMP7)

in VM and in chemoresistance to cisplatin in CC by acting on endothelial cells. This work aimed to evaluate the direct role of BMP7 in the formation of VM in CC. Initially, we generated CC-derived HeLa cells with BMP7 overexpression via CRISPR/dCas9 activation system and confirmed the efficiency of overexpression by qRT-PCR and Western blot. Subsequently, using qRT-PCR, we observed that BMP7-overexpressing HeLa cells increase mRNA levels of VM-associated markers, such as VE-cadherin, EphA2, and vimentin. Furthermore, BMP7 overexpression also promoted the formation of tube-like structure in CC cells. The elucidation of BMP7 regulation in this process is crucial and of great interest for understanding the various roles of this cytokine in CC. Researching VM in CC is vital for identifying new therapeutic targets and improving patient outcomes.

**359. 180 BRAF MUTATIONS POSITIVELY CORRELATE WITH ACSL4 AND COX-2 EXPRESSION IN COLORECTAL CANCER**

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Transition from adenoma to adenocarcinoma in colorectal cancer (CRC) is associated with an increase in mutations in key genes such as APC, KRAS, TP53, and SMAD4. In addition, BRAF mutations and microsatellite instability are implicated in colorectal carcinogenesis in around 30% of patients. In this context, enzymes ACSL4 and COX-2 –known to participate in tumor progression in other cancer types including breast, prostate, and head and neck cancer– also show an increase in CRC transition. Indeed, the accumulation of mutations in APC, KRAS, and TP53 is associated with higher ACSL4 expression. In this context, this study explores the impact of BRAF mutations on ACSL4 and COX-2 expression and their relationship with tumor progression. Bioinformatic analyses of primary CRC samples revealed that tumors with BRAF mutations had higher ACSL4 and COX-2 expression (p<0.05). Further studies were conducted in HT-29 cells (BRAFV600E, overexpressing ACSL4 and COX-2) treated with BRAF mutation inhibitors vemurafenib and dabrafenib to determine ACSL4 and COX-2 expression by Western blot, and in combination with ACSL4 inhibitor PRGL493 to determine cell proliferation through MTT assays. Results indicate that BRAF mutation inhibition reduces ACSL4 expression and blocks COX-2 expression. In addition, BRAF mutation inhibition (vemurafenib 0.5µM, dabrafenib 1µM) combined with ACSL4 inhibition (PRGL493 1µM) showed a synergistic effect on cell proliferation as compared to single treatment (p<0.01). These findings suggest that BRAF mutations may positively correlate with ACSL4 and COX-2 expression and activity in CRC.

**360. 277 POTENTIAL EFFECT OF OLIGO-FUCOIDAN AND BORON NEUTRON CAPTURE THERAPY ON SALIVA PRODUCTION IN HAMSTERS**

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Boron Neutron Capture Therapy (BNCT) is a tumor selective particle radiotherapy that combines preferential tumor uptake of  $^{10}\text{B}$  compounds and neutron irradiation. Mucositis is a common adverse effect induced by BNCT. As saliva is a natural mucoprotector, increasing saliva in the mouth would mitigate mucositis. In this study, we evaluated saliva production in non-cancerized hamsters and animals exposed to oral carcinogenesis. We also assessed Oligo-Fucoïdan (O-Fuco) and BNCT+/-O-Fuco effect on saliva production and mucositis in tumor bearing animals. O-Fuco is a seaweed extract, with anti-tumoral and anti-inflammatory effects. Saliva production was measured during 10 min with endodontic absorbent cones placed inside the hamster's mouth. (GROUP 1) Non-cancerized hamsters at T0, T6, T8, T12, T14, T16, T18 weeks. (GROUP 2) During the oral cancerization protocol (DMBA in mineral oil -0.5%- twice a week, 12 weeks), same time-points as in GROUP 1. (GROUP 3) Idem GROUP 2+O-Fuco (200 mg/kg). (GROUP 4 and 5) Tumor bearing animals exposed to BPA/BNCT+/- O-Fuco (applied during 14 days before and 14 days after BNCT), followed during 28 days. Saliva production in GROUP 1 was independent of sex and age (5.4 to 6.7 mg, 20 animals). In GROUP 2 (10 animals), saliva production tended to reduce from 5.2±2.7mg (T0) to 3.2±1.9mg (T8). After the end of the cancerization protocol, saliva production was similar or higher than in GROUP 1, apparently correlating with tumor development (8.8±4.6mg, T18). In GROUP 3 (10 animals), O-Fuco tended to increase saliva production in all evaluated time-points vs GROUP 2. When comparing GROUPS 4 and 5, at 7 days after BNCT, O-Fuco tended to increase saliva production from 5.5±2.9mg to 8.3±1.3mg and reduce the % of animals with severe mucositis at 10-14 days (20%, 5 animals vs 67%, 3 animals). We reported a tendency of O-Fuco to increase saliva production in cancerized animals, together with a reduction in severe mucositis after BNCT.

### 361. 286 CHARACTERIZATION OF AN INVASIVE ORAL CANCER MODEL IN HAMSTERS TO STUDY BORON NEUTRON CAPTURE THERAPY

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Oral tumors are the most common disease in Head and Neck cancer. Surgery is a conventional treatment, however, sometimes could be mutilant or tumors could not be resected completely. Boron Neutron Capture Therapy (BNCT) is a tumor selective particle radiotherapy that combines preferential tumor uptake of  $^{10}\text{B}$  compounds

and neutron irradiation. We demonstrated the BNCT therapeutic effect in non-invasive, exophytic tumors in the hamster cheek pouch. Previous studies showed that an incision in these tumors could promote invasion of epithelial cells into the underlying connective tissue (invasive oral cancer model). In this study we standardized and characterized this invasive model to study BNCT. We described 3 groups: (A) 2 incisions (14 days apart), 28 days (d) follow up (n= 3 animals), (B) 1 incision, 28 d (n= 6), (C) 1 incision, 14 d (n= 6). Incision was made perpendicularly to the pouch, in sufficient depth to reach the underlying connective tissue (under anesthesia and analgesia). We evaluated animal death and welfare, being the humane end-point to euthanize the animals weight loss  $\geq 20\%$  (WL20%) and tumor volume  $> 1000 \text{ mm}^3$ . At the end of the follow up we assessed tumor re-growth vs T0 (tumor remnant) and, microscopically, tumor cell invasion in connective tissue (H&E). Almost no deaths were reported (A: 0%, B: 17%, C: 5%). Group B exhibited higher, but not significant, percentage of animals with WL20% (40%) compared to A (0%) and C (11%). No complete remissions were observed and tumor invasion in connective tissue was detected (A: 3/3 animals, B: 5/6, C: 4/6). The average of tumor re-growth in A was higher than in B and C (490±542%, 173±98%, 154±115%, respectively). Based on these results and considering that the number of incisions (2 vs 1) and longer times of evaluation (28 days vs 14 days) could affect negatively the animal clinical status and welfare (at the time of irradiation and after BNCT), we decided to continue our future studies with Group C.

### P10 POSTERS

FECHA Y HORA: 21/11/2024 11:00-12:00 H

COORDINADORES: ROJAS PAOLA, PÉREZ PIÑERO CECILIA

### 362. 020 EFFECT OF NICOTINE ON THE RESPONSE OF TRIPLE NEGATIVE BREAST CANCER CELLS TO METRONOMIC THERAPY. NITRIC OXIDE SYNTHASE PARTICIPATION

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Breast cancer is the type with the highest incidence and mortality in women. The Triple Negative (TNBC) subtype has a poor prognosis and metronomic chemotherapy (MC) treatment combining low doses of carbachol (Carb) and paclitaxel (PX) has reported promising results. However, it is not known whether chronic nicotine (NIC) would modulate this effect. Since breast tumor tissue expresses nicotinic receptors and NIC has been associated with resistance to conventional oncological treatment, the aim of this work is to evaluate the signaling pathways involved in the effect of MC in the absence or presence of NIC in TNBC MDA-MB231 cells. By MTT assays we determined that NIC at a concentration similar to that of the smoking patients' plasma (10-7M) increased cell viability (basal: 100±12.7%; NIC: 147.8±13.1%). Treatment with MC (PX10-8M+Carb10-11M) decreased cell viability (MC: 67.6±1.8%) and the presence of NIC did not reduce the effectiveness of the treatment (MC+NIC: 76.4±4.7%). The effect of MC was due to a mechanism dependent on PLC, PKC, Ras, MEK and NF- $\kappa$ B, since its selective inhibitors reduced the effect (105.1±9.8%; 93.5±10.5%; 91.8±12.3%; 90.9±10.1% and 90.0±11.0% respectively). These values did not vary significantly when cells are treated in presence of NIC. Since the mediators involved in the effect of MC are associated with the increase of activation/expression of nitric oxide synthase (NOS), using selective inhibitors we determined the NOS2 and NOS3 isoforms participation, which produce an increase of 114% in nitric oxide (NO) level that would be at least partially responsible for the increased levels of apoptosis determined. These results indicate that in TNBC MDA-MB231 cells, MC exerts its proapoptotic antitumor effect by increasing the NO levels produced by the NOS2 and NOS3



isoforms in the presence or absence of NIC, highlighting the benefits of MC in these conditions.

**363. 193 CULTURE OF CANCER STEM CELLS IN MICRODEVICES FROM VETERINARY TUMOR SAMPLES AS PREDICTIVE ASSAY**

Silvia Gómez<sup>1</sup>, Matías Tellado<sup>3</sup>, Gina Layedra<sup>2</sup>, Betiana Lerner<sup>2</sup>, Maximiliano Pérez<sup>2</sup>, Denise Belgorosky<sup>1\*</sup> & Ana María Eiján<sup>1\*</sup>

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The sphere formation (SF) technique within Lab-On-a-Chip Microdevices (MDs) enables the isolation and quantification of cancer stem cells (CSC) using minimal sample volumes. These CSC populations are linked to treatment resistance, and tumor recurrences. Establishing of cell lines derived from primary tumors, could be valuable in predicting patients' outcomes and treatment responses. MDs provide a miniaturized platform for isolating and monitoring CSC. **Objective:** To develop an assay within MDs to study CSC therapeutic responses, to create a predictive test for patient treatment responses. **Methods:** Four veterinary tumor samples (solid thyroid carcinoma (TCA), thyroid sarcoma (TS), forelimb sarcoma (FS) and mammary adenoma (MA)) were obtained, cultured in 2D and in low adhesion conditions forming spheres. A limiting dilution analysis (ELDA) to determine CSC frequency and sphere forming efficiency were used. **Results:** Three tumor were successfully established as 2D cell lines, and all samples were able to grow as spheres. ELDA analysis revealed the estimated number of CSC increases in relation to tumor aggressiveness (TCA1/726, TS1/626, FS1/811, MA1/1395). In the TCA sample, cell viability was assessed under carboplatin and an inhibitory dose of 150 µM (580 mg/kg) was determined ( $p < 0.0001$ ). With this dose, a SF assay was conducted within MDs to evaluate the response to treatment; however, no reduction in the number of spheres was observed. Notably, this dose was higher than what is typically applied in veterinary medical practice (300 mg/kg), suggesting a potentially sub-optimal treatment response. Despite the treatment, patient died 6 months after surgery due to lung metastasis. The successful growth of CSC from different tumor types in MDs, along with the increase in their CSC estimated number, could be related to the degree of tumor aggressiveness. This method may serve as a valuable tool for analyzing and predicting individual patient responses to treatment.

**364. 206 UNLOCKING THE POTENTIAL: HOW COPPER(II) METALLODRUGS WITH DIPEPTIDES COULD REVOLUTIONIZE BREAST CANCER TREATMENT**

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Several copper(II)-phenanthroline complexes have been studied in both *in vitro* and *in vivo* models, showing that their anticancer activity is exerted through ROS accumulation which causes mitochondrial dysfunction, DNA damage, and apoptosis induction. Some of these derivatives have been selected as candidates for Phase I clinical trials. One of the challenges today is to search for new metal drugs that minimize adverse effects and toxicity is a challenge nowadays. We compared the effect of a copper(II) complex with tetramethylphenanthroline [CuCl<sub>2</sub>(tmp)] (1) with the ternary counterpart with a dipeptide (Alanine and Phenylalanine) [Cu(Ala-Phe)(tmp)]·4H<sub>2</sub>O (2) in human breast cancer cells (MCF-7 cell line) in 2D and 3D models.

These complexes demonstrated an antiproliferative effect with IC<sub>50</sub> values of  $1.8 \pm 0.27$  and  $2.2 \pm 0.35$  µM in the monolayer model and values of around 50-60 µM in MCF-7 spheroid (preliminary data). Both compounds affected colony formation in a dose-dependent manner from 1.5 to 10 µM, with a total inhibition at 2 µM for 2 and 5 µM for 1 ( $p < 0.005$ ). Moreover, ROS levels increased at 1 µM of both complexes. However, exogenous antioxidant scavenger NAC or a mixture of vitamins C and E could not recover cell viability, indicating that ROS production is unrelated to cell injury. Additionally, 1 induced early apoptosis and necrosis, while 2 only induced cell death by apoptosis ( $p < 0.01$ ). The comet assay did not disclose any direct DNA damage. Nevertheless, the complexes interfere with tumor growth by inhibiting the Na<sup>+</sup>/H<sup>+</sup> exchanger isoform1 (NHE1) ( $p < 0.05$  and  $p < 0.001$  for 1 and 2, respectively), although 2 reduces migratory capacity (wound healing assay) and could decrease MMP-9 activity (preliminary data). Overall, we conclude that adding Ala-Phe dipeptide improves the antitumor action in copper(II) phenanthroline complexes.

**365. 243 DIFFERENT POPULATIONS OF EXTRACELLULAR VESICLES EXERT DISTINCT EFFECTS ON THE ACTIVITY OF NK CELLS**

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive disease that induces immunotolerance where extracellular vesicles (EVs) act as intercellular messengers, carrying molecules from tumors to immune cells. EVs can be classified by size as small (sEVs), medium (mEVs), and large (lEVs). With different pathways involved in their biogenesis, EVs are expected to have different effects on immune cells. The aim of this work was to investigate the impact of EVs released by PANC-1 cells treated with gemcitabine (Gem), IFNα2b, and IFNβ on NK cell activity. l/m/sEVs were obtained from differential centrifugation of PANC-1 cells supernatants treated with 1mg/ml Gem, 1000 UI/ml IFNα2b or IFNβ for 2h. For characterization, EVs were captured with magnetic beads conjugated to anti-CD9. The expression of CD63 was performed by flow cytometry. NK cells from Buffy-Coats of healthy donors (RESCD-2024-468-UBA-DCT-FFYB) were purified by magnetic negative selection. Memory-like phenotype was induced by IL12/IL15/IL18. NK cytotoxicity was evaluated by CFSE/PI stain on K562 cells in a ratio of 5:1 (NK:K562). IFNγ release was assessed in supernatants of cytotoxicity assays by ELISA. Our results demonstrated that expression of CD63 in CD9<sup>+</sup>-sEVs-IFNβ decreased 36% ( $p < 0.01$ ), suggesting that treatment of PANC-1 cells with IFNβ affects the biogenesis of sEVs. l/mEVs obtained by culturing PANC-1 cells under basal conditions increased the activity of NK cells meanwhile sEVs diminished it, as well as l/sEVs-Gem and sEVs-IFNα2b. The decrease in IFNγ release accompanied these results. Moreover, all populations of EVs decreased the cytotoxicity of memory-like NK cells ( $p < 0.01$ ). The results suggest that pancreatic tumor cells condition NK cell function through the cargo of the extracellular vesicles they release. Furthermore, chemotherapy might trigger resistance mechanisms in tumor cells that negatively impact immune surveillance.

**366. 299 CHARACTERIZATION OF A NOVEL M46 TRIPLE NEGATIVE BREAST CANCER MURINE PRECLINICAL MODEL**

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Breast cancer (BC) is the most common tumor in women worldwide. However, the availability of preclinical murine models for BC research is limited. In this study, a novel M46 BC murine model that spontaneously developed in a BALB/c female at the Instituto Roffo was analyzed. **Objective:** To characterize the immunophenotypic subtype of the M46 tumor and its clinical progression to establish a new valuable model for preclinical research. **Methods:** The tumor was subcutaneously transplanted into female and male BALB/c mice using a trocar needle. Tumor growth was measured with a caliper. Blood was drawn before and after transplantation to evaluate the leukocyte formula. Anatomy and immunohistopathological tumor classification was performed. Lung and liver macrometastases were quantified under 10X magnification. **Results:** The anatomicopathological diagnosis indicates that M46 is an undifferentiated high-grade invasive tumor (GIII), with a Nottingham Score of 8. It was negative for estrogen (ER), progesterone (PR) and Her2 receptors, as well as for GATA3, CK7, Cromogranin, and Synaptophysin markers. The tumor was positive for SOX10 (90%), with a 60% of Ki67+ cells. Twenty days after inoculation, animals were sacrificed. Tumor volume was 445 mm<sup>3</sup> (IQR: 188.5-1239) in females and 425 mm<sup>3</sup> (IQR: 248.5-1055) in males. Incidence of lung metastasis was 100%, with a median of 12 (IQR: 8-69) in females and 18 (IQR: 6-65) in males per lung. Incidence of liver metastasis was 50% in both sexes. Tumor-induced leukocytosis was observed (p<0.0001) with variation in the leukocyte formula (an increase in neutrophils and a decrease in lymphocytes). No significant differences were found between the sexes regarding tumor size, growth kinetics, incidence or number of metastases, leukocytosis, or variations in the leukocyte formula. These results demonstrate that the M46 preclinical model represents a novel and promising tool for the study of TN BC, potentially valuable for future research.

**367. 416 THE ANTIHELMINTIC FLUBENDAZOLE AS AN ANTI-TUMOR DRUG IN BREAST CANCER. EFFECTS ON EXTRACELLULAR MATRIX REMODELING**

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Breast cancer (BC) is one of the most frequently diagnosed cancers and the leading cause of cancer death among women worldwide. Despite early diagnosis and treatment, recurrence and metastasis remain major challenges. Flubendazole (Flu), a benzimidazole used as a safe and effective anthelmintic, has shown potential in inhibiting cell growth in leukemia and intestinal cancer. The present study analyzes the effects of Flu on BC extracellular matrix remodeling associated with cell migration, using two BC cell lines: MDA-MB-231, with a less differentiated phenotype and MCF-7, differentiated and less invasive. Cytotoxicity was assessed by MTT after 48h; morphological changes linked to epithelial dysfunction were analyzed by fluorescence microscopy after phalloidin-FITC and Hoechst stain-

ing, with or without Flu (0.5 and 2.5 µM). Migration was assessed by wound healing assay, while extracellular matrix remodeling was evaluated by zymography for Metalloproteinase (MMP) activity and molecular biology techniques for biglycan expression. 1 µM Flu reduced viability by 50% in both cell lines. 0.5 µM Flu arrested cell cycle at the G2/M phase (p<0.0001 for MDA-231; p<0.001 for MCF-7 vs control), accompanied by morphological changes and an increase in the number of apoptotic cells (8% in MDA-231 and 7.2% in MCF-7). Migration inhibition with 0,5uM Flu was significant in MDA-231 cells (p<0.001 vs control) but not in MCF-7. Regarding extracellular matrix remodeling, Flu decreased MMP-2 activity (p<0.01 vs control) and reduced biglycan expression, only in MDA-231 cells. Our results show a differential effect of Flu on MDA-231 cells, suggesting an increased efficacy against less differentiated cells. Given Flu's poor solubility and low bioavailability, a nanotechnology delivery platform is needed to develop Flu as a potential breast cancer therapy.

\*Same contribution

**P11 POSTERS**

FECHA Y HORA: 21/11/2024 11:00-12:00 H

COORDINADORES: NOVARO VIRGINIA, DE LUCA PAOLA, MAINETTI LEANDRO

**368. 027 iNOS-MEDIATED NITRIC OXIDE MAINTAINS THE STEM NICHE IN GLIOBLASTOMA AND THEIR INHIBITION SENSITIZES RESISTANT CELLS TO TEMOZOLOMIDE**

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Instituto de Oncología Ángel H. Roffo

Glioblastoma (GBM, WHO grade 4) has a poor survival rate despite treatment (temozolomide, TMZ), hence the importance of developing new therapeutic strategies. Inducible nitric oxide synthase (iNOS), a nitric oxide (NO) producer, could be a therapeutic target as NO is linked to cancer stem cell (CSC) maintenance, responsible for therapy resistance. **OBJECTIVE:** To analyse TMZ effects in combination with the iNOS inhibitor S-methylisothiourea (SMT) on cell viability, migration, and CSC on GBM cell lines (GL26, LN229). **METHODS:** Two approaches were used: simultaneous (Sim) and sequential (Seq). Viability (2D, by MTS): Sim 5 days(d) TMZ+SMT, Seq 4d TMZ followed by 2d TMZ+SMT (4d+2d). Vimentin (Vim) and E-cadherin (Ecad) expression (qPCR). Migration: wound healing. Spheroids (3D): diameter monitoring, Sim 25d, Seq 17d+4d. Spheres (3D, high dilution): CSC frequency (F) by ELDA, sphere forming efficiency (SFE), Sim 10d, Seq 4d+2d. **RESULTS:** In 2D TMZ alone decreased viability (p<0.05), while SMT did not in both cell lines. Seq approach decreased it (p<0.05), while Sim had similar effect compared to TMZ (LN229). Migration decreased with TMZ (LN229 p<0.0001, GL26 p<0.01), SMT (GL26 p<0.01) and Seq (LN229 vs TMZ p<0.0001, vs SMT p<0.0001; GL26 vs TMZ p<0.05, vs SMT p<0.05). Vim decreased with individual treatments (p<0.05), while E-cad increased with SMT (p<0.05), without been affected by Sim nor Seq. Spheroids diameter was not affected by SMT, decreased with TMZ (LN229 p<0.001; GL26 p<0.001) and with combined approaches (GL26 Sim p<0.05, LN229 Seq p<0.001). SMT reduced the CSCF compared to control (LN229 p<0.001). Sim and Seq presented similar results. **CONCLUSION:** iNOS inhibition affects a pluripotent minority cell population, that appears to be TMZ resistant, suggesting that iNOS expression/NO production could be involved in maintaining this resistant subpopulation. Therefore, its inhibition could be a promising therapeutic target to enhance the response to TMZ.

**369. 253 INTERACTION OF T4 AND E2 ON MAMMARY CARCINOGENESIS (EX VIVO AND IN VITRO)**

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Hypothyroidism seems to be a protective factor against breast cancer (BCa) though long-term exposure or high doses of thyroxine (T4) may increase BCa risk. Our previous studies indicated that hypothyroidism prolonged the latency, reduced the incidence, and retarded the growth of tumors in rats, and that T4 may influence mammary carcinogenesis by interacting with other hormone pathways. In this study, our aim was to investigate the biological impact of T4 alone or combined with estradiol (E2) on mammary tumors of hypo- and euthyroid rats, as well as human BCa cell lines. Female Sprague-Dawley rats were treated with a single dose of dimethylbenzanthracene and were divided into two groups: hypothyroidism (HypoT; 0.01% 6-N-propyl-2-thiouracil -PTU- in drinking water, n = 20) and EUT (untreated control, n = 20). At sacrifice, tumor explants were obtained and treated either with 10<sup>-9</sup> M T4, E2, or a combination, or DMEM/F12 only as control for 15 min to evaluate intracellular signaling pathways, and after 24 h to evaluate changes in the expression of proteins related to apoptosis and proliferation by immunohistochemistry and Western Blot. In vitro, MCF-7 cells were treated with the same treatments. We assessed cell proliferation via MTT assays, and cell viability using Trypan Blue and the expression of proteins related to proliferation and apoptosis by immunocytochemistry and Western Blot. In HypoT tumor, T4+E2 stimulated proliferation, as evidenced by increased expression of Ki67 and mitotic/apoptotic index. In addition, suppressed apoptotic processes, as indicated by reduced expression of caspases 3 and PARP. In vitro, T4 induced MCF7 cell proliferation (p=0.012), viability (p=0.025) after 24h treatment. T4+E2 also enhanced the proliferation (p= 0.013) and viability of MCF-7 after 48 h, and activated the pERK/ERK pathway similarly to T4 alone (p < 0.05). In conclusion, T4 tends to enhance the action of E2 in proliferation and the effect of their interaction depends on the thyroid status.

**370. 271 NORCANTHARIDINE: A PROMISING NATURAL THERAPY FOR TRIPLE-NEGATIVE BREAST CANCER**

Lizeth Aixa Ariza Bareño, Aldana M. Schey, Diego Britz Neira, Andrés Bechis, Luciana Cañonero, Laura B. Todaro, Alejandro J. Urteger.

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Triple negative breast cancer (TNBC) is a highly aggressive subtype characterized by the absence of estrogen and progesterone receptors, as well as the lack of HER2 overexpression. The absence of specific targeted therapies, underscores the need to explore novel therapeutic strategies. Norcantharidin (NCTD), a natural compound with known antitumor effects in lung and liver malignancies, has not been extensively studied in TNBC context. Therefore, our work aimed to assess the potential therapeutic implications of NCTD in TNBC. We evaluated the antiproliferative effects of NCTD on human TNBC cell lines (HS578T, MDA-MB231) and a murine cell line (4T1), finding significant inhibition with IC50 values of 56 µM, 15 µM, and 35 µM, respectively, as determined by the MTS assay. Moreover, fluorescence microscopy (acridine orange/BrEt staining), flow cytometry (Annexin V/PI staining) and Western blot assays (monitoring cleaved caspase 3, Parp and LC3-II/LC3II levels) revealed the induction of apoptosis and autophagy processes. In vivo assays using BALB/c mice further supported these findings. Systemic administration of NCTD (2.5 and 3.75 mg/kg) significantly reduced both tumor size and local recurrence (p<0.001 and p<0.01, respectively). This effect is likely attributable not only to a reduction in tumor mass but also to an impact on cancer stem cells, as NCTD also impairs in vitro oncosphere formation. With the aim of exploring a combined therapy, we examined the antiproliferative effect of Doxorubicin on the same TNBC cell lines, finding IC50 values of 0.3 µM, 0.4 µM, and 0.071 µM, respectively. However, the addition of NCTD resulted in an antagonistic effect. In conclusion, our study highlights the antitumor activity of NCTD in TNBC, offering promising prospects as a therapeutic option. Nevertheless, additional research is required to optimize its efficacy and fully elucidate the molecular mechanisms involved in its action.

**371. 273 MOLECULAR ALTERATIONS ANALYSIS IN PEDIATRIC WILMS TUMOR ASSESSED BY FISH AND MLPA**

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Wilms tumor (WT) is the most common renal tumor in children with an ASR of 6.2/million in Argentina. About 40 genes are related to WT development but SIOP and COG are still compiling molecular data from the northern hemisphere. Our aim was to characterize molecular alterations on pediatric WT from our region and compare the performance of two stratification approaches. Thirty one FFPE-biopsies of WT were assayed by FISH covering 7 chrom. regions and MLPA, the latter covering 4 additional chrom. regions. Median age was 3.7 years (0.5-13 years) and the male:female ratio was 1.4:1. Tumor stage (TS) I presented in 16/31 (51.6%) cases, TS II in 7/31 (22.6%), TS III in 6/31 (19.4%) and TS IV and TS V in 1/31 (3.2%) cases each. Initial SIOP risk was high in 5/31 (16.1%) and intermediate in 26/31 (83.9%) cases. By FISH, 27/31 (87.1%) cases presented at least one genetic alteration, by MLPA 29/31 (93.5%). Either gains or losses in ChrXp, Xq, 4q, 1p and 17p were the most frequent. Considering FISH results 18/31 (58.1%) cases had alterations in 1p and in 17p, 13/31 (41.9%) in 1q, 12/31 (38.7%) in 2p and 11p15.5, 11/31 (35.5%) in 11p13, 10/31 (32.3%) in 16q. Regarding MLPA 23/31 (74.2%) cases had alterations in chr X, 22/31 (71%) in 17p, 20/31 (64.5%) in 4q, 18/31 (58.1%) in 1p, 15/31 (48.4%) in 11p15.5, 13/31 (41.9%) in 2q, 11p13 and 16p, 12/31 (38.7%) in 1q and 2p, 8/31 (25.8%) in 16q. Considering the 7 common chrom. regions analyzed by both techniques, there was full concordance in 2/7 (28.6%) and partial concordance in 5/7 (71.4%) regions. The average sensitivity of FISH was 92.35% and of MLPA was 96.04%. No significant differences were found between SIOP risk or TS, in relation to any individual genetic alterations. Even though MLPA is a high-throughput technique, FISH allows the assessment of the amount of tumoral cells in the context of the tissue architecture. Sensitivity was similar; however, techniques should complement each other for an accurate diagnosis.

**372. 276 STUDY OF NEW INHIBITORS OF HEAT SHOCK PROTEIN 90 KDA**

Valentina Hansen<sup>1,2</sup>, Iara S Santa Cruz<sup>2</sup>, Nicolás Bruno<sup>1</sup>, Alejandra Erlejman<sup>1,2</sup>, Mario Galigniana<sup>1,3</sup>, Gisela Mazaira<sup>1,2</sup>

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Hsp90 maintains the active form of specific client proteins that already have a stable tertiary structure, including steroid receptors and various oncoproteins. Due to their high proteotoxic stress, cancer cells rely heavily on chaperones. Hsp90 inhibitors are the only chemotherapeutic agents known to strongly impact all hallmarks of cancer, making Hsp90 a promising target for cancer therapy. Numerous Hsp90 inhibitors are currently undergoing clinical and preclinical trials with varying results but have shown nephro- and hepatotoxicity. This study aimed to examine the biological actions of synthetic compounds designed via computational modelling for their potential inhibitory effect on Hsp90's intrinsic ATPase activity, which is crucial for its function. We evaluated these compounds on Hsp90 ATPase activity in vitro, as well as on cell viability and migration in prostate and breast cancer models. Additionally, their oxidative capacity and potential to inhibit glucocorticoid receptor (GR) nuclear translocation was tested. Geldanamycin (GA), a known Hsp90 inhibitor, served as a positive control. Pyrazoline-derivative compounds (C3, C6, and 4F) confirmed in silico predictions by effectively inhibiting Hsp90 ATPase activity. As anticipated, GA treatment inhibited nuclear import of the steroid receptor in normal cells and reduced cell viability and migration in both types of cells: prostate and breast. The synthetic

drugs similarly inhibited cell viability and migration but did not affect GR nuclear import in normal cells. Interestingly, the compounds were observed to affect cytoskeletal stability, resulting in a more adherent cell phenotype, and exhibited a significantly higher oxidative capacity. This lack of effect on steroid receptor inhibition suggests these drugs could have significant pharmacological benefits, avoiding certain side effects. The study provides new insights that could aid in the development of more effective and less toxic drugs.

- 373. 310 EFFICACY OF METRONOMIC CHEMOTHERAPY TARGETING MUSCARINIC RECEPTORS IN TRASTUZUMAB-RESISTANT HER2+ BREAST CANCER CELLS**  
Yamila Sanchez, Laura Sapere, Lucía Vázquez, Sofía Volpi, Francisco Pérez Kasten, Alejandro Español  
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Breast cancer has the highest incidence and mortality rates among women. The HER2+ subtype is typically treated with trastuzumab (TZB), a HER2-targeted monoclonal antibody, combined with chemotherapy. However, resistance to TZB is a frequent problem, leading to the use of high doses of chemotherapeutics as paclitaxel (Px), which lacks selectivity and causes adverse effects. Searching for new therapies or selective therapeutic targets is key to improving antitumor treatments. Metronomic chemotherapy (MC) involves the continuous administration of low-dose chemotherapeutic agents, reducing adverse effects. On the other hand, our research group determined the presence of muscarinic receptors (MR) in breast tumors and its absence in normal breast tissue, which makes them a potential therapeutic target. Combining both approaches, we demonstrated the efficacy of a MC with Px and the muscarinic agonist carbachol (Carb) in luminal A and triple-negative breast cancer cells. This study aimed to evaluate the efficacy of this MC in TZB-resistant HER2+ breast tumor cells. We identified a clone of HER2+ SKBR3 cells resistant to TZB, as it did not reduce cell viability in MTT assays. These cells express functional MR, as Carb reduced cell viability in a concentration-dependent manner. Additionally, MC with Px 10-8 M and Carb 10-6 M significantly reduced cell viability (control: 100.0±7.2%; MC: 20.2±3.6%, p<0.0001) similarly to conventional chemotherapy (CC) used in TZB-resistant tumors (Px 10-6 M: 9.8±1.5%, p>0.05 vs. MC). MC also reduced cell migration, a malignancy parameter, in wound healing assays (control: 46.2±19.4%; MC: 12.3±6.7%, p<0.01) similarly to CC (9.1±3.1%, p>0.05 vs. MC). In conclusion, Px+Carb MC effectively reduces cell viability and migration in TZB-resistant HER2+ SKBR3 cells, suggesting potential benefits of replacing CC with MC in TZB-resistant HER2+ breast cancer, offering a less toxic treatment option, possibly improving overall prognosis and quality of life.

- 374. 511 ARYL HYDROCARBON RECEPTOR LIGANDS AS POTENTIAL ADJUVANTS OF ENDOCRINE THERAPY IN THE TREATMENT OF ESTROGEN RECEPTOR POSITIVE BREAST CANCER**

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Most women diagnosed with breast cancer have tumors expressing the estrogen receptor (ER) and are prescribed antiestrogens tamoxifen (TMX); 40% of these patient's present resistance to treatment. M05 is a murine breast cancer model ER+. TMX induces a selection of breast cancer stem cells (BCSC) in the M05 tumor, however, AhR ligand Aminoflavone (AF) decreases BCSC in M05. A preliminary in vivo experiment showed that treatment of M05 tumors with TMX, or TMX +AF induced a decreased in tumor's growth and weight. In addition, smaller size of mammospheres was obtained from tumors treated with AF or AF+TMX respect to the controls. **Our Goal** was to evaluate the activity of AF+TMX on mammospheres development in the three breast cancer cell lines ER+, human MCF-7 and murine LM05 E and LM05 Mix. **Materials and Methods:** MCF-7 cells were cultured in 6 wells agar coated plates, with BCSC selection medium

B27+EGF (1uM) during 5 days, after 24 hs cells were treated: Control, TMX (1uM), AF (2uM) and TMX+AF (1 y 2 uM respectively), mammospheres were counted (>50um diameter) under light microscope. Three independent experiments were performed. For statistical analysis Kruskal Wallis and Mann Whitney test of the Graph Pad 8 were used. For murine cells we performed extreme limiting dilution analysis (ELDA). **Results:** MCF-7 mammospheres: C=56 ±18,25; TMX=53 ±12,9; AF=13 ±14,53 and TMX+AF=13 ±13,12. K-W p<0,0001. M-W performed in C vs TMX and AF vs TMX+AF was ns. C vs AF p=0,0001 and C vs TMX+AF, TMX vs AF and TMX vs TMX+AF p<0,0001. ELDA studies showed that number of LM05 E cells needed to form one mammosphere was 35% higher when treated with TMX, is 87% and 67% higher when treated with AF, and AF+TMX respectively compared to control. LM05 Mix cell line ELDA showed equivalent results. **Conclusion:** AF alone or in combination with TMX decreases BCSC and mammospheres formation in the three cell lines studied. Therefore AF, can be a potential adjuvant of TMX in the clinic setup.

## REPRODUCCIÓN

### O1 COMUNICACIONES ORALES

FECHA Y HORA: 20/11/2024 16:10-17:10 H

LUGAR: AUDITORIUM

COORDINADORES: ROSSANA RAMHORST, VERONICA BOSQUIAZZO

- 375. 008 THE SODIUM-PROTON EXCHANGERS SNHE AND NHE1 CONTROL MOUSE SPERM PLASMA MEMBRANE HYPERPOLARIZATION DURING CAPACITATION**

Analia G Novero<sup>1</sup>, Paulina Torres Rodriguez<sup>2</sup>, José L De la Vega Beltran<sup>2</sup>, Liza J Schiavi-Ehrenhaus<sup>3</sup>, Guillermina M Luque<sup>3</sup>, Micaela Carruba<sup>1</sup>, Cintia Stival<sup>1</sup>, Inaki Gentile<sup>1</sup>, Carla Ritagliati<sup>1</sup>, Celia M Santi<sup>4</sup>, Takuya Nishigaki<sup>2</sup>, Diego Krapf<sup>6</sup>, Mariano G Buffone<sup>3</sup>, Alberto Darszon<sup>2</sup>, Claudia L Trevino<sup>2</sup>, Dario Krapf<sup>1</sup>.

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Sperm capacitation endows sperm with fertilizing competence after ejaculation. Hyperpolarization of the sperm plasma membrane potential ( $E_m$ ), promoted through opening of SLO3 K<sup>+</sup> channels, is a key event associated with capacitation and correlates with success in human-assisted reproductive techniques procedures. Previous evidence showed the dependence of SLO3 on alkalization and implicated the role of cAMP/PKA in this process. This study aimed to unveil the molecular mechanisms leading to hyperpolarization. Sperm from sNHE, Slo3 and CatSper KO mice were incubated under capacitating conditions with or without pharmacological inhibitors.  $E_m$  measurements were assessed using population fluorimetry, while pH by single cell microscopy and flow cytometry. Specific PKA inhibition by sPKI did not block  $E_m$  hyperpolarization (p<0.001). However, inhibition of cAMP synthesis impaired this event, further rescued by the addition of 8Br-cAMP (p<0.001), supporting the role of cAMP in  $E_m$ . Based on the dependence of Slo3 on intracellular pH, we analyzed the role of NHE exchangers in  $E_m$ . Sperm from the sperm specific NHE (sNHE) KO mice lacked hyperpolarization, which was not rescued by 8Br-cAMP (p<0.05), probably due to the absence of the Cyclic Nucleotide Binding Domain (CNBD) in sNHE. In addition, pharmacological inhibition of a second exchanger presents in sperm, NHE1, also blocked membrane hyperpolarization, similar to what was observed in the calcium channel CatSper KO

mice ( $p < 0.005$ ), probably due to the stimulation of NHE1 by calcium. As expected, a pulse of intracellular calcium promoted sperm alkalization. In both CatSper KO and NHE1 inhibition, Em hyperpolarization was restored by 8Br-cAMP. Our results show that two parallel pathways synergistically modulate alkalization driving hyperpolarization: cAMP via sNHE probably through its CNBD and NHE1 modulated by CatSper derived  $Ca^{2+}$ , to ultimately reach alkalization threshold to promote SLO3 activation.

**376. 056 HIGH-THROUGHPUT SCREENING METHOD FOR DISCOVERING NOVEL CATSPER INHIBITORS USING FLUORESCENT CELL BARCODING AND INTRACELLULAR CALCIUM INCREASE CAUSED BY MEMBRANE DEPOLARIZATION AND pH ALKALINIZATION**

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Freshly ejaculated mammalian sperm are unable to fertilize the egg. To gain this ability, the gamete must undergo a series of biochemical and physiological changes during its passage through the female reproductive tract, collectively known as capacitation. These changes involve the acquisition of hyperactive motility and the ability to undergo acrosomal exocytosis. The sperm-specific calcium ( $Ca^{2+}$ ) channel CatSper activates during capacitation by pH alkalization leading to  $Ca^{2+}$  influx and subsequent sperm hyperactivation. The lack of CatSper results in infertility. The exclusive expression of CatSper in sperm and its critical role in sperm function makes this channel an attractive target for contraception. However, the structural and functional complexity of this channel difficulties the development of strategies to find a specific inhibitor. In this work, we developed a high-throughput method to screen drugs with the capacity to block CatSper in mammalian sperm. The assay to measure CatSper opening extent is based on the stimulation of CatSper by incubating live mouse sperm cells in an alkaline high potassium solution (K8.6) that induces membrane depolarization and pH alkalization. Immediately upon CatSper activation, intracellular  $Ca^{2+}$  concentration was measured by flow cytometry using Fluo4-AM dye. This assay was combined with fluorescent cell barcoding, allowing higher throughput flow cytometry by using unique fluorescent signatures to differentiate sperm samples, enabling the simultaneous acquisition of differentially labeled samples incubated with distinct compounds in a single tube. In this way, acquisition times are highly reduced, which is essential to perform larger screening experiments for drug discovery. This method was validated using CatSper1 KO sperm and pharmacological CatSper non-suitable contraception targets inhibitors. Altogether, we developed a high-throughput drug screening for finding new CatSper blockers and potential contraception targets.

**377. 118 EXPLORING THE IMPACT OF METFORMIN ON TESTICULAR AGING IN SYRIAN HAMSTERS**

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Earlier, we described that testicular aging in the Syrian hamster is associated with increased inflammatory-oxidative events and reduced autophagy, and *in vitro* incubations of old hamster testes with metformin improved the physiological status. *In vivo* experiment offer long-term observations and greater physiological relevance than *in vitro* studies. Thus, in this work, 20-months old hamsters were supplemented for 2 months with 170 mg/kg body weight/day of metformin (equivalent to that 1.500 mg oral dose usually administered to patients with *type 2 diabetes* mellitus, kindly provided by Fund. Roemmers and Montpellier S.A) dissolved in drinking water. Control group received only drinking water. Body and testicular weights and blood glucose levels decreased in metformin-treated hamsters. Unexpectedly, metformin supplementation significantly increased the expression of inflammatory indicators (NLRP3, Nek7, caspase 1 and IL1 $\beta$  evaluated by RT-qPCR and Wb) and the levels of oxidative stress markers (lipid peroxidation determined by TBARS assay, catalase expression assessed by Wb and catalase activity quantified by a colorimetric assay). Metformin also reduced autophagy (increased p62 expression and diminished LAMP2 levels determined by Wb) and further impaired testicular histology (increased Sertoli cell vacuolization and premature detachment of spermatocytes and spermatids from seminiferous tubules) and steroidogenesis (diminished protein and mRNA StAR expression, as well as serum levels of LH and testosterone assessed by RIAs). Therefore, although our previous *in vitro* studies suggest that metformin may exert beneficial effects in the testis reducing local inflammatory-oxidative status and stimulating autophagy, contradictory results were seen in the *in vivo* experiment. Thus, testicular impact of metformin administration deserves further investigation in order to establish whether its anti-aging therapeutic potential in the testis should be reconsidered and/or disregarded.

**378. 231 SIMULTANEOUS HIGH-RESOLUTION LIVE IMAGING OF CYTOPLASMIC AND INTRACELLULAR CALCIUM STORE DYNAMICS IN PROGESTERONE-INDUCED CAPACITATED MOUSE SPERM**

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Progesterone (Prg) is linked to various physiological processes in sperm, such as the stimulation of acrosomal exocytosis (AE), which requires an increase in intracellular calcium ( $[Ca^{2+}]_i$ ). In mouse sperm, the main  $Ca^{2+}$  channel, CatSper, is not activated by Prg. Our previous research showed that Prg induces AE via a transient rise in  $[Ca^{2+}]_i$  in the sperm head, but it remains unclear whether this rise is due to extracellular calcium influx or release from internal reservoirs. To clarify this, we analyzed the dynamics of cytoplasmic and stored  $[Ca^{2+}]_i$  in Prg-induced capacitated mouse sperm using high-resolution live cell imaging with two  $Ca^{2+}$  dyes, Fluo-5N AM and Calbryte 590 AM. These dyes fluoresce in different subcellular locations and exhibit distinct patterns upon sperm capacitation. Calbryte fluorescence increases uniformly throughout the sperm after capacitation, while Fluo-5 shifts from a compartmentalized to a homogeneous distribution. This indicates that capacitation not only elevates  $[Ca^{2+}]_i$  but also redistributes it within sperm. Only capacitated sperm showed a Prg-induced  $[Ca^{2+}]_i$  rise, primarily with an oscillatory pattern. Calbryte displayed global oscillations, whereas Fluo-5 oscillations were confined to the sperm head, suggesting a calcium store. The Prg-induced  $[Ca^{2+}]_i$  increase was reduced in the absence of extracellular  $Ca^{2+}$ , and the oscillatory response was abolished. Additionally, Prg still induced a  $[Ca^{2+}]_i$  rise in sperm from CatSper KO mice, suggesting that CatSper is not essential for this process. By visualizing Prg-induced cytoplasmic and stored  $[Ca^{2+}]_i$  dynamics

simultaneously, our findings underscore the importance of live-cell nanoscopy in sperm physiology.

### 379. 525 MODULATION OF LIPID TRANSPORT AND ACCUMULATION IN THE PLACENTA FROM OVERWEIGHT RATS BY BUTYRATE

Florencia Heinecke, Marina Labiano, Victoria Dechamps, Jeremías Pablo Flores Quiroga, Rocio Alejandra Galarza, Verónica White.

*Centro de estudios Farmacológicos y Botánicos (CEFYO-BO-CONICET-UBA), Facultad de Medicina, Buenos Aires, Argentina*

Butyrate, a postbiotic from the gut microbiota, improves lipid metabolism and prevents inflammation. In a rat model of maternal overweight, we previously observed increased maternal triglyceridemia and placental lipid overaccumulation, whereas maternal butyrate treatment did not prevent placental overaccumulation but did prevent maternal hypertriglyceridemia. Our aim was to evaluate whether butyrate was able to modulate placental lipid transport from overweight rats. **Methods:** Female Wistar rats were fed standard (CT rats) or saturated fat-rich (FD rats, 26% fat) diet for 8 weeks and mated with control males. Pregnant FD rats were orally administered with vehicle (CTB rats) or butyrate (3%, FDB rats) daily during gestation. On day 21 of gestation, all rats were euthanized, and fetuses and placentas removed. Protein levels and mRNA levels of lipid transporters were determined by WB and RT-qPCR. **Results:** Butyrate administered to CT rats increased Cd36 mRNA levels only in female placenta. In placentas from FD fetuses compared to controls, Lpl mRNA levels was increased in placenta from females (114%,  $p < 0.001$ ), and Cd36 mRNA levels was decreased in placenta from males. (44%,  $p < 0.05$ ). In an environment without hypertriglyceridemia, in FDB female placenta, butyrate did not prevent the increase in Lpl mRNA levels, induced an increase in CD36 protein and mRNA levels, and Lip g mRNA levels. Differently, in male placenta, butyrate prevented the decrease in Cd36 mRNA levels and showed an increase in CD36 protein levels and Lpl mRNA levels. **Conclusion:** FD induced placental lipid overaccumulation due to high maternal triglyceridemia and facilitated by sex-specific differences in lipid transporter expression. The combination of FD and butyrate induced placental lipid overaccumulation even at low triglyceridemia with sex differences in lipid transporter expression. CD36 appears to be modulated by butyrate in a sex-dependent manner and to be responsible for lipid accumulation.

#### P1 POSTERS

FECHA Y HORA: 19/11/2024 11:00-12:00 H

COORDINADORES: DAIANA VOTA, GABRIELA JAITA

### 380. 052 TRACKING HUMAN SPERM USING OPEN-SOURCE MACHINE LEARNING

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**Introduction & Objective:** Sperm motility analysis is crucial for assessing sperm quality and predicting their fertilization potential. Although Computer Assisted Sperm Analysis (CASA) systems are valuable for detecting and tracking sperm using imaging technology, they are expensive and not always a priority for clinics due to their limited predictive value for treatment outcomes. This study aims to develop a new open-source method to accurately segment and track human sperm cells, providing complete trajectories and new descriptive parameters. **Methods:** Videos of sperm cells were

captured using a phase-contrast microscope with a 10x objective. Cell segmentation was performed using open-source deep learning algorithms. Initially, built-in models were tested on the images, and incorrect regions of interest (ROIs) were manually corrected. The updated ROIs were then used to train a new model. This process was repeated iteratively: the model was applied to new images, ROIs were adjusted, and the model was retrained with the current and previous images. **Results:** After training the base model, the final system accurately detected sperm heads while disregarding other particles present in the medium. This segmentation can be used in conjunction with cell tracking software to monitor sperm cell motion and obtain individual and detailed trajectories for each sperm cell. **Conclusions:** We demonstrate that human sperm cells can be accurately detected using open-source deep learning software. By combining this software model with other open-source cell tracking platforms, we have optimized a user-friendly system to analyze human sperm cell motility without the need for a CASA system. A notable advantage of this detection and tracking system is its versatility, as it works with both positive and negative phase-contrast microscopy. This makes it adaptable for use in various laboratories.

### 381. 096 ENDOMETRIAL EXPRESSION OF ADHESION MOLECULES INVOLVED IN DELAYED CONCEPTION OF DAIRY COWS

Sofía Cainelli<sup>1</sup>, Astrid Rivas<sup>1</sup>, Lucía Villalba<sup>1</sup>, María Belén Peralta<sup>2</sup>, Emmanuel Angeli<sup>1,3</sup>, Hugo H. Ortega<sup>1,3</sup>, Melisa ML. Velázquez<sup>2,3</sup>, Antonela F. Stassi<sup>1,3</sup>.

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In humans and rodents, the presence of adhesion molecules (AM) and their ligands in both trophoblast and maternal endometrium plays fundamental roles in early events of reproduction. However, in cattle, there are no reports of these molecules in relation to pregnancy recognition. The aim of this study was to analyze the protein expression of osteopontin (OPN), mucin-1 (MUC-1), intercellular AM 1 (ICAM-1), vascular cell AM 1 (VCAM-1) and platelet endothelial cell AM 1 (PECAM-1) in endometrium, and their possible association with delayed conception. Endometrial biopsies were obtained from multiparous Holstein cows ( $n = 12$ ) at 60 days in milk (DIM) following procedures approved by the Ethics Committee (FCV-UNL). All dairy cows returned to their normal cycles before 45 DIM and their voluntary waiting period was 70 days. Histological sections were processed and the protein expression levels of OPN, MUC-1, ICAM-1, VCAM-1 and PECAM-1 were evaluated by indirect immunohistochemistry. Dairy cows were grouped according to conception parturition interval (CPI): CPI fewer than 100 DIM ( $CPI_{<100}$ ), and CPI greater than 130 DIM ( $CPI_{>130}$ ). A Generalized Linear Model was used to analyze the protein expression levels of each AM in the endometrial luminal epithelium (LE), stroma (S) and glandular epithelium (GE). The protein expression levels of OPN and PECAM-1 in the S showed significant differences between  $CPI_{<100}$  and  $CPI_{>130}$  ( $P < 0.05$ ). Specifically, the  $CPI_{<100}$  group showed higher levels of OPN and lower levels of PECAM-1 than the  $CPI_{>130}$  group. Furthermore, the Kaplan–Meier test was used to evaluate the potential association between the protein levels of each AM and delayed conception. Cows with higher levels of OPN in S and lower levels of MUC-1 in GE conceived earlier than those with lower levels of OPN in S and higher levels of MUC-1 in GE ( $P < 0.05$ ). Our findings suggest that OPN, MUC-1 and PECAM-1 could have a role in determining the time to conception in dairy cows.

### 382. 117 BSA-MEDIATED CATSPER-INDEPENDENT RAPID CALCIUM UPTAKE INITIATES SAC ACTIVATION DURING

**MOUSE SPERM CAPACITATION**

Rita C. del Prado<sup>1</sup>, Martina Jabłoński<sup>1</sup>, Liza J. Schiavi-Ehrenhaus<sup>1</sup>, Ana Romarowski<sup>1</sup>, Alberto Darszon<sup>2</sup>, Diego Krapf<sup>3</sup>, Mariano G. Buffone<sup>1\*</sup>, Guillermina M. Luque<sup>1\*</sup>.

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Mammalian sperm require capacitation to fertilize oocytes, which can be induced in vitro using capacitating medium (CAP) containing energy sources, albumin, Ca<sup>2+</sup>, and HCO<sub>3</sub><sup>-</sup>. Previous studies have shown that only a subpopulation of mouse sperm exhibits a rapid increase in intracellular Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>) during CAP incubation, becoming evident within 1 minute. This study investigated early [Ca<sup>2+</sup>]<sub>i</sub> changes by analyzing live sperm loaded with the Ca<sup>2+</sup> dye Fluo-4 AM to determine if this early [Ca<sup>2+</sup>]<sub>i</sub> is required for the initial activation of soluble adenylyl cyclase (sAC). When extracellular Ca<sup>2+</sup> was removed, either by using a medium with no added Ca<sup>2+</sup> or by adding EGTA, this early Ca<sup>2+</sup> influx was abolished, indicating that Ca<sup>2+</sup> is incorporated from the extracellular environment through an ion channel or transporter. The rapid rise was independent of the CatSper channel, as CatSper1 KO sperm incubated in CAP showed a significant increase in [Ca<sup>2+</sup>]<sub>i</sub> similar to wild-type sperm. To identify which CAP component is responsible for this initial Ca<sup>2+</sup> uptake, CatSper1 KO sperm were exposed to either HCO<sub>3</sub><sup>-</sup> or bovine serum albumin (BSA), revealing that the Ca<sup>2+</sup> increase occurred only in response to BSA. As an indirect assessment of sAC activation, we measured substrates phosphorylated by PKA (pPKAs) in CatSper1 KO sperm after BSA addition and found that BSA induced a significant increase in pPKAs. Additionally, pharmacological inhibition of sAC with 11861 did not affect the early [Ca<sup>2+</sup>]<sub>i</sub> increase, indicating that sAC activation occurs downstream of this Ca<sup>2+</sup> uptake. These findings suggest that a subpopulation of mouse sperm rapidly increases [Ca<sup>2+</sup>]<sub>i</sub> during capacitation due to a CatSper-independent influx of extracellular Ca<sup>2+</sup> induced by BSA. This rise in [Ca<sup>2+</sup>]<sub>i</sub> is critical for the initial activation of sAC, offering new insights into the molecular mechanisms behind sAC regulation and the early events that trigger capacitation signaling pathways.

**383. 132 INFLUENCE OF HEAT STRESS DURING INTRA-UTERINE DEVELOPMENT ON THE EXPRESSION OF TRANSCRIPTION FACTORS IN OVARIAN DOMINANT FOLLICLES IN DAIRY COWS**

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The mammalian ovary is a complex organ essential for reproductive functions, including folliculogenesis and steroidogenesis. These processes are regulated by signaling factors which mediate communication at both the cellular and tissue levels. Within this cross-talk, transcriptional factors SF-1, DAX-1 and FOXL2 are involved, regulating specific genes. Intrauterine life can be influenced by harmful stimuli, such as adverse environmental conditions, which can affect the fertility of offspring in adulthood. This study aimed to analyze the expression of SF-1, DAX-1 and FOXL2 in preovulatory follicles of cows gestated under heat stress. Adult Argentinean Holstein cows (n=24) that were gestated under different environmental conditions were used in this study. Ovarian samples were obtained by ovariectomy and protein expression of SF-1, DAX-1 and FOXL2 in granulosa and theca interna layers of preovulatory follicle was determined by immunohistochemistry. Gestation was divided into two periods (P1: 0-150 days; P2: 151 days-birth); and three trimesters (T1: 0-90 days; T2: 91-180 days; T3: 181-birth days) and temperature-humidity index (THI) was calculated for each stage. SF-1 expression in theca showed positive association trend with the percentage of days

with THI ≥72 throughout gestation. FOXL2 expression in granulosa cells was positive associated with heat stress variables in P1, particularly in T1; but showed negative association in P2, specifically in T3. Additionally, DAX-1 expression in granulosa cells showed negative association with THI variables in P1, especially in T1, and positive association in P2, particularly in T3. The results suggest that exposure to high THI levels during pregnancy could alter the expression of transcription factors involved in critical mechanisms of folliculogenesis and gonad development. Consequently, changes during in utero development can potentially impact the fertility of cows in their adult life.

**384. 274 EVALUATION OF THE EFFECT OF LACTOFERRIN ON OXIDATIVE DAMAGE PARAMETERS IN HUMAN SPERM**

Lautaro Pretto<sup>1</sup>, Macarena Mendiondo<sup>1</sup>, Rocío Malandra<sup>1</sup>, Aldana Gola<sup>2</sup>, Eugenia Vich<sup>1</sup>, Liliana Rateni<sup>3</sup>, Cecilia Basiglio<sup>1,2</sup>, Estefanía Massa<sup>3</sup>, Sergio Ghersevich<sup>1,4</sup>

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Reactive Oxygen Species (ROS) play a crucial role in sperm capacitation, but excessive levels can induce oxidative damage to DNA and sperm membranes. Lactoferrin (LF), a protein found in the female reproductive tract, has been shown to possess antioxidant properties. This study aimed to evaluate the effects of LF on oxidative damage parameters in human sperm. Motile sperm obtained from normozoospermic donors (n=34) were incubated in the presence or absence of LF and/or an oxidizing agent for 1 hour at 37°C. After incubation, lipid peroxidation (TBARs assay), ROS production (dichlorofluorescein and fluorometry), and genotoxic damage (comet assay) were assessed. The average value for the controls was set at 100%, and the result of each treatment was compared to the control value. Statistical analysis was performed using ANOVA. Spermatozoa were exposed to increasing concentrations of LF, with or without tert-butyl hydroperoxide (HPT, 25 µM). HPT significantly increased lipid peroxidation (186.4±31.8%, p<0.001) compared to controls and LF-only treatments. LF at concentrations of 5 µg/ml (LF5+HPT: 136.6±20.1%; p<0.05), 10 µg/ml (LF10+HPT: 132.3±37.9%, p<0.01), and 100 µg/ml (LF100+HPT: 140.7±25.8%, p<0.05) significantly reduced HPT-induced lipid peroxidation. HPT also increased ROS production (154.5±36.9%, p<0.001), and LF at 10 µg/ml reduced this production in the presence of HPT (117.3±9.9%, p<0.05). However, the incubation with 200 µg/ml of LF also caused an increase in ROS (164±11.5%, p<0.001). In spermatozoa incubated with increasing doses of LF and H<sub>2</sub>O<sub>2</sub>, the 100 µg/ml LF dose significantly reduced H<sub>2</sub>O<sub>2</sub>-induced genotoxic damage (p<0.01). These results indicate that certain concentrations of LF can protect sperm from oxidative damage at both the DNA and membrane levels. Since the concentration of LF in the female tract can vary widely, its presence could help modulate genotoxic and liperoxidative damage in that environment.

**385. 331 EFFECT OF POLYCYSTIC OVARY SYNDROME ON UTERINE HISTOMORPHOLOGY**

María Virginia Acosta, Aldana Nerea Ramis, Gisela Soledad Bracho, María Alejandra Cardozo, Laura Kass, Verónica Lis Bosquiazzo

Instituto de Salud y Ambiente del Litoral (ISAL, UNL-CONICET), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral.

In a rat model of polycystic ovary syndrome (PCOS), we previously demonstrated changes in uterine steroid hormone metabolism that suggest increased estrogen levels. Estrogens have been shown to stimulate vascular permeability and the expression of molecules that regulate uterine angiogenesis. The aim of this study was to evaluate uterine angiogenesis in a PCOS rat model. Female Wistar rats were treated subcutaneously with sesame oil (control) or dehydroepiandrosterone 6 mg/100 g body weight (PCOS) from 21 to 40 days of age. After 24 hours, the uterine horns were collected. Vascular endothelial growth factor (Vegf) expression was analyzed by real-time PCR and immunohistochemical (IHC). Endothelial cell proliferation

was assessed by double IHC staining using Ki-67 for detection of proliferating cells and nestin for identification of the subpopulation of endothelial cells capable of proliferation. In uterine tissue, Vegf mRNA expression was decreased in PCOS animals. Vegf protein expression was quantified in all uterine compartments, with an increase observed in the myometrium of PCOS animals. However, the uterine endothelial cell proliferation was similar between experimental groups. In PCOS rats, additional nestin staining was observed in cells surrounding the basement membrane of the luminal epithelium (LE). Further, vimentin staining for detection of fibroblasts was positive in this area and co-expression of nestin and vimentin proteins was confirmed by immunofluorescence. As no changes in endothelial cell proliferation were observed, we suggest that PCOS would not affect uterine angiogenesis. Additionally, PCOS could induce post-transcriptional modifications of Vegf. In the uterus of PCOS rats, a distinctive phenotype of fibroblasts was identified below the LE, characterized by the expression of nestin and vimentin intermediate filaments.

**386. 380 EFFECTS OF CHRONIC ADMINISTRATION OF CANNABIS DURING PREGNANCY: AN IMPACT ON METABOLISM AND DEVELOPMENT OF OFFSPRING IN ADULTHOOD**

Ayelén Aixa Mirón Granese, Carolina Marvaldi, Julieta Aisemberg, Fernando Correa, Daniela Sedán, Dario Andrinolo, Ana María Franchi, Camila Martínez Calejman, Manuel Luis Wolfson.

*Cannabis* is the most widely used substance globally, particularly among individuals of reproductive age. Its biological effects are mediated by the endocannabinoid system (eCS), a complex lipid signaling network comprising endogenous ligands, their receptors (CB1 and CB2), and the enzymes involved in their biosynthesis and catabolism. Several studies indicate that the endocannabinoid system plays a role in both metabolic and reproductive processes. Given that  $\Delta^9$ -tetrahydrocannabinol (THC), the main psychoactive component of *Cannabis sativa*, is highly lipid-soluble and can cross the placenta to accumulate in fetal tissues, prenatal *cannabis* exposure might disrupt the eCS, potentially impacting offspring development. This study aimed to explore whether prenatal *cannabis* exposure affects offspring in adulthood. We administered THC oil (0.3  $\mu\text{g}/\mu\text{l}$ ) intragastrically to female BALB/c mice from day 1 (determined by vaginal plug observation) until the day before the onset of labor. After delivery, we assessed the offspring on postnatal day 60 using the open field test. We observed that males prenatally exposed to *cannabis* spent significantly more time in the center of the arena compared to controls, while this behavior was not noted in females. We subsequently euthanized the animals and extracted brown fat to analyze metabolic parameters by measuring the protein levels of ATGL, HSL, ACC and UCP1. Our results showed lower HSL protein levels in THC-exposed offspring compared to controls, while ACC, ATGL, and UCP1 levels were elevated. Additionally, we examined ovaries from the females and found that those prenatally exposed to *cannabis* had a reduced proportion of primordial follicles compared to the control group. In conclusion, our findings highlight the potential long-term impacts of chronic prenatal *cannabis* exposure with high THC content, revealing significant effects on behavior, metabolism, and reproductive health that warrant further investigation.

**387. 496 CELL-FREE DNA FOR THE MONITORING OF HIGH-RISK PREGNANCIES IN WOMEN WITH THROMBOPHILIA**

Julieta Cepeda<sup>1</sup>, María Emilia Racca<sup>1,2</sup>, María Alejandra Cardozo<sup>1,2,6</sup>, Luisa Gaydou<sup>1,2</sup>, Stefania D'lorio<sup>5</sup>, Mónica Muñoz-de-Toro<sup>1,4</sup>, María Mercedes Milesi<sup>1,3</sup>, Jorgelina Varayoud<sup>1,3</sup>, María Florencia Rossetti<sup>1</sup>, Jorge Guillermo Ramos<sup>1,2</sup>  
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Cell-free DNA (cfDNA) has emerged as a promising tool for monitoring pregnancy and predicting adverse obstetric outcomes. We investigated cfDNA levels in women with thrombophilia (TBP), a condition associated with an increased tendency to thrombosis and pregnancy complications. An anonymous survey was conducted among women from Argentina diagnosed with TBP (n=74) to assess its impact on their obstetric history. Pregnant women with (n=30) and without (n=83) TBP were recruited from BLUT Laboratories (Santa Fe, Argentina) and other regional laboratories. Clinical information and pregnancy outcome data were collected from medical records. Healthy pregnant women without TBP who had term pregnancies without complications were selected as healthy controls (HC, n=37). cfDNA was isolated from blood plasma using a commercial kit and quantified by quantitative PCR. The association of cfDNA levels with maternal conditions and pregnancy outcomes was evaluated by univariate and multivariate statistical analyses, including multiple correspondence analysis (MCA) and multiple linear regression. Survey results indicated a negative impact of TBPs on pregnancy outcomes, with high incidence of obstetric complications despite standard treatment. Univariate analysis showed significantly increased median cfDNA levels in the 1st (p<0.001) and 2nd (p<0.01) trimesters in women with antiphospholipid syndrome (APS), an acquired TBP, compared to HC. In APS patients, complicated pregnancies showed increased cfDNA levels in the 2nd trimester compared to uncomplicated (p<0.05). Multivariate analysis including all patients revealed significant associations between 1st and 2nd trimester cfDNA levels and combined variables related to TBP and pregnancy complications. In conclusion, elevated cfDNA levels were associated with TBP and adverse pregnancy outcomes, highlighting the potential of plasma cfDNA as a non-invasive biomarker for identifying high-risk pregnancies, enabling early interventions.

**P2 POSTERS**

FECHA Y HORA: 19/11/2024 16:00-17:00 H

**COORDINADORES: MÓNICA FRUNGIERI, JULIETA AISEMBERG**

**388. 058 MATERNAL ORAL ADMINISTRATION OF BUTYRATE AMELIORATES THE ALTERATIONS INDUCED BY MATERNAL OVERWEIGHT IN THE OFFSPRING OF RATS**

Victoria Dechamps<sup>1</sup>, Marina Labiano<sup>1</sup>, Florencia Heinecke<sup>1</sup>, Jeremías Pablo Flores Quiroga<sup>1</sup>, Rocío Alejandra Galarza<sup>2</sup>, Verónica White<sup>1</sup>

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We have observed that maternal overweight programmed alterations in lipid metabolism in the rat offspring. We also observed that the maternal oral treatment with butyrate, sub-product of the fiber digestion by intestinal microbiota, prevented fetal overgrowth and liver lipid overaccumulation, while in mothers, prevented hypertriglyceridemia. Our aim was to evaluate the effects of maternal overweight on lipid metabolism and liver and reproductive function, and whether butyrate could prevent the alterations. **Methods:** Female Wistar rats were fed standard (CT rats) or high saturated fat (FD rats) chow for 8 weeks and mated with CT males. Butyrate (3%) or vehicle was orally administered daily during gestation and 3 days per week during lactation (FDB rats). Mothers were euthanized after weaning and the offspring were euthanized at 140 days of age. The offspring were fed a control diet. The offspring and their fat pads were weighted. Circulating levels of triglyceride (TG) cholesterol (C) and activity of hepatic transaminases (ALT and AST) by the IFCC method. Finally, we analyzed offspring reproductive function by the



evaluation of sperm count, motility and the duration of estrous cycle.

**Results:** Female and male offspring showed no changes in weight when comparing CT, DG and DGB. Adipose tissue accumulation was also unchanged by maternal overweight although we found a decrease in males and females from the DGB group (DG vs DGB  $p < 0.05$ ). Circulating levels of TG and C showed no changes although ALT and AST were increased in DG males and females (CT vs. DG  $p < 0.05$ ). Importantly, Butyrate prevented the increase in males (DG vs. DGB  $p < 0.05$ ). Also, maternal overweight induced a decrease in sperm motility and alterations in the estrous cycle, both prevented by maternal treatment with Butyrate. **Conclusions:** Maternal overweight programmed metabolic alterations that were prevented partially by maternal treatment of Butyrate in the rat offspring.

**389. 060 IDENTIFICATION AND CHARACTERIZATION OF TWO NOVEL CATSPER INHIBITORS AS POTENTIAL NON-HORMONAL MALE CONTRACEPTIVES**

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During capacitation, sperm acquire the ability to develop a change in the motility pattern called hyperactivation (HA), which is critical for fertilization. HA requires an increase in intracellular  $Ca^{2+}$ , facilitated by sperm-specific CatSper channels in the flagellum. Given its exclusive expression in sperm and critical role, CatSper is a promising target for non-hormonal male contraception. However, the potential of blocking CatSper as a contraceptive has not been fully explored due to inadequate screening methods for identifying novel, specific inhibitors, a challenge arising from the channel's structural and functional complexity. We developed and conducted a membrane potential assay combined with fluorescent cell barcoding, enabling a high-throughput screening method to find drugs that block CatSper using flow cytometry. We have performed a screening of 6924 compounds and we identified two novel Catsper inhibitors, compound 2 and compound 8. IC<sub>50</sub> values were 4.120  $\mu$ M for compound 2 and 5.103  $\mu$ M for compound 8. Sperm viability remained unchanged with increasing compound concentrations compared to control (only vehicle). The most pronounced effect of CatSper malfunction is altered sperm motility, particularly HA. We assessed motility parameters using a computer-assisted sperm analysis (CASA) system with and without these compounds. Key kinetic parameters related to HA were significantly affected, similar to the phenotype observed in CatSper1 knockout sperm. Furthermore, the impact of both compounds on sperm motility continued even after the inhibitors were removed, indicating that the effect is not immediately reversible. The identified compounds show promise for developing non-hormonal male contraceptives. Their ability to inhibit CatSper and alter sperm motility indicates potential effectiveness in preventing fertilization. Further research should optimize these inhibitors and evaluate their efficacy and safety in advanced biological models.

**390. 069 PRENATAL HYPERANDROGENIZATION AFFECTS UTERINE RECEPTIVITY IN A MURINE PCOS MODEL**

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Polycystic Ovary Syndrome (PCOS) is a common reproductive disorder in women of reproductive age. Although the etiology is unknown, prenatal androgen excess is considered a key factor in its

development. PCOS is a leading cause of infertility, linked not only to ovarian dysfunction but also to altered uterine functionality.

We aimed to investigate the effect of fetal programming mediated by androgen excess on uterine receptivity and the establishment of pregnancy in adulthood. In this study, pregnant Sprague Dawley rats (F0) were injected with testosterone or vehicle to obtain prenatally hyperandrogenized (PH) and control groups, respectively. At 90 days of age, the adult female offspring (F1) were either euthanized in estrous (AD90, n=10) or mated with control males and euthanized on gestational day 14 (GD14, n=6). At AD90, uterine protein expression of PPAR $\gamma$  and PPAR $\alpha$  (master regulators of uterine function), and key steroidogenic enzymes, were quantified by Western Blot. A uterine histomorphologic analysis was conducted. At GD14, the number of implantation sites were evaluated. At AD90, results showed that PPAR $\alpha$  protein levels were significantly lower ( $p < 0.05$ ) in the PH group compared to controls, while PPAR $\gamma$  levels remained unaffected. The protein levels of steroidogenic enzymes were also altered; specifically, 3 $\beta$ -HSD and CYP17 levels were higher ( $p < 0.05$ ), while 17 $\beta$ -HSD levels were lower ( $p < 0.05$ ) in the PH group compared to controls. Histomorphologic analysis revealed an increased number of uterine glands and gland conglomerates in the PH group. At GD14, the number of implantation sites was significantly decreased in the PH group ( $p < 0.05$ ). In conclusion, fetal programming induced by excess androgens may adversely affect uterine functionality in adulthood, evidenced by altered uterine histomorphology and key steroidogenic enzyme levels, leading to decreased uterine receptivity and compromised fertility.

**391. 081 EFFECT OF 2,4-DICHLOROPHOENOXYACETIC ACID (2,4-D) ON MATURE SERTOLI CELL FUNCTIONS**

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In the last decades, a gradual decrease in male reproductive function has been evidenced, which has given rise to numerous epidemiological and experimental studies. Several reports state that the exposure to xenobiotics contributes to altered fertility in men. 2,4-Dichlorophenoxyacetic (2,4-D) is a widely used herbicide, however, its potential effects on testicular function remain obscure. Particularly, Sertoli cells (SCs) are essential to the adequate development of the germ cells (GCs), because they provide an optimal environment through the formation of junctions that constitute the blood-testis-barrier (BTB), and produce lactate, GC's main energetic source. This work aimed to evaluate the effect of low doses of 2,4-D on different functions of SCs. To this end, SC cultures obtained from 20-day-old rats were incubated in the absence (B) or presence of 2,4-D 10 nM or 10  $\mu$ M. Results are expressed as mean $\pm$ SD (\* $p < 0.05$ ). Transepithelial Electrical Resistance (TER), which indicates the integrity of the junctions was determined. Both doses of 2,4-D decreased TER (B:45,0 $\pm$ 1,0; 2,4-D 10 nM:15,7 $\pm$ 1,2\*; 2,4-D 10  $\mu$ M:12,0 $\pm$ 3,5\*,  $\Omega$ .cm<sup>2</sup>). However, 2,4-D in both doses tested did not modify mRNA levels of claudin11, occludin, and connexin43, proteins that constitute BTB junctions. Regarding SC nutritional function, 2,4-D did not modify lactate production and glucose consumption. In addition, it did not alter lactate dehydrogenase (LDH) activity and glucose transporter 1 (GLUT1) mRNA levels. In summary, the results suggest that 2,4-D can affect the junctions of BTB but not the lactate production in SC. Nevertheless, more studies are necessary to elucidate if this herbicide alters other SC functions and the possible mechanisms involved in its effects.

**392. 095 DEVELOPMENT OF AN IN VITRO MODEL FOR THE STUDY OF FOLLICULAR PERSISTENCE IN CATTLE**

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Follicular persistence in cattle is an early event that can lead to the development of Cystic Ovarian Disease (COD). We developed an *in vitro* granulosa cell model to study the mechanisms leading to COD. The objectives were: a) To characterize the presence of receptors and enzymes in a bovine granulosa cell line (BGC-1). b) To evaluate the effects caused by progesterone (P4) stimulation in BGC-1. BGC-1 cells were exposed for 12, 24 and 48 h to 20 ng/mL of P4. Culture medium without P4 was used as basal control. We evaluated the expression of CYP19, 3 $\beta$ HSD, PR, ER $\alpha$ , ER $\beta$ , and LHR by immunocytochemistry (ICC). Additionally, we evaluated the co-expression of PR and STAT5 through immunofluorescence (IF). Cell viability was analyzed using the MTT cytotoxicity assay and apoptosis and ROS production by flow cytometry. The data was analyzed using ANOVA with Duncan's post-test and T-student test. The expression of different functional molecules in BGC-1 cells was evaluated by ICC. CYP19A1, 3 $\beta$ -HSD, PR, ER $\alpha$  y  $\beta$  and LHR protein expression were detected in BGC-1 cells. The mean fluorescence intensity (MFI) of STAT5, PR, and the percentage of simple and double-positive cells (STAT5/PR) were lower in basal BGC-1 cells than in BGC-1 cells stimulated with 20 ng/mL of P4 after 12, 24, and 48 h of stimulation. P4 does not affect the cellular proliferation of BGC-1 cell line after 12, 24, and 48 h of stimulation. P4 displayed a high percentage of living cells after 12, 24, and 48 h of stimulation, and a low number of apoptotic and necrotic cells. Additionally, P4 induce ROS production after 24 and 48 h. The BGC-1 granulosa cell line expresses the evaluated markers related to steroidogenesis, as well as estrogen, progesterone, and luteinizing hormone receptors. Finally, BGC-1 cells are capable of responding to P4 stimulation without incurring damage, stimulating ROS production.

### 393. 103 EXPOSURE OF MINIMUM DOSES OF GLYPHOSATE DURING THE POSTNATAL PERIOD ALTERS GENES EXPRESSION

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Previously, we have demonstrated that chronic oral exposure to the pesticide glyphosate during the postnatal period impacts ovarian function and the estrous cycle, even at a low dose. Therefore, to further study the effect of this pesticide on ovarian gene expression, one month old female Sprague Dawley rats (n=16) were randomly assigned to one of the following groups: Control (receiving water) or Treated (receiving an oral dose of glyphosate 1mg/kg per day). After eight weeks of treatment, the ovaries of 5 representative animals from each group were dissected and stored in RNA later for further RNA extraction to perform RNAseq. To determine the differential expression of genes, we used the gene-level edgeR analysis package. We performed an initial trimming and adapter removal pass using Trimmomatic. Reads that passed Trimmomatic processing were aligned to the GRCr8 build of the rat genome with Bowtie2/TopHat2 and assigned to gene-level genomic features with the Rsubread featureCounts package and expressed as Reads per Kilobase per Million mapped Reads (RPKM). Differential expression was analyzed using the generalized linear modeling approaches implemented in edgeR. Lists of differentially expressed genes were identified based on unadjusted p-value<0.05 and used as input for gene set analysis over-representation analysis using Webgestalt.org. Over-represented gene sets showing adjusted p-value <0.05 were used for posterior RT-qPCR validation. Eight over-represented genes (*Ccnb1*, *Ccnd2*, *Dhh*, *Ihh*, *InhB*, *Angpt2*, *AMH*, and *Plk1*)

as well as some steroidogenesis enzymes (3 $\beta$ -HSD, Aromatase, P450scc, and STAR) were selected to validate by RT-qPCR and normalized with 18s and  $\beta$ -Actin. Interestingly, *Ccnb1*, *Ccnd2*, *InhB*, and *Plk1*, showed a significant decrease in expression in the treatment group (p<0.05), supporting our previous findings showing that chronic oral exposure to low glyphosate during the pubertal period acts as an endocrine disruptor.

### 394. 192 EVALUATION OF FERTILITY IN MALE MICE TREATED WITH PROGRAMMED CELL DEATH PROTEIN 1 (PD-1) INHIBITOR

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Immune checkpoint molecules play an important role in down regulating the immune system to reduce autoimmunity and promote self-tolerance. Programmed cell death protein 1 (PD-1)/PD-ligand 1 costimulation is a mechanism underlying testis immune privilege and long-term survival of intratesticular islet allografts. Antibodies (Abs) blocking PD-1 can trigger antitumor immunity and have changed the therapeutic landscape in many cancer patients. However, the understanding of the potential impact of immune checkpoint inhibitors (ICIs) on testicular function and male fertility remains limited. In the present work we compared different parameters of testicular function in male mice treated with anti-PD-1 Abs to mice injected with isotype control Abs (control group). Anti-Mouse PD-1 or IgG isotype control (100  $\mu$ g/mouse) was administered i.p twice a week for 6 weeks to adult male C57BL/6J mice. Blood, testes, epididymis and testicular draining lymph nodes (TLN) were collected 15 days after the final treatment. Sperm analysis showed that mice from anti-PD-1 group exhibited a significant decrease in cauda epididymal sperm count compared to control group (sperm count ( $\times 10^6$ /ml): 44.0 $\pm$ 10.2 vs 85.6 $\pm$ 14.5, p<0.05), whereas similar values of progressive sperm motility were observed in both groups. Since ICIs could impair fertility by injury to the hypothalamic-pituitary-gonadal axis, as well as damage to the reproductive tract organs, seric testosterone quantification and testis and epididymis histopathology were assessed. No difference was observed for testosterone profiles in mice from both groups studied. In contrast, histopathology showed that PD-1 inhibition induces male genital tract inflammation, which was concomitant with an increase of IL-6 mRNA level within TLN. Our results suggest a down-regulation of PD-1-based tolerance evidenced by testis functional decline and genital tract inflammation. Additional research is required to elucidate immunological mechanisms involved.

### P3 POSTERS

FECHA Y HORA: 19/11/2024 16:00-17:00 H

COORDINADORES: DEBORA COHEN,  
MARIANA FARINA

### 395. 011 TROPHOBLAST FACTORS MODULATE LIPID METABOLISM IN MACROPHAGES CONTRIBUTING TO THE ACQUISITION OF AN ANTIINFLAMMATORY PROFILE

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In normal pregnancy, extravillous trophoblast cells (Tb) support the differentiation of monocytes into M2-like decidual macrophages (MA). Metabolic reprogramming in MA is linked to functional polarization. We recently reported that monocytes present increased glucose dependence and long chain fatty acid (LCFA) uptake during pregnancy and that Tb conditioned media (Tb-CM) promote anti-inflammatory markers along with LCFAs uptake in vitro. Our aim was to deepen into the effect of Tb factors on lipid metabolism in MA and the association with an M2-like profile. Peripheral blood mononuclear cells were isolated from fertile non-pregnant women by Ficoll-Paque/Percoll, differentiated into MA with M-CSF and incubated with Tb-CM from HTR8/SVneo cell line (MA+Tb-CM) as a model of maternal-fetal interface. Metabolic inhibitors of glucose utilization (2DG), mitochondrial respiration (oligomycin) and fatty acid oxidation (FAO) (etomoxir) were used. ATP production, gene expression, lipid droplet localization and cell phenotype were assessed by bioluminescent assay, RT-qPCR, confocal microscopy or flow cytometry, respectively. Oligomycin, etomoxir and 2DG reduced ATP production individually in MA+Tb-CM, with greater effect if they were combined ( $p < 0.05$ ). Inhibition of FAO in MA had no effect. Tb-CM increased the expression of FAO-rate-limiting step importer CPT1 and lipid droplet formation enzyme DGAT ( $p < 0.05$ ) in MA and enhanced the colocalization of lipid droplets with mitochondria (Manders M1/M2,  $p < 0.05$ ). Since LPS induced LCFA uptake which mediates prostanoid synthesis, we evaluated COX1/2 expression. LPS induced COX2 and reduced COX1 whereas Tb-CM tended to induce COX1 suggesting differential lipid utilization. Also, inhibiting FAO with etomoxir prevented the increase in CD209 in MA+Tb-CM ( $p < 0.05$ ) and tended to increase CD86 expression. Our results reveal the involvement of trophoblast factors modulating macrophage metabolism thus contributing to M2-like profile acquisition.

**396. 124 MATERNAL DIETS ENRICHED IN EXTRA VIRGIN OLIVE OIL PREVENT A PROOXIDANT ENVIRONMENT IN THE FETAL UTERI IN DIABETIC PREGNANT RATS**

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**Introduction:** We previously observed an increased prooxidant environment and reduced prolactin (PRL) levels in the uteri of the offspring of diabetic rats, alterations prevented by a maternal diet enriched in extra virgin olive oil (EVOO). Whether there are alterations in the fetal uterus of diabetic pregnant rats is unknown. **Aim:** To evaluate PRL levels and the prooxidant environment in the uterus of 21-day-old fetuses from diabetic rats fed or not with an EVOO-enriched diet during pregnancy. **Methods:** A mild pregestational diabetic rat model was induced in females by neonatal administration of streptozotocin (90 mg/kg sc). Control and diabetic females were mated with healthy males and fed a standard diet enriched or not in 6% EVOO during pregnancy. Fetal uteri were collected on day 21 of pregnancy to evaluate *rPrI*, *Cat* and *Mnsod* mRNA levels by qRT-PCR, and 4HNE (a marker of lipoperoxidation) and PRL levels by immunohistochemistry. **Results:** mRNA levels of *rPrI* were reduced in the fetal uterus of diabetic rats fed the standard diet (0.57 fold change,  $p < 0.05$  vs. controls), an alteration that was prevented by the EVOO-enriched maternal diet. PRL levels were decreased in the fetal uterus of diabetic rats (0.37 fold change,  $p < 0.05$  vs. controls) an alteration that was no longer significant when the dams

were fed the EVOO-enriched diet. 4HNE levels were increased in the fetal uterus of diabetic rats (+50%  $p < 0.05$  vs. controls), an alteration that was prevented by the maternal EVOO-enriched diet. No changes in *Cat* and *Mnsod* mRNA levels were observed in diabetic rats compared to controls, but their expression was increased in the fetal uteri of diabetic rats fed the EVOO-enriched diet (0.38 and 0.39 fold change,  $p < 0.05$  vs. control and diabetic groups - standard diet, respectively). **Conclusion:** Uterine alterations induced by maternal diabetes are evident from the fetal stage and the EVOO-enriched maternal diet could protect the uterine development from the pro-oxidant environment.

**397. 184 OUTER MEMBRANE VESICLES FROM THE PERIODONTAL PATHOGEN PORPHYROMONAS GINGIVALIS REDUCE THE AUTOPHAGIC FLUX IN TROPHOBLAST CELLS**

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*Porphyromonas gingivalis* (Pg) is one of the main pathogens causing periodontitis. Pg releases outer membrane vesicles (OMV) that allow it to interact with the host promoting its pathogenesis. There is an association between oral infections and defective placentation, but the mechanisms involved are elusive. During placentation, low levels of nutrients and oxygen are linked to an induction of autophagy in trophoblast cells (Tb) in order to provide energy and contribute to cellular homeostasis. Alterations in autophagy and mitochondrial function are associated with pregnancy complications related to placental insufficiency. The aim of this work was to evaluate the effect of PgOMV on Tb metabolism and functions focusing on the regulation of autophagy. OMVs were isolated by ultracentrifugation from the supernatant of Pg culture. HTR-8 human first trimester trophoblastic cell line was treated with 1 µg/ml PgOMV. The migratory capacity, mitochondrial membrane potential and oxygen consumption rate (OCR) were measured by wound healing assay, fluorescence microscopy and Seahorse® Extracellular Flux Analyzer, respectively. Autophagic flux markers, such as LC3 and p62, were evaluated by fluorescence microscopy. Lysosome quantification was performed using the fluorescent dye LysoTracker and LAMP1-2 markers. PgOMV treatment impaired trophoblast function and metabolism by inhibiting migration capacity, increasing both basal respiration and maximal respiration capacity ( $p < 0.05$ ) and enhancing mitochondrial potential membrane ( $p < 0.05$ ). PgOMV also reduced autophagic flux, as evidenced by the accumulation of LC3 and p62-positive vesicles in presence of Bafilomycin A1, an autophagy inhibitor. Additionally, PgOMV diminished the lysosomal area per cell compared to control cells ( $p < 0.05$ ). In conclusion, our results indicate that PgOMV impairs autophagy flux and mitochondrial activity in Tb cells, which could compromise placentation and contribute to adverse pregnancy outcome.

**398. 185 PROINFLAMMATORY EFFECT OF GINGIVAL FLUID FROM PREGNANT WOMEN ON PLACENTAL CELLS AND NEUTROPHILS: CORRELATION WITH PERIODONTAL STATUS**

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Periodontal disease associates with adverse pregnancy outcomes but the underlying mechanisms are still unclear. Inflammation of placental tissues and activation of maternal neutrophils by periodontal pathogens is proposed as a conditioning factor. To deepen into the role of periodontal factors in placental inflammation, we studied pregnant women at 16-20 weeks and term regarding their periodontal status and the *in vitro* effect of their gingival fluid (GCF) on placental cells and neutrophils. Periodontal variables including bacteria content in the GCF were measured at a single time-point 24h after caesarean surgery or at 16-20 weeks at their dental care control. A control sample of periodontal healthy nonpregnant women was analyzed in parallel. Cytokines and vascular mediator expression was assessed by RT-qPCR in placental explants and endothelial cell line EA.hy926 incubated with individual GCFs. Reactive oxygen species (ROS) production in volunteer donors' neutrophils upon GCF stimulation was measured with a fluorescent probe by flow cytometry. Compared to control GCF, term GCF enhanced proinflammatory cytokine expression in placental explants, which correlated with the periodontal status and bacterial count in the fluids ( $p < 0.05$ ). Term GCF reduced endothelial cell migration vs. control GCF ( $p < 0.05$ ). Neutrophils exposed to term GCF produced higher levels of ROS vs. control samples. This effect correlated with patients' periodontal status ( $p < 0.05$ ). Of note, gingival inflammation and bacterial count in term GCF were greater than in GCF of 16-20 weeks' pregnant women ( $p < 0.05$ ). The worsening in the periodontal condition at term paralleled a higher ROS production trend in neutrophils treated *in vitro* with term vs. 16-20 weeks GCF. These findings are consistent with a cumulative proinflammatory and deleterious effect of periodontal fluid components on the placenta throughout pregnancy providing new clues to the pathogeny of pregnancy complications.

### 399. 203 IMPACT OF ASPIRIN TREATMENT IN NORMAL AND PREECLAMPTIC PREGNANCIES: SEXUAL DIMORPHISM IN NEONATAL WEIGHT

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**Introduction** Low-dose aspirin (LDA) is proposed to reduce the severity of preeclampsia, although its effectiveness remains a topic of ongoing debate. **Objective** To assess the impact of LDA on newborn weights in both normotensive (NP) and preeclamptic (PE) pregnancies. **Methods:** We conducted a retrospective study at Hospital Posadas, approved by the Institutional Review Board, involving 229 pregnant women. Participants were classified into 4 groups based on LDA intake (150 mg/day from weeks 16-36) and pregnancy outcome (NP or PE). ANCOVA was used to evaluate LDA impact on newborn weights. Post hoc analysis was performed using Bonferroni's test with a significance level of 0.05. **Results:** In non-treated groups, PE newborns had lower weights than NP ones (2953.88 g vs. 3269.63 g  $p = 0.0304$ ). Female (F) newborns from PE weighed less than those from NP (2813.88 g vs. 3237.98 g  $p = 0.0176$ ), while male (M) newborn weights did not differ significantly. In LDA-treated groups, newborn weights in PE were similar to NP.

F newborns weighed less than M in both NP (3012.61 g vs. 3250.37 g  $p = 0.0205$ ) and PE (2819.09 g vs. 3468.20 g  $p = 0.0208$ ). No significant weight difference was found between F newborns in NP vs. PE or M newborns. In NP, LDA-treated newborns weighed less than those untreated (3131.49 g vs. 3269.63 g  $p = 0.0198$ ). F newborns were lighter than M (3012.61 g vs. 3250.37 g  $p = 0.0205$ ) and those from untreated pregnancies (3012.61 g vs. 3237.98 g  $p = 0.0141$ ). No significant difference was noted for M. In PE, LDA did not significantly affect newborn weights. F and M newborns had similar weights in untreated pregnancies. In LDA-treated PE, F weighed less than M (2819.09 g vs. 3468.20 g  $p = 0.0208$ ). No significant weight differences were observed for F or M. **Conclusion:** Female newborns from preeclamptic pregnancies are most affected by lower weights, and this condition is not improved by low-dose aspirin.

### 400. 461 IMPACT OF SPERM NUCLEAR STATUS ON THE SUCCESS OF ASSISTED REPRODUCTION TREATMENTS

Valentina Munilla Lacasa<sup>1</sup>, Vanina Andrea Fontana<sup>1</sup>, Gastón Rey Valzacchi<sup>2</sup>, Juan Carlos Calvo<sup>1</sup>, Maite Yael Cambiasso<sup>1</sup>  
<sup>1</sup> Instituto de Biología y Medicina Experimental (IBYME-CO-NICET). <sup>2</sup> Procreate.

Assisted reproductive treatments (ART) aim to overcome infertility by eliminating most obstacles to fertilization. However, there are instances where these techniques fail. During spermiogenesis, the sperm nucleus undergoes significant changes that provide chemical and mechanical stability to the sperm, rendering them transcriptionally inactive and thereby preventing potential defects. However, alterations in the replacement of histones with protamines during this process can lead to errors that potentially affect the integrity of sperm chromatin. In the present study, we aimed to evaluate how sperm DNA damage might be associated with the decondensation process and how it affects ART success. Sperm samples were obtained from 25 patients undergoing ART. The fragmentation of sperm DNA has been measured using the TUNEL technique and the study of *in vitro* head decondensation was performed incubating sperm with 10 mM GSH and 46  $\mu$ M Heparin for 60 minutes. Some of the samples that were TUNEL-positive were magnetically separated using Annexin V columns (MACS) as a method for selecting non-apoptotic spermatozoa. *In vitro* decondensation was studied on these samples, both before and after MACS. The success of ART has been studied by classifying embryo quality on day 3. We found a positive correlation between TUNEL parameter and % of sperm head decondensation ( $p = 0.0015$ ,  $r = 0.60$ , Spearman correlation coefficient) and a positive correlation between TUNEL and poor embryo quality ( $p = 0.0027$ ,  $r = 0.66$ , Spearman correlation coefficient). In case of sperm samples selected with MACS we found that post selection, the % of decondensation increased ( $p = 0.0156$ , Wilcoxon test). In addition, we found that embryo quality does not differ with the MACS sperm selection. These results suggest that the integrity of sperm head is related to a successful ART and the findings suggest that the MACS technique might not significantly improve embryo quality in ART.

### 401. 491 ANTIOXIDANT RESPONSE TO URIC ACID EXPOSURE: IMPLICATIONS IN PREECLAMPSIA PATHOGENESIS

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**Introduction:** Before clinical symptoms of preeclampsia appear,

elevated serum uric acid levels can trigger stress in trophoblastic cells, leading to increased production of reactive oxygen species (ROS) and hydrogen peroxide ( $H_2O_2$ ). It has been reported that Aquaporin-3 (AQP3), which is essential for the migration and endovascular differentiation of trophoblastic cells, significantly decreases in preeclamptic placentas. Elevated uric acid levels are also associated with altered placentation. **Objectives:** Our objective is to evaluate if uric acid can induce oxidative stress in placental cells and how it correlates with AQP3 expression. **Methods:** Normal human placental explants were cultured in DMEM/F12 medium with different uric acid concentrations (0, 3, 5, and 7 mg/dL). Viability was assessed using the MTT assay. The expression of antioxidant enzymes SOD2 and catalase was evaluated by Western Blot. In silico analyses of the AQP3 promoter sequence (Gene ID: 360) identified potential antioxidant responsive elements (ARE). AQP3 expression was also assessed by Western Blot. **Results:** SOD2 and Catalase protein expression, Both increased significantly starting from 5 mg/dL ( $n=6$ ;  $p < 0.05$ ). In Silico Analysis: Identified ARE-like sequences in the AQP3 promoter, indicating potential binding sites for antioxidant response transcription factors. AQP3 Expression: Also significantly increased from 5 mg/dL ( $n=6$ ;  $p < 0.05$ ). **Conclusion:** Starting at 5 mg/dL, uric acid induces oxidative stress in placental explants, evidenced by increased SOD2 and catalase expression. This stress seems to regulate AQP3 expression, which also increased significantly at the same concentration. In silico analysis identified ARE-like sequences in the AQP3 promoter, indicating these elements might regulate AQP3 expression in response to oxidative stress. These findings suggest that uric acid may induce oxidative stress, potentially modulating AQP3 expression in placental tissue.

#### P4 POSTERS

FECHA Y HORA: 20/11/2024 11:30-12:30 H

COORDINADORES: VANESA HAUK,  
MATZKIN MARÍA EUGENIA

#### 402. 255 EVIDENCE OF PATERNAL PROGRAMMING OF OXIDATIVE AND ENDOPLASMIC RETICULUM STRESS IN THE LIVER OF FETUSES Sired BY DIABETIC MALE RATS

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Background: Male gametes exposure to paternal diabetes before conception can lead to permanent epigenetic marks that influence fetal development and, consequently, the health of the offspring. The increase in oxidative stress has been linked to endoplasmic reticulum stress (ERS) and fetal development alterations. Aim: to study the paternal programming of increased oxidative and endoplasmic reticulum stress in the fetal liver in a model of diabetic male. Methods: Male control (C) and diabetic rats (D, obtained by neonatal streptozotocin injection, 90 mg/kg) were mated with healthy females ( $n=8$ ). On day 21 of gestation, the fetuses sired by C and D groups were sexed under stereomicroscope and the fetal liver was stored at  $-80^{\circ}\text{C}$  to measure lipid peroxidation (by determination of tiobarbituric acid reactive species, TBARS), and the mRNA expression of the antioxidant enzymes Manganese Superoxide Dismutase (Mn-Sod) and Catalase and of the Glucose-regulated protein 78 (GRP78) that regulates ERS (by qPCR). Results: Male fetal liver from D group showed an increase in TBARS (13.4%,  $p < 0.05$ ) compared to controls, but no differences were found in females. Male fetuses from D group also exhibited higher hepatic mRNA expression of MnSOD and GRP78 (95% and 125% respectively,  $p < 0.05$ ) than controls, with no significant changes in Catalase mRNA expression. Female fetuses from D group showed increased GRP78 mRNA levels (138%,  $p < 0.05$ ) than controls, while MnSOD and Catalase mRNA levels showed no significant differences. Conclusions: Paternal diabetes induces oxidative and endoplasmic reticulum stress and alters gene expression of antioxidant enzymes in the fetal liver in a sex-specific manner.

#### 403. 281 EXPLORING THE THERAPEUTIC POTENTIAL OF ROSEMARY EXTRACT IN ENDOMETRIOSIS: ANTIOXIDANT AND ANTI-PROLIFERATIVE ACTIVITIES

Sofia del Valle<sup>1</sup>, Ignacio Ruiz<sup>2</sup>, Julieta Simone<sup>1</sup>, Mariela Bilotas<sup>1</sup>, Gustavo Leirós<sup>2</sup>, Analía Ricci<sup>1</sup>, Gabriela Meresman<sup>1</sup>.  
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Background: Natural therapeutic alternatives are being explored in endometriosis research due to the limitations of current treatments, which are relatively ineffective, often associated with significant side effects, and do not prevent disease recurrence. This study was conducted to characterize an ethanolic rosemary extract (RE) and evaluate its therapeutic potential in in vitro models of endometriosis. Methods: The total phenolic content was determined using the Folin-Ciocalteu method, and the main active compounds, carnosic acid (CA) and rosmarinic acid (RA), were quantified using high-performance liquid chromatography (HPLC). The antioxidant properties of the extract were assessed using DPPH and ABTS radical scavenging capacity assays, as well as the ferric reducing antioxidant power (FRAP) assay. The effects of various concentrations of RE on cell viability were examined in two human endometrial cell lines—stromal (t-HESC) and epithelial (ECC-1)—as well as in an endometriotic cell line (12-z). Additionally, the impact of RE on cell migration was assessed in T-HESC and 12-z cells. Results: The phenolic content of RE was found to be  $18.3 \pm 1.83 \mu\text{g EAG/g}$ , with  $37.2 \mu\text{g CA/g}$  and  $3.968 \mu\text{g RA/g}$ . The extract exhibited significant antioxidant activity, with DPPH values of  $35.36 \pm 4.13 \text{ mM Trolox equivalent (TE)}$ , ABTS values of  $38.45 \pm 0.92 \text{ mM TE}$ , and FRAP values of  $43.13 \pm 0.96 \text{ mM TE}$ . Furthermore, RE was found to significantly inhibit cell viability in ECC-1 at 5 mg/mL, in t-HESC at 8 mg/mL, and in 12-z at 7 mg/mL, while also reducing cell migration in 12-z and T-HESC cells. Conclusion: This study highlights the potential of ethanolic RE as a natural treatment for endometriosis, demonstrating significant antioxidant activity and effective inhibition of cell viability and migration in endometriosis models; however, more clinical research is required to fully validate its efficacy and safety.

#### 404. 326 HUMAN ENDOMETRIAL STROMAL CELL-DERIVED CONDITIONED MEDIUM CONFERS A METABOLIC PHENOTYPE TO MACROPHAGES THAT IS MODIFIED BY ANGIOTENSIN II TREATMENT

Rosario Macchi<sup>1,2</sup>, Marisa Castro<sup>1,2</sup>, Guillermo Blanco<sup>2</sup>, Andrea Canellada<sup>1,2</sup>

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Endometrial stromal cell (ESC) secretome may influence decidual macrophage (dMac) phenotype. Placental Renin-Angiotensin System may impact on ESC and modify their effects on dMac through functions related to mitochondria, metabolism, extracellular vesicles and vesicle acidification. We evaluated the effect of angiotensin (Ang) II conditioning of decidualized and non-decidualized ESC secretome, on macrophages (Mac) derived from peripheral blood monocytes regarding those functions. Human ESC cell line ThESC were decidualized for 6 days (d) or not (nd) and treated (+) or not (-) with Ang II for 48 h. Conditioned medium (CM) was collected. Monocytes were separated from PBMC and cultured for 6 days with CM in the presence of M-CSF. Mac were live-stained with mitotracker (MTK), lysotracker (LTR), monodansylcadaverine (MDC), Hoescht and tetramethylrhodamine ethyl ester (TMRE), scanned on an EVOS M7000 Imaging System and analysed with a single cell (sc) approach. ndCM- Mac showed large size, extensive cytoplasmic projections, enlarged mitochondrial network, high mitochondrial membrane potential (MMP), expansion of lysosomes and intracellular vesicles. MTK+MDC+LTR- extracellular structures and their uptake by Mac were noted. ndCM+ Mac located in separated clusters from ndCM- upon dimensionality reduction of fluorescent and morphometric sc parameters, mainly directed by the increase in mitochondrial mass (MM). dCM- Mac showed intercellular pro-

jections with cell-cell contacts and formed large size multinucleated cells with a shared mitochondrial network and high MMP. dCM+ Mac located in clusters separated from dCM-. LPS Mac appeared round, small, with high MM but without MMP, and located away from CM clusters. ThESC secretome conferred a differential phenotype to Mac that was modified by pretreatment of ThESC with Ang II. A fine tuning of dMac differentiation could be associated with the adjustment of embryo selectivity and quality control of implantation.

**405. 327 LOCAL PLACENTAL RAS DEREGULATION IS ASSOCIATED TO ALTERED METABOLISM AND MITOCHONDRIA-RELATED FUNCTIONS IN DECIDUAL MACROPHAGES IN PATIENTS WITH A HISTORY OF RECURRENT MISCARRIAGE**

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Recurrent miscarriage (RM) can result from high receptivity to low quality embryos. Placental Renin-Angiotensin System (RAS) can potentially influence metabolic reprogramming of decidual cells (DSC) and macrophages (dM) as a fine-tuning mechanism to selectivity. Here we explored the association of placental RAS deregulation and cell function alterations in dM and DSC through transcriptomics. Publicly available scRNAseq decidual samples from 3 RM patients and 3 normal controls (NC) (GSE214607) were analysed using Seurat5, Monocle3, DAVID, GSEA, G profiler and CellPhoneDB. We explored 72 endometrial biopsies (GSE165004) through CIBERSORT. We identified 17 cell types that included dM, DSC and extravillous trophoblast. We found expression of several RAS components among cell types, as well as four subtypes of dM that showed a phenotypic transition to mature dM, which expressed genes related to efferocytosis and resolution of inflammation. Function exploration of dM showed enrichment of extracellular vesicles, cytoskeletal rearrangement, vesicle secretion and extracellular space functions. Messages between DSC and dM included MerTK, GAS6, AXL and PROS1, as well as CXCL8, CCL2 and NR3C1. RAS gene expression was altered in RM vs. NC samples. Differentially expressed genes between samples were associated to functions such as oxidative phosphorylation, electron transport chain, ATP synthesis, mitophagy, cell growth, lipid and carbohydrate metabolism, endocytosis, vesicle acidification and cell migration. DSC abundance decreased and dM increased in RM patients vs. NC in bulk endometrial biopsies transcriptomes. Deregulated RAS genes were associated with RM group. Placental RAS was found deregulated in dM and DSC in RM patients, which was accompanied by deregulation in cell metabolism and motility functions. We conclude that deregulation of placental RAS is associated to functional alterations in RM patients. RAS intervention on DSC may impact these functions.

**406. 379 COMPARISON BETWEEN LEVELS OF ENDOCANNABINOIDS IN DECIDUA AND SERUM IN A MURINE MODEL OF PRETERM BIRTH**

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Preterm birth (PTB) is the leading cause of morbimortality in neonates. The decidua is an essential tissue that before the onset of labor, it thins to facilitate the labor in progress. A premature regression of the decidua is associated with preterm parturition. The endocannabinoid system (ECs; consisting of GPCRs, lipid ligands

and enzymes) is one of several signaling pathways implicated in maternal-fetal interface, and endocannabinoids are implicated in different aspects of the physiopathology of reproduction. Using a CB1-KO mice, we developed a murine model of PTB induced by LPS and we observed that CB1-KO mice show lower PTB rate than WT mice (56% CB1-KO vs. 81.5% WT) (Ethical Committee approval N° 2021-1942). The aim of this study was to evaluate if the levels of the main endocannabinoid and ECBs related molecules are modulated in serum of pregnant mice in the same direction that in decidual tissue using a model of PTB. We performed a mass spectrometry analysis on deciduas explants and serum obtained from WT or CB1-KO mice on day 15 of gestation. We found that decidual content of Anandamide (AEA; the main endocannabinoid) was increased in WT deciduas treated with LPS and this increment was not observed in CB1-KO deciduas. In serum from WT mothers treated with LPS we observed the same pattern, and this increment was not observed in serum from CB1-KO mice. Regarding *N*-acyl ethanolamines (NAEs), known as endocannabinoid related molecules, the serum content of PEA, SEA, OEA and LEA was increased with LPS treatment, with no differences between genotypes. However, this increase was not observed when we analyzed NAEs in decidual tissue. Our results suggest that AEA measured in serum could be a possible indicator of inflammation in maternal-fetal interface. Further studies are needed to postulate ECBs as biomarkers of PTB.

**407. 389 ROLE OF ENDOCANNABINOIDS IN THE REGULATION OF OXYTOCINERGIC SYSTEM IN HUMAN PLACENTA**

Yilena Canela Delás<sup>1</sup>, Tomás Etcheverry<sup>1</sup>, Marisol Murillo Murillo<sup>1</sup>, Natalia Szpilbarg<sup>2</sup>, Jorge Sar<sup>3</sup>, Andrea De Laurentiis<sup>1</sup>, Mariana Farina<sup>1</sup>

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Endocannabinoids have been implicated in reproductive processes, from implantation to childbirth. A significant increase in anandamide (AEA) levels has been reported, suggesting a central role for this endocannabinoid in the onset of labor. Among the essential signals in the initiation of labor, oxytocin and its binding to the oxytocin receptor (OTR) are well known; however, the relevance of the oxytocinergic system (OT/OTR) in the human placenta remains unknown. Therefore, this study aimed to investigate the role of endocannabinoids in the expression of the OT/OTR in both normal and pathological human placentas. Human placenta obtained from term singleton pregnancies were collected from three groups: vaginal deliveries VD (n=8), cesarean section CS (n=15) without uterine contractile activity and preeclamptic placentas PE (n=8) from women with elevated blood pressure and proteinuria after 20 weeks of gestation. In addition, explants from CS placentas were treated with AEA, URB-597 a selective FAAH inhibitor, R-(+)-Methanandamide (Met-AEA), a stable AEA analogue, and/or SR141716A, a CB1 receptor antagonist, to assess the impact of endocannabinoids on OT/OTR. OTR expression was analyzed by WB and immunofluorescence, while OT levels were measured by Dot blot. Our findings reveal that VD placentas expressed significantly higher OT levels and protein expression of OTR compared to CS and PE placentas (p<0.05). We observed a significant increase in OTR expression when CS placentas were incubated with Meta-AEA or sequential pulses of AEA, suggesting that prolonged stimulation of AEA is necessary to increase OTR protein levels. Furthermore, these effects were prevented with SR141716A (p<0.05), a selective CB1 receptor antagonist. In summary, our results demonstrate that changes in AEA levels modulate OTR expression and OT release in human placenta, suggesting a prominent role of endocannabinoids as a mediator of labor signaling.

**P5 POSTERS**

FECHA Y HORA: 20/11/2024 16:10-17:10 H

**COORDINADORES: EVANGELINA CAPOBIANCO,  
MARÍA LAURA RIBEIRO**

**408. 002 SPERM-BORNE mRNAs: POTENTIAL ROLES IN ZYGOTE GENOME ACTIVATION AND EPIGENETIC INHERITANCE**

**Betina González, Candela R. González.**

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Despite growing recognition of sperm-borne mRNAs and their delivery to the oocyte during fertilization, the exact roles of paternal mRNAs in fertilization and embryonic development remain unclear. We curated transcriptomic data of murine sperm and performed network and functional analysis with the upper quartile of the most enriched mRNAs in the sperm pool, meeting two criteria: higher levels in the zygote vs the oocyte pool, indicating paternal contribution, and evidence of positive translation in the zygote. The analysis identified 52 sperm-borne mRNAs prominently involved in chromatin organization and regulation of gene expression. Functional enrichment highlighted key proteins linked to chromatin-binding complexes, with a significant focus on chromatin remodeling and epigenetic regulation. Histone H3.1 emerged as a central hub in the network, interacting with proteins like Nuclear Receptor Co-Repressor 1 (NCOR1) and MLLT3, involved in histone modification. This network also revealed proteins involved in histone acetylation, such as MBTD1 and PHF20, which are part of the NuA4 histone acetyltransferase complex, NSD1, a histone methyltransferase that regulates H3K36me2, and SPIN1, a reader of specific histone marks. Additionally, the analysis highlighted proteins crucial for mRNA processing and export, such as SLBP, YBX1, and components of the exon junction complex like MAGOH, essential for mRNA stability and proper gene expression in the developing embryo. Our study reveals that sperm cells carry a distinct set of mRNAs crucial for chromatin organization, DNA metabolism, mRNA processing, and gene expression regulation during the maternal-to-zygotic transition and zygotic genome activation. These findings underscore the significant role of sperm-borne RNAs in early embryonic development and epigenetic inheritance, highlighting the need for further research to fully understand their functions.

**409. 048 POTENTIAL EFFECTS OF JUVENILE LIRAGLUTIDE TREATMENT ON TESTICULAR FUNCTION IN ADULT ANIMALS**

Marina Ercilia Dasso<sup>1</sup>, Cecilia Lucía Centola<sup>1</sup>, Florencia Nerea Tabares<sup>1</sup>, Cristian Sobarzo<sup>2</sup>, María Gabriela Ballerini<sup>1</sup>, María Noel Galardo<sup>1</sup>, Silvina Beatriz Meroni<sup>1</sup>, María Fernanda Riera<sup>1</sup>.

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Blood-testis barrier (BTB) is essential to maintain spermatogenesis by providing an adequate environment for germ cell development. Several animal models have shown that defects in the BTB lead to detrimental effects on spermatogenesis. Liraglutide (Lira), an analog of glucagon-like peptide 1, is widely used for treating adult patients with obesity and type 2 diabetes. Recently, Lira was approved for use in children over 10 years old. We have previously observed that Lira treatment of juvenile rats increases BTB permeability. Therefore, we proposed to analyze whether Lira alters testicular cell apoptosis in juvenile rats and the impact of juvenile treatment on testicular function in adult animals. To this end, SD male rats were assigned to the following groups: Lira (receiving daily 0,2 mg/kg Lira s.c.) and control (C; sterile saline solution s.c.) from Pnd20 to Pnd33, a period that is essential to complete a functional BTB.

On Pnd34, cell apoptosis was evaluated by TUNEL. Lira treatment increases cell apoptosis (C:5.5±2.1%; Lira:12.7±4.8% tubules with TUNEL-positive cells; n=6; \*p<0.05). To evaluate whether juvenile exposure to Lira has consequences during adulthood, a set of animals treated as mentioned was allowed to grow until Pnd90. Testicular histological analysis showed normal cellular associations and complete spermatogenesis. The BTB permeability, analyzed using a biotinylated tracer, was similar in both groups (C: 3.2±1.0; Lira: 2.8±1.3 % permeated tubules; n=6). Besides, juvenile Lira treatment did not modify daily sperm production and serum testosterone levels in adult animals. Altogether, these results indicate that the harmful effect of Lira on BTB integrity was accompanied by an increase in cell apoptosis in juvenile rats. Considering the results obtained in adult animals, it is possible to speculate that Lira treatment during the juvenile period does not affect testicular function in adulthood.

**410. 082 REGULATION OF LIPID METABOLISM BY LACTATE IN MATURE SERTOLI CELLS**

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Sertoli cells (SCs) actively metabolize glucose into lactate (LAC), the major energy source for germ cells. In addition, SCs use fatty acids (FAs) as their own energetic fuel, which are stored as triglycerides (TAGs) in lipid droplets (LDs), crucial for maintaining ATP levels. On the other hand, new evidence suggests that LAC is no longer considered merely as a byproduct of anaerobic glycolysis or a secondary energy metabolite. Instead, it is emerging as a multifunctional signalling molecule exerting diverse biological actions. Indeed, LAC can suppress lipolysis in adipocytes through direct activation of GPR81 receptor, which is also expressed in SCs. Since the regulation of mechanisms involved in LD formation can be relevant for SC physiology, we aimed to evaluate the effect of LAC on the capacity of SCs to store lipids. SC obtained from 20-day-old rats were maintained under basal conditions (B) or incubated with 20 mM LAC for different periods of time. The expression levels of genes encoding proteins involved in lipid metabolism and TAG and LD content were examined. It was observed that LAC increased glycerol-3-phosphate acyltransferase 3 (GPAT3) (LAC48h: 1.37±0.06\*) while it decreased adipose triglyceride lipase (ATGL) (LAC4h: 0.68±0.01\*, fold-variation, mean±SD, n=3; \*p<0.05 vs B) expression levels. An augmentation of TAG content was also observed (B: 1.83±0.18, LAC48h: 2.43±0.29\* µg/µgDNA, n=3; \*p<0.05 vs B). Besides, LAC increased LD content and the effect was inhibited by 3-hydroxybutyrate (3-OHB), an antagonist for GPR81 (B: 0.54±0.1<sup>a</sup>; 3-OHB: 0.49±0.11<sup>a</sup>; LAC48h: 0.87±0.07<sup>b</sup>; LAC+3-OHB: 0.60±0.12<sup>a</sup> number of LD/cell, n=3, different letters indicate statistically significant differences, P<0.05). These results suggest that LAC regulates lipid metabolism in mature SCs, probably through GPR81 activation.

**411. 213 POLYCYSTIC OVARY SYNDROME: LONG-TERM EFFECTS ON UTERINE LESIONS AND TISULAR STEROID METABOLISM**

Gisela Soledad Bracho, Inri Iñiguez, María Virginia Acosta, Gabriela Anahí Altamirano, Verónica Lis Bosquiazzo.

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Polycystic ovary syndrome (PCOS) is an endocrinopathy that affects women of reproductive age and could cause long-term endometrial alterations. In this work, the aim was to investigate the long-term effects of PCOS on uterine lesions and endocrine environment. We evaluated: a) the incidence and multiplicity of uterine lesions and b) the endocrine state evaluating serum steroid hormone levels, uterine steroid receptor and steroidogenic enzyme expression at 6 and 24 months. PCOS was induced in female Wistar rats through sc. injection of dehydroepiandrosterone (6mg/100g bw) from 21 to 40

days of age. The CONTROL group received injections of sesame oil. After treatments, female rats were euthanized at 6 and 24 months. Blood was collected for serum steroid hormones quantification and uterine tissues were processed for histomorphology analysis and qRT-PCR. At 6 months, the results demonstrated that PCOS rats showed similar uterine lesions but with a higher 17- $\beta$ -estradiol (E2)/progesterone (P4) ratio than control rats ( $p < 0.05$ ). At 24 months, the PCOS rats showed a higher incidence of luminal hyperplasia and a higher multiplicity of glands with metaplasia and conglomerates of glands, as well as higher E2/P4 ratio ( $p < 0.05$ ). In addition, an increase in the protein expression of estrogen receptor alpha (ESR1) and mRNA for steroid sulphatase and steroid (Sts) and 5 $\alpha$ -reductase type I (*Srd5a1*) was observed. These results show that early induced PCOS alters the uterine endocrine status in the long-term, with an increased E2/P4 ratio and changes in steroidogenic enzyme and ESR1, suggesting a higher exposure and sensitivity of the uterus to estrogens, which together could explain the higher incidence/multiplicity of uterine lesions observed in PCOS rats.

**412. 393 HIGH-SUGAR DIET NEGATIVELY IMPACTS MALE FERTILITY: POTENTIAL ROLE FOR THE SMALL GTPASE RAC1 AS A METABOLIC STATE TRANSDUCER IN THE TESTIS OF DROSOPHILA MELANOGASTER**

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Obesity is considered a relevant risk factor for reproductive disorders. However, little is known about the effect of diet on male fertility. Rac1 is a member of the Rho GTPase family, which has a prominent role in metabolic homeostasis. We sought to determine the effect of diet on male fertility and the role of Rac1. To that end, adult male *OregonR Drosophila melanogaster* flies were kept under a high-sugar diet (HSD) or a standard diet (SD) diet for 14 days before performing fertility assays. To determine the involvement of Rac1, we knocked down (KD) Rac1 in the testis hub (stem cell niche) using the UAS-Gal4 system and *dsRNAi*. The *Rac1-KD* flies (control line: *mCherry<sup>dsRNAi</sup>*) were fed a HSD or a SD for 14 days, before performing fertility assays. For the assays, the male flies kept under the different diets were mated with *OregonR* virgins (1:1 ratio) for 3-5 days. Fertility rate (FR) was determined as the number of *pupae*/vial (mean $\pm$ SEM), and it was compared to the control through a non-parametric unpaired t-test (Mann-Whitney, significance  $P < 0.05$ ). Testis cell composition was analysed by immunofluorescence staining for somatic (traffic jam, Tj) and germ (vasa) cell testis markers. A 26.9% reduction in FR was seen for flies kept under HSD (17.75 $\pm$ 1.52, n=61) compared to those fed SD (24.27 $\pm$ 1.98, n=56,  $P=0.016$ ). Upon *Rac1* KD, FR decreased by 70.1% (6.62 $\pm$ 3.06) compared to the control line (22.13 $\pm$ 5.83, n=16,  $P=0.031$ ), suggesting a role for Rac1 in male fertility maintenance. A similar trend was observed under HSD (control line: 16.36 $\pm$ 5.50, n=14, vs *Rac1-KD*: 4.00 $\pm$ 2.09, n=16,  $P=0.083$ ). We found a dramatic over-proliferation of Tj+ cells and vasa+ cells in the testes of *Rac1-KD* flies under both diets. Our results show that both, HSD and loss of *Rac1* in the testis hub, have a negative impact on male fertility. The absence of *Rac1* in the hub could prevent testis adaptation to changes in the nutritional status, suggesting a role for Rac1 as a metabolic state transducer.

**413. 406 EXPLORING NORMALITY TO UNDERSTAND INFERTILITY: ANALYSIS OF ENDOMETRIAL MICROBIOME IN HEALTHY REPRODUCTIVE AGE ARGENTINE WOMEN**

Constanza Nazario1,2\*, Leticia Solari1,2\*, Mariela Sciarra3, Carlos Daniel De La Vega Elena1  
1Instituto de Investigaciones IUNIR, Rosario, Argentina 2 Servicio de Reproducción, Sanatorio Centro, Rosario, Argentina. 3CIBIC, Rosario, Argentina

Background: The human microbiome, comprising trillions of microorganisms, varies due to geographical and individual factors. The endometrial microbiome plays a crucial role in female reproductive health, influencing menstrual cycles, pregnancy, and childbirth. However, specific compositional data from different geographic re-

gions remain understudied. Objectives: This study aimed to characterize the endometrial microbiome in healthy women of reproductive age from Rosario, Argentina, to establish a baseline for comparative pathological studies. Materials and Methods: We conducted a prospective observational study with 100 healthy women aged 22 to 50, recruited from August 2022 to May 2023. Endometrial samples were collected from 98 participants using an endometrial aspiration cannula during the follicular phase or under hormonal contraceptive treatment. Microbiome analysis was performed by sequencing the V4 region of the 16S rRNA gene using the NextSeq 500/550 platform (Illumina) with 2x150 bp Paired End chemistry. Results: The endometrial microbiome was dominated by the phylum Firmicutes, specifically the Lactobacillaceae family. The most prevalent species identified were *Lactobacillus helveticus* and *Lactobacillus iners*. Conclusion: The study successfully mapped the endometrial microbiome composition in healthy women from Rosario, revealing a predominance of lactobacilli, consistent with previous findings in other regions. These results provide a critical reference for future research on the role of the microbiome in gynecological reproductive pathologies. The geographic specificity of these findings highlights the need for similar studies across different regions to fully understand microbiome variability and its implications on fertility.

**414. 449 PROGRESS IN THE DEVELOPMENT OF AN INNOVATIVE THERAPY USING THERMOSENSITIVE HYDROGELS AS SERTOLI CELL CARRIERS**

Denisse Ferrer Viñals1, Patricia Verónica Jacobo1, María Belén Maio1, Thaisy Munduruca Pires1, Cecilia Lucía Centola2, María Noel Galardo2, Mónica Beatriz Frungier3, Cristian Marcelo Sobarzo1, Romina Glisoni4, María Susana Theas1  
1 Instituto de Investigaciones Biomédicas (INBIOMED, UBA-CONICET), Facultad de Medicina. Buenos Aires, Argentina.  
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4 Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Nanobiotecnología (NANOBIOTEC UBA-CONICET), Buenos Aires, Argentina.

Experimental orchitis in rats is a well-established model for studying autoimmune (EAO) infertility. Traditional pharmacological treatments have had limited success in restoring testicular function affected by chronic inflammation. A novel approach involves intratesticular therapy using immunosuppressive Sertoli cells, offering a promising alternative for treating epididymitis-orchitis. This study aimed to explore the use of the biocompatible linear triblock copolymer, Pluronic® F127, as a hydrogel for intratesticular transplantation of mature Sertoli cells in EAO rats. Additionally, we sought to refine protocols for isolating Sertoli cells from healthy gonads for therapeutic applications. F127 (25% w/v) forms a gel at physiological temperature (37°C), allowing for localized delivery of Sertoli cells. Intratesticular injection of the TM4 Sertoli cell line (2x10<sup>6</sup> cells) dispersed in F127 hydrogel (22% and 25% w/v) significantly increased cell density near the injection site compared to cells dispersed in saline after 2 days. Transplanted Sertoli cells in F127 (25% w/v) were identified as Sox-9+ cells in the testicular interstitium at 19, 33, and 51 days post-injection. These cells exhibited round and elongated morphologies, with some forming circular structures. A reliable protocol for the isolation and culture of adult therapeutic Sertoli cells has been established. After 4-5 days in culture several parameters were determined: cell viability was 83% (trypan blue exclusion), purity was 95% (vimentin+ cells). Lactate production under basal and stimulated (FSH 0.1  $\mu$ g/ $\mu$ l) conditions was 40.86  $\mu$ g/ $\mu$ g DNA and 61.20  $\mu$ g/ $\mu$ g DNA after 24 h, respectively. Our findings suggest that F127 is an effective hydrogel matrix for Sertoli cell therapy, as it enhances cell concentration and the therapeutic impact of secreted soluble factors. Moreover, this approach could reduce the number of Sertoli cells required for treatment.



## P6 POSTERS

FECHA Y HORA: 21/11/2024 11:00-12:00 H  
 COORDINADORES: MANUEL WOLFSON,  
 SUSANA VALDEZ

**415. 104 DIRECT EFFECTS OF ANTI-MULLERIAN HORMONE ON CUMULUS-OOCYTE COMPLEXES**

Leda Mailen Vera<sup>1</sup>, Luciana Judith Bogado<sup>1</sup>, Philippe Godin<sup>2</sup>, Yamila Gamaleri<sup>1</sup>, David Pepin<sup>2</sup>, Marina Cinthia Peluffo<sup>1</sup>  
<sup>1</sup>Centro de Investigaciones Endocrinológicas "Dr. César Bergadá" (CEDIE) -CONICET-FEI-División de Endocrinología, Hospital de Niños "Ricardo Gutiérrez".  
<sup>2</sup>Massachusetts General Hospital, Boston, MA, United States.

Anti-Müllerian hormone (AMH) is involved in several physiological processes in the ovary, including folliculogenesis. Recent studies have shown that supraphysiological AMH causes contraception in cats. However, its mechanism of action remains unknown. Hence, this study aimed to assess if AMH has a direct effect on cumulus-oocyte complexes (COC) and, in consequence, on periovulatory events, such as cumulus-oocyte expansion (C-OE). To evaluate this objective, ovaries from adult domestic cats (*Felis catus*, n=22) were used, and COCs were isolated from antral follicles (0.5–2 mm). Isolated COCs (n=108) were randomly assigned and cultured in the presence of AMH (1 or 10 µg/ml) or absence (Control, media alone). COCs were cultured at 38°C under 5% CO<sub>2</sub> for three hours to evaluate gene expression. Representative COCs for each treatment (n=3 COCs/treatment/culture, n=4 cultures; n=36 total COCs) were used for individual RNA extraction using an RNA Nanoprep Kit and quantitative RT-PCR using Taqman probes. AMH target genes (*ID3* and *IGFBP5*) and essential ovulatory genes (*AREG*, *HAS2*, and *TNFAIP6*) were assessed. Normalized mRNA expression levels with ribosomal RNA protein 18S levels showed a significant increase in *ID3* expression in the AMH group (10 µg/ml, p<0.05). Higher levels of *IGFBP5* were also observed in the presence of both concentrations of AMH, but it did not reach statistical significance. In contrast, no significant changes were observed in the expression of any of the assessed ovulatory genes. Also, a preliminary 24-hour culture (n=53 COCs) showed that addition of exogenous AMH to the culture media may promote the maintenance of compact COCs and reduce the IF signal of hyaluronic acid, a key marker of C-OE. In summary, our results demonstrate a direct role for AMH in the feline COC, suggesting a possible inhibition of periovulatory processes.

**416. 163 MATERNAL DIETS ENRICHED IN EXTRA VIRGIN OLIVE OIL AMELIORATES SERUM MARKERS OF LIVER AND HEART DAMAGE IN DIABETIC RATS AT POSTPARTUM**

Virginia Taylor, Florencia Schibert, Evangelina Capobianco, Romina Higa, Alicia Jawerbaum.  
 Laboratory of Reproduction and Metabolism. CEFYBO-CO-NICET. Faculty of Medicine, University of Buenos Aires, Buenos Aires, Argentina.

**Introduction:** Pregnancy is a challenge for metabolic and cardiovascular health in pregestational diabetes. Diets enriched in extra virgin olive oil (EVOO) improve metabolic and cardiovascular health in metabolic diseases. Whether EVOO-enriched diets improve maternal postpartum outcomes in diabetic mothers is unknown. **Aim:** To evaluate circulating markers of hepatic, cardiac and tissue damage one month after delivery in diabetic rats fed or not in with an EVOO-enriched diet from preconception to parturition. **Methods:** A mild pregestational diabetic rat model was induced by neonatal administration of streptozotocin (90 mg/kg sc). Control and diabetic females were mated with healthy males and fed a standard diet supplemented or not with 6% EVOO from preconception to parturition (n=8 per group). Maternal serum was collected one month after parturition for evaluation of markers of liver and tissue damage (alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities) and of cardiac damage (creatinine kinase-MB (CK-MB) activity) by colorimetric assays (Wiener Lab).

**Results:** At postpartum, diabetic dams showed an increase in serum ALT and ALP activities compared to controls (+79% and +118%, respectively, p<0.02), alterations that were no longer significant when the diabetic dams received the EVOO-enriched diet. Serum LDH activity was increased in the diabetic dams compared to controls (+100%, p<0.02), an alteration that remained when the dams received the EVOO-enriched diet. Serum CK-MB activity was increased in the diabetic dams compared to controls (+42%, p<0.05), an alteration that was prevented by the maternal EVOO-enriched diet. **Conclusion:** Increased serum markers of tissular damage, hepatic damage and cardiac damage were evidenced in diabetic dams at postpartum. The observed metabolic alterations were partially prevented by the maternal EVOO-enriched, a diet that seems to protect diabetic dams from cardiac damage.

**417. 212 DYSBALANCE BETWEEN MARKERS OF CELLULAR SENESCENCE AND PROLIFERATION IN DECIDUA FROM ADVANCED MATERNAL AGE RATS**

María Laura Leonardi, Alejandro Ranieri, Irune Pirrone, Alicia Jawerbaum, Romina Higa  
 Laboratorio de Reproducción y Metabolismo, CEFYBO-CO-NICET, Facultad de Medicina, Universidad de Buenos Aires, Argentina.

**Background:** Pregnancy at Advanced Maternal Age (~35 years, AMA) induces obstetric complications and neonatal adverse outcomes. During early pregnancy, an optimal decidual function is essential for a proper placenta and embryo development. In our rat model of AMA, decreased embryonic growth and altered placental morphology were previously observed in day 12 of pregnancy. FoxO1 is a transcription factor that plays a role in decidualization and placentation and, at a cellular level, in senescence by modulating the expression of genes related to cell cycle withdrawal, as p21 and p16. Phosphorylation of FoxO1 by AKT and SGK1 induces its inactivation. Previously, we have shown that the activity of FoxO1 was increased in decidua from AMA. Here, we aim to study phosphorylation of AKT and SGK1 and levels of p21, p16 and PCNA in decidua from control and AMA rats. **Methods:** Three-month-old (Control) and ten-month-old (AMA) Wistar rats were mated with three-month-old males. At day 12 of pregnancy, decidua was obtained for Western blot and RT-qPCR assays (n=5-7 per group).

**Results:** A reduced phosphorylation of SGK1 (-48%, p<0.05) without changes in total SGK1, P-AKT and total AKT were observed in decidua from AMA rats compared to controls. mRNA levels of p21 and p16, FoxO1 target genes, were increased in decidua from AMA rats compared to controls (+54% and +35% respectively; p<0.05). PCNA, a cellular proliferation marker, was reduced (-32%, p<0.05) in decidua from AMA rats compared to controls. **Conclusion:** The reduced phosphorylation of SGK1 may contribute to the reduced phosphorylation of FoxO1 found in decidua from AMA rats that leads to an increased activity of this transcription factor. Target genes of FoxO1, p21 and p16, are increased probably leading to a dysbalance between cellular senescence and proliferation in decidua from AMA rats. These alterations are likely related to the decreased embryonic growth and altered placental morphology previously observed in AMA rats.

**418. 242 MATERNAL OVERWEIGHT INFLUENCES THE RESPONSE OF MALE OFFSPRING TO 3-METHYLCHOLANTHRENE EXPOSURE**

Jeremias Pablo Flores Quiroga<sup>1</sup>, Victoria Dechamps<sup>1</sup>, Marina Labiano<sup>1</sup>, Florencia Heinecke<sup>1</sup>, Rocio Alejandra Galarza<sup>2</sup>, Verónica White<sup>1</sup>, Alicia Graciela Faletti<sup>1</sup>.  
<sup>1</sup>CEFYBO-CO-NICET-UBA, Facultad de Medicina, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina. <sup>2</sup>CEREGeo-FCyT-UADER, Facultad de Ciencia y Tecnología, Universidad Autónoma de Entre Ríos, Oro Verde, Entre Ríos, Argentina.

**Objective:** This study aimed to investigate whether male offspring from overweight rats are more susceptible to the impact of 3-Methylcholanthrene (3MC), an environmental pollutant with reproductive toxicity and obesogenic effects. **Methods:** Rats were fed either a

standard (SD) or fat-rich (FD) diet for eight weeks and then mated with control males. Progenitors remained on their respective diets until weaning. The offspring that consumed SD were orally exposed to either a vehicle (V) or 3MC (0.5mg/kg) three times per week for seven weeks starting at 60 days of age. After treatment, they were euthanized and the body, fat pad, testicles and epididymis were weighed. Sperm was obtained from the cauda of the epididymis and sperm count and motility were determined. **Results:** Exposure to 3MC significantly decreased body weight ( $p < 0.01$ ) in FD offspring, although the adipose tissue index remained unchanged. In contrast, SD offspring exposed to 3MC exhibited a reduced adipose tissue index without any significant changes in body weight. No differences were observed in the relative weights of the testis and epididymis. However, 3MC exposure significantly increased testicular length in offspring from both dietary groups ( $p < 0.01$ ). Interestingly, in the SD group, 3MC led to a decrease in epididymal length ( $p < 0.05$ ), while in the FD group, it caused an increase ( $p < 0.05$ ). Finally, 3MC affected sperm quality, resulting in a decrease in sperm motility and count in offspring from both the SD and FD groups ( $p < 0.05$ ). The 3MC showed different obesogenic effects in the offspring from control and overweight rats. **Conclusion:** Gonads morphometry was differently affected between dietary groups, although 3MC similarly affected sperm quality in males born to control and overweight rats. Our results indicate that maternal metabolic history plays an important role in determining the susceptibility of offspring to metabolic and reproductive alterations caused by exposure to environmental pollutants.

**419. 492 FEMALE OFFSPRING OF OVERWEIGHT RATS ARE MORE SUSCEPTIBLE TO THE OBESOGENIC EFFECTS OF 3-METHYLCHOLANTHRENE**

Labiano Marina, Dechamps Victoria, Heinecke Florencia, Flores Quiroga Jeremias Pablo, Galarza Rocio Alejandra, Faletti Alicia Graciela, White Veronica, CEFYBO-CONICET-UBA Buenos Aires, Argentina

Polycyclic aromatic hydrocarbons are common toxic pollutants with obesogen effects because they interfere with lipogenic and adipogenic processes. Offspring from obese rats show lipid liver overaccumulation. We aimed to investigate whether maternal obesity predisposes female offspring to an increased susceptibility to 3MC-induced obesogen and toxic effects. Female Wistar rats were fed with standard chow (control) or a fatty diet from weaning (FD rats). After 8 weeks, control and FD rats were mated with control males and allowed to give birth and breast-feed their pups. Progenitors maintained their respective diets until weaning. All offspring were fed a standard diet. At 60 days of age, one female from each progenitor was orally administered 3MC (0.5mg/Kg) and another with vehicle three days a week for seven weeks and then euthanized. Body, adipose tissue, and liver were weighted. Circulating liver enzyme-activity was measured as a parameter of liver-function. Body weight was unchanged between control and FD offspring and decreased by 3MC only in the FD offspring (12%,  $p < 0.05$ ). Also, gonadal adipose tissue (GAT) weight and index (weight relative to body weight) and total adipose tissue index were unchanged between control and FD offspring and decreased by 3MC only in the FD offspring (40%, 28%, and 20%,  $p < 0.05$ ). Aspartate transaminase serum activity was increased in the offspring from the FD group compared to the control offspring (39%,  $p < 0.05$ ). Bilirubin levels were increased by 3MC only in the offspring from FD rats (39%,  $p < 0.05$ ). After exposure to 3MC, FD offspring displayed obesogen and toxicity-related alterations that were not evident in the controls, suggesting an increased vulnerability of the offspring from obese rats to the contaminant and highlighting the role of the maternal metabolic health in the programming of the susceptibility to obesogen compounds.

**420. 494 ETHANOL INTAKE INDUCE DAMAGE TO REPRODUCTIVE BIOLOGY IN MALE MICE: PROTECTIVE ROLE OF RESVERATROL**

Lucila Gotfryd<sup>1</sup>, Maite Yael Cambiasso<sup>1</sup>, Juan Carlos Calvo<sup>1</sup>, Vanina Andrea Fontana<sup>1</sup>

<sup>1</sup> Instituto de Biología y Medicina Experimental (IBYME) - CONICET, Vuelta de Obligado 2490, Ciudad Autónoma de

Buenos Aires, Argentina

Ethanol consumption is known to have detrimental effects on male fertility, including impairments in sperm quality and testicular function. The potential of resveratrol to counteract these adverse effects remains unclear. This study aimed to investigate the effects of ethanol consumption on sperm quality, testicular histology, and reactive oxygen species levels, and to determine whether resveratrol can reverse these effects. Adult male mice (*Mus musculus*, CrIFcen:CF1) were divided into 4 groups: water + vehicle (Control), ethanol 15% + vehicle (Et), water + resveratrol 20 mg/kg (Res), and ethanol 15% + resveratrol 20 mg/kg (EtRes). Ethanol was administered *ad libitum* in the drinking water, while the vehicle and resveratrol were administered every 24 hours via esophageal gavage for 12 days, followed by two days of water only. After sacrifice, sperm motility, concentration, viability, membrane integrity (hypo-osmotic swelling test), and presence of reactive oxygen species (CellROX Green assay) were assessed. Testicular DNA fragmentation (TUNEL assay) and histology (after fixation and staining with hematoxylin-eosin) were also evaluated. A significant reduction in sperm membrane integrity was observed in the Et group compared to the Control group ( $p < 0.05$ ), with resveratrol showing a potential protective effect. No significant differences were observed in sperm concentration, motility or viability between the groups. DNA fragmentation analysis in seminiferous tubules and Leydig cells revealed higher levels in the Et group ( $p < 0.001$ ), with resveratrol reducing this damage in both the Res and EtRes groups. Histological analysis showed that resveratrol prevented ethanol-induced histopathologic damage in the seminiferous epithelium. In conclusion, ethanol consumption impairs sperm membrane integrity, increases DNA fragmentation in testicular cells, and damages seminiferous tubules histopathology. These adverse effects are partially mitigated by resveratrol treatment.

**421. 504 PORPHYROMONAS GINGIVALIS OUTER MEMBRANE VESICLES IMPAIR TROPHOBLAST ENDOVASCULAR FUNCTION**

Matías Sassot, Brenda Lara, Daniel Papparini, Ailén Fretes, Paula Tribelli, Rosanna Ramhorst, Claudia Pérez Leirós, Daiana Vota, Vanesa Hauk

Universidad de Buenos Aires - CONICET. Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN). Buenos Aires, Argentina.

Periodontitis is a chronic inflammatory oral disease affecting up to 50% of pregnant women that has been associated with pregnancy complications like preeclampsia (PE) and fetal growth restriction (FGR). *Porphyromonas gingivalis* (Pg), a key pathogen in periodontal inflammation, releases outer membrane vesicles (OMVs) with virulence factors crucial for its pathogenicity. During early pregnancy, trophoblast cells (Tb) undergo changes that enable them to proliferate, migrate, invade the decidual stroma, and participate in vascular remodeling while modulating the immune response. Chronic maternal infection with oral pathogens like Pg could disrupt placental homeostasis, leading to PE and FGR. The aim of this work is to study the effect of PgOMVs on Tb endovascular function and their impact on vascular remodeling. For *in vitro* experiments the HTR-8 trophoblast cell line and EA.hy926 endothelial cells were used. The migratory capacity, reactive oxygen species (ROS) production and mRNA expression, were measured by wound healing assay, flow cytometry using DCFH-DA probe and qPCR, respectively. For *in vivo* studies in C57BL/6 mice treated with PgOMVs on day 6.5 of gestation and implantation sites were obtained at 8.5 gestation day. Results show that PgOMVs disrupt the balance of angiogenic mediators in Tb and impair endothelial cell function, evidenced by reduced migration ( $p < 0.05$ ), increased immune cell adhesion, and higher ROS production ( $p < 0.05$ ). *In vivo*, PgOMVs treatment induce histological changes in the implantation sites compared to control and decreases the mRNA expression of key markers of trophoblast differentiation such as Prl3d1, angiogenesis, and immune regulation ( $p < 0.05$ ). Furthermore, implantation sites treated with Pg OMVs show increased ROS production compared to control implantation sites ( $p < 0.05$ ). These findings suggest a pathogenic role for PgOMVs in early pregnancy, disrupting trophoblast contributions to vascu-

lar remodeling and placental homeostasis.

## TOXICOLOGÍA / ECOTOXICOLOGÍA

### 01 COMUNICACIONES ORALES

FECHA Y HORA: 19/11/2024 11:00-12:00 H

LUGAR: SALA DE CÁMARA

COORDINADORES: PABLO ANDRES EVELSON,  
MARÍA DEL CARMEN MARTINEZ,  
MARÍA TERESA PINO

#### 422. 007 EFFECTS OF DERMAL EXPOSURE TO BENZOPHENONE-3 DURING PREGNANCY ON MOUSE MAMMARY GLAND INVOLUTION

Ayelen L. Gomez<sup>1,2</sup>, María Belén Beckley<sup>1</sup>, Gonzalo Schierano-Marotti<sup>1,2</sup>, Sofia Oddi<sup>1</sup>, Verónica L. Bosquiazzo<sup>1,3</sup>, Gabriela A. Altamirano<sup>1,2</sup>, Laura Kass<sup>1,2</sup>

<sup>1</sup> Instituto de Salud y Ambiente del Litoral (ISAL, UNL-CONICET), Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. <sup>2</sup> Cátedra de Patología Humana, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral. <sup>3</sup> Departamento de Bioquímica Clínica y Cuantitativa, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral.

Mammary involution is characterized by intense tissue remodeling, and its alteration could lead to inadequate lactation or predispose to tumor development. In this sense, the UV-filter benzophenone-3 (BP3) has been shown to have estrogenic activity and to alter apoptosis, which could affect this process. Here, we assessed whether exposure to BP3 during pregnancy alters the involution of the mammary gland parenchyma in mice. Pregnant C57BL/6 mice were dermally exposed to vehicle (sesame oil; Control), 5 (BP3.5) or 50 mg BP3/kg/day (BP3.50) from gestation day 8.5 to 18.5. On lactation day 10 (L10), mammary involution was forced by removal of the pups and mammary gland samples were collected at 0 h (L10) and 48 h (I48) post-weaning. Alveolar development, relative alveolar area (area occupied by alveoli / total mammary gland area) and epithelial apoptosis were evaluated in histological sections; TUNEL-positive cells and luminal cells with apoptotic morphology were considered as apoptotic cells. Also, the mRNA expression of Stat3, Bax and Igfbp5 was assessed by real-time RT-PCR at I48. As expected, at I48 alveolar lumens were dilated as a result of milk stasis and flattened epithelial cells were observed. Also, the epithelial apoptosis was increased at I48 compared to L10. However, BP3 exposure did not alter relative alveolar area or epithelial apoptosis at L10 or I48 ( $p > 0.05$ ). In addition, no differences in the mRNA expression of Stat3, Bax and Igfbp5 were observed between experimental groups at I48 ( $p > 0.05$ ). In conclusion, dermal exposure to BP3 during pregnancy does not alter early mammary involution, at least at the doses tested here.

#### 423. 036 EXPOSURE TO IMIDACLOPRID INSECTICIDE INDUCES CHANGES IN MAMMARY GLAND AND MAMMARY EPITHELIAL CELLS CONTRIBUTING TO TUMORIGENESIS

Noelia V. Miret<sup>1</sup>, M. Agustina Leguizamón<sup>1</sup>, Sol Buján<sup>1</sup>, Yamila Sánchez<sup>2</sup>, Carolina Pontillo<sup>1</sup>, Florencia Chiappini<sup>1</sup>, Alejandro Español<sup>2</sup>, Andrea Randi<sup>1</sup>

<sup>1</sup> CONICET- Universidad de Buenos Aires, Instituto de Investigaciones Biomédicas (INBIOMED), Laboratorio de Efectos Biológicos de Contaminantes Ambientales, Buenos Aires, Argentina.

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The neonicotinoid insecticide Imidacloprid (IMI) is one of the most used insecticides worldwide and binds to the nicotinic acetylcholine receptor (nAChR). IMI acts as an endocrine disruptor (ED), enhancing the synthesis of estradiol. EDs can trigger functional and morphological alterations in the breast, raising the susceptibility to

generating tumors. Increasing evidence shows the potential risk to humans of IMI exposure. Our aim was to study IMI action in the mammary gland, analyzing the role of G protein-coupled estrogen receptor (GPER) and nAChR signaling, which are deregulated in breast cancer. Environmental doses of IMI (0.01-10  $\mu$ M) did not alter the viability of mammary epithelial cells NMuMG (MTT assay, 24h) but promoted proliferation at 0.1 and 1  $\mu$ M (clonogenic assay, 7 days). In addition, IMI boosted cell migration at 1 and 10  $\mu$ M (wound healing assay) and the activity of metalloprotease (MMP)2 at 10  $\mu$ M and MMP9 at 0.1 and 10  $\mu$ M (gel zymography, 24h). Moreover, 10  $\mu$ M IMI increased GPER and  $\alpha$ 7-nAChR protein levels after 24 h of treatment, as well as c-Src phosphorylation, a kinase downstream of GPER and nAChR, after 1, 2 and 4 h (western blot). When cells were preincubated with specific inhibitors -G15 for GPER, mecamylamine for nAChR, methyllycaconitine citrate for  $\alpha$ 7-nAChR or PP2 for c-Src- results showed that IMI-promoted wound healing was blocked. In contrast, the increase in MMP2 activity only involves GPER, whereas MMP9 activity depends on GPER and nAChR. Finally, female pre-pubertal BALB-c mice were treated with IMI (0.01, 0.1 and 10 mg/kg) orally for 4 weeks and the whole mammary gland was mounted and hematoxylin-eosin stained sections were examined. IMI (10 mg/kg) raised ductal hyperplasia and the number of terminal end buds. IMI (0.1 mg/kg)-treatment induced ductal growth but reduced branch density. These results support our hypothesis that IMI exposure could produce mammary gland alterations, favoring processes that contribute to tumorigenesis.

#### 424. 261 OBSTETRIC COMPLICATIONS AND ENVIRONMENTAL EXPOSOME: PESTICIDE EXPOSURE IN A SUSCEPTIBLE POPULATION FROM SANTA FE PROVINCE

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Pesticides, as components of the environmental exposome, may contribute to the development of pregnancy related complications. This study aims to investigate potential associations between obstetric complications, urinary pesticides concentrations, and rural residence among pregnant women in Santa Fe province. A cross-sectional study was conducted with pregnant women from urban (n=38) and rural (n=51) areas. Sociodemographic and lifestyle data was collected via questionnaires. Pregnancy risk factors, health status and recent pregnancy outcomes were obtained from postpartum medical records. Pesticides residues in urine samples were analyzed using a multi-residue method involving solid phase extraction with Oasis HLB Prime cartridges, followed by gas chromatography-tandem mass spectrometry for identification and quantification. Pregnancy outcomes were classified as fetal complications (n=7, intrauterine growth restriction and low birth weight), maternal complications (n=13, gestational hypertensive disorders and diabetes), and uncomplicated pregnancies (n=49). Statistical analysis included Fisher's exact test and Mann Whitney-U test, with significance set

at  $p < 0.05$ . Women from rural areas exhibited a significantly higher frequency of fetal complications ( $p = 0.003$ ). Some rural women with maternal and fetal complications showed shorter gestation lengths ( $p = 0.007$ ). Rural residents also had a higher prevalence of pre-existing conditions ( $p = 0.01$ ), including a history of obstetric complications ( $p = 0.039$ ). Notably, participants with fetal complications had a higher median number of pesticide residues in urine (median: 4,  $p = 0.02$ ) and were associated with the presence of triazole fungicides ( $p = 0.022$ ). These results suggest a higher prevalence of obstetric complications in rural areas. Furthermore, the presence and quantity of pesticides residues in maternal urine may be associated with fetal health outcomes. Further research is needed to elucidate these associations.

#### 425. 403 ASSESSMENT OF RECOVERY FROM AIR POLLUTION-INDUCED ADVERSE EFFECTS ON THE OLFACTORY BULB

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Several epidemiological studies suggest that the brain is a target of particulate matter (PM) present in air pollution. Neuroinflammation and oxidative stress are considered the main mechanisms leading brain alterations. This study aimed to evaluate the recovery of adverse effects caused by urban air pollution on the olfactory bulb (OB), focusing on oxidative stress and inflammation parameters. BALB/c mice of 8-week-old were exposed to either filtered air (FA, control) or urban air (UA) in whole-body chambers located in a polluted area of Buenos Aires city, for up to 4 weeks. To assess the potential recovery of adverse effects, a washout (WO) period of 4 weeks was performed, in which the animals were exposed to FA. In UA exposed mice, a decrease in GSH levels ( $p < 0.001$ ) was observed, indicating a disruption in redox homeostasis that may explain the increased levels of 4-HNE protein adducts ( $p < 0.001$ ). On evaluating the inflammatory response, TNF $\alpha$ , IL6 and IL10 levels and Iba-1 expression, showing an increase after UA exposure ( $p < 0.05$ ;  $p < 0.001$ ). Additionally, OB from UA-exposed mice exhibited reduced MBP expression levels ( $p < 0.001$ ). Finally, olfactory discrimination test was found to be altered in the habituation/dishabituation pattern to different odor stimuli. When assessing whether these alterations were reversible, the findings indicated that WO period restored GSH levels ( $p < 0.001$ ) and reduced 4-HNE protein adducts formation ( $p < 0.01$ ). However, TNF $\alpha$ , IL6 and IL10 levels remained elevated compared to UA-exposed mice ( $p < 0.01$ ). Interestingly, UA mice showed a recovery in the habituation/dishabituation pattern to different odor stimuli. Overall, these findings suggest that a WO period following exposure to UA can mitigate oxidative stress-related changes and revert olfactory odor capability changes. These results contribute to our understanding of the mechanisms by which environmental PM induces neurotoxicity.

#### 426. 409 EFFECT OF IMIDACLOPRID ON THE REGULATION OF LIVER CELL GROWTH IN VIVO, IN TUMOR AND VIRAL IN VITRO MODELS

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Exposure to environmental pollutants may develop hepatocarcinoma. In livers with hepatitis B it can be more severe because it contains protein X(HBx), a transactivator of pathways. Endocrine dysregulation (ED) is relevant in the early stages of neoplasia and hundreds of pollutants act as ED, altering thyroid hormones (HT). Among the pollutants is Imidacloprid (IMI), a neonicotinoid whose mechanism of action is unknown. Objective: To study the effect of IMI on the regulation of liver cell growth. Mice or cells were treated with IMI (0,01; 0,1 and 10 mg/kg or 0,01; 0,1,1 and 10  $\mu$ M), 24hs and times course were carried out. In hormone-depleted cells, with different T<sub>3</sub> doses (10<sup>-9</sup>M-10<sup>-6</sup>M) or TGF-BRI inhibitor (SB-431542) were pretreated, followed by IMI. Statistic, ANOVA 2 way, \* $p < 0.05$ . In vivo: Hepatic TH(RIA) and body weight; histology and cell number/area(H-E). In vitro, Huh-7: Cell migration (wound test); Cox-2(WB); MMP2/9 (activity/WB); B3, PCNA and TGF-B1 levels (Times/doses curve). In vitro, Huh-7 transfected: PCNA(WI). Hepatosomatic index, cell number and PCNA did not change; IMI 10 mg/kg decreased (28%) T<sub>3</sub> levels, and histology shows necroinflammatory foci. The B3 receptor decreased 75% and 32% at 0.01 and 10 mg/kg; TGF-B1 decreased at all doses (37, 33 and 60%). In vitro, cell migration increased (29, 51%), 0.01-0.1 $\mu$ M; PCNA decreased (55%), 10 $\mu$ M; TGF-B1 at 0.01-10 $\mu$ M increased (50, 53%) and Cox-2 (62, 63%) respectively. MMP2 protein increases (55, 56%) and MMP2 and MMP9 activity (30, 45% and 21, 67%) at 0.01-10  $\mu$ M. PCNA decreased 15-45 min (79, 71 and 90%), increased 1-4 hs (20, 80, 120%) and decreased (46%) at 24 h, IMI (10 $\mu$ M). In Huh-7 transfected, IMI decreased PCNA (60%). Inhibition of RI-TGF-B1 did not prevent decrease of PCNA by IMI. Exogenous T<sub>3</sub> (10<sup>-7</sup>M) potentiates the decrease in PCNA by IMI (74%). Conclusion: IMI deregulates cell growth in vivo and in vitro models. T<sub>3</sub> could be involved in its mechanism of action.

#### 427. 502 IMPACT OF BP3 AND DEET PRENATAL EXPOSURE ON MATERNAL AND F1 LITTER PARAMETERS IN MICE: A COMPARATIVE STUDY IN A MULTIPARITY MODEL

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The sunscreen benzophenone-3 (BP3) and the insect repellent N, N-diethyl-m-toluamide (DEET) are widely used topical compounds known as endocrine disruptors (EDs). Despite their extensive use, critical gaps remain, particularly regarding the synergistic effects of BP3 and DEET on pregnancy. Additionally, most studies have focused on first pregnancies, though evidence suggests ED effects may emerge only after multiple pregnancies. This study aimed to evaluate the effects of BP3, DEET, and their combination (BP3+DEET) on maternal and offspring parameters in a multiparity model. To achieve this, we performed a two-pregnancies protocol: pregnant C57BL/6 mice (F0) were exposed dermally to BP3 (50 mg/kg/d), DEET (40 mg/kg/d), or BP3+DEET from gestation day (gd) 0 to gd 10, only during first gestation. Then F0 were re-mated without further exposure to the compounds. Maternal weight, gestation length, sex ratio, and litter size were assessed, along with offspring (F1.1 and F1.2) parameters using one-way ANOVA and unpaired t-tests. We found no changes in maternal weight, length of gestation, sex ratio and litter size in both pregnancies. On the other hand, BP3 and BP3+DEET increased offspring weight in both F1.1 and F1.2, while DEET and BP3+DEET shortened estrous cycles in F1.1 and reduced days in estrus in F1.2. These effects were significant

compared to controls but showed no difference between individual compounds and the mixture. No changes were observed in vaginal opening, sperm count and percentage of seminiferous tubules with active spermatogenesis. Our findings indicate: a) BP3 and DEET have distinct biological targets, with BP3 specifically affecting offspring weight and DEET altering the estrous cycle; b) there is no synergy between BP3 and DEET; c) these effects can manifest in subsequent pregnancies after first-gestation exposure.

#### P1 POSTERS

FECHA Y HORA: 20/11/2024 11:30-12:30 H

COORDINADORES: LAURA ALVAREZ,  
MARISA GABIELA REPETTO, NOELIA MIRET

#### 428. 029 BREAST CANCER HER2 (+) PROGRESSION AND ACTIVATION OF G PROTEIN -ASSOCIATED ESTROGEN RECEPTOR (GPER) PATHWAY INDUCED BY INSECTICIDE IMIDACLOPRID

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Breast cancer is one of the leading cancers among women worldwide. Recently, interest has grown into studying the pesticide impact on disease progression. Neonicotinoids are insecticides used for fruits and vegetables. Imidacloprid (IMI) is among the 10 most detected agrochemicals in Argentina and Brazil, accumulating due to its repeated application, potentially affecting human health. IMI increases aromatase levels and estradiol secretion in breast cancer cells, which may contribute to tumor development by stimulating estrogen receptor (ER) pathways such as G protein-associated ER (GPER). Several endocrine disruptors activate the aryl hydrocarbon receptor (AhR), modulating inflammation, proliferation and migration. The indoleamine 2,3-dioxygenase (IDO) degrades tryptophan producing kynurenine, involved in tolerogenic responses. Our aim was to study whether exposure of HER2(+) LM3 breast cancer cells to IMI (0.01, 0.1, 1 and 10  $\mu$ M) modify cell viability, proliferation and migration, as well as metalloprotease (MMP)2 and 9 activities, and whether these effects are mediated by the AhR, IDO and GPER pathways using specific inhibitors. In addition, we studied its action on AhR and GPER expression, and its downstream pathway ERK1/2. Our results showed that IMI (10  $\mu$ M) increases cell viability at 48 h ( $p < 0.05$ ) (MTT assay) through AhR and GPER pathways. Low doses of IMI (0.01 and 0.1  $\mu$ M) enhance cell migration (wound healing assay) ( $p < 0.01$ ), while cell proliferation (clonogenic assay) ( $p < 0.05$ ) is increased at 10  $\mu$ M, dependent on AhR, GPER and IDO. The activity of MMP-2 shows an enhancement at IMI (0.01-0.1  $\mu$ M), while MMP-9 at IMI (1-10  $\mu$ M) (gel zymography). Furthermore, exposure to IMI enhances GPER and ERK1/2 expression by Western blot at 0.1  $\mu$ M ( $p < 0.05$ ), showing a tendency to reduce AhR levels at all doses. These results demonstrate that exposure to environmentally relevant concentrations of IMI activates the GPER pathway, collaborating in HER2(+) breast cancer progression.

#### 429. 317 MENSTRUAL CUP'S CYTOTOXICITY TEST BY DIRECT CONTACT

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<sup>2</sup> CLAIM®. Organización de Investigación por Contrato.

Menstrual cups are receptacles placed in the vagina to collect menstrual flow, are made of durable materials so that they can be used repeatedly. Due to advances in international regulations regarding the limitation of testing on animals and to the general applicability of *in vitro* cytotoxicity tests and their widespread use in evaluating a large range of medical devices and materials, to testing new approaches is needed. The ISO 10993 standards series themselves do not prescribe any specific testing but are employed worldwide as a framework to establish regulatory compliance needed for initiating clinical investigations to medical devices. This work aims to set the bases and the protocol of a direct contact cytotoxicity test applicable specifically to a menstrual cup but it could also be applied to test other devices. Our assay was performed using the mouse fibroblast L929 cell line in a six well cell culture plate. We exposed the monolayer directly to 10x10mm cuts of a menstrual cup, following by a 24 hours incubation under growing conditions and then a 2 hours incubation with Neutral Red to test the ability of viable cells to incorporate and bind the dye neutral red in the lysosomes (neutral red intake). Qualitative evaluation included observing the cells under a microscope and assigning a cytotoxic grade ranging from zero to four, based on an estimated percentage of lysis and cell morphology. Results indicated that the menstrual cup do not generate cytotoxic effects under the evaluated conditions. There was a physical monolayer's rupture but this was due to the manipulation of the plate. We are currently working on the validation of the quantitative method, which is based on the evaluation of the metabolic activity of the cells.

#### 430. 343 BREATHING POOR QUALITY URBAN AIR EXACERBATES THE LUNG OXINFLAMMATORY RESPONSE IMPAIRING TISSUE REPAIR FOLLOWING ACUTE LUNG INJURY

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Air pollution is a significant risk factor for respiratory diseases, driving global morbidity and mortality. We have shown that air particulate matter (PM) exposure induces 'oxi-inflammation,' the interplay between oxidative stress and inflammation. Our study aims to investigate if the PM-induced oxi-inflammation on lung increases the tissue damage impairing the proper repair. To characterize PM exposure, BALB/c mice were exposed to filtered air (FA) or urban air (UA), in whole-body exposure chambers for 8 weeks. We observed that UA did not activate the Nf $\kappa$ B pathway, as its nuclear expression was not increased. When bronchioalveolar lavage cells were analyzed, UA-alveolar macrophage activation was found by flow cytometry an increased in nitric oxide (NO) production ( $p \leq 0.05$ ) without changes in the cellular redox status. Then, we evaluate tissue repair 5 days after an acute lung injury induced by intratracheal instillation of 0.1 N hydrochloric acid (HCl). After UA exposure and lung injury, NADPH oxidase activity and mitochondrial H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>•-</sup> production were augmented ( $p \leq 0.05$ ). In this scenario, GSH/GSSG ratio decreased ( $p \leq 0.05$ ) due to increased GSSG. The resulting shift towards a more oxidized environment in the UA-HCl group, may be responsible for the altered inflammatory response observed, since Nf $\kappa$ B pathway was activated showing an increased nuclear translocation ( $p \leq 0.05$ ), together with increased level of pro-inflammatory cytokines TNF- $\alpha$  ( $p \leq 0.001$ ) and IL-6 ( $p \leq 0.01$ ), responsible for the local inflammatory response. Redox homeostasis plays a crucial role in maintaining a healthy physiological cellular state. However, a prooxidative challenge, resulting in a loss in redox homeostasis along with the progression of an inflammatory response leads to and

inadequate feedback response, that may end up delaying alveolar repair, hindering the restoration of alveolar epithelium architecture, extending the loss of lung function.

**431. 369 MULTIVARIATE ANALYSIS OF THE CYTOTOXIC EFFECTS OF HYDROCARBONS ON BREAST TUMORIGENIC AND NON-TUMORIGENIC CELLS.**

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We previously showed that the water-soluble fraction of oil (WAF) decreased clonogenicity (Clon) and cell viability (Viab) in tumorigenic MDA-MB-231 and MCF-7 breast cancer cells. Anthracene (ANT) decreased Viab and Clon only in non-tumorigenic MCF10-A cells ( $p < 0.05$ ). WAF led to an increase in catalase (CAT) activity in tumorigenic cells ( $p < 0.01$ ). ANT reduced CAT in all cell lines ( $p < 0.05$  to  $p < 0.001$ ) and GST activity decreased in MDA-MB-231 and MCF10-A cells ( $p < 0.05$  and  $p < 0.01$ ) but increased in MCF-7 cells ( $p < 0.001$ ). Here, we performed a multivariate statistical approach analyzing the mean values of all evaluated parameters to explore correlations between cytological and biochemical responses. Principal Component Analysis (PCA) of WAF exposure revealed that the first component C1 explained 64.5% of the total variability, with Clon and Viab exhibiting a positive correlation and GST a negative correlation. C2 explained 24.8% of the variability, with the only contribution of CAT. For ANT, C1 explained 48.4% of the variability, with Clon and GST showing opposing contributions, while C2 explained 25.1%, influenced by Viab and CAT in the same direction. We also computed an integrated biomarker response (IBR) index, which corroborated PCA findings. The IBR index revealed a biphasic, concentration-dependent response to WAF in both tumorigenic cell lines, and a concentration-dependent ascending response to ANT for all cell lines. This increasing IBR profile with concentration aligns with the behavior of biomarkers, indicating that a combination of viability biomarkers and antioxidant/detoxifying enzymes provides a robust assay for assessing hydrocarbon toxicity. Our findings suggest that cellular sensitivity to contaminants is modulated by tumorigenicity and exposure to WAF or ANT may exacerbate malignant conditions. The integration of multiple biochemical and cellular parameters, analyzed through multivariate methods, offers a comprehensive evaluation of toxicant effects.

**432. 443 PRE AND POST-PUBERTAL EFFECTS OF EARLY EXPOSURE TO CHLORPYRIFOS ON MAMMARY GLAND DEVELOPMENT IN FEMALE RATS**

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We have previously demonstrated that chronic exposure to the endocrine disruptor chlorpyrifos (CPF) in adult rats promotes the development of benign mammary lesions, such as hyperplasia and adenosis. The aim of this study was to assess in an in utero exposure model in female Sprague-Dawley rats, if environmentally relevant concentrations of CPF (NOAEL: 0.1 mg/kg/day - CPF 0.1 and ADI: 0.001 mg/kg/day - CPF 0.001) can affect normal mammary gland (MG) development and/or hormone levels in female offspring exposed from gestation to weaning. We evaluated mammary gland development in pre (PRE) and post-pubertal (POST) rats exposed to CPF or vehicle by analyzing whole mounts (WM) of the 4th MG and by histopathological examination of mammary tissue. PCNA expression was determined by immunohistochemistry in PRE rats, and

hormonal levels of E2, LH, and FSH were determined by ELISA and RIA, respectively, in POST animals. In PRE rats, CPF 0.1 induced a significant decrease in the number of terminal end buds (TEB) ( $p < 0.01$ ) and in PCNA expression ( $p < 0.05$ ) compared to controls, whereas histopathological analysis revealed an increase in the relative glandular area and periductal fibrous stroma. In rats exposed to CPF 0.001, WM analysis showed a significant increase in the distance between the 4th and 5th MG ( $p < 0.05$ ), along with a reduction in the glandular area and periductal fibrous stroma upon microscopic observation. In POST rats exposed to both CPF concentrations, we observed a significant increase in the number of TEB compared to controls ( $p < 0.05$ ). However, circulating levels of E2, LH and FSH did not show significant variations. These results suggest that early-life exposure to environmentally relevant concentrations of CPF may cause an early disruption in mammary gland development, altering several of the parameters analyzed. It is important to consider that these changes could have implications not only for the exposed individuals but also for future generations.

**433. 475 MULTIPARITY EXPOSURE TO BP3 AND BPA LEADS TO IMPAIRED OVARIAN FUNCTION IN OFFSPRING**

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We are frequently exposed to chemicals in personal care products, such as benzophenone-3 (BP3), a UV filter in cosmetics, and bisphenol A (BPA), commonly found in plastics and resins. Both BP3 and BPA are endocrine disruptors that can affect reproductive health. Previously, we showed that exposure to BP3, BPA, or their combination across two sequential pregnancies in a multiparity model significantly reduces ovulation rates. BP3 was applied dermally, and BPA was administered orally. To further investigate into the mechanisms driving these effects, we examined whether reduced ovulation is linked to changes in follicular dynamics, steroidogenic enzyme expression, and hormone levels. Pregnant C57BL/6 females mated with BALB/c males were treated with: a) Control (olive oil/ethanol-water); b) BP3 (50 mg/kg/day); c) BPA (4 µg/kg/day); or d) BP3 + BPA from gestation day 0 to day 9 in both pregnancies. Serum and ovarian samples were collected from the offspring of the second gestation at key stages during gonadotrophin stimulation. We quantified follicle populations, measured serum estradiol, testosterone, and progesterone levels, and assessed mRNA expression of steroidogenic enzymes. Although enzyme levels remained unchanged, estradiol was altered 14 hours post-hCG in the BP3 and BP3+BPA groups. Primordial follicle populations were consistent across groups, but a significant reduction in antral follicles was observed in BP3, BPA, and BP3+BPA groups. Besides, BPA group had a lower total oocyte count. In conclusion, BP3 and BPA, whether individually or combined, negatively affect ovarian response to exogenous gonadotropins by inhibiting follicular development in offspring of the second gestation. Lower antral follicle counts have been reported in women undergoing in vitro fertilization with high BPA levels, or in younger women with elevated BP3 levels. Our findings emphasize the need to consider multiparity as a contributing factor in the emergence of EDC-related effects.

**P2 POSTERS**

FECHA Y HORA: 21/11/2024 11:00-12:00 H

COORDINADORES: ISMAEL BAROSSO, JIMENA PAULA CABILLA

**434. 037 IMIDACLOPRID EXPOSURE PROMOTE BREAST CANCER CELL PROLIFERATION AND MOTILITY**

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Exposure to endocrine disrupting pesticides has been pointed out as a risk factor that increases the incidence and aggressiveness of breast cancer, thus trying to explain in part the fact that this disease is the leading cause of cancer death among women. Imidacloprid (IMI) is a neonicotinoid insecticide widely used which modulates estradiol secretion in breast cancer cells, therefore it has been suggested that it could promote this disease. Our objective was to evaluate the impact of IMI exposure on cell viability, proliferation and motility in MCF-7 (ER+) and MDA-MB-231 (ER-) breast cancer cells. Furthermore, we studied IMI action on estrogen receptors (ER) expression, such as ER $\alpha$  and G protein-coupled ER (GPER), since both have been linked to breast cancer. Results showed that IMI does not produce a cytotoxic effect (MTT assay) when cells were exposed to environmentally relevant concentrations of IMI (0.01-10  $\mu$ M) for 24 or 48 h. On the contrary, IMI increases cell proliferation in MCF-7 and MDA-MB-231 at the lowest dose (0.01  $\mu$ M) after 7 days of exposure (clonogenic assay). Western blot results showed a reduction in the levels of ER $\alpha$  at 0.01  $\mu$ M and GPER at 10  $\mu$ M in MCF-7. In MDA-MB-231, IMI decreased GPER expression at all assayed doses. Finally, metalloprotease-9 activity was increased in MCF-7 at all concentrations (gel zymography), whereas cell migration was enhanced in MDA-MB-231 at 0.1  $\mu$ M (wound healing assay). In conclusion, IMI exposure induces alterations in ER-positive and -negative breast cancer cells, promoting cell proliferation and motility, suggesting that it may be involved in breast cancer progression. Future studies are necessary to examine the role of ER $\alpha$  and GPER on these effects.

**435. 043 NEONICOTINOID INSECTICIDE IMIDACLOPRID INDUCES CHANGES IN HORMONAL PROFILE, INFLAMMATORY RESPONSE AND CELL MIGRATION RATE IN HUMAN ENDOMETRIAL STROMAL CELLS**

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Endometriosis is a hormone-dependent, inflammatory disease which is defined by the presence of endometrial tissue outside of the uterine cavity. The major symptoms are chronic pelvic pain and infertility. The development of endometriosis depends on factors that facilitate migration, adhesion, proliferation and invasion of endometrial cells. Environmental endocrine-disrupting chemicals (EDs) interfere with the synthesis, secretion, transport, signaling, or hormone metabolism. Endometriosis has been potentially linked to EDs exposure. Imidacloprid (IMI) is a neonicotinoid insecticide; currently the most widely used agricultural insecticides. Studies report that EDs, such as IMI, affect aromatase expression and interfere with estrogen signaling. Estrogen receptors mediate the effects of estrogenic compounds. G protein-coupled estrogen receptor (GPER) expression was significantly increased in the stroma of endometriosis. This study aimed to evaluate the effect of IMI on alterations associated with endometriosis progression. Stromal endometrial cells were exposed to different doses of IMI (0.01, 0.1, 1 and 10  $\mu$ M) *in vitro*. The results showed that IMI increases the cell migration ratio (15 and 30% IMI 1 and 10  $\mu$ M) (scratch motility assay) and proliferation (48% IMI 10  $\mu$ M) (MTT and PCNA expression). Moreover, we observed that IMI (1 and 10  $\mu$ M) induces aromatase and

GPER expression (western blot) (74 and 67%; 62 and 71%). We also examined the activity of MMP-2 and MMP-9 (gel zymography), which participate in migration and invasion processes. IMI increased MMP-9 (17, 26 and 23% IMI 0.1, 1 and 10  $\mu$ M) and MMP-2 activities (41 and 28% IMI 0.01, 1  $\mu$ M). Finally, we observed that IMI induces cyclooxygenase-2 expression (western blot) (74% IMI 1), which participate in the inflammatory process. In conclusion, our results provide experimental evidence that IMI induces alterations associated with endometriosis development and progression. Our results show that IMI exposure could contribute to endometriosis development by disrupting hormonal homeostasis in human endometrial cells.

**436. 072 ROLE OF ESTROGEN RECEPTOR BETA (ER $\beta$ ) IN CADMIUM (Cd) AND ARSENIC (As)-INDUCED PROLIFERATION AND MIGRATION OF HUMAN CERVICAL CARCINOMA CELLS**

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Endocrine disruptors (EDCs) are chemicals that interfere with the endocrine system by mimicking or blocking natural hormones, leading to significant health risks. Among these, cadmium (Cd) and arsenic (As) are classified as metalloestrogens due to their ability to imitate estrogenic activity. Cd is a known type I carcinogen, and As is prevalent in Argentina soils and groundwater. While the estrogenic effects of Cd and As through estrogen receptor alpha (ER $\alpha$ ) have been well characterized, the role of estrogen receptor beta (ER $\beta$ ) in EDC-mediated signaling remains largely unexplored, despite its presence in several hormone-responsive tumors. This study aims to determine whether Cd, As, or their combination can trigger estrogenic signaling via ER $\beta$ . HeLa cervical carcinoma cells, which express ER $\beta$  and GPER1 but lack canonical ER $\alpha$ , were exposed for 72 hours to 10 nM Cd, 10 nM As, or a combination of both, in the presence or absence of 17 $\beta$ -estradiol (E2) or an ER $\beta$  antagonist (PHTPP). The results showed that Cd and As increased the G2/M cell cycle phase (flow cytometry,  $p < 0.05$ ), mitotic index (nuclear morphology,  $p < 0.05$ ), and wound closure (scratch motility assay,  $p < 0.05$ ). Additionally, Cd and As altered the expression of E- and N-cadherin (western blot,  $p < 0.05$ ). The presence of PHTPP partially prevented Cd- and As-mediated effects, suggesting that both metalloestrogens can exert xenoestrogenic actions through ER $\beta$ .

**437. 293 BIOCHEMICAL CHARACTERIZATION OF MICROCYSTIN-INDUCED BRAIN AREAS DAMAGE IN RATS**

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*Microcystis aeruginosa*, a prevalent cyanobacterium in Argentina's freshwater bodies, produces harmful cyanotoxins known as microcystins (MC). Among these, MC-LR stands out due to its high toxicity and significant environmental risk. Beyond its established liver damage and tumor-promoting properties, MC-LR's potential neurotoxicity is a growing concern. Consequently, MC can induce severe behavioral, morphological, and neurological alterations, including neuronal loss and oxidative stress. **This study aimed to assess the impact of [Dleu<sup>3</sup>]MC-LR and MC-LR on various areas of the rat brain (striatum, cortex, cerebellum -CB-, and hippocampus -HC-) in terms of oxidative stress. A single dose of [Dleu<sup>3</sup>]MC-LR at 150  $\mu$ g/kg (total dose, *i.p.*) was given every 4 days for 21 days in acute exposures.** Both MC variants were detected in all brain regions examined, with MC-LR concentrations exceeding

those of [**Dleu**]**MC-LR**. **MC** levels in HC were the lowest. Consequently, reactive species, measured by DCFH-DA oxidation, significantly increased in the CB and striatum, with notably high levels in the cortex but no change in the HC compared to controls. Lipid damage, assessed by **thiobarbituric acid reactive species (TBARS)**, occurred in the cerebellum and striatum, but not in the HC or cortex. Additionally, catalase (CAT) activity was significantly reduced in the CB, with no changes observed in other brain regions relative to controls. MC exposure induces neurotoxicity in rats, emphasizing the role of oxidative stress in this process. Despite a higher **proportion of [**Dleu**]**MC-LR** in the administered toxin, **MC-LR concentrations in the brain were** significantly greater. These results diverge from previous low-dose studies, suggesting potential differences in blood-brain barrier permeability. Notably, CAT activity remained unchanged at this high MC dose, contrasting with prior findings. These discrepancies highlight the complex interplay of factors influencing the body's response to MCs.**

**438. 399 ALTERATION OF THE MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 2 (MRP2) BARRIER FUNCTION BY OXIDATIVE STRESS IN CACO-2 CELLS: IMPACT ON OCHRATOXIN A SECRETION AND TOXICITY**

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**Introduction:** The intestinal tract is exposed to external agents that generate reactive oxygen species (ROS). If not neutralized, it causes oxidative stress (OS), triggering cellular protein alterations, intestinal barrier disruption, and gastrointestinal disorders. MRP2, an ABC transporter, plays a crucial role in restricting the absorption of toxic dietary contaminants, thus modulating their toxicity. **Objectives:** To evaluate the long-term effect of OS on MRP2 in Caco-2 cells, and investigate the apical secretion and toxicity of Ochratoxin A (OTA), a mycotoxin, substrate of MRP2, in cultures treated with TBH 250  $\mu$ M for 24h. **Methods:** MRP2 expression was evaluated by western blot in total cell membranes (TCM). Real time RTq-PCR was performed to evaluate changes in mRNA expression. The MRP2 transport activity was determined by quantifying the efflux of dinitrophenyl-S-glutathione (DNP-SG) or OTA into the incubation medium by HPLC. Statistical analyses were performed using one-way ANOVA followed by the post hoc Tukey-test and results were expressed as a % of control (C). **Results:** We confirmed that TBH generated OS at 24 h, as indicated by increased lipid peroxidation end products (+140%) and reduced SOD activity (-29%) ( $p < 0.05$ ,  $N=6$ ). MRP2 protein expression and DNP-SG efflux decreased significantly in TBH group (-42% and -55% respectively) with respect to C ( $p < 0.05$ ,  $N=3$ ), without change in MRP2 mRNA levels. We observed a decrease in OTA apical secretion in the TBH group (-13%) compared to C, consistent with MRP2 impairment. MTT assay showed that TBH 250  $\mu$ M and OTA significantly reduced cellular viability (-10%) ( $p < 0.05$ ,  $N=3$ ). **Conclusion:** Long-term OS led to the post-transcriptional down-regulation of MRP2, impairing its barrier function and resulting in decreased excretion and increased toxicity of OTA; confirming MRP2's toxicological relevance in this context.

**439. 473 EFFECT OF THE ENVIRONMENTAL POLLUTANT IMIDACLOPRID ON THE CARDIOVASCULAR SYSTEM**

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**Introduction:** According to the World Health Organization, cardiovascular diseases are the leading cause of death. Among the factors that increase the risk of developing them is exposure to environmental pollutants, which can act as endocrine disruptors (EDs). Both the organochlorine pesticide hexachlorobenzene (HCB) and the neonicotinoid insecticide Imidacloprid (IMI) are deleterious to human health and alter thyroid hormones (TH). It is known that TH and estrogen receptors (ER $\alpha$ ) regulate the cardiovascular system. We previously demonstrated that HCB in rats alters vascular pressure, morphology and function and that TH, deiodinase II and ER $\alpha$  are altered. **Objective:** to evaluate the effect and mechanism of action of IMI on the cardiovascular system in vivo and its possible role as an ED. **MyM:** Different doses of IMI (0.01, 0.1, 1 and 10 mg/kg b.w.) were administered orally by micropipette to Balb/c nude mice for 30 days. The following were analyzed: Mouse weight and hepatosomatic index. In the aorta: wall width; number of cells/area, PCNA, ER $\alpha$  (Western Blot) and DII mRNA levels (RT-PCR). In the heart: total area and left ventricle (LV) cavity. **Results:** Mouse weight and hepatosomatic index were not altered. In the aorta: the number of cells/area decreased at doses of 0.01 and 10 mg/kg b.w., (43.4% and 39.5% respectively;  $p < 0.001$ ). The wall width increased with 10 mg/kg b.w. (35.5%,  $p < 0.01$ ). PCNA levels did not change, and ER $\alpha$  levels decreased with 0.01 and 10 mg/kg b.w., (23.5% and 33% respectively;  $p < 0.05$ ,  $p < 0.01$ ). DII mRNA decreased by 31%, ( $p < 0.01$ ) with 10 mg/kg b.w. In heart: total LV area did not change, LV cavity/total area increased by 26.8% ( $p < 0.05$ ) compared to control. **Conclusion:** IMI administration generates morphological and molecular alterations in mouse heart and arteries. DII and ER $\alpha$  could be involved in these alterations, suggesting, in addition, a role as ED of IMI.

**440. 490 SUBCHRONIC EXPOSURE TO AIRBORNE PARTICULATE MATTER AND UNDERNUTRITION IN YOUNG RATS: AN HISTOPATOLOGICAL STUDY IN LUNG AND EXCRETORY ORGANS**

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Air pollution (gases and particulate matter-PM) and undernutrition, are two environmental factors that compromise public health, particularly affecting vulnerable populations such as children. While PM primarily affects the respiratory system, it can also harm distant organs such as the excretory organs (liver and kidneys), vital for xenobiotic detoxification. The aim of this study was to evaluate histopathological changes in the lungs, liver and kidneys in an animal model of nutritional growth retardation (NGR) after subchronic exposure to ROFA (Residual Oil Fly Ash). Wistar weanling rats were divided in two groups: NGR animals were fed during 4 weeks with a restricted diet 20% compared to *ad libitum* intake of Control (C) and were intranasally instilled with 0.17mg/kg BW of ROFA or its vehicle 3 times a week. Histopathological changes were assessed by histomorphometry and H&E or PAS stainings. In the lungs, ROFA exposure resulted in a reduction of airspace (C 55.8 $\pm$ 4.4 vs. ROFA 38.7 $\pm$ 6.6,  $p < 0.01$ ; NGR 61.3 $\pm$ 2.5 vs. NGR+ROFA 44.2 $\pm$ 9.9,  $p < 0.001$ ), a decrease in the number of alveoli per area (C 492 $\pm$ 70 vs. ROFA 247 $\pm$ 20,  $p < 0.001$ ; NGR 481 $\pm$ 62 vs. NGR+ROFA 302 $\pm$ 42,  $p < 0.001$ ), and changes in size distribution in ROFA and NGR+ROFA. Additionally, it caused inflammation and cellular infiltration. At the hepatic level, NGR showed an increase in binucleated cells.



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ROFA exposure further elevated this parameter and induced microvesiculation in C and NGR. NGR and NGR+ROFA exhibited a patchy pattern of PAS-positive cells indicating depletion of glycogen. In the kidneys, NGR exhibited alteration at the glomerular level and in the filtration space. ROFA induced inflammatory cells infiltration

in the interstice and focal hemorrhages in C and NGR. In summary, these results indicate that NGR animals exhibit histopathological alterations in the lungs, liver and kidneys, and that subchronic exposure to ROFA exacerbates these alterations, potentially compromising the detoxification function of these organs.

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