XXIII CONGRESSO NAZIONALE A.I.B.G.

A.I.B.G Associazione Italiana di Biologia e Genetica Generale e Molecolare

REGIONE ABRUZZO

Comune di Città Sant'Angelo

18 – 20 settembre 2025

AUDITORIUM DEL RETTORATO
Campus Università Gabriele d'Annunzio
Chieti-Pescara

CHIETI

Con il patrocinio di:

CAST
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Con il contributo di **Fiorella Altruda**

Care Colleghe e Cari Colleghi,

desideriamo ringraziarVi per la partecipazione al **XXIII Congresso Nazionale A.I.B.G. 2025** che si terrà a Chieti. Questo appuntamento rappresenta un momento unico per rafforzare le connessioni scientifiche tra i nostri gruppi, favorendo la condivisione delle nostre ricerche e l'avvio di nuove collaborazioni stimolanti.

Il programma del Congresso include presentazioni orali seguite da brevi discussioni e una sessione poster, offrendo a tutti i partecipanti l'opportunità di condividere i propri studi. I contributi, raccolti nel libro degli abstract, saranno disponibili online per garantire la massima diffusione delle idee e dei risultati.

Durante il Congresso premieremo i vincitori del Premio Guido Tarone, un riconoscimento istituito nel 2018 per onorare la memoria di un collega che ha lasciato un segno indelebile nella nostra comunità scientifica. Inoltre, la Società mette a disposizione borse di studio per giovani ricercatori non strutturati, con l'obiettivo di sostenere e valorizzare talenti emergenti nel campo della Biologia e della Genetica.

Condividiamo con Voi la passione per la scienza, l'impegno nel costruire un futuro di innovazione e siamo certi che questo Congresso sarà un'occasione preziosa per arricchire il nostro percorso scientifico e umano.

Benvenuti a Chieti!

Abstract Book

A.I.B.G

18 settembre 2025
Giovedì

11:30 **Apertura congresso e registrazioni**

12:00 **Cocktail di benvenuto/light lunch**

14:00 **Saluti istituzionali e presentazione del congresso**

14:30 **Role of forces in membrane dynamics and liver tissue morphogenesis**
Marino Zerial (Human Technopole, Milano)

15:15 **Gli insegnamenti BIOS-10/A nei corsi di laurea triennale e magistrale in Psicologia**
Arturo Bevilacqua (Università La Sapienza, Roma)
Maria Teresa Fiorenza (Università La Sapienza, Roma)

I SESSIONE: Meccanismi di difesa cellulare e risposte cellulari allo stress (15:45 - 18:00)
Chairs: **Riccardo Alessandro** (Università di Palermo)
Ornella Parolini (Università Cattolica del Sacro Cuore, Roma)

15:45 **Three-dimensional cell culture models reveal oncoprotective effects of lifestyle interventions in breast cancer survivors**
Elena Barbieri (Università degli Studi di Urbino "Carlo Bo")

16:00 **Natural proteoglycan-like molecules from Dugesia japonica mucus activate ferroptotic death in cancer cells**
Gaetana Gambino (Università di Pisa)

16:15 **D-chiro-inositol and LPS, but not myo-inositol, induce a hyperandrogenic response in human KGN granulosa cells**
Cristiano Giuliani (Università La Sapienza, Roma)

Coffee Break (16:30 - 17:15)

17:15 **Interorganellar cross-talk in cellular stress response signaling**
Nicoletta Guaragnella (Università degli Studi di Bari "Aldo Moro")

17:30 **Natriuretic peptides: a novel mechanism controlling dendritic cells inflammatory phenotype**
Letizia Mezzasoma (Università degli Studi di Perugia)

17:45 **Adipose tissue inflammation and senescence in aging: a key role for the complement 3a receptor**
Bianca Vezzani (Università degli Studi di Parma)

Trasferimento in Hotel e serata libera

19 settembre 2025
Venerdì

COMUNICAZIONI PREMIO TARONE (08:30 - 09:50)
Chairs: **Paola Riva** (Università degli Studi di Milano)
Fernanda Martini (Università degli Studi di Ferrara)

Premio Tarone under 35

08:30 **Expulsion of iron-rich ferritin via CD63-mediated exosome drives ferroptosis resistance in ovarian cancer cells**
Anna Martina Battaglia (Università degli Studi di Catanzaro "Magna Graecia")

08:50 **Dysregulation of fatty acid metabolism by EZH2 inhibitors uncovers novel therapeutic approaches for ACC**
Marta Claudia Nocito (Università della Calabria)

Premio Tarone under 45

09:10 **A new mouse model of JMML highlights differential susceptibility of embryonic hematopoietic stem/ progenitor cells to the Kras G12D mutation**
Emanuele Azzoni (Università degli Studi di Milano-Bicocca)

09:30 **Extracellular vesicles from second trimester human amniotic fluid as candidate therapeutics against skeletal and cardiac muscle injury**
Sveva Bollini (Università degli Studi di Genova)

Coffee Break (09:50 - 10:30)

II SESSIONE: Epigenetica e regolazione dell'espressione genica (10:30 - 11:45)
Chairs: **Rosanna Chianese** (Università degli Studi della Campania "Luigi Vanvitelli", Napoli)
Daniela Caporossi (Università degli Studi di Roma "Foro Italico")

10:30 **Unveiling the origin and functions of diagnostic circulating microRNAs in lung cancer**
Tommaso Colangelo (Università degli Studi di Foggia)

10:45 **Role of MECP2 in inflammaging-related ovarian dysfunctions: insights from a genetically-induced deficient mouse model**
Valeria Cordone (Università degli Studi dell'Aquila)

11:00 **MLL1-driven H3K4me3 establishes epigenetic memory of vascular inflammation and oxidative stress triggered by chronic hyperglycemia**
Nadia Di Pietrantonio (Università degli Studi "G. d'Annunzio", Chieti-Pescara)

11:15 **Multi-SINEUP: a novel RNA therapeutic approach for 22q11.2 microdeletion syndrome**
Stefano Espinoza (Università del Piemonte Orientale, Novara)

19 settembre 2025
Venerdì

11:30 **Leucine-rich repeat kinase 2 controls clusterin translation via mir-22-5p: implication for parkinson's disease**
Alice Filippini (IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia)

III SESSIONE: Controllo della proliferazione cellulare e del differenziamento (11:45 - 15:00)
Chairs: **Luca Tiberi** (Università degli Studi di Trento)
Provvidenza Maria Abruzzo (Università degli Studi di Bologna)

11:45 **Engineering the microenvironment to modulate macrophage polarization and anti-tumor activity**
Naym Blal (Università degli Studi di Salerno)

12:00 **Studying molecular mechanisms leading to STAG2-driven medulloblastoma**
Antonella Lettieri (Università degli Studi di Milano)

12:15 **Endothelial response to hormonal stimuli in the window of implantation: insights from a 3D human endometrium model**
Francesca Paola Luongo (Università degli Studi di Siena)

Lunch e Sessione Poster (12:30 - 14:30)

14:30 **The protective role of GSH in mesenchymal stem cell senescence**
Francesca Cristiana Piritore (Università degli Studi di Verona)

14:45 **Mechanism of action of extracellular vesicles in neuronal-muscle regeneration**
Michela Pozzobon (Università degli Studi di Padova)

15:00 **Assemblea dei Soci**

17:00 *Trasferimento in Hotel*

18:30 *Partenza per cena sociale presso Ristorante Villa Chiara, Città Sant'Angelo (Pescara)*

20 settembre 2025
Sabato

IV SESSIONE: Biogenesi, funzioni e alterazioni di organelli e strutture cellulari ed extracellulari (09:00 - 10:15)
Chairs: **Angelo Poletti** (Università degli Studi di Milano)
Simona Paladino (Università degli Studi di Napoli Federico II)

09:00 **Evidence of an unprecedented cytoplasmic function of DDX11, the warsaw breakage syndrome DNA helicase, in regulating autophagy**
Raffaella Bonavita (Università degli Studi di Napoli Federico II)

09:15 **Erythrocyte-derived nanoparticles as drug delivery vehicles to target the tumor microenvironment: in vitro evaluation of their therapeutic effect**
Maria Chiara Ciferri (Università degli Studi di Genova)

09:30 **The role of lactate in mitochondrial metabolism of DOX-induced senescent AC16 cells**
Rosamaria Militello (Università degli Studi di Firenze)

09:45 **Investigating the role of mTORC2 and Rictor in autophagy using dictyostelium discoideum**
Cristina Panuzzo (Università degli Studi di Torino)

10:00 **TUSC3, a component of the OST complex, mediates endoplasmic reticulum triage of signaling glycoproteins**
Thomas Vaccari (Università degli Studi di Milano)

Coffee break reinforced (10:15 - 13:00)

V SESSIONE: Genomica strutturale e funzionale (11:00 - 12:15)
Chairs: **Carla Olivieri** (Università degli Studi di Pavia)
Paola Piomboni (Università degli Studi di Siena)

11:00 **A circRNA derived from PUMILIO 1 gene regulates transcripts related to female reproduction by a possible feedback mechanism involving PUM1 protein**
Angela Caponnetto (Università degli Studi di Catania)

11:15 **Towards customized allele-specific CRISPR/Cas gene editing for the treatment of ocular surface disorder in EEC syndrome**
Laura De Rosa (Università degli Studi di Modena e Reggio Emilia)

11:30 **Targeted inhibition of SMAD3 reduces senescence in aged ovaries**
Osama Elsallabi (Università degli Studi “G. d’Annunzio”, Chieti-Pescara)

11:45 **An inducible iPSC-Cas9 platform for allele-specific normalization of Hsa21 gene dosage in trisomic cells**
Antonella Izzo (Università degli Studi di Napoli Federico II)

20 settembre 2025
Sabato

12:00 **Targeted siRNA delivery via functionalized nanoparticles for the treatment of FGFR-related syndromic craniosynostosis**
Martina Salvati (Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma)

12:15 **Consegna Premi Tarone**
Assegnazione borse congressuali

13:00 **Chiusura del congresso**

Con il contributo
non condizionante di:

COMECER 50 YEARS

DiaGAMMA
di Giampiero Romano
Forniture per il laboratorio biologico e biotecnologico

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ZANICHELLI

Comunicazioni Orali

THREE-DIMENSIONAL CELL CULTURE MODELS REVEAL ONCO-PROTECTIVE EFFECTS OF LIFESTYLE INTERVENTIONS IN BREAST CANCER SURVIVORS

E. Barbieri [1], G. Annibalini[1], M. De Santi[1], G. Baldelli[1], V. Gentilini [1], F. Lucertini [1], M. Bocconcelli[1], D. Sisti[1], S. Barocci[2], V. Catalano[2], A. Villarini[3], R. Emili[2]

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[2]*Ospedale Santa Maria della Misericordia, AST Pesaro Urbino, Italy*

[3]*Department of Medicine and Surgery, Section of Hygiene and Public Health, University of Perugia, Perugia, Italy*

Background:
The use of three-dimensional (3D) cell culture models in exercise oncology has gained increasing attention due to their ability to replicate better tumor behavior, particularly processes like recurrence and dormancy. While lifestyle interventions have shown benefits on health-related quality of life (HRQoL) in breast cancer survivors (BCS) , their role in modulating biochemical pathways, such as the insulin-like growth factor (IGF-1) system, and limiting tumor progression remains unclear.

Methods:
This study investigated the biological impact of a 3-month home-based lifestyle intervention, combining progressive aerobic training (40–70% heart rate reserve) and Mediterranean diet adherence, in 30 BC survivors from the MoviS randomized clinical trial (NCT04818359). Serum samples were collected before and after the intervention to assess circulating levels of IGF-1, IGFBP3, metabolic markers (e.g., glycemia, insulin, lipids), cardiorespiratory fitness (VO_2 max), and HRQoL. These samples were tested *in vitro* on triple-negative breast cancer (TNBC) cells (MDA-MB-231), using a dual 3D culture system to evaluate microtumor formation and spheroid development.

Results:
3-month lifestyle intervention significantly improved cardiometabolic health in BCS: reductions in BMI, glucose, insulin, HOMA Index, IGFBP3, total cholesterol, LDL, and hs-CRP levels were observed, along with increased VO_2 max. A non-linear U-shaped relationship was found between baseline IGF-1 and changes in both IGF-1 and VO_2 max, emphasizing the complex role of IGF-1 as both a potential pro-tumorigenic and a marker of overall health in BCS, with a strong inverse correlation between pre- and post-intervention values ($r = -0.62$, $p < 0.001$). Serum collected after the lifestyle intervention impaired TNBC spheroid viability and structural integrity compared to baseline samples. Moreover, lifestyle-conditioned serum induced a reduction in microtumor formation in 16 out of 30 participants (>5% decrease), with an average reduction of 10.2% across the cohort. The analysis

revealed that circulating IGF-1 was the only predictor significantly identified (b, 0.112; $p = 0.001$), whereas the other parameters were not significantly associated with spheroid formation.

Conclusions:
These findings support the use of 3D cell models as a valuable tool for translational exercise oncology research. Importantly, they demonstrate that lifestyle modifications can exert anti-tumor effects, potentially mediated through IGF-1 pathway modulation. This work highlights the potential of structured physical activity and dietary interventions in improving physiological health and controlling tumor progression in breast cancer survivors, offering insight into personalized cancer prevention strategies.

NATURAL PROTEOGLYCAN-LIKE MOLECULES FROM DUGESIA JAPONICA MUCUS ACTIVATE FERROPTOTIC DEATH IN CANCER CELLS

G. Gambino[1], *G. Marcelli*[1], *L. Benvenuti*[2], *C. Bertini*[1], *P. Iacopetti*[1], *L. Giambastiani*[3], *L. Pozzo*[3], *A. Salvetti*[1], *L. Rossi*[1]

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Cancer progression arises from a complex interplay of genetic, metabolic, and microenvironmental alterations. While chemotherapy remains central to cancer treatment, new strategies are needed to overcome resistance and improve selectivity. Ferroptosis, a regulated form of cell death driven by iron-dependent lipid peroxidation, has recently emerged as a promising therapeutic avenue. However, the clinical translation of ferroptosis inducers (FINs) is hampered by toxicity and limited in vivo efficacy.

We have recently demonstrated that mucus secreted by *Dugesia japonica* (a freshwater planarian) exerts potent anticancer effects by activating both by cytostatic and reactive oxygen species (ROS)-dependent cytotoxic mechanisms and can induce multiple forms of cell death, with a preferential activation of ferroptosis.

Planarian mucus is rich in sulphated glycosaminoglycans, a common feature of peptidoglycans and notably, the active fraction of planarian mucus has a molecular weight between 150 and 200 KDa, which is compatible with the class of small leucine-rich proteoglycans (SLRPs). These ECM components, such as Decorin, act as a pan-receptor tyrosine kinase (RTK) inhibitor thereby suppressing downstream signaling pathways. Planarian mucus proteoglycans might act with a similar mechanism, likely inhibiting multiple RTKs. Indeed, mucus treatment leads to an initial reduction in intracellular glutathione (GSH) levels, probably mediated by the relieves of STAT3-mediated suppression of CHAC1 activity. As a result, a self-amplifying loop is triggered, involving oxidative endoplasmic reticulum (ER) stress with ATF4 activation, which in turns potentiate CHAC1 expression.

Our findings position planarian mucus as a sustainable and bioaccessible source of natural FINs that warrant further characterization and evaluation for cancer therapy.

D-CHIRO-INOSITOL AND LPS, BUT NOT MYO-INOSITOL, INDUCE A HYPERANDROGENIC RESPONSE IN HUMAN KGN GRANULOSA CELLS

C. Giuliani[1], *G. Casoli*[2], *G. Di Emidio*[2], *C. Tatone*[2], *A. Bevilacqua*[1]

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[2]*Department of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila*

Polycystic ovary syndrome (PCOS), affecting 10-13% of women of reproductive age[1], is linked to insulin resistance, inflammation, oxidative stress, hyperandrogenism, and infertility. Reduced aromatase activity in ovarian granulosa cells is a major cause of hyperandrogenism. PCOS treatment benefits from the administration of myo-inositol (MI) and D-chiro-inositol (DCI), combined at a 40:1 molar ratio. The balance between DCI and MI is critical for ovarian health, and we have previously found that DCI reduces aromatase expression and produces androgenic effects if administered to female mice at high doses[2]. Since bacterial lipopolysaccharide (LPS) has been shown to induce oxidative stress and inflammation in KGN tumor-derived human granulosa cells[3], we treated these cells for 24 hours with 2µg/mL LPS or 20nM, 50nM, 100nM DCI, or MI as the negative control, to establish a PCOS-like cellular model. We investigated molecular and cellular functions of KGN cells, including cell proliferation, oxidative stress and inflammation, *CYP19A1* expression, aromatase abundance and activity. While neither treatment affected cell proliferation, DCI showed antioxidant activity and no inflammatory effects, but LPS induced oxidative stress and inflammation. However, both molecules decreased *CYP19A1* expression and aromatase abundance, reducing estradiol secretion. Like DCI, MI exerted antioxidant effects but did not affect aromatase expression and activity. We confirmed known effects of LPS[3,4] and showed that DCI, at the tested concentrations, maintains cellular homeostasis but induces a hyperandrogenic response. We propose that both DCI and LPS produce distinct PCOS-like alterations in KGN cells, providing useful *in vitro* models for studying PCOS pathophysiology.

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[2] DOI:10.3390/ijms22115691

[3] DOI:10.1007/s10863-022-09942-7

[4] DOI: 10.3389/fendo.2021.629554

INTERORGANELLAR CROSS-TALK IN CELLULAR STRESS RESPONSE SIGNALING

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Cells experience a variety of stress during their life, thus they need to mount adaptive strategies for survival or succumb through the activation of cell death programs. A dynamic and complex asset of interactions between and within molecules and pathways will determine the ultimate cell fate. In this scenario, the communication among organelles can play a major role and deserves further attention. Our group has been working on the interorganellar cross-talk between mitochondria and peroxisomes in a model of *Saccharomyces cerevisiae* yeast cells stressed with sodium chloride. We have characterized a tight communication between these two organelles occurring through the activation of the mitochondrial retrograde pathway, named RTG. This pathway sustains cell adaptation acting downstream of the short-term stress response mediated by Hog1, the master regulator of the High Osmolarity Glycerol pathway. Metabolomic analysis identified citrate levels and its cellular distribution as a potential regulators of the RTG-mediated adaptive process. This is consistent with the increased expression of *CIT2,* encoding for the peroxisomal isoform of citrate synthase and considered prototypical of RTG pathway activation, and of citrate synthase activity. Interestingly, an osmosensitive phenotype has been found for the mitochondrial carrier encoded by *YHM2*, which has been reported to transport citrate and oxoglutarate. These data highlight how citrate trafficking can contribute to the metabolic reprogramming of yeast osmo-stressed cells. Elucidating the molecular basis of interorganellar coordination with the role of proteins, pathways and/or selected metabolites will help to clarify possible mechanisms of adaptation for the maintenance of cell homeostasis upon stress.

NATRIURETIC PEPTDES: A NOVEL MECHANISM CONTROLLING DENDRITIC CELLS INFLAMMATORY PHENOTYPE

L. Mezzasoma[1], *G. Manni*[1], *E. C. Silva Barcelos*[2], *D. Ricciuti*[1], *B. Pierioni*[1], *M. Gargaro*[2], *G. Mencarelli*[1], *H. Acha-Orbea*[3], *F. Fallarino*[1], *V. N. Talesa*[1]

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The NLRP3 inflammasomes is a cytoplasmatic multiprotein complex playing a crucial role in mediating the innate immune responses to dangerous signals and cellular stress. In dendritic cells (DCs), specialized antigen-presenting cells that coordinate both immune activation and tolerance, NLRP3 activation is tightly associated to their maturation and functional programming. However, NLRP3 activation in DCs can lead to both beneficial and harmful outcomes depending on the context of the disease, suggesting that modulating this pathway could offer a valuable pharmacological approach for controlling immune responses.

Natriuretic Peptides (NPs), such as atrial (ANP) and brain (BNP) types, exert diverse immunoregulatory functions through Natriuretic Peptide Receptor-1 (NPR-1), which is broadly expressed across immune cells. Although NPs are known to inhibit NLRP3 inflammasome activation and IL-1β release in human monocytes, their specific role in DCs remains poorly characterized.

This study investigated the role of NPs in regulating NLRP3 inflammasome activity in two conventional DCs subsets: cDC1 and cDC2. We found that both subsets exhibit basal expression of NPR-1, which is further upregulated upon inflammatory stimulation. Moreover, under inflammatory conditions, cDCs actively produce ANP and BNP. Although both subsets exhibit basal expression of NLRP3 pathway components, cDC2 shows markedly stronger activation of the NLRP3/IL-1β axis following LPS and ATP stimulation, compared to cDC1. Importantly, the NPs/NPR-1 axis more potently suppresses NLRP3 activation in cDC2 by acting at translational and post-translational levels.

These findings identify NPs as key modulators of the inflammatory phenotype in cDCs and highlight the NPs/NPR-1 signaling axis as a promising therapeutic target for the selective regulation of DCs subset-mediated immune response.

ADIPOSE TISSUE INFLAMMATION AND SENESCENCE IN AGING: A KEY ROLE FOR THE COMPLEMENT 3A RECEPTOR

B. Vezzani[1], *E. Tamburini*[1], *A. Terenziani*[1], *N. Oliverio*[1], *L. Baier* [2], *A. Bartolomucci*[3]

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Obesity, characterized by excessive adipose tissue (AT) accumulation, is a major global health issue linked to accelerated aging-associated diseases. The association between body mass index (BMI), chronological age, and age at disease onset is well established. Augmented levels of pro-inflammatory mechanisms in the AT of the elderly and people having high BMI has been shown to drive accumulation of senescent cells (SNC), a recognized driver of aging-associated diseases. However, how SNC accumulation is mediated remains poorly understood. Innate immunity, and the Complement 3a receptor (C3aR; encoded by *C3aR1*) in particular, is gaining increased interest, due to its high expression in AT and its modulation during aging. We identified a positive association between *C3aR1* and other pro-inflammatory pathways with BMI and chronological age in human AT. Since the AT is composed of multiple cell types, we first investigated expression in the major AT parenchymal cell types. qPCR analysis revealed that macrophages show the strongest expression of *C3aR1* among the AT resident cells, while its expression decreases during adipogenesis in mesenchymal stromal cells (MSC), suggesting that its up-regulation in obese-AT might be due to infiltrating macrophages. Gain of function experiments in 2D cultures demonstrated that C3aR activation promotes the expression of pro-inflammatory cytokines in monocytes, macrophages, and adipocytes. Under the same conditions, C3aR agonists caused senescence markers in MSC, but not in the other cell types. However, using conditioned media and 3D adipose spheroids, we identified a cellular crosstalk in that C3aR activation in macrophages caused a pro-inflammatory and senescent phenotype in adipocytes. Overall, our results identify C3aR as a new mediator of AT inflammation and senescence, having significant implications for aging-associated diseases.

EXPULSION OF IRON-RICH FERRITIN VIA CD63-MEDIATED EXOSOME DRIVES FERROPTOSIS RESISTANCE IN OVARIAN CANCER CELLS

A. M. Battaglia[1], A. Sacco[1], E. Giorgio[1], L. Petriaggi[1], J. Elzanowska[2], A. R. Cruz[2], L. Rocha[2], C. Esteves Pereira[2], M. C. Strano Moraes[2], L. Palazzo[3], C. De Vitis[4], B. Costa-Silva[2], F. Biamonte[1]

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Ferroptosis is a promising therapeutic target in ovarian cancer (OVCA). Recent evidence highlights that OVCA sensitivity to ferroptosis is closely linked to the accumulation of intracellular and redox-active labile iron pool (LIP), with ferroptosis resistance occurring when LIP remains limited, even in the presence of ferroptosis inducers (FINs). However, the mechanism underlying this intrinsic resistance is poorly understood. Here, we show that the FIN erastin (8µM, 8h) fails to induce ferroptosis in OVCA cells COV318 and PEO4, as assessed by PI flow cytometry assay. Mechanistically, erastin causes the upregulation of CD63, a tetraspanin involved in forming multivescicular bodies (MVBs) and exosomes (EVs), along with an increase of MVBs quantity as observed by transmission electron microscopy (TEM). EVs isolated via ultracentrifugation and, then, characterized by nanoparticle tracking analysis (NTA), revealed a significant increase in EVs/cell in erastin-treated COV318 and PEO4. Notably, Western blot of EV fractions highlighted the presence of CD63 and the main iron storage protein FtH. In agreement, intracellular LIP, measured by FerroOrange immunofluorescence, resulted unaltered. Similarly, lipid peroxidation and mitochondrial ROS, analyzed by BODIPY-C11 and MitoSOX flow cytometry assays, did not show significant alterations. Notably, siRNA-mediated *CD63* knockdown or pharmacological inhibition of EV biogenesis with GW4869 prevented FtH release and restored LIP accumulation, lipid peroxidation, and ferroptosis sensitivity in both cell lines. Overall, our results suggest that OVCA cells can resist ferroptosis by modulating their iron metabolism through a non-canonical iron expulsion pathway, specifically by utilizing $CD63^+$ EVs to secrete iron-rich FtH. These data propose that combining erastin with EV inhibitors could improve therapeutic efficacy in resistant OVCA.

DYSREGULATION OF FATTY ACID METABOLISM BY EZH2 INHIBITORS UNCOVERS NOVEL THERAPEUTIC APPROACHES FOR ACC

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Epigenetic changes are present in all human cancers and are responsible for switching on or off genes, thus controlling tumor cell transcriptome. These changes occur through DNA methylation, histone modifiers and readers, chromatin remodelers, and microRNAs. The histone H3 methyl-transferase EZH2 gene is overexpressed in several cancer types, including adrenocortical carcinoma (ACC), a rare cancer still lacking a targeted therapy. EZH2 inhibitors (EZH2i) have been tested in several clinical trials, but their effectiveness was limited by the toxic effects of the therapeutic doses. We tested several EZH2i on ACC cells, and observed a significant reduction in cell growth only with doses much higher than those required to prevent H3 methylation. We found that all tested EZH2i doses affected lipid metabolism genes, ROS, and glutathione production. Transcript changes correlated with metabolic data, which suggested the effects of EZH2i on ferroptosis. We found that EZH2i dose-dependently increased SLC7A11/glutathione axis and glutathione peroxidase-4 (GPX4), required to counteract lipid peroxidation and ferroptosis. A GPX4 inhibitor synergized with EZH2i, making low doses - which otherwise do not affect cell viability - able to significantly reduce ACC cell growth in vitro and in vivo. Importantly, we found that the anti-ferroptosis defense mechanism induced by EZH2i is a common response for several aggressive tumor phenotypes, uncovering a general co-targetable mechanism that could limit EZH2i effectiveness. Correcting this antioxidant response by ferroptosis inducers may be a new combination therapy that will easily find clinical applications.

A NEW MOUSE MODEL OF JMML HIGHLIGHTS DIFFERENTIAL SUSCEPTIBILITY OF EMBRYONIC HEMATOPOIETIC STEM/ PROGENITOR CELLS TO THE KRAS G12D MUTATION

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Juvenile myelomonocytic leukemia (JMML) is a rare, clinically heterogenous myeloproliferative neoplasm of early childhood, caused by mutations in the Ras pathway. One of the significant challenges in JMML is developing faithful experimental models. This may stem from the critical importance of the cellular context in which driving mutations occur, especially considering that half of JMML cases originate *in utero*.

To generate a model of JMML with prenatal onset, we selectively targeted the $Kras^{G12D}$ mutation to distinct subset of hematopoietic stem/progenitor cells (HSPCs) emerging from Cdh5+ hemogenic endothelium during embryonic development using a strategy we recently validated.

We show that embryonic HSPCs exhibit differential susceptibility to $Kras^{G12D}$. Targeting the mutation to erythro-myeloid progenitors (EMPs) resulted in a late-onset low penetrance myeloproliferative disorder, suggesting that EMPs may not be involved in JMML. Mosaic activation of $Kras^{G12D}$ in either fetal-restricted HSPCs or adult-fated HSCs caused a fully penetrant, JMML-resembling leukemic phenotype in adult mice, more aggressive when $Kras^{G12D}$ was targeted to HSCs. As E14.5 fetal liver (FL) cells showed defining features of JMML such as GM-CSF hypersensitivity, to confirm the *in utero* origin of the disease, we transplanted these cells into recipient mice. Notably, only HSC-, but not fetal-restricted HSPCs- targeted cells could induce an aggressive JMML-like disease. Analysis of the transcriptomic and epigenetic changes following the acquisition of $Kras^{G12D}$ at fetal and postnatal stages is ongoing.

These data suggest that JMML heterogeneity could be, at least in part, explained by variability in the cell of origin. By providing a faithful model of prenatal-onset JMML, our approach offers a valuable platform for studying the early pathogenic events underlying disease initiation and progression.

EXTRACELLULAR VESICLES FROM SECOND TRIMESTER HUMAN AMNIOTIC FLUID AS CANDIDATE THERAPEUTICS AGAINST SKELETAL AND CARDIAC MUSCLE INJURY

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We showed that II trimester human amniotic fluid (hAF) is a source of stromal progenitors (hAFSC) actively secreting extracellular vesicles (EVs) with trophic modulatory effects in skeletal and cardiac muscle injury models. Here we characterized EVs directly separated from hAF (hAF-EVs) and explored their paracrine potential against oxidative stress and chemotherapy-induced cardiotoxicity. hAF was obtained as leftover material from prenatal screening amniocentesis with informed consent from healthy donors. hAF-EVs were obtained by size exclusion chromatography and characterized by nanoparticle tracking analysis, transmission electron- and super-resolution microscopy, proteomics, and metabolic assays. hAF-EVs paracrine potential was evaluated on murine C2C12 myoblasts, on 3D human cardiac microtissue (hMT) under H_2O_2 and TGFb stress and on murine neonatal cardiomyocytes (mNCMs) and hiPS-derived cardiomyocytes (iCMs) exposed to Doxorubicin (Dox) cardiotoxicity. hAF-EV yield was 5.0±0.5x10^9particle/ml, with round cup-shaped morphology and 224.1±4.5 nm size and expression of CD81, CD63 and CD9 markers. hAF-EVs were enriched in CD133/1, CD326, CD24, CD29, and SSEA4 indicating their heterogeneous origin. hMT primed with hAF-EVs and experiencing H_2O_2 stress and TGFβ stimulation showed improved survival, with decreased fibrosis onset ($p<0.0001$). While oxidative stress significantly reduced C2C12 survival and Dox increased apoptosis in mNCMs ($p<0.0001$) and doubled premature senescence in iCMs ($p<0.01$), hAF-EVs rescued C2C12 and decreased cardiomyocyte apoptosis ($p<0.5$). EV cargo was found enriched in peptides related to

anti-oxidant defense pathways; hAF-EVs were also able to produce ATP by oxygen consumption. In light of such findings, II trimester hAF can be then exploited as direct source of EVs with promising therapeutic capacity against skeletal and cardiac muscle injury.

UNVEILING THE ORIGIN AND FUNCTIONS OF DIAGNOSTIC CIRCULATING MICRORNAS IN LUNG CANCER.

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BACKGROUND: Circulating microRNAs (c-miRs) were shown to be effective biomarkers for lung cancer early detection. However, the understanding of c-miRs origin and their biological functions still remains elusive.

METHODS: We analysed miRNA expression in a large panel of lung cancer (LC) and hematopoietic cell lines (N = 252; CCLE database) coupled with c-miR profile of a large cohort of serum samples (N = 975), from high-risk subjects underwent annual LD-CT for 5 years. Furthermore, we examined intracellular and extracellular miR-29a-3p/223-3p expression profile in lung adenocarcinoma (LUAD) tissues, in matched serum samples and in LC and stromal cell lines. Lastly, through the modulation of expression of selected c-miRs by using mimic (OE) or antisense microRNA (KD), we explored their impact on lung cancer transcriptome and cancer/ immune phenotypes.

RESULTS: Here, we investigated the origin of an extensively validated 13 c-miRs signature diagnostics for asymptomatic lung cancer (LC) in high-risk subjects (smokers, >20 packs/y; >50 y old). Overall, we found a mixed origin of these c-miRs, originating both from tumour cells and the tumour microenvironment (TME). Intriguingly, we revealed that circulating miR-29a-3p and miR- 223-3p are abundantly released from LC epithelial cells and immune cells, respectively. In particular, we found that miR-223-3p triggered several lung cancer related phenotypes such as invasion, migration and tumour-promoting inflammation.

CONCLUSIONS: Our study highlights a mixed tumour epithelial and stroma-associated origin of LC c-miRs with new evidences on the multifaceted role of miR-223-3p in LC pathogenesis and immune modulation.

ROLE OF MECP2 IN INFLAMMAGING-RELATED OVARIAN DYSFUNCTIONS: INSIGHTS FROM A GENETICALLY-INDUCED DEFICIENT MOUSE MODEL

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MECP2 is an X-linked gene, that encodes an ubiquitous epigenetic regulator, functioning both as a repressor or an activator of gene transcription, and being also involved in chromatin compaction. *MECP2* gene dosage is critical, since its deficiency leads to Rett syndrome (RTT), and the duplication of its gene locus results in *MECP2* duplication syndrome, which are two rare neurodevelopmental disorders. Although most papers focused on MeCP2 role in the brain, emerging evidence describes the importance of MeCP2 in non-neurological compartments. Recently, a possible role played by this epigenetic reader in reproductive function and oocyte maturation has been described, although providing conflicting results. Hence, the aim of this project is to investigate how MeCP2 deficiency could affect ovarian functionality and whether this alteration might be linked to premature (inflamm)aging of the tissue. To this end, ovaries and oocytes from *Mecp2*$^{+/-}$ (Het) female mice [B6.129P2(C)-*Mecp2*$^{tm1.1Bird/J}$] (12- and 24-week-old mice) and wild-type (WT) littermates (*i.e.*, controls) were collected. Twenty-four-week-old Het mice exhibited significantly elevated serum levels of interleukin (IL)-1β, IL-18, and ASC—key components of the inflammasome-associated response— accompanied by impaired performances in cognitive and muscular behavioural tests, as compared to control mice. At the ovarian level, Het mice showed increased levels of ASC protein, augmented mRNA levels of 5 NAD^+ consuming enzymes in oocytes, and a reduced ovarian reserve. However, NLRP3 and IL-18 levels were comparable between *Mecp2*$^{+/-}$ and WT mice. These preliminary findings suggest that MeCP2 deficiency can affect the ovarian microenvironment, promoting aging, as revealed by dysregulated NAD^+ metabolism and low-grade inflammation.

MLL1-DRIVEN H3K4ME3 ESTABLISHES EPIGENETIC MEMORY OF VASCULAR INFLAMMATION AND OXIDATIVE STRESS TRIGGERED BY CHRONIC HYPERGLYCEMIA

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The epigenetic mechanisms triggered by chronic hyperglycemia during gestational diabetes (GD), which may predispose both mothers and their offspring to future cardiometabolic diseases, remain poorly understood. Therefore, our aim was to investigate the potential involvement of MLL1-mediated trimethylation of histone 3 at lysine 4 (H3K4me3) as a driver of proinflammatory and pro-oxidative cellular states.

Peripheral blood mononuclear cells (PBMC) were collected from GD and control (C) women and also from adolescents born to women of both groups. Endothelial human umbilical vein endothelial cells (HUVEC) and cord blood mononuclear cells (CBMC) were from C- and GD-umbilical cords. The NF-κBp65 and NOX4 expressions were evaluated by RT-PCR reaction and immunofluorescence (IF). MLL1 and H3K4me3 were studied by immunoblotting and IF. H3K4me3 on NF-κBp65 and NOX4 promoters was studied by chromatin immunoprecipitation. Superoxide anion generation was measured by electron spin resonance spectroscopy. Plasma cytokines were measured by ELISA assay. To investigate the role of MLL1, HUVEC were exposed to inhibitor MM102 or siRNA.

PBMC, CBMC, and HUVEC from GD women showed increased expression of inflammatory and pr-oxidant markers, together with enhanced H3K4me3 at NF-κBp65 and NOX4 promoters. These alterations persisted in adolescents born to GD

women, indicating an epigenetically imprinted phenotype. Importantly, MLL1 inhibition reversed these pathological features.

Our findings provide a first proof of concept that hyperglycemia induces an 'epigenetic memory' in endothelial cells through MLL1-dependent H3K4me3 mark. This mechanism may explain the persistence of vascular dysfunction and elevated cardiovascular risk in diabetes, even after glycemic control is achieved. Targeting MLL1 could offer a novel therapy to reprogram adverse vascular memory and mitigate cardiovascular risk.

MULTI-SINEUP: A NOVEL RNA THERAPEUTIC APPROACH FOR 22Q11.2 MICRODELETION SYNDROME

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SINEUPs are a functional class of natural and synthetic antisense long non-coding RNAs that enhance the translation of partially overlapping sense mRNAs. Their activity depends on the combination of two domains: the overlapping region that dictates the specificity (binding domain, BD), and an embedded inverted SINEB2 element that acts as the effector domain (ED), controlling the enhancement of mRNA translation. Previous studies demonstrated the efficacy of SINEUPs *in vitro* and *in vivo* in enhancing the translation of the target genes. SINEUPs are potentially curative for many serious genetic diseases called haploinsufficiency. Among them are cases of microdeletions of an entire portion of one of the homologous chromosomes leading to haploinsufficiency of multiple genes. One example is 22q.11.2 deletion syndrome, which manifests as multi-organs dysfunctions, ranging from cardiac defects to neuropsychiatric symptoms. With the current knowledge, only one gene at a time is usually targeted, thus leaving more complex diseases, such as microdeletions, without therapeutic options. In this study, we designed and synthesized the first multi-BD-SINEUP targeting multiple genes as a therapeutic strategy for 22q.11.2 deletions syndrome. By targeting TBX1, COMT and DGCR8, we demonstrated that the multi-BD-SINEUP could increase the protein levels of the three genes *in vitro* in cells and *in vivo* in the mouse brain. Moreover, the multi-BD-SINEUP was able to rescue the cognitive impairments present in the LgDel mice, a mouse model of 22q11.2 deletion syndrome. In conclusion, we described the first multi-BD-SINEUP that could target and increase the translation of multiple mRNAs, with a proof-of-concept therapeutic application for 22q11.2 deletion syndrome.

LEUCINE-RICH REPEAT KINASE 2 CONTROLS CLUSTERIN TRANSLATION VIA MIR-22-5P: IMPLICATION FOR PARKINSON'S DISEASE

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Leucine Rich Repeat Kinase 2 (LRRK2) is a kinase protein associated with the genetic and idiopathic form of Parkinson's Disease (PD). LRRK2 is highly expressed in the brain, particularly in astrocytes and microglia, and has been connected to several cellular functions, such as protein translation. Here, we showed that LRRK2 controls the protein levels of the chaperone Clusterin (Clu), which we recently showed to limit PD-related α-Synuclein (α-Syn) aggregates uptake by astrocytes. Specifically, we observed that LRRK2 Knock-out (KO) mouse brains and primary astrocytes exhibited a reduced global protein synthesis with a diminished levels of Clu protein compared to the relative wild type (WT), indicating that LRRK2 regulates protein translation in astrocytes. We then explored whether LRRK2 controls Clu levels *via* microRNAs (miRNA). By analysis of the striatum and primary astrocytes from LRRK2 KO mice, we identified miR-22-5p as a putative miRNA for Clu regulation. We confirmed that Clu is targeted by miR-22-5p through a luciferase reporter assay in HEK293 cells and by overexpressing miR-22-5p mimic in primary astrocytes, where we found a significant reduction of Clu levels. Intriguingly, when we investigated the impact of PD-associated LRRK2 G2019S mutation on Clu we observed an opposite scenario with increased level of protein translation and Clu protein, and reduced level of miR-22-5p. Moreover, of relevance, we found that the treatment with miR-22-5p mimic improved the ability of LRRK2 G2019S primary astrocytes to take up α-Syn fibrils.

Overall, our study reveals that LRRK2 controls Clu levels *via* miRNA-22-5p in astrocytes, and that LRRK2-Clu pathway is involved in the clearance of pathological α-Syn. Future studies will allow us to understand whether the modulation of astrocytic LRRK2 G2019S-Clu pathway might attenuate the neuronal spreading of α-Syn pathology in PD.

ENGINEERING THE MICROENVIRONMENT TO MODULATE MACROPHAGE POLARIZATION AND ANTI-TUMOR ACTIVITY

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The extracellular matrix (ECM) is a key regulator of immune cell function, influencing not only adhesion and migration but also macrophage activation and polarization. In this context, in vitro platforms that mimic ECM properties offer valuable tools to study immune modulation in pathological conditions such as cancer and chronic inflammation.In this study, we investigated how ECM-like substrates modulate macrophage polarization in response to nanomaterial-based inflammatory stimuli. We developed scaffolds composed of denatured collagen (gelatin) functionalized with increasing concentrations of carbon nanotubes (CNTs), used as model pro-inflammatory fibers. Human THP-1-derived macrophage-like cells were cultured on these substrates for up to five days. Cell viability remained unaffected after 24 hours, while morphological changes emerged at higher CNT concentrations and prolonged exposure, indicating a shift in activation state.

The evaluation of macrophage immunological polarization using DCF staining and flow cytometry suggest polarization toward a pro-inflammatory M1 phenotype.To assess the functional consequences of macrophage activation in this model, we collected conditioned media from THP-1 cells exposed to CNT-containing substrates and evaluated their effect on tumor spheroid growth. Conditioned media from these macrophages significantly reduced the growth and size of glioblastoma spheroids, suggesting the release of anti-tumoral factors.Altogether, our results demonstrate that ECM-mimicking substrates functionalized with CNTs can modulate macrophage polarization toward an M1 phenotype. This platform provides a promising in vitro tool for investigating nanomaterial–immune system interactions and may support the development of novel immunomodulatory strategies in cancer therapy.

STUDYING MOLECULAR MECHANISMS LEADING TO STAG2-DRIVEN MEDULLOBLASTOMA

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Medulloblastoma (MB) is a cerebellar embryonal tumor likely caused by impaired neural progenitor differentiation, however, the underlying genetics, molecular mechanisms, and predisposing factors leading to MB onset are still unclear. Recently, somatic alterations in cohesin genes have emerged as oncogenic drivers in hematologic and solid tumors. The cohesin complex's role in cerebellum development, gene expression regulation, and DNA repair suggests this structure as a possible candidate in MB onset. Among cohesins, STAG2 is the most frequently affected subunit in cancers, and its loss of function is believed to promote tumorigenesis by altering 3D genome architecture and transcription. We studied *in vitro* the impact of *STAG2* silencing on a cell model and, by cerebellum organoids, on cell differentiation. In addition, we investigated tumor-related phenotypic features in the *in vivo* fly model, obtained by *SA1* (*STAG2* ortholog) silencing. Specifically, *in vitro*, *STAG2*-deficient cells present increased DNA damage, while *STAG2*-silenced cerebellum organoids exhibit a deregulated gene expression related to development. *In vivo*, we showed a defective neuroblast differentiation during larval development, and mass formation within adult fly brains, with a lowered life expectancy consistent with the malignant phenotype. These models aim to elucidate molecular mechanisms underlying *STAG2*-driven MB pathogenesis but also represent a basis for testing targeted therapies, such as PARP inhibitors (PARPi). This treatment has been recently implemented in clinical trials for cohesin-mutated tumors, demonstrating enhanced efficacy in models with defective DNA repair. The use of PARPi in our *STAG2*-depleted *in vitro* and *in vivo* models resulted in increased apoptosis and rescue of life expectancy, respectively, supporting the potential of PARPi as a new patient-specific therapy for MB.

ENDOTHELIAL RESPONSE TO HORMONAL STIMULI IN THE WINDOW OF IMPLANTATION: INSIGHTS FROM A 3D HUMAN ENDOMETRIUM MODEL

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Successful implantation depends on the precise remodeling of the endometrial vasculature during the window of implantation (WOI). Human uterine microvascular endothelial cells (HUtMECs) were cultured and treated with estradiol, medroxyprogesterone acetate, and cAMP to induce decidualization, with or without Ulipristal acetate (UPA) at 5 and 25 μM. Cells were integrated into a 3D endometrial model and analyzed for viability, structure, metabolism, and decidualization markers using qPCR/ddPCR, ELISA, and imaging techniques. Gene expression revealed increased levels of FKBP5, IGFBP1, ZBTB16, and PTGES2 after hormonal treatment, confirming endothelial responsiveness. IGFBP1 protein levels decreased upon UPA exposure Proteomic analysis was also performed using two-dimensional Western blotting (2D-WB) and Melanie software to identify differentially expressed proteins.. Proteomic data confirmed modulation of key proteins involved in vascular adaptation. Endothelial cells actively contribute to endometrial receptivity by supporting vascularization and decidualization processes. This study provides novel molecular and proteomic insights into endothelial dynamics during WOI and highlights potential biomarkers for implantation success.

THE PROTECTIVE ROLE OF GSH IN MESENCHYMAL STEM CELL SENESCENCE

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Glutathione (GSH), a key intracellular antioxidant, maintains redox balance and neutralizes reactive oxygen species. Altered GSH homeostasis is linked to diseases such as diabetes, obesity, and neurodegeneration. Mesenchymal Stem Cells (MSCs), despite their regenerative potential, exhibit reduced proliferative capacity due to replicative senescence. Given its antioxidant capacity, GSH may help delay senescence, a state of irreversible growth arrest. The p53 pathway mediates stress responses by activating proteins like p21 and sestrin 2, involved in cell cycle arrest and oxidative protection, respectively. Chronic inflammation also contributes to senescence through the release of pro-inflammatory factors known as the senescence-associated secretory phenotype (SASP).

Thus, given the critical role of mesenchymal stem cells (MSCs) in regenerative medicine, this study aims to elucidate the effects of glutathione (GSH) on the senescence-associated phenotypes of these cells.

Cells were treated with GSH, H_2O_2 (to induce inflammation), and a combination of both. Protein analysis was performed. In cells treated with both H_2O_2 and GSH, p53 was overexpressed compared to the control while p21 showed no significant changes, unlike cells treated with H_2O_2 alone, where p21 was overexpressed. Sestrin-2 responded to H_2O_2 with increased expression, but GSH treatment, alone or combined, lowered it compared to control. The protective effect of GSH was confirmed by the CCK-8 assay, which showed an increase in viability in cells treated with GSH. Furthermore, a ROS assay was conducted to confirm the antioxidant effect of GSH, revealing decreased ROS levels in cells treated with GSH alone and with GSH and H_2O_2.

In conclusion, the study suggests that GSH has a protective effect against oxidative stress and potentially against senescence.

MECHANISM OF ACTION OF EXTRACELLULAR VESICLES IN NEURONAL-MUSCLE REGENERATION

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Muscle defects resulting from trauma, tumor resection, or congenital abnormalities impact both pediatric and adult patients. In this context, our group demonstrated the neuro-muscular regenerative potential of human GMP mesenchymal stromal cell-derived extracellular vesicles (EVs). Specifically, mice that underwent volumetric muscle loss damage in tibialis anterior, were transplanted with muscle extracellular matrix and treated with either PBS or EVs. Flow cytometry, tissue analysis, qRT-PCR, and physiological testing were conducted in mice 7 and 30 post nanoparticle administration. EV treatment significantly enhanced angiogenesis and myogenesis, while reducing fibrosis. In addition, inflammation was shifted toward a tissue-repairing profile in the EV-treated group compared to controls. Notably, the protein expression in tissue was paralleled by enhanced functional recovery.

However, despite these encouraging outcomes, the mechanisms by which EVs exert their effects remain elusive. Building on these findings, our latest study sought to investigate the underlying mechanisms of EV-mediated neuro-muscular regeneration, utilizing three-dimensional (3D) multicellular in vitro models.We employed (1) decellularized human muscle ECM seeded with human muscle precursor cells and THP1-derived macrophages (M0), and (2) organotypic spinal cord cultures from rat fetuses.

In the 3D functional muscle model, miRNA, protein and gene expression analyses revealed that EVs exert their regenerative effects through the downregulation of TNFα pathway. Similarly, in spinal cord injury model, EVs modulated neuroinflammatory responses by inhibiting TNFα and promoting neural axon sprouting. These recent results underscore the strong regenerative capacity of EVs,

chiefly through the modulation of TNFα, supporting the repair of both muscular and neural compartments.

EVIDENCE OF AN UNPRECEDENTED CYTOPLASMIC FUNCTION OF DDX11, THE WARSAW BREAKAGE SYNDROME DNA HELICASE, IN REGULATING AUTOPHAGY

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DDX11 is a DNA helicase involved in critical cellular functions, including DNA replication/repair/recombination as well as sister chromatid cohesion establishment. Bi-allelic mutations of *DDX11* lead to Warsaw breakage syndrome (WABS), a rare genome instability disorder marked by significant prenatal and postnatal growth restriction, microcephaly, intellectual disability, and sensorineural hearing loss. The molecular mechanisms underlying WABS remain largely unclear. In this study, we uncover a novel role of DDX11 in regulating the macroautophagic/autophagic pathway. Specifically, we demonstrate that knockout of *DDX11* in RPE-1 cells hinders the progression of autophagy. DDX11 depletion significantly reduces the conversion of MAP1LC3/LC3 (microtubule associated protein 1 light chain 3), suggesting a defect in autophagosome biogenesis. This is supported by imaging analysis with a LC3 reporter fused in tandem with the red and green fluorescent proteins (mRFP-GFP-LC3), which reveals fewer autophagosomes and autolysosomes in *DDX11*-knockout cells. Moreover, the defect in autophagosome biogenesis, observed in DDX11-depleted cells, is linked to an upstream impairment of the ATG16L1-precursor trafficking and maturation, a step critical to achieve the LC3 lipidation. Consistent with this, DDX11-lacking cells exhibit a diminished capacity to clear aggregates of a mutant HTT (huntingtin) N-terminal fragment fused to the green fluorescent protein (HTTQ74-GFP), an autophagy substrate. Finally, we demonstrate the occurrence of a functional interplay between DDX11 and SQSTM1/p62, an autophagy cargo receptor protein, in supporting LC3 modification during autophagosome biogenesis. Our findings highlight a novel unprecedented function of DDX11 in the autophagy process with important implications for our understanding of WABS etiology.

ERYTHROCYTE-DERIVED NANOPARTICLES AS DRUG DELIVERY VEHICLES TO TARGET THE TUMOR MICROENVIRONMENT: IN VITRO EVALUATION OF THEIR THERAPEUTIC EFFECT

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In the last two decades, fibronectin (FN) has emerged as a relevant player in tumor progression, fostering the development of FN-targeted therapeutic strategies. Among FN isoforms, the extra domain B-containing fibronectin (ED-B FN) variant has been widely studied as a selective marker for tumor targeting. Natural nanoparticles have recently gained attention as biotechnological tools for drug delivery due to their biocompatibility, cargo-loading capacity, and surface modifiability for ligand attachment. In this study, we engineered erythrocyte-derived extracellular vesicles (NanoEs) by functionalizing their membrane with a fluorescent peptide targeting ED-B FN and loading them with Paclitaxel (PTX). These engineered NanoEs (eNanoEs) were assessed *in vitro* on ED-B-positive cancer cells for targeting specificity, internalization efficiency, and therapeutic efficacy. Internalization studies performed under static conditions and using an "organ-on-chip" approach based on a dynamic multi-organ millifluidic platform simulating human circulation, revealed significantly higher uptake of eNanoEs compared to unmodified particles, as quantified by flow cytometry. Internalization studies on primary colorectal cancer organoids revealed that eNanoEs effectively penetrate and accumulate within 3D cellular structures, confirming their targeting capabilities beyond monolayer cultures. Therapeutic evaluation through MTT assays in static conditions demonstrated a 50% reduction in cell viability upon eNanoE treatment. Furthermore, under dynamic conditions, eNanoEs loaded with ~170-fold less PTX than the free drug exhibited superior cytotoxicity, suggesting improved drug potency through nanoparticle encapsulation. These results highlight the potential of these intercellular communicators to act as natural nanocarriers able to enhance the therapeutic index while minimizing drug dosage.

THE ROLE OF LACTATE IN MITOCHONDRIAL METABOLISM OF DOX-INDUCED SENESCENT AC16 CELLS

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Senescent cells accumulate with age in organ and tissue causing the decline of functionality and various pathological conditions including cardiovascular disease. Regular exercise induces continuous exposure to lactate that contribute to adaptive process through mitochondrial biogenesis and improve of metabolic process. Its accumulation during exercise also appears to be associated with exercise-induced mitochondrial adaptation. Improvement of mitochondria function through lactate exposure could be a tool to prevent cardiomyocytes senescence and cardiac aging. The aim of the following article is to investigate the role of lactate in DOX-induced senescent AC16 cells mitochondrial metabolism. With this in mind, we assessed the metabolic behaviour in senescent cardiomyocytes after chronic lactate exposure and provided a discussion of the effect of this metabolite in regulating mitochondrial physiology during cardiac aging.

INVESTIGATING THE ROLE OF MTORC2 AND RICTOR IN AUTOPHAGY USING DICTYOSTELIUM DISCOIDEUM

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Abstract

Autophagy is essential for recycling damaged components and plays dual roles in cancer progression and survival. While mTORC1 is a well-known inhibitor of autophagy, the role of mTORC2 remains unclear. Here, we used Dictyostelium discoideum, a powerful model for dissecting mTORC2 involvement in autophagy.

Aim

Explore the contribution of mTORC2 to autophagy, with a focus on the structural and functional role of Rictor.

Methods and Results

We analysed autophagy in wild-type Dictyostelium, an LST8 knockout strain, and a Rictor mutant strain. Both mutants showed increased ATG8 and ATG18 puncta, indicating enhanced autophagosome formation, which was confirmed using a commercial autophagosome detection kit. Upon electroporation with the GFP-PGK autophagy reporter and treatment with NH_4Cl, a lysosomal inhibitor commonly used to block autophagosome degradation, we observed a reduced accumulation of free GFP in the mutants' strains, suggesting impaired flux rather than formation. Consistently, these cells displayed a notable reduction in lysosome abundance, reinforcing the hypothesis of impaired autophagic degradation. Furthermore, Rictor protein analysis revealed that upon NH_4Cl treatment, wild-type Rictor undergoes degradation via the autophagic pathway. In contrast, mutant Rictor forms large aggregates, suggesting altered folding and solubility. These mutations lie within intrinsically disordered regions (IDRs) of Rictor, which are known to be sensitive to structural instability and may promote aggregation under stress.

Conclusion

Our data reveal a previously underappreciated role for mTORC2 in promoting autophagic degradation and highlight Rictor as a structurally sensitive component. Given its tendency to aggregate when mutated in intrinsically disordered regions, it would be interesting to further investigate the role of Rictor in neurodegenerative diseases.

TUSC3, A COMPONENT OF THE OST COMPLEX, MEDIATES ENDOPLASMIC RETICULUM TRIAGE OF SIGNALING GLYCOPROTEINS

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One-third of the human-secreted proteome undergoes glycosylation, a post-translational modification that attaches sugar moieties to proteins. Attachment to the amide nitrogen of asparagine residues (*N*-glycosylation) occurs mainly in the endoplasmic reticulum (ER), where most secretory proteins are subjected to stepwise quality control ensuring structural integrity. Newborn proteins are *N*-glycosylated by the oligosaccharyltransferase (OST) complex that enzymatically catalyzes the attachment of the glycan precursor G3M9GlcNAc2. G3M9GlcNAc2-bound proteins are further modified by glycosidases and glycosyltransferases and assisted by chaperones for maturation and folding. However, little is known about how the OST complex helps newly glycosylated proteins to interact with lectin chaperones. Here, we establish that TUSC3, a well-conserved component of the OST complex, has a role in the triage of glycoproteins destined for ER quality control. Biochemical data in murine cells and genetic data *in vivo* using the fruit fly *Drosophila melanogaster* showed that TUSC3 (Ostgamma in *Drosophila*) ensures trimming of the second glucose on G2M9GlcNAc2-BMP4 molecules required for activation of ER stress and acts a positive regulator of BMP signaling. Gene dosage and genetic interaction studies suggest that the level of *Tusc3/Ostgamma* serves as a selective gatekeeper for protein secretion of BMP4 (Dpp in flies). Our study redefines the role of TUSC3 in the OST complex with implications for congenital disorders of glycosylation and cancer.

A CIRCRNA DERIVED FROM PUMILIO 1 GENE REGULATES TRANSCRIPTS RELATED TO FEMALE REPRODUCTION BY A POSSIBLE FEEDBACK MECHANISM INVOLVING PUM1 PROTEIN

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CircRNAs regulate gene expression at multiple levels. They are involved in physiological and pathological processes; however, little is known about their role in female reproduction.

We assessed the expression of several circRNAs in human cumulus cells (hCCs) and focused on circPUM1 derived from the PUM1 host gene. PUM1 is a member of the Pumilio RNA-binding protein (RBP) family, ubiquitously present and evolutionary conserved across eukaryotes that play an essential regulatory role in embryogenesis and germ cell development.

We assessed the high expression of circPUM1 and its linear counterpart in hCCs and in different tissues. Moreover, we demonstrated a significant positive correlation not only in hCCs (r=0.84, p=0.002) but also in follicular fluid, oocytes, liver, brain and heart tissues (r=0.94, p<0.0001), highlighting their ubiquitous regulation.

Among targets of miRNAs predicted to be sponged by circPUM1, we focused on PTEN, a critical regulator of follicle maturation. CircPUM1 and PTEN were positively correlated (r=0.92, p=0.004), pointing out the important role of circPUM1 in the pathways related to female reproduction.

CircRNAs are known to sponge also RBPs and by RIP assay, we demonstrated that circPUM1 physically binds PUM1 protein. The latter, in turn, may decrease the half-life of PUM1 mRNA by binding its 3'-UTR as suggested by the identification of several binding sites within this region. Accordingly, we suggest that circPUM1 can oppose PUM1 mRNA downregulation by sponging PUM1 protein. In turn, increased level of PUM1 mRNA may promote the production of circPUM1 fostering a positive loop that contributes to increased level of PUM1 mRNA. The comprehension of this regulatory mechanism paves the way to a wider investigation of the interaction among circRNAs and their host genes in the pathways related to female human reproduction.

TOWARDS CUSTOMIZED ALLELE-SPECIFIC CRISPR/CAS GENE EDITING FOR THE TREATMENT OF OCULAR SURFACE DISORDER IN EEC SYNDROME

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Ectodermal Dysplasia-Cleft Lip (EEC) syndrome is a rare, dominantly inherited genetic disorder caused by specific TP63 missense mutations in the DNA Binding Domain (DBD). Patients with EEC often exhibit skeletal abnormalities such as polysyndactyly and cleft lip palate, and some mutations can result in the depletion of limbal stem cells, leading to progressive blindness. While surgical interventions can address skeletal deformities, there remains a critical unmet need for effective treatments targeting corneal blindness, a condition that severely diminishes the quality of life for those affected. We propose an innovative allele-specific gene editing approach to correct the R280C (C>T) TP63 mutation, which is associated to both EEC and related ocular defects. By leveraging the proximity of an-NGG PAM sequence near the C>T mutation, we have designed a guide RNA (gRNA) for SpCas9 to precisely target the mutant allele. The ribonucleoprotein (RNP) complex was then electroporated into primary keratinocytes derived from EEC patients, as well as into healthy human keratinocytes for comparison. Since the only distinguishing feature between the two alleles is the R280C mutation, we considered the genomic context of healthy keratinocytes to represent a stringent test for potential off-target effects of SpCas9. Our analysis revealed no relevant off-target editing in healthy human keratinocytes, achieving high efficiency disruption of the mutant allele in EEC-derived keratinocytes. This promising result underscores the ability of our SpCas9+gRNA complex to selectively and efficiently edit the mutated allele. Furthermore, considering the critical role of p63 in regulating stem cell proliferation and differentiation in keratinocytes, we functionally validated that the genome editing specifically targeted the mutated allele in EEC primary epidermal stem cells, leading to the restoration of its functional activity. Finally, to ensure the safety and specificity of our approach, potential off-target sites were examined using different approaches, which confirmed the absence safety of this approach. Collectively, these findings demonstrate the feasibility of using CRISPR/Cas9 technology to specifically disrupt the R280C TP63 mutation, thereby restoring p63 activity in epithelial stem cells, hence offering a potential therapeutic route for addressing the limbal stem cell deficiency in EEC syndrome. Our current and future research aims to broaden the range of targetable TP63 mutations, improve editing efficiency using group-tailored, mutation-specific CRISPR nucleases, and accelerate the development of an in vivo gene therapy for EEC syndrome.

TARGETED INHIBITION OF SMAD3 REDUCES SENESCENCE IN AGED OVARIES

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The ovary is among the first organs to show signs of aging, significantly impacting both fertility and overall health. Cellular senescence a state of permanent growth arrest triggered by DNA damage is a key contributor to aging and related diseases. Interestingly, a genetic variant commonly found in centenarians is associated with lower SMAD3 expression and reduced inflammation, implying a potential role for SMAD3 in longevity. We proposed that phosphorylated SMAD3 (pSMAD3) levels rise in senescent cells and aged tissues, and that inhibiting this pathway could exert senolytic effects. To test this hypothesis, we measured pSMAD3 levels in etoposide-induced senescent IMR90 cells and evaluated the effects of SMAD3 inhibition using both genetic approaches and the pharmacological inhibitor SIS3. We also conducted bulk RNA sequencing on ovarian tissue and examined the expression of pSMAD3, SMAD3, SMAD4, and SMAD7 in ovaries and other organs from aged mice. Chronic treatment with SIS3 in old mice mirrored these findings, while Smad3 haploinsufficiency in a progeroid mouse model led to a reduction in senescence markers and the senescence-associated secretory phenotype (SASP) across multiple tissues. Altogether, our results show that SMAD3 signaling increases with age, and its inhibition alleviates senescence and fibrosis. These findings support the potential of targeting SMAD3 as a therapeutic approach for ovarian aging and systemic age-related pathologies.

AN INDUCIBLE IPSC-CAS9 PLATFORM FOR ALLELE-SPECIFIC NORMALIZATION OF HSA21 GENE DOSAGE IN TRISOMIC CELLS

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Down syndrome represents the most prevalent cause of intellectual disability, with a presumed etiology involving defects in neuronal development. The trisomy of chromosome 21 (T21) results in the overexpression of Hsa21 genes, which leads to the transcriptional dysregulation of genes on other chromosomes, linking their 1.5-fold overexpression to discrete cellular phenotypes. We previously found mitochondrial dysfunction and neuritic and synaptic defects in T21 iPSC-derived neurons and identified candidate Hsa21 genes likely to cause significant dysregulation during neurogenesis.

We developed an experimental model of T21-iPSCs in which a single allele of one or more Hsa21 genes can be selectively silenced using a doxycycline-inducible Cas9. To ensure the specific deletion of the extra copy of Hsa21 selected genes, we designed sgRNAs exploiting SNPs that either created or eliminated a PAM only in one or two alleles, respectively. In addition, a second sgRNA, targeting all three alleles, was designed in a non-functional region. The optimal results for potentially efficient sgRNA activity were obtained for two Hsa21 genes, *NRIP1*and *BRWD1*, and, in a parallel approach, for a larger genomic region, thereby resulting in the simultaneous attenuation of five out of the Hsa21 genes of interest (*BRWD1, DYRK1A, RCAN1, RUNX1* and *SYNJ1*).

T21-iPSC-Cas9 cells were transfected with gRNAs to target the *NRIP1* gene. The efficiency of the editing process was evaluated by combining PCR and Sanger sequencing in bulk iPS cells. The edited iPSC cells were purified by single-cell cloning and several clones are currently undergoing molecular screening and characterization.

Temporal control of Cas9 in this inducible platform will enable a systematic, gene-by-gene dissection of Hsa21 dosage during neuronal differentiation, an essential step to identify key regulators and pinpoint pathogenic drivers.

TARGETED SIRNA DELIVERY VIA FUNCTIONALIZED NANOPARTICLES FOR THE TREATMENT OF FGFR-RELATED SYNDROMIC CRANIOSYNOSTOSIS

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Syndromic craniosynostoses (SCS) are rare, genetically driven craniofacial disorders marked by premature fusion of skull sutures, often with additional anomalies. Most forms (e.g. Crouzon, Apert, Pfeiffer syndromes) arise from heterozygous gain-of-function mutations in *FGFR1*, *FGFR2*, or *FGFR3*. Current treatment requires multiple high-risk surgeries, with reoperation rates up to 50%, especially in monogenic cases.

This study explores a non-invasive therapeutic strategy aimed at re-educating patient-derived calvarial mesenchymal stromal cells (CMSCs) through *FGFR2* silencing, using functionalized poly(d,l-lactide-co-glycolide)–polyethylene glycol–bis-sulfone nanoparticles (PPB NPs) loaded with therapeutic siRNA.

CMSCs were isolated from cranial bone fragments of Crouzon syndrome patients undergoing surgery (Ethical protocols #4876 and #6830, UCSC). A selected siRNA pool achieved 90% *FGFR2* transcript knockdown and 30% protein reduction, with decreased phospho-ERK1/2 and RUNX2 activation.

PPB NPs, produced via double emulsion solvent evaporation, showed spherical morphology (~174 nm). Cellular uptake and intracellular trafficking were analyzed by live imaging and confocal microscopy, revealing 71% cytoplasmic and 28% lysosomal localization. Viability and proliferation assays confirmed excellent NP biocompatibility. siRNA encapsulation efficiency within PPB NPs obtained was 38.5%. siRNA-loaded NPs maintained *FGFR2* silencing in CMSCs for up to 7 days. Findings were validated in vitro using CS murine osteoblast cultures.

To enhance cell-specific targeting, we are engineering NP surfaces with FGF2-derived peptides that bind FGFR2 without activating downstream signaling. This RNA delivery platform offers a promising therapeutic avenue for FGFR-driven craniofacial disorders.

Poster

P- 1

TERT-BUTYL HYDROPEROXIDE IN HUMAN ADULT MESENCHYMAL STEM CELLS ISOLATED FROM DERMIS: A CELLULAR SENESCENCE MODEL

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Stem cell (SC)-based therapy exploits the ability of cells to migrate to damaged tissues and repair them. In this context, there is a strong interest in the use of mesenchymal stem cells (MSCs), multipotent SCs able to differentiate into various cell lineages. They are easy to obtain with minimal risk to donor health, have no tumorigenic properties and exibit immunomodulatory activity. However, MSCs undergo cellular senescence during their in vitro expansion, as well as they may become senescent in vivo being influenced by multiple molecular, cellular and environmental interactions. Senescence causes irreversible block of cell proliferation, but can also affect survival and differentiation potential. Therefore, it is crucial to study the mechanisms of senescence in MSCs to counteract this process and ensure greater success of cell therapy. Among MSCs, human dermal MSCs (hDMSCs) are recently discovered and are a promising tool for tissue repair. The aim of this study was to establish a novel in vitro cellular senescence model using for the first time tert-butyl hydroperoxide (t-BHP) as a senescence inducer in hDMSCs. Therefore, the effects of different concentrations of t-BHP were investigated and compared with those of H_2O_2, another well-known pro-senescent molecule. t-BHP caused oxidative stress as evidenced by the increase in ROS production. Although, the enhanced expression of antioxidants preserved cell from death, they still experienced cellular damage. This damage, in turn, promoted the expression of p21, leading to a block in cell proliferation as well as specific morphological and cytoskeletal changes, which are characteristic of a senescent phenotype. Therefore, t-BHP showed a pro-senescent role in hDMSCs and its effects were greater than H_2O_2.

P- 2

AIRWAY EPITHELIAL STEM CELL RENEWAL AND DIFFERENTIATION: OVERCOMING CHALLENGING STEPS TOWARDS CLINICAL-GRADE TISSUE ENGINEERING

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Despite their life-saving potential, tissue engineering approaches for the treatment of large tracheal and bronchial defects still face significant limitations. A major challenge is the inability to regenerate a functional airway epithelium containing the appropriate amount of stem cells required for long-term tissue renewal following transplantation of the bioengineered graft. In this scenario, extensive cell culture characterization, validation assays and quality controls are needed to guide each step of the regeneration process. Stem cell depletion is often due to suboptimal culture conditions; therefore, we tested the ability of a clinical-grade culture system to support the safe and efficient *in vitro* expansion and differentiation of primary human tracheobronchial epithelial cells. Single-cell clonal analysis was employed to dissect airway basal cell heterogeneity and elucidate tissue-specific regenerative mechanisms. Functional assays assessed wound healing capacity and epithelial integrity under the selected culture conditions. Primary tracheobronchial epithelial cells demonstrated robust proliferative capacity, enabling the formation of a mature, functional epithelium without signs of immortalization. Analysis at the single cell level identified a basal cell subpopulation with *in vitro* self-renewal potential, distinguishing it from transient amplifying cells. This approach has further defined the hierarchy of cellular differentiation and its correlation with regenerative and differentiation potential. Overall, our study show that airway cells can be safely and effectively used in autologous tissue engineering approaches, and outlines validation

assays and possible quality controls to enhance therapeutic success and maximise patient safety in future clinical applications.

P- 3

HUMAN HEALTHY HEPATOCYTE SPHEROIDS AS A MODEL TO INVESTIGATE THE ROLE OF SMALL EXTRACELLULAR VESICLES IN HEPATIC PRE-METASTATIC NICHE ESTABLISHMENT

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Liver metastases represent the leading cause of colorectal cancer (CRC)-related deaths. The establishment of metastasis is preceded by the formation in distant organs of the pre-metastatic niche (PMN), a fertile “soil” supporting the next tumor cells growth. In the liver, the role of the extracellular vesicles (EVs) released by primary cancer cells in promoting the formation of the PMN has been studied by evaluating their ability to affect the activity of the non-parenchymal cells while few data are available about the involvement of hepatocytes (Heps). Recently, we showed that CRC_EVs induce epithelial to mesenchymal transition (EMT) of Heps, early event leading to liver fibrosis, pivotal feature of liver PMN formation. Fibrosis is a complex process that cannot be investigated using an *in vitro* bidimensional (2D) system. Therefore, to investigate the pro-fibrotic activity of the CRC_EVs-educated Heps, we developed a homotypic three-dimensional model of hepatocytes (3D_Heps) that better can reflect the liver structure. Our data show for the first time the ability of CRC_EVs to impact the morphological, physical, and functional properties of 3D_Heps. Beside confirming the ability of CRC_EVs to trigger EMT of Heps, shown by the reduced expression of epithelial markers (E-cadherin and CK8/18) and by the increased expression of mesenchymal markers (Vimentin and N-cadherin), the 3D model allowed us to demonstrate the pro-fibrotic properties of the CRC_EVs-educated Heps. Indeed, in CRC_EVs treated 3D_Heps we observed a significant increased deposition of the ECM proteins, as fibronectin and collagen IV. The obtained data for the firs time support the active role of the CRC_EVs educated-Heps in shaping the hepatic PMN, drawing attention to Heps as new potential cell targets for designing and developing novel therapies for liver metastasis prevention or early treatment.

P- 4

MEOX2 INTERACTS WITH PARP1 IN GLIOBLASTOMA STEM CELLS, AND ITS KNOCK-DOWN SENSITIZES THEM TO DNA-DAMAGING TREATMENTS

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Homeodomain transcription factors are increasingly recognized for their roles in tumor biology, including glioblastoma (GBM), an aggressive and currently incurable primary brain tumor with poor survival outcomes. Mesenchyme Homeobox 2 (MEOX2), encoded on chromosome 7p21.2, is overexpressed across glioma subtypes and correlates with decreased patient survival, especially in radiotherapy-resistant cases. Our work and that of others have shown that MEOX2 is particularly enriched in glioblastoma stem cells (GSCs), which are responsible for tumor initiation, resistance to therapy, and recurrence. In GSCs, MEOX2 supports both self-renewal and viability.

To investigate the mechanistic basis of MEOX2 function in GSCs, we performed mass spectrometry on MEOX2 immunoprecipitates and identified PARP1, a key DNA repair factor, as a potential interactor. This prompted us to examine the role of MEOX2 in DNA damage response. We compared GSCs with MEOX2 knockdown (KD) to controls following treatment with temozolomide (TMZ) or ionizing radiation. DNA damage was assessed by western blot, immunofluorescence, and Comet assays. The results we obtained showed that MEOX2 KD increased sensitivity to both TMZ and radiation and delayed recovery from TMZ-induced damage.

To further evaluate the translational relevance of our findings, we analyzed the contribution of Meox2 to GSC tumor growth in human embryonic stem cell-derived cerebral organoids, where we showed that MEOX2 KD cells failed to sustain growth over time, and notably that, at 7 day post-treatment, TMZ induced a statistically significant reduction in tumor growth of MEOX2 KD cerebral organoid tumor compared to control cells.

Our findings uncover a novel role for MEOX2 in facilitating DNA repair and promoting therapeutic resistance in GSCs, and they suggest MEOX2 as a functional partner of PARP1 and a potential therapeutic target in GBM.

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DIFFERENTLY RECURRENT HOTSPOT MUTATIONS IN HRAS Q61 DELINEATE DIVERSE GENE NETWORK SIGNATURES AND BIOLOGICAL FEATURES IN PAPILLARY THYROID CANCER

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H-Ras signalling is a pivotal regulator of several cellular functions. Its impairing by different types of activating mutations, occurring in many cancers, is deeply investigated. For the most frequent mutation it has been shown their impact on Ras structure/other protein interaction/function but the difference of the less frequent activating mutations within H-RAS affecting the same codon are poorly characterized at biological level.

Here, we defined the biological differences between the rare and constitutively activating mutations of H-RAS in thyroid papillary carcinoma, *H-RAS*Q61R and *H-RAS*Q61K,by adapting an already available pipeline to a reduced and unbalanced number of data sets available in TCGA. Differentially regulated gene networks were associated to *H-RAS*Q61R and *H-RAS*Q61K and were “wet” validated in rat immortalized thyrocytes over-expressing both proteins. We detected a stronger, more rapid and more transient activation of the ERK and AKT pathways in *H-RAS*Q61R than in *H-RAS*Q61K. We also revealed that *H-RAS*Q61R was associated with a more severe cancerous phenotype than *H-RAS*Q61K in terms of cell proliferation, stemness and cell migration, the last tested by zebrafish embryos xenograft assay. Furthermore, we showed the diverse regulation of CREB phosphorylation and of its stability by PKA. Indeed, the PKA inhibitor reduced the aggressiveness of cancer phenotype of *H-RAS*Q61R, reducing their ability to form thyrospheres and blocking their migration.

The reported results reveal the need to characterize not only the driver mutations but also the associated biological utilities since predicting the cancer cell behaviour in regards to metastatic ability and suggesting mutation-dependent therapeutic options.

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BROKEN MICROTUBULES, FAILING SPERM: HOW TYPE 1 DIABETES DRIVES MALE SUBFERTILITY

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Metabolic disorders, including type 1 diabetes (T1D), adversely affect male reproductive health through chronic hyperglycemia and elevated oxidative stress (OS). This study provides novel insights into how T1D-induced OS impairs microtubule (MT) dynamics, the expression of microtubule-associated proteins (MAPs), and overall sperm quality in adult Wistar rats using a streptozotocin-induced T1D model. Evaluation of sperm parameters revealed a significant reduction in concentration, motility, and viability, along with increased morphological abnormalities, DNA fragmentation, apoptosis, and chromatin organization, hallmarks of defective spermatogenesis. Notably, elevated levels of 4-hydroxynonenal (4-HNE), a well-established marker of lipid peroxidation, were particularly evident in the sperm head and flagellum. T1D also induced mitochondrial dysfunction, as demonstrated by a marked reduction in ATP production. Moreover, altered expression and immunolocalization of key Ca^{2+} channel proteins, CatSper and VDAC3, led to impaired Ca^{2+}signaling and reduced motility. Additionally, dysregulation of key MAPs—PREP, DNAL1, and RSPH6A—was observed, along with decreased levels of acetylated tubulin (K-TUB) resulting from aberrant expression of ATAT1 and HDAC6, two enzymes essential for proper sperm motility. Collectively, these findings establish a mechanistic link between T1D, impaired cytoskeletal architecture, defective germ cell maturation, and male subfertility, highlighting MAPs and MT as potential therapeutic targets.

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OBESITY INDUCED BY A HIGH-FAT DIET TRIGGERS ER STRESS AND APOPTOTIC PATHWAYS IN MOUSE TESTES AND SPERMATOZOA

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Obesity is a widespread health issue with systemic repercussions, including significant effects on male reproductive function. C57BL/6 mouse model was used to investigate the metabolic, morphological and molecular alterations induced by a high-fat diet (HFD). After five weeks of HFD, a significant increase in blood glucose levels was detected, indicating metabolic impairment. Moreover, ecographic evaluation of testis showed a notable enlargement of the testicular area in obese mice compared to normoweight controls. Also, to examine cellular stress mechanisms, we performed immunofluorescence analysis for endoplasmic reticulum (ER) stress proteins (BIP, CHOP, and PDI) on testicular tissue, revealing increased ER stress particularly in Leydig cells. This localized stress response may disrupt the testicular microenvironment and hormonal signalling, potentially affecting spermatogenesis. In line with this, gene expression analysis in spermatozoa showed dysregulation of apoptosis-related genes (*BAX, BCL2, CASP3*) and ER stress molecules (BIP, CHOP, PEK). These findings demonstrate that HFD-induced obesity leads to both morphological and molecular alterations in testis and spermatozoa, potentially contributing to reduced male fertility through ER stress and apoptotic pathways.

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TET2-INACTIVATING MUTATIONS INCREASE INFLAMMATORY ACTIVITY IN MACROPHAGES BY ACTIVATION NOTCH 1 PATHWAY

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Somatic mosaicism involving hematopoietic precursors has been associated with an increased risk of mortality from specific cardiovascular diseases. The best-characterized forms of somatic mosaicism are due to mutations in genes involved in cell proliferation, including *TET2* (*TET methylcytosine dioxygenase 2*) and *DNMT3A* (*DNA methyltransferase 3 alpha*), which play a role in DNA methylation. These mutations promote clonal expansion of hematopoietic cells in the absence of overt hematologic malignancy, a condition known as Clonal Hematopoiesis of Indeterminate Potential (CHIP). Loss-of-function mutations in the *TET2* gene, the most frequently mutated gene in CHIP carriers, have been shown to increase atherosclerotic plaque size and expression of pro-inflammatory mediators. Thus, restoring *TET2* function in CHIP carriers may represent a therapeutic strategy to mitigate cardiovascular risk. In macrophages, activation of the Notch pathway regulates transcription of genes encoding pro-inflammatory cytokines. In this study, we investigated the effect of *TET2* inhibition on Notch-1 expression in murine macrophages RAW 264.7. We found that treatment with TFMB-(S)-2-HG, a *TET2* activity inhibitor, led to elevated levels of interleukin-6 (IL-6) and increased Notch-1 expression and activity. Further research is needed to determine whether IL-6 upregulation is a downstream consequence of Notch-1 activation. If confirmed, Notch-1 inhibition using gamma-secretase inhibitors—currently under clinical evaluation in oncology— could represent a novel strategy to reduce the inflammatory state of macrophages with TET2 loss-of-function mutations in individuals with CHIP.

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PHOSPHOGLYCERATE DEHYDROGENASE (PHGDH): A POTENTIAL THERAPEUTIC TARGET FOR ADRENOCORTICAL CARCINOMA TREATMENT

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Adrenocortical cancer (ACC) is a rare endocrine neoplasia for which surgical removal remains the main treatment option. Pharmacological interventions include the use of the adrenolytic drug mitotane, either as monotherapy or in combination with standard cytotoxic drugs. However, therapeutic response is very limited, and patients are at high risk of recurrence and metastasis. This rare carcinoma is characterized by several genetic alterations that contribute to significant intratumoral heterogeneity and drives profound metabolic changes responsible for disease progression, therapeutic failure and chemoresistance. 3-phosphoglycerate dehydrogenase (PHGDH), the primary rate-limiting enzyme in the serine biosynthesis pathway, is altered in several cancers, and its pharmacological inhibition or intervention with gene editing techniques have highlighted its direct involvement in molecular mechanisms that regulate important biological processes such as proliferation, metastasis, and resistance to therapy. Serine and its downstream metabolites (e.g., glycine, one-carbon units) are critical for nucleotide synthesis, redox homeostasis, and methylation reactions, all of which support rapid cancer cell proliferation and survival. Therefore, PHGDH represents a compelling therapeutic target for rare, aggressive, and therapeutically orphan cancers, including ACC. In this study we evaluated the effects of pharmacological inhibition of PHGDH, using NCT-503, on the proliferation, motility and redox state of two experimental models of ACC and its ability to sensitize tumor cells to ferroptosis inducers, a cell death process closely linked to redox imbalance and cell metabolism. The mechanisms activated by ACC cells in response to NCT-503 have relevance to the defining new therapeutic perspectives involving the use of PHGDH inhibitors in combinations with ferroptosis-inducers.

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IMPACT OF DOPAMINE D2 RECEPTOR IN THE NEURO-IMMUNE CROSSTALK IN EXPERIMENTAL MULTIPLE SCLEROSIS

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Alterations of the neuro-immune crosstalk, including inflammation-driven synaptopathy, play a central role in the pathophysiology of Multiple Sclerosis (MS), a chronic inflammatory and neurodegenerative disease of the central nervous system (CNS). Both infiltrating autoreactive lymphocytes and activated microglia are deeply

implicated in MS-associated synaptic pathology, by altering synaptic function through inflammatory mediators, as observed in MS and its animal model, experimental autoimmune encephalomyelitis (EAE). Among neuro- and immune-modulators involved in MS pathogenesis, catecholamines—particularly dopamine (DA)—are emerging as key players. Dopamine receptors (DRs) can exert both 'pro- and anti-inflammatory' effects on microglia and lymphocytes depending on the context; however, their role in immune-mediated synaptic alterations in MS remains poorly understood.

To explore the impact of D2R in neuro-immune interactions, we used two experimental models of MS: a T cell-based MS chimeric model and a microglia-specific D2R knockout EAE mouse line (mic $D2R^{-/-}$). In the chimeric model, T cells isolated from EAE mice were applied to healthy cortico-striatal slices; synaptic activity was assessed by patch-clamp recordings of spontaneous excitatory postsynaptic currents (sEPSCs). Pre-treatment of EAE T cells with the D2R agonist quinpirole reduced synaptic dysfunction, indicating a protective role of D2R activation in T cells. In vivo, mic $DR2^{-/-}$ and control mice showed similar clinical EAE scores, but mic $DR2^{-/-}$ mice displayed reduced anxiety-like behavior and normalized sEPSC kinetics, suggesting that microglial D2R deletion mitigates synaptic alterations.

Overall, our findings highlight distinct and cell-specific roles of D2R in modulating neuro-immune interactions, with D2R activation in T cells and inhibition in microglia both exerting beneficial effects on synaptic integrity in EAE

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IN SILICO PREDICTION OF A NEW POSITIVE FEEDBACK GENE EXPRESSION LOOP MEDIATED BY COMPETITIVE ENDOGENOUS RNA NETWORK IN RECURRENT GRADE 2 MENINGIOMAS

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Meningiomas (MNGs) are the most common intracranial brain tumors originating in the meninges. Recently, a biomarker based on the expression of 34 RNA transcripts (34HR-MNG) in MNG samples has been described to be able to predict their outcome, comprising recurrence, better than other previously used biomarkers. To deepen the molecular mechanisms potentially regulating the expression of some transcripts among the 34HR-MNG, here we built a competitive endogenous RNA (ceRNA) network, through an *in silico* approach. MiRNAs targeting 34HR-MNG transcripts and corresponding sponging circRNAs were retrieved through MiRTarbase and ENCORI databases, respectively. The RNA-seq dataset GSE189672 has been used to correlate the expression of candidate circRNA host genes with specific molecular and clinical features of 109 WHO grade 1 and 2 MNGs. The expression of candidate circRNAs and their linear counterparts has been validated through qRT-PCR. Among the 34HR-MNG transcripts, we focused on PIM1 that was significantly upregulated in (i) grade 2 recurrent *vs* not-recurrent, (ii) WHO grade 2 *vs* grade 1 and (iii) chromosome 1p loss-harboring MNGs and positively correlated with Ki-67 level. CircRNAs 0076215 and 0076216, both generated from PIM1 host gene, were predicted to sponge miRNAs 16-5p and 195-5p, two tumor suppressors in MNG showing in turn PIM1 among their targets. The expression of circRNAs 0076215 and 0076216 was validated in a set of 19 physiological human tissues and positively correlated with that of PIM1 mRNA. Our data suggest oncogenes PIM1 as critical player of MNG recurrence and the upstream ceRNA network made of circRNAs 0076215 and 0076216 and miRNAs 16-5p and 195-5p as responsible for its upregulation, through a positive feedback loop.

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LNCH19 AND ALTERNATIVE SPLICING: A NEW EVIL AXIS IN COLORECTAL CANCER ONCOGENESIS

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Alternative Splicing (AS) plays a critical role in several aspects of cancer biology, with its deregulation closely linked to cancer progression, metastasis, and drug resistance. In colorectal cancer (CRC), AS is essential for transcriptomic variations and cancer deregulation. Exon inclusion/exclusion during splicing events depends on the gene's regulatory sequences bound by splicing factors (SFs) such as hnRNPs, RBFOX1, and RBFOX2. RBFOX2, a master regulator of tissue-specific AS, is proven to be involved in tumor onset, epithelial-mesenchymal transition, and CRC metastasis. Long non-coding RNAs (lncRNAs) can influence AS by acting as trans-regulatory agents, recruiting SFs, or directing them to specific genes. LncH19, an ncRNA dysregulated in many tumors and classified as an oncogene, plays a significant role in CRC pathogenesis. We demonstrated that lncH19 interacts with immature RNAs and SFs like hnRNPM and RBFOX2 in two CRC cell lines (SW620 and HCT116). Combining data obtained from RNA sequencing of lncH19 antisense precipitation with the database of splicing factors (SFs) binding sites, we identified 57 lncH19-related transcripts with hnRNPM and RBFOX2 binding sites. Notably, among these we found the mRNA of RAC1, a GTPase whose alternatively spliced isoform RAC1B is implicated in malignant transformation. We demonstrated that lncH19 acts as a shuttle, driving the two SFs to RAC1 mRNA, facilitating its AS and RAC1B expression, and the consequent induction of RAC1B downstream genes: cMyc and cyclin-D. Moreover, data from CRC patient biopsies confirmed the correlation between lncH19 and RAC1 expression, supporting our in vitro model. These findings provide the first evidence of new mechanisms by which lncH19 may promote CRC through AS modulation.

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SPHINGOSINE 1-PHOSPHATE-DRIVEN INTERPLAY BETWEEN NEUTROPHILS AND ENDOMETRIOTIC CELLS FUELS FIBROSIS IN ENDOMETRIOSIS

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Endometriosis is a chronic inflammatory/fibrotic disease affecting ~190 million reproductive age women worldwide, characterized by ectopic endometrial like tissue. Although its pathogenesis remains unclear, recent evidence highlights that increased neutrophil infiltration and extracellular trap (NET) formation are linked to lesion progression. However, the molecular mechanisms orchestrating neutrophil activation and their involvement in the endometriotic lesion progression remain uncertain. In the lesion microenvironment, we recently showed that the signaling of the bioactive lipid sphingosine 1-phosphate (S1P) is implicated in fibrosis development.

Here, we investigated the role of S1P in the interplay between neutrophil and endometriotic cells.

We demonstrated that S1P treatment enhanced NET release in differentiated HL60 cells (dHL60). NET, isolated and characterized, were used to treat human epithelial endometriotic cells. NET treatment upregulated different markers of epithelial mesenchymal transition (ZEB1, Snail), demonstrating NET involvement in the establishment of fibrosis. In addition, to characterize the role of S1P in endometriotic cell-neutrophil interplay, sphingosine kinase isoforms (SK1, SK2), which catalyze S1P synthesis, were silenced in endometriotic cells. Interestingly, the conditioned media obtained by SK-silenced cells significantly reduced NET release, with SK2 depletion exerting stronger effects. Overall, these findings reveal an interplay between neutrophils and endometriotic cells that promotes fibrosis in an S1P rich microenvironment.

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NEURONAL CROSSTALK MODULATES FERROPTOSIS SENSITIVITY AND IMMUNE REMODELING IN ORAL CANCER: MOLECULAR, FUNCTIONAL, AND TRANSLATIONAL INSIGHTS

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Ferroptosis is a promising therapeutic vulnerability in oral carcinoma and may impact disease progression by remodeling the tumor microenvironment (TME). We analyzed RNAseq data from 360 OSCC samples (TCGA) and identified two subtypes based on 9 ferroptosis-related genes (FRGs). A subtype with high expression of *TFRC*, *PARP3*, *GCLC*, *USP35*, *SRC*, *CD44* and low expression of *GOT1*, *HSF1*, *AGPAT3* showed a better overall survival (OS) (low-risk), while the inverse FRG signature was associated with a worse OS (high-risk). This signature was validated in 50 primary OSCCs and *in vitro*, where we found that SCC154 OSCC cell line, showing the FRGs-signature, had low proliferation, reduced migration, and high sensitivity to RSL3 (low-risk) while OT1109, with no FRGs-signature, was more aggressive and RSL3-resistant (high-risk). Differential gene expression analysis and GSEA in the 50 primary tumors revealed enrichment in cancer–neuro and cancer–immune crosstalk in the high-risk OSCC subgroup. H&E and S100 IHC staining showed increased neuronal and tumor-associated macrophage (TAM) infiltration in high-risk tumors. Co-culture of OSCC with iPSC-derived neural stem cells showed that conditioned media (CM) from high-risk cells promoted neuronal differentiation, as evidenced by the up-regulation of MAP2 and GFAP markers, while CM from neurons enhanced high-risk OSCC migration and invasiveness in 3D ECM scaffolds (live imaging). Furthermore, neurons reduced RSL3 cytotoxicity. Secretome analysis by mass spectrometry revealed neuronal secretion of antioxidant/stress-buffering proteins (SOD, PRDX2, PDIA3, CLU) and DAMPs (HMGB1, CALR), which may protect tumor cells from oxidative stress and recruit TAMs, supporting a functional neuron–immune–tumor axis. Targeting the neuroprotective TME or disrupting neuron–tumor redox crosstalk may improve ferroptosis-based therapies in high-risk OSCC.

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SHIELDING THE TESTIS: D-ASPARTATE SUPPORTS REPRODUCTIVE RESILIENCE IN A MODEL OF MICROPLASTICS EXPOSURE

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Microplastics (MPs) are widespread environmental contaminants resulting from plastic degradation and are now commonly detected in food, water, and air. As exposure to MPs has become virtually unavoidable, understanding their impact on biological systems is essential. Given the high sensitivity of the testis—where MPs have been associated with impaired spermatogenesis, hormonal imbalance, and tissue disorganization—a deeper understanding of the underlying cellular and molecular mechanisms is crucial to clarify how MPs affect testicular physiology and to identify potential targets for the development of effective protective strategies. This study aimed to evaluate the role of D-Aspartate (D-Asp), an endogenous amino acid with antioxidant and regulatory functions in the testis, in rats exposed to polystyrene MPs (PS-MPs). Adult rats received PS-MPs (0.1 mg/day) and were treated with D-Asp (0.1 mM) administered before, during, or after MPs exposure for 45 consecutive days. PS-MPs caused structural damage to seminiferous tubules, reduced epithelial thickness and sperm quality, and downregulated markers of steroidogenesis (StAR, 3β-HSD, 17β-HSD) and proliferation (PCNA, SYCP3). Oxidative imbalance was confirmed by altered antioxidant enzymes (SOD1, SOD2, CAT) and elevated markers of lipid peroxidation (4-HNE, TBARS), autophagy (LC3B, p62), and apoptosis (CYT C, TUNEL-positive cells). D-Asp counteracted these effects, preserving tissue structure, redox balance, hormone production, and spermatogenic progression. Benefits were observed in all treatment schedules, including under physiological conditions. These results provide one of the first in vivo demonstrations of D-Asp's ability to support testicular homeostasis under environmental stress, highlighting its potential as a natural compound for safeguarding male reproductive function in a contaminated world.

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SHEDDING LIGHT ON THE ROLE OF AGR2 AND ZSCAN4 IN THE PATHOPHYSIOLOGY OF IDIOPATHIC PULMONARY FIBROSIS IN LUNG TISSUE MODELS FROM PATIENTS

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Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive interstitial lung disease characterized by unexplained irreversible pulmonary fibrosis. At the molecular level, the IPF process is described first by recruiting fibroblasts to the damaged site, followed by an increase in their proliferation and extracellular matrix deposition. Consequently, the architecture of the lung is destroyed, resulting in respiratory insufficiency. Although the aetiology of IPF is unclear, studies have shown that miRNAs play a key role in IPF, which influences cell proliferation, apoptosis, motility, and invasion. Two of the most critical miRNAs implicated in IPF are miR-143-3p and miR-34a-3p. Specifically, they are both down-regulated in IPF fibroblast foci. For the first time, our *in silico* analysis reveals two new candidate biomarkers for IPF: Anterior gradient 2 (AGR2) and Zinc finger and SCAN domain containing 4 (ZSCAN4), which are targets of miRNA-143-3p and miR-34a-3p, respectively. In particular, to evaluate the role of AGR2 and ZSCAN4 in IPF, we used public repository data (GSE122960 and GSE128033) of scRNAseq performed on lung tissues obtained from healthy donors and IPF patients, and we observed an up-regulation of both in alveolar epithelial cells correlated to the cluster of AT I and AT

II. At the same time, our bioinformatics studies highlighted that AGR2 and ZSCAN4 are targeted by miRNA-143-3p and miR-34a-3p, respectively. Finally, we validated our findings by qRT-PCR and Western blotting using two biological models: human lung cells (A549) and lung tissue from IPF patients. In conclusion, our studies shed light on the cross-talk between miRNA-143-3p and AGR2 and miR-34a-3p and ZSCAN4 to understand their involvement in the pathophysiology of IPF and identify them as new therapeutic candidates.

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ORGANOIDS AS PRECLINICAL MODEL IN INFLAMMATORY BOWEL DISEASE TREATMENT

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Inflammatory bowel disease (IBDs) is a group of chronic inflammatory disorders of the gastrointestinal (GI) tract, primarily comprising two major forms: Crohn's disease (CD) and ulcerative colitis (UC). These conditions are characterized by abnormal immune responses leading to inflammation in various segments of the intestinal tract, which can result in significant morbidity requiring long-term pharmacological treatment, frequently associated with significant side effects. In the last few years, Patient Derived Organoids (PDOs) have gained recognition as a robust *in vitro* model, accurately mimicking the histological architecture, pathological state and molecular characteristics of the original tissues and therefore of the patient himself. Our study aims to investigate the potential of IBD-derived organoids as a platform that retains molecular profiles and inflammatory signatures observed in corresponding patient tissues, making them a powerful resource for precision medicine approaches. Building upon this model, we explore an alternative therapeutic strategy using a natural-based compound with potential anti-inflammatory properties. Given the high variability in drug response and adverse effects associated with current IBD therapies, our findings suggest that PDOs can serve as a valuable tool for preclinical screening and development of personalized treatments, offering a safer and more effective alternative for managing IBD.

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THE BET INHIBITOR JQ1 DOWNREGULATES IL-20 RECEPTOR A EXPRESSION AND JAK/STAT SIGNALLING PATHWAY IN TRIPLE NEGATIVE BREAST CANCER CELLS

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The dynamic crosstalk between the tumour microenvironment (TME) and triple-negative breast cancer (TNBC) cells plays a critical role in tumor progression and treatment response representing an attractive target for developing new drugs with anticancer activity. Recent studies highlighted the involvement of IL-20 receptor subunit alpha (IL-20RA) signalling in several cancers, including BC, where its overexpression affected several oncogenic and immune pathways contributing to invasion, metastasis and TME activity. Epigenetic dysregulation as imbalances in regulators such as Bromodomain and extraterminal domain family (BET) proteins shape the TME, and their suppression alters multiple key oncogenic pathways and reduces the expression of pro-inflammatory cytokines in TNBC. The aim of this study was to evaluate the interplay between JQ1, a selective small molecule BET inhibitor (BETi), and IL-20RA in *in vitro* and *in vivo* models of TNBC. Viability of MDA-MB 231 and MDA-MB 468 cells was significantly reduced by JQ1 in a dose- and time-dependent way, with an EC50 of about 0.5 μM observed after 48 h and 72 h, respectively. The compound determined a gene and protein downregulation of IL-20RA affecting oncogenic JAK/STAT signalling pathway and PDL-1 expression as well as the expression of genes involved in the epithelial mesenchymal transition (EMT). Intra-peritoneal administration of JQ1 in nude mice hosting a xenograft TNBC after flank injection of MDA-MB-231 cells determined a great reduction in the growth of the neoplasm correlating with a reduced IL-20RA and PDL-1 expression as well as JAK and STAT3 decreased phosphorylation. These results showed, for the first time, a correlation between BETi's activity and IL20RA suggesting JQ1 as therapeutic candidate in the management of TNBC characterized by high levels of IL-20RA expression.

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DEVELOPMENT AND CHARACTERIZATION OF AN HHT-IPSCS MODEL FOR DRUG TESTING

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The latest gene editing technologies and the use of iPSCs is leading to the development of novel cell-based disease models that allow to reproduce and characterize the phenotypic alterations implied in the pathogenesis of specific disorders. Hereditary Hemorrhagic Telangiectasia (HHT) is a rare genetic vascular disease caused by mutations in *ENG*, *ACVRL1, SMAD4*, or *GDF2*, leading to an impairment of the TGFβ/BMPs pathway. Here we present an iPSCs-based cell line carrying a homozygous truncating variant in *ENG*, obtained by CRISPR-Cas9 technology.

CRISPR-Cas9 was performed on a well-estabioshed iPSCs line. The quality of the gene editing was attested by restriction enzyme digestion, Sanger sequencing, STR, karyotyping, pluripotency and multi-lineage assays. The lines were mycoplasma free. Angiogenesis studies were performed on both parental and mutated lines, which were further differentiated in endothelial cells and blood vessels organoids (BVOs). Proliferation assays and migration under laminar flow were conducted.

The quality assessment of LUMCi029-A *ENG*$^{mut/mut}$ line was successful. The *in vitro* HHT model showed defective angiogenic features, including flow-induced cell migration and response to VEGFR2 signalling, comparable to the already published data on Eng-KO mice retina.

The development of a reliable and highly characterized *in vitro* HHT model represents an important tool for deepening the cellular and molecular processes modulating angiogenesis, leading to the pathological phenotype. Furthermore, this may provide a valuable support for drug testing.

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INVESTIGATING THE INFLUENCE OF ENDOTHELIAL CELLS FROM GESTATIONAL DIABETIC UMBILICAL CORDS ON EXTRACELLULAR MATRIX REMODELLING BY DERMAL FIBROBLASTS

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Endothelial cells (ECs) and fibroblasts resident in the dermis show an intense bidirectional molecular crosstalk both in physiological and pathological conditions. In delayed wound healing situations associated with diabetes, fibroblast migration and extracellular matrix (ECM) remodelling is impaired, displaying reduced collagen (Col) levels and increased production of metalloproteases (MMP). However, the influence of ECs on these imbalances and the mechanisms that underlie the interaction between dermal fibroblasts and ECs in diabetes are not completely understood.

Thus, the aim of this study is to investigate the influence of human umbilical vein endothelial cells (HUVEC) from gestational diabetes (GD) affected women, where cells reproduce diabetic endothelium features through epigenetic modifications, on ECM remodelling by human dermal fibroblast (HDF).

Using an indirect co-culture system of HDF and GD-HUVEC, levels of Col1, MMP-2 and its inhibitor (TIMP-2) were assessed by RT-PCR, flow cytometry, immunofluorescence and zymography. Moreover, mitogen-activated protein kinase (MAPK) and mothers against decapentaplegic homolog 2-3 dimer (SMAD2-3), key mediators of Col1 production, were evaluated by flow cytometry.

HDF co-incubated 3 days with GD-HUVEC showed impaired ECM remodeling, revealed by reduced Col1 and TIMP-2 expression as well as increased MMP-2 production and activity compared to HDF co-incubated with control cells (C-HUVEC). In addition, HDF co-incubation with GD-HUVEC downregulated MAPK activation compared to HDF co-incubated with C-HUVEC, while no differences were detected for SMAD2-3 protein expression.

These results suggest that GD-HUVEC negatively affect ECM remodeling by HDF through altered MAPK-signaling without affecting the TGF-b/SMAD2-3 pathway, unraveling new insight connected to delayed wound healing mechanisms in diabetes.

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NKTR CONTROLS CRANIAL NEURAL CREST-DERIVED CARTILAGE DEVELOPMENT BY REGULATING SPLICING MACHINERY

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By analysing the exome of patients with a syndrome mainly characterised by global developmental delay and craniofacial malformations, we identified mutations in the Natural Killer cell Triggering Receptor (NKTR) gene.

Very little is known about the role of NKTR during cellular and developmental processes. Yet the presence of a domain involved in the splicing of messenger RNA and the existence of overlapping syndromes due to defective splicing in neural crest cells (NCCs) suggest that NKTR might be a novel gene implicated in this class of genetic disorders so called spliceosomapathies. NCCs are unique group of cells that originate at the neural tube border and participate to the development of several organs including face structures. Our data reveal that: NKTR associates with the spliceosome (i.e. the nuclear region where RNA splicing processes occur), when overexpressed in cells; NKTR expression is regulated by the transcription factor SOX9 during craniofacial development in mouse and zebrafish embryos; NKTR inactivation in zebrafish induces defects in face cartilage formation. Overall our results revealed NKTR as a novel gene implicated in splicing processes of craniofacial development and as new gene involved in the pathogenesis of a novel spliceosomopathy.

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IKBA-MEDIATED MITOCHONDRIAL INTERACTION WITH TUFM DRIVES ENDOTHELIAL ACTIVATION AND LUNG CANCER METASTASIS

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Mitochondria play a pivotal role in cancer development and metastasis, with the NF-κB signaling axis regulating both processes.

Traditionally, the NF-κB pathway is understood to operate within the cytoplasmic and nuclear compartments, where it is tightly regulated by its inhibitor, IκBα.

However, our research provides novel evidence that IκBα also partially localizes within the mitochondria. To specifically investigate the function of mitochondrial IκBα, we engineered a construct that targets IκBα to the mitochondria of lung cancer cells. Expression of mitochondrial IκBα led to increased cell proliferation, enhanced migratory capacity, and reduced apoptosis in response to chemotherapy. with a reduced apoptotic response following chemotherapy exposure.

Mechanistically, mitochondrial IκBα interacts with TUFM (Tu translation elongation factor, mitochondrial), impairing its role in mitochondrial protein synthesis. These mitochondrial alterations trigger a metabolic reprogramming in lung cancer cells, which in turn activates endothelial cells (ECs) and promotes cancer-associated thrombosis (CAT). CAT is marked by the aberrant activation of the coagulation cascade, often through the release of procoagulant factors such as von Willebrand factor (vWF), as well as increased platelet activation and aggregation. These thrombo-inflammatory changes not only facilitate vascular complications but also play a crucial role in promoting metastatic dissemination by aiding tumor cell extravasation and colonization of distant sites.

In summary, our findings uncover a previously unappreciated function of IκBα within mitochondria, positioning it as a novel regulator of mitochondrial translation through TUFM inhibition. This mitochondrial dysfunction initiates a cascade of events linking altered cancer cell metabolism with endothelial activation and thrombotic complications.

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MECHANISTIC INSIGHTS ON THE IMPACT OF THE ATG7 P.V471A VARIANT IN THE PROGRESSION OF STEATOTIC LIVER DISEASE.

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Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) is the most common chronic liver disorder and a major cause of liver-related morbidity and mortality worldwide. Autophagy dysfunction, mainly due to chronic hepatic lipid accumulation, has emerged as a key pathogenic mechanism in MASLD. Our previous study observed strong association between the ATG7 p.V471A variant and hepatocellular ballooning. This study aimed to investigate the pathogenic role of the ATG7 p.V471A variant by evaluating its impact on autophagy, focusing on mitophagy, lipophagy, and fibrogenesis using HepG2 cell lines and 3D spheroids composed of HepG2 and hepatic stellate cells (HSCs) treated or not with fatty acids. By CRISPR/Cas9 system homozygous ($V471A^{A/A}$) and wild-type ($V471A^{V/V}$) lines were generated; HepG2 cells were naturally heterozygous ($V471A^{V/A}$). The study found that ATG7 variants exhibited an accumulation of lipid droplets (LDs) and an increase in their size, as revealed by Nile Red staining. Seahorse analysis showed no changes in mitochondrial respiration. Conversely, ATG7 variants affect mitochondrial abundance, as detected by Mitotracker probe and cyclophilin D staining, particularly under chloroquine treatment, and increase mtROS, as assessed by the MitoSOX probe. Total ROS levels, detected by DCF probe, were elevated in ATG7 variants, resulting in hepatocellular damage. The ATG7 variants are also associated with increased collagen deposition observed in 3D spheroids by immunofluorescence. Antioxidants were used as a strategy to mitigate LD accumulation and fibrogenic response. Overall, the ATG7 p.V471A variant amplifies oxidative stress and fibrogenic signaling, promoting a self-reinforcing cycle of mitochondrial dysfunction

and liver injury. These findings provide new insights into MASLD pathogenesis and suggest autophagy and oxidative stress as potential therapeutic targets.

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VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2 ACTIVATION IN EXPERIMENTAL RAT ULCERATIVE COLITIS

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Ulcerative colitis (UC), one of the two main forms of inflammatory bowel disease (IBD), is a chronic, non-specific inflammatory condition affecting the colon. The disease primarily involves mucosa and submucosa. Although its etiology and pathogenesis remain unclear, there is still a lack of specific and effective therapeutic options for UC.

The Vascular Endothelial Growth Factor-A (VEGF-A)/Vascular Endothelial Growth Factor-2 (VEGFR-2) axis, which plays a key role in angiogenesis associated with severe disorders, has been extensively studied in vivo in various diseases. However, its characterization in the context of UC remains largely unexplored.

To investigate the involvement of VEGF-A/VEGFR-2 pathway in UC, we have used an animal model of acute colitis induced via intrarectal administration of trinitrobenzene sulfonic acid solution (TNBS) in ethanol which recapitulates the macroscopic, histological, and immunological features of human colitis. Western blot analyses have revealed a significant upregulation of VEGF-A and VEGFR-2 expression in vascular endothelium of colon of TNBS-treated rats compared to controls. These molecular findings were supported by immunofluorescence showing marked colocalization of VEGFR-2 and RECA-1, a rat endothelial cell marker, as well as evidence of neovascularization in colitis tissue. Furthermore, immunoprecipitation assays showed increased levels of phosphorylated VEGFR-2 in colitis samples accompanied by upregulation of the p-Akt/Akt signaling axis, consistent with enhanced angiogenesis activity.

In conclusion, our study provides novel evidence of VEGFR-2 activation and downstream p-Akt/Akt signaling in experimental colitis model supporting their potential role in pathological angiogenesis in UC. These findings suggest that the VEGF-A/VEGFR-2 pathway may represent a promising therapeutic target for IBD.

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A NEW RNA-BASED THERAPY FOR THE FRAGILE X SYNDROME

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The Fragile X Syndrome (FXS) is the most common form of monogenic intellectual disability and autism, caused by the absence of the Fragile X Messenger Ribonucleoprotein (FMRP). Currently, no cure is available for FXS. Recently, mRNA therapeutics arise as a novel approach with a potential application in drug development. Preclinical studies have successfully explored mRNA therapy in different human conditions, including genetic disorders, and current clinical efforts are corroborating these findings. mRNA therapeutics could be used in a wide range of diseases. Here, we explored mRNA-based therapy as a new category of compounds for FXS. We demonstrated a successful delivery of *FMR1* mRNA in mouse and human brain cells, leading to the efficient and correct FMRP synthesis. Moreover, the delivery of *FMR1* mRNA rescued those cellular and molecular deficits that are considered hallmarks of FXS in both mouse primary neurons and in Fragile X patient-derived fibroblasts. These last are a valuable human cellular model that strengthens the translational potential of our findings. Together, these results shed light on a strong potential for the development of an innovative therapeutic intervention for FXS, based on the reintroduction of *FMR1*-mRNA in brain cells, with an immediate translational outcome.

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CUMULUS CELLS MOLECULAR BIOMARKERS CAN BE INDICATIVE OF HUMAN OOCYTE DEVELOPMENTAL COMPETENCE

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In ART procedures, oocyte selection occurs on parameters based on the morphological observation of ooplasm, polar body and cumulus cells. As such criteria are subjective and not indicative of intrinsic oocyte quality, we aim to define more objective markers predictive of oocyte competence and of positive clinical outcomes. To this end, it is possible to utilize granulosa and cumulus cells that actively contribute, via gap junctions and paracrine interactions, to acquisition of oocyte developmental competence but are usually discarded after cumulus-oocyte complexes (COCs) pick-up. Previous results demonstrated that cumulus cell apoptosis rate was lower in women who achieved a pregnancy in comparison to nonpregnant women. In this study, we selected patients (n=14; all participants signed informed consent) with similar clinical characteristics (age 30-40 y, BMI<25, hyporesponders) and undergoing the same stimulation protocol. We selected cumulus cells obtained from COCs retrieved from follicles with a diameter >18 mm, whose oocytes were then utilized for ICSI. We analyzed phosphoAKT and antioxidant enzyme (SOD 1/2; Catalase; GPx1) levels by ELISA assay as well as phosphoERK1/2 intracellular localization by confocal microscopy to correlate results with the clinical outcome of the related embryos. Experimental data evidenced that only cumulus cells surrounding oocytes capable of developing good-quality embryos and positive clinical outcomes evidenced phosphoERK1/2 translocation into cell nucleus and a significantly higher intracellular accumulation of phoshoAKT and of antioxidant enzymes. These preliminary results suggest that the activation of efficient prosurvival pathways in cumulus cells could be important to create/maintain a suitable microenvironment for the development of competent oocytes.

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CYTOTOXIC EFFECTS AND LYSOSOMAL ALTERATIONS OF FUCOIDAN NANOPARTICLES ON ANAPLASTIC THYROID CANCER CELLS

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Anaplastic thyroid cancer (ATC) is a rare and aggressive form of thyroid disease characterized by rapid growth and presence of metastasis at the time of diagnosis. ATC is resistant to conventional therapies and for this reason novel targeted therapies are necessary. Recently it has been reported that fucoidan (FU), a biopolymer derived from algae, prevents cancer development and enhances the efficacy of conventional treatments. The aim of this study was to investigate the role of FU on the growth and lysosomal vulnerability on *in vitro* models of anaplastic thyroid cancer cells (SW1736) and non-tumorigenic thyroid cells (NTHY-ORI 3.1) used as a control. Moreover, FU-based nanosystems (NanoFU) were developed in order to increase the cell localization of the biopolymer. The incubation of FU and NanoFU (0.001, 0.01 and 0.1 mg/ml) with cells demonstrated an increase of cell growth reduction promoted by the colloidal systems after 72 h incubation with respect to the free form of the biopolymer when lowest concentration of the polysaccharide was employed (0.001 mg/ml). Cancer cells are vulnerability to lysosomal stress and fucoidan seems to be involved in the alteration of the lysosomal protein expression. In this study, for the first time to the best of authors' knowledge, immunofluorescence analysis of LAMP1 showed an alteration in lysosomal morphology and number in SW1736 cells when the FU formulations (especially as nanoparticles) were tested at a biopolymer concentration of 0.01 mg/ml. Our preliminary data highlighted the significant cytotoxic effects exerted by NanoFU on ATC cells probably as a consequence of lysosomal alterations. The described phenomena may represent novel targeted-mechanism to be exploited for the treatment of thyroid tumors.

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MOLECULAR MECHANISMS UNDERLYING THE TOXIC EFFECTS OF ZEARALENONE IN MOUSE SPERMATOCYTE-DERIVED CELL LINE GC-2

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Spermatogenesis is regulated by an organized network of signals controlling the balance between cell proliferation and death. Alteration of these mechanisms, which can lead to infertility, depends on several factors such as exposure to environmental pollutants, including mycotoxins. Among them, zearalenone (ZEN), a non-steroidal estrogenic compound produced by several Fusarium species, compromises spermatogenesis through mechanisms not well defined. This study investigated ZEN effects on GC-2 cells, a mouse spermatocyte-derived cell line. Our results demonstrated that ZEN high concentrations inhibit cell growth and colony-forming ability. ZEN cytotoxicity is related to induction of oxidative stress and ferroptosis as evidenced by increase in the reactive oxygen species, lipid peroxidation and ferrous iron (Fe2+) levels in ZEN-treated cells. Moreover, ZEN up-regulated the mRNA expression of ferroptosis-related genes such as heme oxygenase-1 (HO-1). The observation of increase in mRNA levels of PTGS2 and IL6 genes, which encode for cyclooxygenase-2 and interleukin 6 respectively, also confirmed ZEN ability to trigger inflammation. Furthermore, we verified in ZEN-mediated toxicity the involvement of GPER (G protein-coupled estrogen receptor), a transmembrane estrogen receptor of which ZEN is reported to be an agonist. We found that ZEN induces phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) which is reversed by G15, a GPER antagonist. Interestingly, ZEN-mediated lipid peroxidation and HO-1, PTGS2 and IL6 mRNA expression increase were abrogated by G15 use, confirming GPER-mediated pathways in both ferroptosis and inflammatory state triggered by ZEN. Our results contribute to clarify the molecular mechanisms underlying ZEN-dependent testicular toxicity and confirm the pivotal role of GPER and estrogens in regulating spermatogenesis progression.

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INVESTIGATION OF INTELLECTUAL DISABILITY CAUSATIVE FEATURES IN DOWN SYNDROME BY MACHINE LEARNING ANALYSIS

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Down Syndrome (DS) or trisomy 21 is the most frequent chromosomal abnormality in humans. DS is characterized by the presence of numerous congenital defects and comorbidities. In particular, individuals with DS typically have cardiovascular malformations, growth retardation, craniofacial dysmorphism and intellectual disability (ID). ID represents the most significant symptom in DS and is always present, although with varying severity from individual to individual. Nevertheless, no pathogenic or molecular mechanism has yet been identified as causative. Studying a complex and not monogenic condition such as DS and finding a clear correlation between cause and effect might be difficult through classical analysis methods, thus different approaches need to be used.

A pilot study on 106 DS individuals has shown how the use of Artificial Intelligence (AI) can provide reliable results in identifying variables likely involved in cognitive impairment in DS, such as immune system dysfunction, gastrointestinal disorders, thyrotropin concentration and hearing loss. On this basis, we have collected molecular, clinical and cognitive data of 152 subjects with DS and we have created a new dataset following FAIR principles (Findable, Accessible, Interoperable, and Reusable), We have developed a tree-based Machine Learning (ML) algorithm to analyze all these different types of data (continuous, categorical and discrete variables) and to find linear as well as non-linear correlations between our variables and cognitive impairment in DS.

These ML models lead to uncovering unexpected information that would not be observable by human analysts or more canonical statistical approaches. They are

essential to identify new molecular or clinical variables that play a crucial role on the onset of ID in DS and to determine what biological mechanisms are actually involved in this particular condition.

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HDL-MIMETIC REPROGRAMS TRYPTOPHAN METABOLISM AND ASTROCYTE REACTIVITY TO COUNTERACT NEUROINFLAMMATION

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Inflammation-driven modulation of tryptophan metabolism via kynurenine pathway (KP) activation promotes the synthesis of neurotoxic metabolites, notably quinolinic acid (QA), contributing to neuroinflammatory processes. In a swine model of lipopolysaccharide (LPS)-induced acute kidney injury (AKI), we investigated an engineered HDL-mimetic as a molecular strategy to selectively reprogram KP flux and attenuate neuroinflammation.

CER-001 significantly reduced brain IDO1 mRNA expression ($p<0.005$), while upregulating DDC ($p<0.05$), thus diverting tryptophan metabolism towards serotonin biosynthesis. Other KP enzymes (AFMID, KMO, KYAT3, KYNU, HAAO) were unchanged, indicating selective modulation. ELISA analyses revealed decreased QA and kynurenine levels ($p<0.05$), increased kynurenic acid and tryptophan ($p<0.05$), and reduced KYN/Trp ratio ($p<0.05$) from 6 to 24 hours.

At the cellular level, CER-001 reduced astrocytic GFAP and Cx43 expression ($p<0.05$), while AQP4 remained unaffected, suggesting targeted modulation of astrocyte reactivity. Moreover, CER-001 upregulated SR-BI expression ($p<0.05$), facilitating HDL transcytosis and neuroprotective lipid signaling.

Clinical data corroborated preclinical findings, confirming sustained modulation of KP metabolites with decreased QA and KYN/Trp ratio and increased 5-HT levels up to 30 days ($p<0.05$).

Overall, CER-001 selectively reshapes inflammatory tryptophan metabolism, suppresses neurotoxic KP outputs and astroglial activation, and restores neuroimmune balance, offering a novel biotechnological approach to mitigate inflammation-associated neurotoxicity.

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SYNJ1 CONTROLS PI(4)P HOMEOSTASIS AT ERES TO SUPPORT COPII VESICLE FORMATION AND EARLY SECRETORY TRAFFICKING

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The early secretory pathway (ESP) relies on the precise coordination of membrane lipid composition and protein machinery to ensure efficient COPII vesicle formation at endoplasmic reticulum exit sites (ERES). Here, we identify the phosphoinositide phosphatase SYNJ1 as a critical regulator of PI(4)P homeostasis in the ESP. High resolution confocal microscopy and proximity ligation assays revealed SYNJ1 localization at ERES and its interaction with core COPII components, Sec16a and Sec23a. Co-immunoprecipitation analyses further suggest that SYNJ1 preferentially binds an unmodified form of Sec23a, potentially reflecting an active or membrane-associated pool relevant for vesicle budding. Moreover, SYNJ1 depletion led to aberrant PI(4)P accumulation at ERES, ER, and ERGIC, as shown by the increased fluorescence of the PI(4)P biosensor P4C-GFP. Despite the enrichment of PI(4)P in these compartments, the number of PI(4)P/Sec23a proximity signals was markedly reduced in SYNJ1i cells, indicating impaired Sec23a recruitment or retention into budding vesicles. This was confirmed by altered Sec23a partitioning between membrane and cytosolic fractions, consistent with disrupted coat assembly or recycling. These results indicate that unbalanced PI(4)P may alter membrane properties at ERES. Functionally, SYNJ1 knockdown impaired ER-to-Golgi trafficking of a panel of secretory cargos with distinct topologies and sizes—including luminal proteins, transmembrane proteins, and GPI-anchored cargos—highlighting a general role in ER export. These defects collectively point to a general dysfunction of early trafficking steps due to disrupted PI(4)P turnover. We propose that SYNJ1 acts at ERES to locally dephosphorylate PI(4)P, maintaining membrane lipid composition required for optimal curvature, COPII component dynamics, and vesicle release. Our data uncover a previously unrecognized role for SYNJ1 in coordinating lipid and protein factors to support early secretory trafficking.

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ER STRESS INDUCED BY GLIADIN IN INTESTINAL EPITHELIAL CELLS VIA THE CXR3/PLC/IP3/IP3R AXIS REPRESENTS A KEY STEP IN THE PATHOGENESIS OF CELIAC DISEASE

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Background and aims: Celiac Disease (CD) is an autoimmune disorder primarily affecting the gut of genetically predisposed individuals, triggered by gliadin peptides (PT) produced by the digestion of wheat gluten. Although inappropriate activation of the immune system is considered the main cause of intestinal dysfunction and tissue damage, the interaction between PT and intestinal epithelial cells (IECs) remains an important step in the pathogenesis of CD. Therefore, understanding the molecular mechanism(s) activated in IECs exposed to PT represents a unique opportunity to define new potential therapeutic targets.

Methods: Three models were used to investigate the molecular basis of PT-induced damage. CaCo-2 cells were used as an *in vitro* model. As an *ex vivo* model, we used our Gut-*Ex*-*Vivo* System (GEVS), which allowed us to culture the gut of our CD mouse model, enabling controlled stimulation with PT and selected drugs. Finally, biopsies from pediatric CD/non-CD patients were used to verify our results.

Results: Our study demonstrated a prompt induction of ER stress in IECs upon PT exposure through a CXCR3/PLC/IP3/IP3R-axis responsible for calcium release through the ER both *in vitro* and *ex vivo*. Inhibition/buffering of PT-stimulated ER stress by pharmacological chaperones or inhibiting the CXCR3/IP3R axis results in complete abrogation of TG2 upregulation, pro-inflammatory cytokine production, and tissue damage. Finally, increased expression of ER stress markers was confirmed in biopsies from CD patients compared to non-CD patients.

Conclusions: Overall, our analysis i) revealed the key role of ER stress in the pathogenesis of CD, ii) identified the molecular mechanism linking PT and the induction of ER stress, and iii) uncovered valuable novel therapeutic targets.

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SEX-DEPENDENT CHAPERONE-ASSISTED SELECTIVE AUTOPHAGY (CASA) ACTIVITY IN ALS GLIAL CELLS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that differentially affects males and females, and in which astrocytes and microglia have been shown to play a pivotal role in disease progression. Autophagy disfunction in both males and females is a common event in ALS, and alterations in CASA complex components, like HSPB8 and BAG3, have been previously described; however, it is unknown whether this system is impaired in a sex-dependent way in astrocytes and microglia, during ALS progression. Adult mouse primary spinal cord astrocytes (P120) RNAseq analyses identified 620 DEGs in females and 187 DEGs in males, including a reduced HSPB8 and HSPB6 expression in SOD1-G93A female primary astrocytes. CASA complex evaluation by qPCR in SOD1-G93A primary astrocytes (P120) showed increased HSPB8 and BAG3 mRNA expression in male mice, meanwhile in females, the opposite effect was observed. Interestingly, no alterations were detected in astrocytes isolated at P2. Quantification of p62 bodies in male astrocytes showed a higher number of p62 bodies per cell in WT versus G93A conditions, and autophagy inhibition through NH4Cl treatment drastically increased this effect. In females, a lower number of p62 bodies per cell was observed in WT versus G93A astrocytes. Moreover, we observed a lower number of p62 bodies in WT female astrocytes compared to males, meanwhile in G93A mice, a significant increase in p62 bodies per cell was observed. Analyses on LC3 puncta showed a decrease in the number of puncta per cell in G93A versus WT astrocytes in males, meanwhile no changes were detected between female samples. Finally, studies in microglia primary cultures, isolated from mouse spinal cord at P2 and stimulated with a cytokine cocktail, only showed a significant decrease in LC3 mRNA expression in G93A microglia, both in female and male samples, meanwhile at the protein level, a significant reduction in BAG3 expression was observed in female microglia, both in WT and G93A conditions. Altogether, data suggests that G93A male astrocytes increase the expression of proteins such as HSPB8 and BAG3, possibly boosting autophagy to counteract misfolded protein accumulation. On the other hand, female astrocytes possess a higher autophagy capacity than male astrocytes; however, this feature is reduced in presence of G93A ALS mutation.

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MESENCHYMAL STEM CELLS AND MICROGRAVITY: INVESTIGATING STRESS RESPONSE FOLLOWING REAC BIOMODULATION

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Mesenchymal stem cells (MSCs) exhibit many of the regenerative and differentiative properties of embryonic stem cells, without raising ethical concerns and being easy to collect. However, under stressful conditions, they can lose these properties, undergoing premature senescence or apoptosis. Simulated microgravity negatively impacts human physiology, influencing cellular behavior. Each cell responds to stress in several ways, typically by activating signaling pathways that promote either survival or apoptosis. The nature of the response also depends on the type and duration of the stressor. Radio Electric Asymmetric Conveyer (REAC) biomodulation, specifically the metabolic optimization-microgravity (MO-MG) protocol, enhances tissue repair through a targeted, integrated cellular response.

In the present study, MSCs were exposed for 24 hours to simulated microgravity (µg) using a 3D random positioning machine (RPM), which provides a simulated microgravity environment of less than 10^{-3} g. Control cells were placed in a static bar at 1 g and subjected to the same vibrations experienced under µg conditions. After 24 hours, cells were cultured and treated with the REAC MO-MG protocol for 6 days. The treatment cycle consisted of 9 sessions, each lasting approximately 30 minutes. Sessions were spaced at least one hour apart, with no more than four sessions administered in a single day. We then evaluated cell morphology following REAC MO-MG treatment and analyzed the expression of stemness-associated genes (Oct-4, Sox2, and Nanog), along with epigenetic factors closely related to stress response (Sirt1, DNMT1, and HSP70). Additionally, the expression of Sirt1, mTOR, and Cytochrome C was assessed using confocal microscopy. Our results clearly demonstrate the role of REAC MO-MG in restoring MSC potency after stress, maintaining higher expression levels of stemness markers and epigenetic factors as compared to MSCs exposed only to µg, suggesting the potential application of this treatment in future space missions.

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PERTURBATION OF SECRETORY PATHWAY IN HEALTH AND DISEASE: NEW PERSPECTIVES

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The perturbation of protein translocation into the secretory pathway using Sec61 translocon inhibitors is a novel and promising strategy for tackling many pathological situations, including cancer and viral infections. However, a highly sensitive and direct screening platform for selecting Sec61 inhibitors is unavailable. Here, we develop a new "*resuming luminescence upon translocation interference"* (RELITE) assay capable of selecting Sec61 inhibitors in a single round of screening. This assay exploits the inactivation of firefly luciferase, once translocated into the endoplasmic reticulum (ER), and the possibility of diverting and "re-lighting" luciferase into the cytosol by a Sec61 inhibitor. Using this method, we selected small molecules capable of hampering the protein expression of the PD-L1 immune checkpoint by interfering with its ER translocation and delivering it for degradation. In conclusion, our screening method will greatly facilitate the selection of Sec61 inhibitors for down-modulating the expression of many disease-relevant proteins.

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KRIT1 REGULATION BY THE PIEZO1 MECHANSENSITIVE RECEPTOR: NOVEL FINDING FOR THE CCM PHENOTYPE

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Mechanotransduction refers to the cell ability to convert mechanical stimuli into molecular responses, and it is mediated by membrane receptors known as mechanoreceptors. Since early vasculogenesis, mechanical cues drive blood vessel morphogenesis and, in this context, the role of PIEZO1 in blood-brain barrier (BBB) development has been confirmed. PIEZO1 is a selective Ca^{2+} cation channel activated by hemodynamic forces. We previously reported evidence of *PIEZO1* involvement in cerebral cavernous malformation (CCM), a vascular anomaly in which a reduced number of pericytes surrounds brain capillaries, that appear enlarged and tangled. Endothelial cells (ECs) show defective junctions and undergo endothelial-to-mesenchymal transition. The three genes *KRIT1*, *CCM2* and *PDCD10* have been linked to CCM development.

Current data suggest that PIEZO1 and KRIT1 guarantee BBB properties by regulating common signaling pathways. Here, we investigate how PIEZO1 dysfunction affects KRIT1 activity in human cerebral microvascular endothelial cells (hCMECs/D3). In detail, hCMECs exposed to both the PIEZO1 agonist Yoda1 and antagonist GsMTx4 showed increased KRIT1 expression and subcellular redistribution, as well as actin microfilament reorganization, as confirmed by western blot and immunofluorescence. In addition, both treatments seem to promote KRIT1 phosphorylation.

Finally, a change in *PIEZO1* expression was detected in *KRIT1* morphant zebrafish larvae, suggesting that a possible forward feedback loop occurs.

Comparison of the transcriptomes obtained from both treated hCMECs and CCM-derived ECs will allow the identification of the key nodes in this signalling.

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IN VITRO MODELLING OF JOUBERT SYNDROME USING PATIENT-DERIVED IPSCS IN 2D AND 3D CULTURES.

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Joubert syndrome is a congenital neurodevelopmental defect characterized by a distinctive cerebellar and brainstem malformation recognizable on brain imaging. This genetically heterogeneous disease is linked to the dysfunction of the primary cilium and thus classified as a ciliopathy. The primary cilium is a sensory organelle present on the cell surface composed of a microtubule scaffold-based core structure (axoneme), a highly specialized plasma membrane, a centriole-derived basal body and an adjoining transition zone sub-compartment that sorts proteins into and out of the organelle.

To better understand the cellular phenotype underpinning this cerebellar pathology, induced pluripotent stem cells (iPSCs) are being used to investigate the impact of patient-relevant gene mutations on cerebellar development. This project sets to model in vitro, in both 2D monolayer and 3D organoid models, the process of cerebellar lineage formation, comparing lines from mutation-bearing iPSCs to healthy iPSC controls over a protocol up to day 35 of cerebellar differentiation. Initial results confirmed the induction of cerebellar lineage marker expression over time, including CALBINDIN and BARHL1, and revealed the presence of significant ciliary defects in patient-derived cultures, visible in both neuroprogenitor and organoid cultures. Further analyses are underway to refine and compare the differentiation kinetics in patient-derived and control cell lines.

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EVALUATION OF THE EFFECTS OF EXPOSURE TO HEAT-NOT-BURN TOBACCO (IQOS), ELECTRONIC DEVICES, AND CIGARETTE SMOKE ON ORAL HEALTH

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The use of electronic nicotine delivery systems (ENDS), including e-cigarettes (e-cigs) and inhaled tobacco products (IQOS), is increasingly being used, especially among adolescents, as an alternative to smoking combusted tobacco products. While these products have gained popularity due to the absence of harmful chemicals typically found in tobacco smoke (TS), their safety, regulation and long-term effects remain controversial.

The present project aims to elucidate the biological effects of IQOS on human oral health compared to those induced by e-cigarettes and TS using oral cell models, specifically dental pulp stem cells (DPSCs). IQOS, e-cig, and TS aerosols will be generated using a 'smoke machine,' where the produced aerosol is absorbed by the culture medium to create the 100% extract for in vitro cell administration.

We evaluated the growth of DPSCs cultured in media exposed to different cigarette smoke for 24/48/72 hours, using two concentrations: 100% and 6.25%. Among the three types of smoke considered, only the 100% TS condition significantly reduced cell growth at all time points. Exposure to 100% IQOS resulted in a significant decrease only at 24 hours. No significant changes were observed using the 6.25% concentration.

Regarding the evaluation of ROS no significant variations were observed at 24, 48, and 72 hours at 6.25% conditions.

To assess the effect of treatments on cell migration, we conducted a wound-healing assay. The results showed a significant reduction in wound closure starting at 15 hours for cells exposed to 100% TS and IQOS extracts. No effects were seen with the 6.25% cigarette smoke condition.

Similarly, in the 3D organoid model, preliminary experiments revealed a marked decrease in organoid growth in both undifferentiated and odontogenically differentiated DPSCs after exposure to 100% e-cig. These preliminary results suggest that “acute” exposure to high concentrations of both TS and e-cig may compromise the regenerative capacity of DPSCs, with potential implications for tissue repair and healing.

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A NEW PLATFORM FOR THE DIRECT PROFILING OF THERAPEUTIC OLIGONUCLEOTIDES

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The explosion of genomic data has led to the discovery of numerous disease-causing genes, opening up new therapeutic opportunities uniquely accessible to oligonucleotide-based drugs. Therapeutic oligonucleotides (TOs), including small interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs), represent a significant breakthrough, being part of an emerging class of treatments. These molecules can inhibit mRNA translation, correct aberrant splicing, and more. Like all drugs, TOs must meet the regulatory standards established by global medicines agencies. Among the requirements, developers must submit pharmacokinetic and pharmacodynamic data. However, one major challenge is the limited performance of current TOs detection technologies (LC-MS and ELISA-based) which have several drawbacks that hinder the development of new therapies.

In this study, we present an innovative platform for TOs profiling. It integrates a novel silicon photomultiplier-based reader with a new chemical detection method for nucleic acids in a semi-automated platform. We designed a set of reagents to analyze previously identified siRNAs and ASOs targeting the MAPT gene in a neuron-derived human induced pluripotent stem cells (hiPSCs) model of frontotemporal dementia with parkinsonism linked to chromosome 17. We differentiated the hiPSCs carrying a disease-associated point mutation (IVS 10+16) into neurons, and then transfected them with the siRNAs and ASOs. Neurons were analyzed at various time points up to 120 days. We profiled both intracellular and extracellular levels of siRNAs and ASOs with our platform. Not only we were able to quantitatively detect the TOs, but we also discovered intriguing and unexpected findings regarding their half-life inside and outside the cells. These insights are now guiding the development of predictive in silico models of TO behavior in neuron-derived iPSCs.

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QUANTUM MOLECULAR RESONANCE MODULATES INFLAMMATION, SENESCENCE, AND AUTOPHAGY IN MONOCYTES AND FIBROBLASTS

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This study investigates the effects of Quantum Molecular Resonance (QMR) technology on inflammation, cellular senescence, and autophagy in an in vitro model using THP-1-derived macrophages and IMR-90 fibroblasts rendered senescent by etoposide treatment. Inflammatory stimulation in monocytes was triggered by lipopolysaccharide and hyaluronic acid fragments. QMR treatment significantly reduced nitrosative stress by downregulating COX-2 and iNOS expression, inhibiting NF-κB activation, and decreasing peroxynitrite production. QMR also suppressed NLRP3 inflammasome activation and promoted M1-to-M2 macrophage polarization. Notably, QMR reduced the expression of the senescence marker p21 not only in IMR-90 fibroblasts but also in THP-1 monocytes, indicating a broader anti-senescent effect. Additionally, QMR modulated autophagic processes, supporting restoration of cellular homeostasis. These findings highlight the therapeutic potential of QMR in regulating key inflammatory and aging-related pathways across different cell types.

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IMPACT OF LACTOBACILLUS-DERIVED METABOLITES ON MICROGLIAL ACTIVATION AND P2Y12 RECEPTOR EXPRESSION IN EPILEPTIC HUMAN BRAIN SLICES

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The bidirectional communication between the gut and the brain is essential for the proper development of the central nervous system. Among the key components of the gut microbiome, *Lactobacillus* species have emerged as important modulators of host physiology. These bacteria not only interact with intestinal epithelial cells to maintain gut barrier integrity but also produce metabolites capable of reaching the brain and influencing neuroimmune function. Microglia, the resident immune cells of the brain, play a central role in neuroinflammation and are regulated in part by purinergic signaling via the P2Y12 receptor.

We hypothesize that metabolites secreted in culture supernatant derived from *Lactobacillus* species modulate P2Y12 receptor signaling in the brain environment, thereby affecting microglial activation and motility. Dysregulation of this pathway may contribute to neurological disorders, including pediatric epilepsy. To investigate this hypothesis, we analyzed P2Y12 expression and microglial morphology in human cortical brain slices obtained from pediatric epilepsy patients, following exposure to specific *Lactobacillus*-derived metabolites. Using immunofluorescence, we observed that these metabolites influence microglial morphology and reduce both P2Y12 receptor expression and neuronal activity. Additionally, we found that *Lactobacillus* culture supernatants modulate the levels of extracellular ATP, a key activator of P2Y12 signaling, suggesting a potential impact on epileptic activity. These findings highlight a novel role for gut microbiota-derived metabolites in modulating

microglial function and suggest a potential microbiota–brain axis mechanism contributing to the pathophysiology of developmental epilepsies.

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UNDERSTANDING NAD+ IN OVARIAN ENERGY BALANCE DURING AGING

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The ovary undergoes an age-dependent functional decline beginning in the fourth decade of life, making ovarian aging the main contributor to female reproductive aging. Nicotinamide-adenine-dinucleotide (NAD^+), a crucial coenzyme in cellular redox reactions, regulating cellular metabolism and longevity. Recently, the beneficial role of NAD^+ precursors on the maintenance of female fertility during aging has emerged, although the underlying mechanisms remain unclear. In this context, we have investigated changes induced by the aging process in the expression level of NAD^+ producing and consuming enzymes, as well as alterations in cellular bioenergetics. Then we explored the beneficial effects of NAD^+ boosting approaches. To this aim we employed mouse oocytes from young and aged mice and established a senescent model of human granulosa cells (hGCs). Ingenuity Pathway Analysis (IPA) revealed that aging down-regulated all cellular pathways for NAD^+ synthesis (Kynurenine pathway, Preiss-Handler pathway and NAD^+ salvage pathway) and influenced NAD^+-dependent enzymes. Considering that NAMPT, the rate-limiting enzyme of NAD^+ salvage pathway, was deregulated, aged oocytes were treated with the NAMPT activator P7C3. Our results showed that P7C3 treatment increased NAD, improved spindle assembly, mitochondrial bioenergetics, and reduced mitochondrial proton leak. It also influenced NAD^+ regulatory network gene expression, with Sirt1 as a central node. Then, senescent hGCs were cultured with NAD^+ boosting molecules (nicotinamide riboside, NR; nicotinamide, NAM and P7C3), with an improvement of proliferation. NR and NAM counteracted the effects of senescence and restored NAD^+/NADH ratio, ATP levels, ROS production and SIRT1 expression. Collectively, these findings offer new mechanistic insights into how NAD^+ restoration may serve as a therapeutic approach to mitigate ovarian aging.

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INDIRUBIN-3′-OXIME (BIO) INTERCEPTS GSK3B PATHWAY AND MITIGATES THE PATHOPHYSIOLOGY OF IDIOPATHIC PULMONARY FIBROSIS IN PRECISION-CUT LUNG SLICES

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Idiopathic pulmonary fibrosis (IPF) is a progressive disease with a 5-year survival rate of 20% of patients, reflecting the lack of effective therapies. At the molecular level, the IPF process is characterized by the recruitment and proliferation of fibroblasts in the damaged site and extracellular matrix deposition. Consequently, the architecture of the lung is destroyed, resulting in respiratory insufficiency. These events are driven by a complex network of inflammatory cytokines, chemokines, and heterogeneous cell types that accumulate within specific foci in lung tissue adjacent to areas of active tissue fibrosis. Currently, the approved drugs for IPF treatment aren't sufficient to block IPF progression. An efficient, accurate diagnostic tool is lacking that allows rapid intervention and easy therapy monitoring. This is mainly due to the lack of a valid preclinical model for testing either molecular pathways or new adjuvant therapies. Among the main actors in the IPF process, we focused on Anterior gradient 2 (AGR2) and the Wnt-GSK3β pathway. To study if the inhibition of GSK3β could modulate the IPF phenotype via AGR2, we generated a 2D lung cell model of lung fibrosis (ihTLF) treating with both LPS at 2ng/ml for 6h and TNFα at 1ng/ml for 12h, evaluating the upregulation of the main fibrotic genes (COL1A1, TGFβ, TIMP1 and MMP2) by RT-qPCR. Moreover, to intercept GSK3β pathway we used BIO, a well-known compound for inhibiting GSK3β, promoting the reduction

and the reversion of IPF phenotype. In that case we treated with BIO ihTLF and human precision-cut lung slices (PCLS) both from donor and IPF patients. Our results show a decrease in the main fibrotic actors (αSMA, GSK3β, AGR2, COL1A1) and suggest that BIO could mitigate the IPF process through GSK3β inhibition.

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CHROMATIN ACCESSIBILITY PROFILING VIA SINGLE-NUCLEUS ATAC-SEQ REVEALS DISTINCT CELLULAR PATTERNS AND EXPRESSION SIGNATURES IN FSHD MUSCLE DEGENERATION

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Skeletal muscle consists of several cell types that finely orchestrate tissue homeostasis. The proportions and subtypes of these cells are modulated during both muscle regeneration and degeneration.

The mechanisms driving muscle degeneration in facioscapulohumeral muscular dystrophy (FSHD), a rare progressive muscle disorder caused by the inappropriate activation of the DUX4 gene, remain only partly understood. Clarifying the complexity of muscle degeneration in FSHD patients remains a critical challenge. We hypothesize that a dysregulated balance among key cell populations contributes to tissue degeneration and disease progression.

To investigate what distinguishes muscles in FSHD, we analyzed biopsies from affected and spared muscles of patients, guided by magnetic resonance imaging, as well as samples from healthy controls. We performed state-of-the-art single-nucleus assay for transposase-accessible chromatin (snATAC-seq) on whole muscle tissue to define cellular heterogeneity and identify gene expression signatures linked to pathogenesis. Our multiomic approach included both qualitative and quantitative assessments of muscle cell populations, the identification of expression patterns within each subtype, and a bottom-up analysis of dysregulated pathways.

Preliminary results revealed at least 10 distinct cellular clusters and muscle-specific variations in cell population composition, suggesting a unique distribution across samples. By integrating public snRNA-seq data, we assigned clusters to specific cell types and identified subpopulations potentially involved in degenerative or regenerative processes. Chromatin accessibility analyses also revealed gene-specific expression variability across clusters.

This integrative approach highlights key cellular and regulatory differences between affected and spared muscles, providing novel insights into FSHD pathogenesis.

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IDENTIFICATION OF MIR-1246 AS A NEW INFECTION MARKER IN HUMAN AND CANINE MACROPHAGE-LIKE CELLS INFECTED BY LEISHMANIA INFANTUM

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In recent years, microRNAs (miRNAs) have emerged as key regulators of inflammation and immune responses. Leishmaniasis is a neglected infectious disease affecting humans and other mammals (mainly dogs), caused by protozoa belonging to the *Leishmania* genus. The parasite primarily infects the macrophages, establishing a niche permissive for its proliferation. Although several studies analyzed the host miRNome during infection with different *Leishmania* species, only a few reports investigated the miRNA dysregulation following infection with *L. infantum*.

In this study, miRNA-seq was performed on human monocytic cells (U937, THP-1), and canine macrophage-like cells (DH82) infected by *L. infantum* for 24h or 48h, alongside culture media at 48h post-infection (for THP-1 and DH82 cells). Differential expression analysis identified miR-1246 as one of the most significantly upregulated miRNAs in infected U937,THP-1 cells, and THP-1 culture media. Although miR-1246 is not annotated in dog, its mature sequence shared 100% identity with a fragment of canine U2 spliceosomal RNA sequence which is identical to human RNU2. Interestingly, the miR-1246 qPCR human assay successfully detected miR-1246 in DH82 samples, revealing a significant upregulation in culture media of infected cells.

To explore the potential functional impact of miR-1246, expression levels of three validated target genes involved in immune regulation (i.e., *GSK3B*, *CD22*, *XK)* were assessed in infected U937 and/or THP-1 cells. While *GSK3B* expression remained unchanged in both in vitro models, *CD22* and *XK* were significantly downregulated in U937 cells. These preliminary findings suggest a potential role for miR-1246 in modulating host responses during *L. infantum* infection in a one health context, highlighting it as a candidate infection marker in both human and canine leishmaniasis.

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CACNG8 AS A MODIFIER OF SYNAPTIC INTEGRITY IN RETINAL DISORDERS: INSIGHTS INTO AMPA RECEPTOR SIGNALING, BIOGENESIS, AND CELLULAR MECHANISMS

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The Transmembrane AMPA Receptor Regulatory Protein Gamma-8 (TARP γ-8), encoded by *CACNG8*, is an essential auxiliary subunit for AMPA receptor (AMPAR) trafficking, gating kinetics, and synaptic stabilization. Although its role in central synapses is well-established, its involvement in retinal neurotransmission and Inherited Retinal Dystrophies (IRDs) remains unexplored. This study investigated *CACNG8* as a potential modifier gene in IRDs, integrating human genetics, molecular dynamics simulations, and cellular biogenesis analysis to understand its impact on AMPAR signaling and synaptic integrity. Whole Exome Sequencing of IRD families —characterized by severe optic atrophy and residual photoreceptor function— identified rare *CACNG8* variants, including the stop-gain p.Arg123Ter and the missense p.Leu96Val and p.Val102Met. Structural analyses revealed that these mutations disrupt AMPAR-centered synaptic complexes, affecting glutamate signaling and plasticity. Through molecular docking and dynamics (MD) simulations, we analyzed the cellular mechanisms and biogenetic pathways altered by these variants. The p.Arg123Ter mutation, by truncating the PDZ-binding domain, impaired cellular anchoring with PSD95, disrupting postsynaptic densities and AMPAR clustering. This led to defective protein trafficking and receptor localization, reducing neurotransmitter efficiency. Hydrogen bond networks and electrostatic interactions crucial for stability were severely compromised. The missense variants induced conformational shifts in the β1–β2 loop and transmembrane helices, affecting ion channel gating and trafficking dynamics. PCA of MD trajectories showed restricted receptor flexibility, impacting mechanotransduction and synaptic adaptation. GIST analyses revealed desolvation at critical interfaces, reducing ion permeability and destabilizing synaptic homeostasis. Thus, *CACNG8* might modulate

AMPAR trafficking, proposing it as a therapeutic target for synaptic restoration in retinal degeneration.

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CHRONIC EXPOSURE TO CADMIUM ALTERS CHEMORSISTENCE TO GEMCITABINE IN PANCREATIC CANCER CELLS

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Cadmium (Cd) is a toxic heavy metal released into the environment mainly through industrial activities. The IARC classifies Cd as a carcinogen by inhalation, while evidence for carcinogenicity via oral ingestion is limited. Epidemiological studies associate Cd with several cancers, including pancreatic cancer (PC), a particularly aggressive tumor with a poor prognosis and <10% 5-year survival. Although Cd accumulates in pancreatic tumor tissues, its link to PC remains unclear (Djordjevic et al., 2019). Several studies have demonstrated that the exposure to heavy metals, such as Cd, influence the response to the treatment in different tumors. Gemcitabine (GCB), a deoxycytidine analog, is the first-line treatment for PC, but only 30% of patients respond, and resistance develops quickly (Koltai et al., 2022). We hypothesized that Cd may influence GCB efficacy. For this purpose, BxPC-3 and AsPC-1 cell lines, representative of primary and metastatic PC, respectively, were chronically exposed to different low concentrations of Cd (2.5 µM, 5 µM, 10 µM for 28days). Then, cells were further treated with a cytotoxic concentration of GCB (5 µM), and the cytotoxic effect and proliferation capacity was investigated. MTT and apoptosis assays showed increased viability in BxPC-3 cells treated with Cd+GCB vs GCB alone ($p<0.01$), suggesting reduced sensitivity. No significant changes were observed in cell cycle distribution. PARP activation supported these results. Conversely, AsPC-1 cells treated with Cd+GCB showed reduced viability vs GCB alone ($p<0.01$), suggesting a synergic effect. Cell cycle analysis revealed an increased number of cells in the sub-G1 and G1 phases. An increase in PARP cleavage reinforced these results. These findings suggest that chronic Cd exposure may modulate the response of PC cells to GCB potentially contributing to chemoresistance or chemosensitization depending on the tumor context.

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ROLE OF SET7 METHYLTRANSFERASE IN METHYLGLYOXAL-INDUCED VASCULAR INFLAMMATION ASSOCIATED TO HYPERGLYCEMIA

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Methylglyoxal (MG) is a metabolite of glucose which can cause endothelial function impairment, playing a key role in the pathogenesis of diabetic vascular complications. High MG levels in diabetes induce the formation of advanced glycation end products (AGEs) resulting in receptor-AGE (RAGE) activation, oxidative stress and stimulation of the NF-κB inflammatory pathway. Methyltransferase Set7 has recently emerged as a potential regulator of NF-κB activity.

This study aimed at investigating the involvement of Set7 in the MG-mediated activation of NF-κB pathway in primary human umbilical vein endothelial cells (HUVECs) derived from women affected by gestational diabetes (GD), as a cellular model of hyperglycemia-induced endothelial dysfunction by epigenetic modifications.

GD-HUVECs and cells derived from control women (C-HUVECs) were employed to evaluate the role of Set7, both in basal condition and after MG treatment, using inhibitors for Set7 and RAGE, (R)-PFI-2 and RAGE229, respectively. Western blot, flow cytometry, activity assay, IncuCyte technology and monocytes-HUVEC adhesion assays were used to evaluate Set7 expression, RAGE protein levels and the activation of inflammatory pathways (NF-κB p65 expression and activity, VCAM-1 expression and membrane exposure and monocytes adhesion).

Results indicated increased Set7 levels, but not enhanced RAGE levels in GD-, compared to C-HUVECs, and this was associated with higher basal and MG-induced NF-κB activity. Of note, the MG-increased inflammatory pathways were significantly suppressed by the inhibition of Set7.

Overall, our results indicate the possible involvement of Set7 in the vascular inflammation mediated by high MG levels in chronic hyperglycemia. Therefore, its inhibition could represent a new therapeutic strategy in pathogenesis of diabetic vascular complications.

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HIGH-INTENSITY INTERVAL TRAINING IMPACTS ON DISEASE ONSET AND PROGRESSION IN SOD1-G93A MOUSE MODEL

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Amyotrophic lateral sclerosis (ALS) is a devastating motor neuron disease that affects upper and lower motor neurons in the brain, brainstem and spinal cord.

Impairment of metabolic homeostasis has been widely described in both patients and animal models of ALS, characterised by weight loss, depletion of energy stores, hypermetabolism and alterations in glucose handling. Given these features, physical activity (PA) may have a protective effect by improving exercise capacity.

To clarify its role, we randomly selected four groups of 35-day-old female SOD1-G93A and wild-type (WT) mice and performed a high-intensity interval training (HIIT) protocol on a treadmill for eight weeks. During the training, we measured muscle strength, weight and motor skills to define the onset of the disease. At the end of the training, we analysed the tibialis anterior, sciatic nerve and spinal cord using histological and molecular techniques.

We found that HIIT training delayed the onset of ALS in SOD1-G93A mice by at least 3 weeks. Compared to sedentary SOD1-G93A mice, the tibialis anterior of trained SOD1-G93A mice exhibited a less oxidative phenotype. Exercise slows down the denervation process in SOD1-G93A mice and delays Wallerian degeneration, also protecting against motor neuron loss. Furthermore, we found that the exercise protocol alleviates neuroinflammation by reducing immune cell infiltration in the sciatic nerve and neuroglia activation in the spinal cord. At the molecular level, we observed that PA modulates extracellular vesicles-associated microRNAs released from the skeletal muscle (SkM-EVs), whose activity was validated on muscle and neuronal *in vitro* models.

Taken together, our findings support the hypothesis that HIIT exercise represents an ameliorative factor for ALS, delaying disease onset and affecting both denervation and neuroinflammation, possibly mediated by SkM-EVs.

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FF-REPAIR PROJECT: ANTI-AGING EFFECTS OF SECRETOME FROM ADIPOSE MESENCHYMAL STEM CELLS IN HUMAN SENESCENT GRANULOSA CELLS

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Age-associated ovarian dysfunctions negatively affect female reproductive environment, and this threats women's fertility mostly in Western societies, where women are giving birth at an increasingly older age. This has powered scientific research to find approaches to improve ovarian health and counteract reproductive aging. Some evidence suggested that mesenchymal stem cell-derived secretome may be used to delay senescence in several contexts with a cell-free strategy, although mechanisms underlying such effects are still largely debated. This project is primarily aimed at exploring whether adipose mesenchymal stem cells (hAMSCs)-derived secretome can revert the "aged" phenotype in a laboratory-developed senescent model of granulosa cells, key cooperative somatic partners for oocyte growth and maturation.

The secretome was obtained culturing commercially available hAMSCs (<10^{th} passage) for 3 days in DMEM w/o FBS to generate the conditioned medium (CM). In parallel, human KGN cells were made senescent through a H_2O_2-based treatment and assessed for senescence phenotype based on specific markers including p21, PCNA, and β-galactosidase. Then, senescent KGN and non-senescent KGN cells were treated for 2 days with either conditioned or non-conditioned medium. The effects of freezing, filtration, and dilution, were evaluated. As shown by morphological evaluation under phase contrast microscope, WB and β-galactosidase assay, antiaging effects of CM treatment were observed, although freezing, filtration, and dilution affected the outcome. This highlighted the importance of storage and processing of CM in determining its efficacy thereby suggesting the need to standardize and optimize protocols. Further experiments will identify the biologically active contents of CM, thus providing useful information for developing new strategies in anti-aging reproductive medicine.

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TARGETING RAN TRANSLATION TO RESCUE PATHOLOGICAL MECHANISMS DRIVEN BY C9ORF72 MUTATION IN FTD/ALS MODELS

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Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are two neurodegenerative disorders with overlapping clinical, genetic, and pathological features. A high percentage of FTD/ALS cases share a genetic cause, specifically the hexanucleotide repeat expansion (HRE) in *C9ORF72* gene. This mutation leads to neurodegeneration through a loss of function and a toxic gain-of-function via RNA foci formation and the production of dipeptide repeat proteins (DPRs) through repeat-associated non-AUG (RAN) translation. The regulation of RAN translation is currently not fully characterized. PKA has recently been shown to be involved, however, it is involved in many other pathways. Therefore, to prevent the pathology, we have identified a kinase downstream of PKA. The present study investigates the modulation of RAN translation using antisense oligonucleotides (ASOs) targeting a specific kinase downstream of PKA, in HEK293T cells and motor neurons differentiated from induced pluripotent stem cells (MN-iPSCs). The efficacy of the ASO was evaluated through molecular and biochemical techniques, including RT-qPCR, Western blot, dot blot, and filter trap assay. In HEK293T, results demonstrate a selective reduction of DPRs produced through RAN-translation and not the canonical translation mechanism. Moreover, we show that the downregulation of the kinase does not impact degradation pathways, reinforcing its role in RAN-translation regulation. Furthermore, the downregulation of the kinase mitigated toxic effects in MN-iPSCs, supporting its potential as a therapeutic strategy. These findings suggest that targeted ASO approaches on RNA translation can attenuate the pathological consequences of *C9ORF72* HRE and provide a promising direction for disease-modifying therapies in FTD/ALS spectrum disorders.

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TARGETING MITOCHONDRIAL DYSFUNCTION IN LYSOSOMAL STORAGE AND OTHER NEURODEGENERATIVE DISORDERS

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Growing evidence implicates mitochondrial dysfunction as a key driver of neurodegeneration in several diseases, including the "juvenile Alzheimer's" Niemann Pick C1 (NPC1)[2], a cholesterol lysosomal storage disorder, where inflammation contributes to neuronal loss[3].
Our findings indicate that mitochondrial cholesterol depletion impairs respiratory complexes and elevates ROS[2], partly *via* downregulating *Mnrr1* and antioxidant genes. *Mnrr1* (*Chchd2*) is a key regulator of organelle function[4], acting in mitochondria to control metabolism and apoptosis, and in the nucleus to activate stress-responsive genes, including itself. Its deficiency phenocopies NPC1 pathology by reducing oxidative metabolism, ATP production, and cell growth while increasing ROS. Moreover, *Mnrr1* is required for mitophagy and the mitochondrial unfolded protein response. *Mnrr1* overexpression reduces cholesterol accumulation and improves mitochondrial function in cells harboring common NPC1 mutations.

We are investigating the impact of drug-induced Mnrr1 overexpression in cellular models of NPC1 disease because we hypothesize that increased Mnrr1 expression could reduce neuroinflammation and reverse metabolic deficits caused by cholesterol dyshomeostasis. Our data show that the metabolic modulator nitazoxanide (NTZ) improves mitochondrial function by stabilizing organelle membrane potential, reducing ROS generation, and optimizing oxidative phosphorylation[5;6]. Additionally, NTZ significantly raises CoxIV expression and recovers Mnrr1 protein levels, with a noticeable perinuclear accumulation suggesting enhanced mitochondrial integrity and function. Meanwhile, NTZ decreases mTorc1, Ampk-α, and p62 protein expression, suggesting a coordinated modulation of autophagy-regulating pathways that may aid in restoring organelle quality control. Thus, NTZ seems to be a mitochondrial resilience enhancer that may repair critical quality control and bioenergetic systems seriously impaired in NPC1.

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MUSCLE SIGNATURE CORRELATES WITH MOTOR IMPAIRMENTS IN FXS

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Individuals with Fragile X Syndrome (FXS) often show difficulties in coordinating motor skills, likely due to impaired balance and gait deficits. While over the past years it became evident and recognized by clinicians that individuals with FXS have motor problems, how impairments in the CNS of individuals with FXS contributes to those motor deficits at the cellular and molecular level is still largely unexplored. Here, we demonstrate that juvenile Fmr1 KO mice have shorter latency to fall in the rotarod test compared to wild-type (WT) mice, indicating impaired motor coordination.

Furthermore, Fmr1 KO mice exhibit a higher frequency of slips when tested in the ladder rung, corroborating the observed deficits in motor balance and coordination. Unexpectedly, Fmr1 KO mice show increased grip strength compared to WT mice.

We next performed a molecular profiling of the muscle fibers in Fmr1 KO mice and detected altered expression patterns of glycolytic muscle fibers in the tibialis anterior specifically during a developmental window.

Our findings indicate that muscle signature is developmentally regulated in FXS and correlates with motor imbalance, providing the first molecular insights into the neurobiological mechanisms underlying motor manifestations in FXS.

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ADIPOCYTE-DERIVED EXTRACELLULAR VESICLES AS NEW MODULATORS OF MELANOMA PROGRESSION

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Melanoma is an aggressive cancer characterized by a rapid metastatic process. Thus, understanding the mechanisms underlying its progression is urgently needed to improve patient outcomes. In this regard, there is consistent evidence of a tumor-sustaining crosstalk between melanoma and subcutaneous adipose tissue; however, the role of extracellular vesicles (EVs) in this communication still needs to be clarified. We demonstrated that the EVs derived from adipocytes did not alter melanoma cell proliferation but significantly promoted tumor cell migration and invasion by determining an enrichment in mesenchymal markers, such as N-cadherin and vimentin. In particular, these changes were accompanied by the transition towards a stem-like phenotype, characterized by enhanced spherogenic ability and ABCG2 upregulation. Interestingly, this led to a reduced response to vemurafenib, with diminished apoptotic rates and decreased caspase 3 and PARP cleavage. Mechanistically, an increase in PGC-1α expression was found, resulting in higher mitochondrial mass and activity and ROS overproduction; of note, treatment of melanoma cells with XCT790 and SR-18292, two specific inhibitors of mitochondrial biogenesis, successfully counteracted the above EV-related effects, suggesting that this process could be targeted to suppress the EV-mediated interactions between subcutaneous adipocytes and melanoma. Taken together, these results highlight the crucial role played by EVs in melanoma stroma, highlighting the ability of adipocyte-derived vesicles to sustain melanoma cell aggressiveness via PGC-1α activation.

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NEW ROLE OF PROTEIN MISFOLDING CORRECTOR IN THE INFLAMMATORY PROCESS: POSSIBLE THERAPEUTIC INDICATION IN NEURODEGENERATIVE DISEASES

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Proteins with aberrant structures that lose their functionality and result in tissue and cellular dysfunction, are the hallmark of protein misfolding disorders. Neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and Huntington's disease, share a common etiopathogenesis based on the presence of misfolded proteins that fold autonomously in neuronal cells, triggering inflammation and cell death. Misfolded protein buildup results in ER stress, which modifies Ca^{2+} homeostasis. This chronic stressor triggers NF-kB pathway activation and ER-resident procaspase 4 cleavage, which results in inflammatory reactions and cell death. In this study, the corrector Vx-445 (Elexacaftor) activity, employed in the pharmaceutical treatment of cystic fibrosis, was assessed on human adenocarcinomic basal alveolar epithelial and neuronal cell lines, in which a reticular stress condition was generated by Thapsigargin.

The aim is to test whether the corrector can reduce ER stress by restoring the folding of misfolded proteins and reducing the inflammatory process triggered by these events. Therefore, IkBα, p-STAT 3 and COXII protein levels were analyzed by flow cytofluorimetry, while Ca^{2+} content was assessed by spectrofluorimetry. Results obtained suggest a significant involvement Vx-445 in restoring cellular homeostasis with consequent reduction of proteins expressed in inflammatory conditions, such as IL-6, tested by ELISA assay. Therefore, even if our theories are still preliminary, they encourage further research aimed at evaluating Vx-445 as a potential drug for the treatment of inflammatory disease related to protein misfolding.

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MAPPING NEURONAL NETWORK IN ANGELMAN SYNDROME – THE ROLE OF UBIQUITIN-PROTEIN LIGASE E3A IN SHAPING SYNAPSES

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Neuronal maturation and plasticity are manifested through dynamic changes in synaptic function and structure. Morphological changes of the pre- and postsynapse reflect its activity and strength. In excitatory synapses, size and shape of dendritic spines are finely regulated during development and experience-dependent remodelling of the neuronal network. Indeed, early and late onset neurological conditions arise as consequences of mutations in genes accounting for synapse maturation and plasticity.

Genomic alterations leading to unbalanced expression of the Ubiquitin Ligase-E3A (UBE3A) cause defects in brain development, leading to neurodevelopmental disorders. Increased expression/activity of UBE3A in neurons is associated to the appearance of autistic-like phenotypes, while its reduction due to the loss of the maternal copy of UBE3A gene (paternally imprinted in neurons) is causing Angelman Syndrome (AS). AS is a rare neurological disorder affecting around 1:20.000 newborn. Features of AS include delayed development, severe learning difficulties, little or no speech, movement and balance impairments, seizures. Most of these symptoms are likely originating from synaptic defects.

To clarify whether neuronal dysfunctions and synaptic alterations in AS are due to a disrupted neuronal network wiring or if they have a cell-autonomous origin, we took advantage of two different mouse models: 1) UBE3A-KO mice in which all neurons are lacking the expression of the ligase, and 2) *in utero*-electroporated mice in which a subset of pyramidal neurons of layer 2/3 of the somatosensory cortex is Ube3a-depleted with CRISPR/Cas9 mediated genome editing.

Synaptic connectivity of excitatory neuronal circuits in AS models were characterized and mapped using advanced imaging technologies as volume Electron Microscopy (vEM) and volume Correlative Light and Electron Microscopy (vCLEM).

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MIR-126-3P FROM EXTRACELLULAR VESICLES PROMOTES RENAL INFLAMMAGING THROUGH SRY (SEX-DETERMINING REGION Y)-BOX 2 (SOX2) IN ACUTE RESPIRATORY DISTRESS S

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Acute Respiratory Distress Syndrome (ARDS) is a severe inflammatory condition characterized by pulmonary epithelial injury and systemic organ dysfunction. Murine studies have revealed a key role of alveolar extracellular vesicles (EVs) in systemic injury, especially in the kidneys. This study elucidates the biological mechanisms by which ARDS-derived EVs can damage renal tubular and endothelial cell.

We enrolled 35 ARDS patients undergoing lung-protective ventilation. EVs were isolated from plasma using ultracentrifugation, characterized through Nanoparticle Tracking Analysis (NTA), and immunophenotyped with MACSplex exosome beads. MicroRNA profiling of ARDS-EVs was performed by TaqMan® Array Human MicroRNA Cards.

To assess the biological effects of ARDS-EVs, we exposed renal proximal tubular epithelial cells (RPTECs) and HUVECs to isolated EVs (5^e+4 EV/ target cells for 24 and 48 h). The role of miR-126-3p was studied using siRNA-mediated knock-down.

NTA analysis enabled stratification of patients into three groups based on EV concentration: low (P25), medium (P50), and high (P75). EV concentration correlated significantly with parameters of ventilator-induced lung injury, including respiratory rate ($R^2 = 0.47$, $p < 0.001$) and the incidence of Acute Kidney Injury. In RPTEC, EVs from higher concentration groups (P50 and P75) arrested cell cycle progression, induced senescence as assessed by SA-βGAL staining, Klotho downregulation, and p16INK4a upregulation ($p<0.05$), without promoting apoptosis (Annexin V/PI).

miRNA profiling revealed upregulation of miR-21-5p, miR-126-3p, miR-27a-5p, and miR-223. In vitro, exposure to EVs from P50 and P75 groups significantly reduced SOX2 gene, a transcription factor from the SRY-box family that is crucial for cell fate determination, whereas inhibition of miR-126-3p restored SOX2 to baseline levels. IPA analysis linked the identified miRNAs to profibrotic pathways involving TGFβ/ERK/Smad signaling.

In conclusion, EVs from ARDS patients promote tubular cell senescence by miR-126-3p that contributes to renal aging and fibrosis by suppressing SOX2 expression.

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HOLOCLAR LESSONS: BIOLOGICAL AND CLINICAL INSIGHTS FROM AUTOLOGOUS CULTIVATED LIMBAL STEM CELLS TRANSPLANTATION FOR REGENERATING CORNEAL EPITHELIUM

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The autologous cultivated human stem-cell based limbo-corneal epithelium is the first regenerative advanced therapy medicinal product in ophthalmology globally, and it is now a therapy with proven safety and efficacy across various regulatory frameworks available worldwide for limbal stem cell deficiency patients. In Europe is Holoclar, applied in 4 studies compliant with current regulatory rules. These include two pan-European prospective studies (HOLOCORE and HOLOCORE FOLLOWUP) on 60 patients, monitored for 1 and 6 years, respectively; a real-world evidence PASS "registry like" 5-year study on commercially-treated patients (HOLOSIGHT); and a 20-year study on patients treated previously (HOLOUP). The critical and integrate analysis of these results has provided valuable biological insights. First, the expanded clinical experience has allowed for identification of the optimal biopsy to achieve an effective therapy. Then, optimization of biological and clinical procedures has enabled the reduction of the minimum required p63 bright stem cells within the cultivated limbal graft (previously set at 3% based on statistical correlations with clinical efficacy in real-world evidence) to 2%. Together with restoring tissue architecture and proper cellular localization, the transplanted cultivated epithelium produced multiple paracrine effects, steadily influencing the surrounding microenvironment with various outcomes. These included partial reabsorption of stromal opacities (suggesting an effect on keratocytes), promotion of physiological wound healing, as evidenced by full success following stromal substitution, regression of neovascularization, maintenance of lacrimal secretion, and improved visual acuity. In conclusion, this evidenced-based acquired knowledge is key for a deeper understanding of the system's biology and for the advancement of future cell therapies.

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REDOX IMBALANCE AND IRON DEFICIENCY COOPERATE TO INDUCE PSORIASIS-LIKE FEATURES IN KERATINOCYTES

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Redox homeostasis plays a critical role in regulating keratinocyte de-differentiation and inflammation, two key hallmarks of the psoriatic phenotype. Iron, a regulator of cellular redox balance, may influence these processes; however, its specific role in psoriasis remains incompletely understood. To this, we perturbed redox status in HaCaT cells, an immortalized human keratinocyte line, using the ferroptosis inducer RSL3 (10 μM, 24h). RSL3 inhibited GPX4 activity, triggered ROS accumulation and lipid peroxidation, and caused ~50% cell death, as assessed by Western blotting, PI and BODIPY-C11 flow cytometry assays, respectively. RSL3 activated the p-NF-κB pathway and upregulated multiple pro-inflammatory genes (IL-6, IL-8, TNF-α, DEFB3), along with a keratin expression switch from KRT1 to KRT17, an established marker of de-differentiation. All these effects were abrogated by co-treatment with either the ferroptosis inhibitor ferrostatin-1 (100 μM) or the IL-17A neutralizing antibody Taltz (1 μM), a clinically approved therapy for psoriasis. Unexpectedly, RSL3 also decrease the intracellular labile iron pool (LIP). Hence, to further investigate the role of iron in psoriatic-like responses, HaCaT cells were treated with either ferric sodium gluconate (Ferlixit, 100 μM, 24h) or the iron chelator deferoxamine (DFO, 200 μM, 24h). Ferlixit did not affect LIP or ROS levels, indicating effective buffering of iron without oxidative stress induction. Ferlixit reduce pro-inflammatory genes without altering keratinocyte differentiation. In contrast, DFO mimicked RSL3 effects, increasing pro-inflammatory genes and promoting the KRT1-to-KRT17 shift. Overall, iron deficiency promotes a psoriatic-like phenotype in keratinocytes by enhancing inflammatory signaling and disrupt differentiation programs, pointing to iron homeostasis as a potential therapeutic target in psoriasis.

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FABP5 IS A KEY PLAYER IN METABOLIC MODULATION AND NF-KB DEPENDENT INFLAMMATION DRIVING PLEURAL MESOTHELIOMA

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Pleural mesothelioma (PM) is a rare but highly aggressive cancer, known for its resistance to therapy and poor clinical outcomes. Metabolic reprogramming and chronic inflammation are recognized as key drivers of tumor progression, yet the molecular mechanisms connecting these processes in PM remain poorly defined. In this study, we identify fatty acid-binding protein 5 (FABP5) as a central regulator linking lipid metabolism and NF-κB-mediated inflammatory signalling in mesothelioma cells.

We first characterized the metabolic and inflammatory profiles of PM cells compared to normal mesothelial cells. PM cells showed increased proliferation, altered cell cycle progression, resistance to apoptosis, and significant accumulation of saturated and unsaturated fatty acids. These changes were accompanied by elevated mitochondrial mass, disrupted ADP/ATP and NAD+/NADH ratios, and increased production of reactive oxygen species (ROS), indicating extensive bioenergetic remodelling.

FABP5 expression was markedly upregulated in PM cells. Silencing FABP5 impaired several cancer-associated hallmarks, including reduced proliferation, enhanced apoptosis, altered mitochondrial bioenergetics (as shown by changes in ADP/ATP and NAD+/NADH ratios), and decreased lipid droplet accumulation and phospholipid biosynthesis enzyme expression. Furthermore, FABP5 knockdown inhibited NF-κB activation and reduced the expression of key NF-κB-dependent pro-inflammatory genes, establishing a direct connection between FABP5 activity and the inflammatory tumor microenvironment.

Collectively, these findings position FABP5 as a key orchestrator of the metabolic and inflammatory pathways that sustain PM aggressiveness. Therapeutically targeting

FABP5 could simultaneously block tumor metabolic adaptations and dampen the chronic inflammation driving disease progression. This dual approach offers promising opportunities for the development of innovative metabolic and immunomodulatory therapies for pleural mesothelioma.

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IRON METABOLIC PLASTICITY AND STEM-LIKE FEATURES SHAPE CLASS IV FERROPTOSIS INDUCERS SENSITIVITY IN OVARIAN CANCER CELLS

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Cancer stem cells (CSCs) drive ovarian cancer (OVCA) aggressiveness, contributing to recurrence, relapse, and resistance to therapy. Iron supports CSC survival and self-renewal but also promotes oxidative stress, making ferroptosis a promising therapeutic target in OVCA. Here, we investigated the effects of iron-based class IV ferroptosis inducers (FINs) in four OVCA cell lines (KURAMOCHI, PEO1, COV362, PEA1) cultured in 2D, and 3D conditions to enrich CSCs. In 3D, KURAMOCHI and PEO1 cells displayed resistance to anoikis and upregulation of CSC markers (*NANOG, OCT4, CD44, ALDH1A1*), as shown by qRT-PCR. These features were accompanied by increased iron demand, evidenced by reduced labile iron pool (LIP), measured using the FerroOrange assay, and enhanced levels of the iron importer CD71 and the iron storage protein FtH1, assessed by Western blotting. In contrast, 3D COV362 and PEA1 cells displayed fewer CSC features, reduced FtH1, increased LIP, lipid peroxidation, and ferroptosis (˜40% PI^+ cells). These findings highlighted two OVCA subgroups with distinct iron-handling phenotypes: a CSC-like, iron-adaptive group and a non-CSC, ferroptosis-prone group with limited iron plasticity. Then, we tested class IV FINs (Ferlixit or FAC, 100 μM, 24h) in both OVCA cell types. 2D KURAMOCHI and PEO1 cells adapted to iron overload by downregulating CD71, maintaining iron homeostasis. Conversely, COV362 and PEA1 cells, with less flexible iron metabolism, underwent ferroptosis. Notably, all 3D-cultured cells, instead, tolerated iron overload, likely due to enhanced iron buffering in CSC-enriched populations. Finally, we found that combining class IV FINs with GPX4-targeting FINs restored ferroptosis sensitivity in CSC-like OVCA cells. These results suggest that ferroptosis-based therapies should be stratified according to the CSC and iron-handling profiles of OVCA subpopulations.

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INDUCED MITOCHONDRIAL DEFICIT BY NDUFS3 TRANSIENT SILENCING IN PANCREATIC CANCER CELLS REDUCES RAB7 EXPRESSION AND CAUSES LYSOSOMAL IMPAIRMENT

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RAB7 is a small GTPase with multiple roles in the cell, among which the regulation of late endocytic trafficking, lysosomal biogenesis, mitochondria-lysosomes crosstalk, and the contribution to several mitochondrial processes. Mitochondrial metabolism and dysfunctions are widely studied in cancer and in Pancreatic Ductal Adenocarcinoma (PDAC), one of the deadliest cancers worldwide, in which metabolism is described as highly heterogeneous. Cancer therapeutic strategies targeting mitochondrial function have gained significant attention in recent years. Moreover, mitochondrial impairment can alter the crosstalk between mitochondria and lysosomes. Here, we used PDAC cell models to induce a transient mild mitochondrial deficit by downregulation of a subunit of mitochondrial Complex I, named NDUFS3, to investigate the consequences on RAB7 and the late endocytic pathway and, thus, the contribution of the mitochondria-lysosomes communication alterations to PDAC progression. Our results showed that *NDUFS3* transient silencing caused not only mitochondrial deficit, slower oxidative metabolism, and mitochondrial morphology alterations but also RAB7 downregulation and impairment of the late endocytic pathway. In addition, *NDUFS3*-silenced RAB7-downregulated cells showed less invasive tumorigenic potential, revealed by reduced levels of vimentin and other Epithelial-to-Mesenchymal Transition proteins, decreased viability, migration, and invasiveness. In this context, the modulation of RAB7 expression causes the restoration of vimentin levels, mitochondrial morphology, and mitochondrial proteins. Overall, our data show that in the PDAC background, mitochondrial deficit determines alterations of the crosstalk with lysosomes, leading to dysfunctions, and that this process is regulated by RAB7, highlighting the synergic role of RAB7 and mitochondrial dysfunction in cancer.

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REPOSITION OF SALINOMYCIN: EVALUATION OF ITS CELL DEATH PROMOTION IN GASTRIC CANCER

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Salinomycin, usually used as an anti-coccidial drug, has recently been shown to possess anti-cancer activity. Based on these evidence, we aimed to evaluate the effect of salinomycin on gastric cancer (GC) going deeper the type of cell death induced. We investigated the salinomycin effects on four GC cell lines characterized by a different sensitivity to cisplatin, a common first-line chemotherapeutic. We performed a dose-response MTS assay at 24h, 48h and 72h, based on the doubling time of all cell lines, to estimate the IC50. This assay highlighted a significant viability reduction for all cell lines in a dose and time dependent manner. The cell line specific IC50 values were then used for the assessment of different type of cell death by flow cytometry, western blotting and immunofluorescence. Interestingly, we observed a cell line specific death mechanism. In particular, two cell lines, NCI-N87 and SNU1, showed a significant activation of apoptosis. The AGS and KATO-III cell lines, instead, were characterized by an increase of reactive oxygen species production that prompted us to hypothesize a possible ferroptosis involvement. Measuring the alteration levels of specific markers, after salinomycin treatment, we confirmed a ferroptosis contribution in these cell lines. Notably, we observed that autophagy represented a cell death mechanisms common to all cell lines, strengthening the assumption that autophagy interacts with and affects the complex cell death machinery. At last, given the known effect of salinomycin against cancer stem cells, we also evaluated the alteration of CD44 and CD133, two recognized gastric cancer stem cell markers. We demonstrated a marked reduction of the cell fractions positive to these markers. Our preliminary data suggested a translational potential of salinomycin toward clinical application in GC.

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SINEUP TARGETING PRPF31 FOR RETINITIS PIGMENTOSA 11

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Retinitis pigmentosa (RP) is the most common inherited retinal disorder. Loss-of-function mutations in PRPF31 gene mainly cause RP11, and it is characterized by progressive degeneration of photoreceptors. The main aim of this project is to test a novel therapeutic approach using SINEUPs, a new functional class of natural antisense long non-coding RNAs that specifically increase protein levels of the target mRNAs by promoting the translation.

In this study, we screened a panel of SINEUP targeting human PRPF31 in a cell line of retinal pigmented epithelium (RPE). We found that two SINEUP significantly increased PRPF31 protein expression in RPE cell lines and rescued defective ciliogenesis present in heterozygous cells. The two state-of-the-art models for RP11 are primary RPE cells and retinal organoids (ROs) derived from patients' induced pluripotent stem cells (iPSCs). To generate patients'-derived RPE, we isolated CD34+ cells from asymptomatic carriers, healthy individuals, and RP11 patients and reprogrammed them into iPSCs.

Then we differentiate them in primary RPE. ROs from both healthy and RP11 patients were infected with AAV2 expressing either SINEUP-PRPF31 or PRPF31 cDNA and transcriptomic and proteomic analysis were performed.

Our study demonstrates the potential of SINEUPs as a novel therapeutic strategy for RP11. By targeting the PRPF31 gene, SINEUPs significantly increase protein expression and rescue defective cellular processes, such as ciliogenesis, in RPE cells. Additionally, transcriptomic analysis revealed that SINEUP-PRPF31 treatment restores the expression of most dysregulated genes in RP11 cells, suggesting a broad therapeutic impact. The use of patient-derived induced pluripotent stem cells and retinal organoids in this study provides a relevant in vitro model to further investigate the efficacy of SINEUPs in a clinically relevant context.

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THE TWO FACES OF EPIGENETIC SIGNATURES: DISEASE MONITORING AND THERAPEUTIC TARGETS FOR HEMATOLOGICAL CONDITIONS

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Main epigenetic mechanisms include DNA methylation, histone modifications and microRNAs. Given their pivotal role in gene expression regulation, it is not surprising that they are frequently dysregulated in human diseases, including both rare genetic disorders and multifactorial diseases such as cancer. Conversely to genetic abnormalities, epigenetic alterations are not permanent offering promising tools in the context of personalized therapy, both as signatures of treatment response and monitoring of disease and as druggable targets.

Our research work fits into this pioneering field, studying this dual perspective of epigenetic changes in haematological disorders.

One goal addresses the need to identify molecular signatures to discriminate responders from non-responders to conventional and innovative CAR-T therapies for blood cancers. Bone marrow aspirates from an explorative group of patients with different clinical outcomes were subjected to a genome-wide methylation analysis. To monitor DNA methylation patterns across the clinical iter, we are currently planning to collect peripheral blood samples from the same cohort at subsequent time points.

In a second aim, we are exploring the potential use of epigenetic drugs for iron overload conditions such as beta-thalassemia. Plasma and serum samples from 75 beta-thalassemia patients and controls were collected and subjected to a strict quality control procedure before evaluating a panel of miRNAs involved in iron homeostasis and ferroptosis by digital PCR. Artificial intelligence will prioritize the most

promising miRNA candidates for the design of anti-miRNA oligonucleotides to be tested in relevant cellular models of iron dysregulation.

These ongoing projects will contribute to a better understanding of epigenetic alterations in these blood diseases also proving their utility as precision medicine tools.

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EVALUATION OF NICOTINAMIDE LEVELS IN AQUEOUS HUMOR AND PERIPHERAL BLOOD MONONUCLEAR CELLS IN GLAUCOMA PATIENTS: CORRELATIONS WITH THE SENESCENCE.

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Glaucoma is a multifactorial neurodegenerative disease characterized by the progressive and irreversible loss of retinal ganglion cells (RGCs). While lowering intraocular pressure (IOP) remains the only proven therapeutic strategy to slow disease progression, many patients continue to experience visual field deterioration despite IOP values within normal limits. Emerging evidence suggests a link between glaucomatous conditions, cellular senescence and mitochondrial damage. The senescence of retinal ganglion cells (RGCs) may contribute to the pathogenesis of glaucoma. Nicotinamide (NAM), a precursor of nicotinamide adenine dinucleotide (NAD+), plays a critical role in cellular energy metabolism and mitochondrial function. Preclinical studies have shown that NAM supplementation may protect RGCs from degeneration, suggesting a potential role in neuroprotection. To date, no study has evaluated NAM levels in ocular fluids of patients with glaucoma at different disease stages. This bicentric, case-control study aims to quantify NAM concentrations in aqueous humor and PBMC of patients with open-angle glaucoma (OAG), both pharmacologically controlled and uncontrolled, as well as healthy controls undergoing cataract surgery. Preliminary results indicate that patients with uncontrolled OAG (Group 1) show lower levels of NAM in the aqueous humor and PBMC and an increase in the senescence markers p21 and p16 compared to both pharmacologically compensated patients (Group 2) and healthy subjects (Group 3). This evidence supports a correlation between NAM depletion, cellular senescence, and glaucoma severity. Taken together, our data suggest that NAM may serve not only as a biomarker of mitochondrial dysfunction and disease progression, but also as a promising target for neuroprotective interventions aimed at preserving retinal ganglion cell integrity in glaucoma.

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OFF-TARGET EVALUATION OF CP ON ANIMAL CELLS: EVIDENCE OF SAFETY AND IMMUNOMODULATORY ACTIVITY

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Cerato platanin (CP) is a small protein (120 amino acids) from the Cerato-platanin family, secreted by the plant pathogenic fungus *Ceratocystis platani*. It is currently under investigation in a PRIN-PNRR project for its potential use as a selective bioherbicide. The project is based on the innovative concept of selectively activating the immune system of weed species without triggering responses in crop plants, thereby enabling targeted weed control with minimal impact on crop.

Treatment of seeds from the weed species *Lolium multiflorum*, *Digitaria sanguinalis*, and *Amaranthus hybridus* with CP resulted in reduced shoot and/or root elongation during germination, while seedlings of *Triticum aestivum* (wheat) remained unaffected, indicating species-specific sensitivity.

To evaluate biological safety, CP was tested on various human cell lines, including colonic adenocarcinoma (Caco-2), cardiomyocytes (AC16), ovarian epithelial cells (A2780) and primary fibroblasts, and murine cell lines such as macrophages (RAW 264.7) and kidney epithelial cells (Renca). No significant cytotoxicity was observed, even at CP dose higher than those used in plant experiments. Interestingly, CP induced measurable antioxidant and anti-inflammatory effects in RAW 264.7 cells, suggesting immunomodulatory properties also in animal cells.

These results confirm the absence of mammalian cytotoxicity and support the continued development of CP as a safe and selective bioherbicide. Furthermore, they demonstrate the feasibility of exploiting species-specific immune responsiveness for precision-targeted weed management.

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IN SILICO AND IN VITRO STUDIES OF NOVEL LIGANDS FOR IL-20 RECEPTOR A WITH ANTIPROLIFERATIVE PROPERTIES

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Interleukins (ILs) are key players in tumour microenvironment (TME) cytokine network contributing to the growth and development of tumour progression. Recent studies revealed the involvement of IL-20 receptor subunit alpha (IL-20RA) signalling in several cancers, including triple-negative breast cancer (TNBC), in which IL-20RA is highly expressed influencing proliferation, cell death, invasiveness and TME activity. This study aimed to elucidate the role of the IL-20RA as a new potential therapeutic target and to identify selective bioactive ligands able to affect IL-20RA activity. After validating IL-20RA signalling pathway in two TNBC *in vitro* cell lines, virtual screening of over 310,000 compounds from both DrugBank and ZINC15 databases identified four hit potential compounds tested against TNBC cell lines. Notably, a well-known Human Immunodeficiency Virus Type 1 (HIV-1) protease inhibitor Ritonavir, significantly inhibited cell proliferation (about 40% at 50 μM, $p<0.001$). At variance, for the other ligands the antiproliferative effect did not appear to be linear and dose and time dependent. To understand whether the activation of IL-20RA was responsible for the Ritonavir-cytostatic effect, TNBC cells were preincubated with IL-20 followed or not by treatment with Ritonavir counteracting Ritonavir's growth-inhibiting action. In addition, IL-20RA knockdown restored significantly the proliferation in Ritonavir-treated TNBC cells. In conclusion, these findings demonstrated that Ritonavir affects the proliferation of TNBC cells influencing IL-20RA activity and suggested its potential repurposing as a therapeutic agent for TNBC management. Further studies in *in vivo* models will be needed in order to study Ritonavir's anticancer application, including the management of TNBC and other tumours characterized by high levels of IL-20RA expression.

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ACETYLCHOLINE PROMOTES THE AGGRESSIVENESS OF LNCAP PROSTATE CANCER CELLS VIA A MECHANISM INVOLVING GLYOXALASE 1/MG-H1 PATHWAY AND OSTEOPONTIN

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The neurotransmitter acetylcholine (ACh) plays a pro-tumorigenic role in various types of cancer, including prostate cancer (PCa). However, our understanding of how ACh promotes tumor growth in PCa is still limited. Glyoxalase 1 (Glo1) is an enzyme involved in metabolism that removes methylglyoxal (MG), a byproduct of glycolysis and a potent post-translational modifier, producing a major advanced glycation end-product, called MG-H1. The Glo1/MG-H1 pathway is linked to the development and progression of PCa. In our study, by using LNCaP and PC3 cells, which model poorly aggressive and bone metastasis PCa, respectively, we found that ACh specifically enhances the proliferation, migration and invasion of LNCaP cells through a mechanism inducing Glo1 enzyme specific activity inhibition, that leads to MG-H1 accumulation and osteopontin (OPN) upregulation. These findings uncover a previously unknown process driven by ACh that contributes to PCa advancement. This opens new possibilities for *in vivo* studies to understand how ACh-driven Glo1/MG-H1 and OPN influence cancer progression, and could lead to new strategies for managing PCa, with the goal of preventing it from becoming incurable.

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NEW INSIGHTS FOR THE ENDOCANNABINOID SYSTEM (ECS) IN GERM CELL PROGRESSION: A COMPARATIVE STUDY IN MALE MICE

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The endocannabinoid system (ECS) – comprising of endogenous ligands, membrane transporters, cannabinoid receptors, and the enzymes responsible for the synthesis and degradation of ligands - actively participates in spermatogenesis regulation. The endocannabinoids anandamide (AEA) and 2-arachydonoylglicerol (2-AG) act as endogenous ligands for their cognate type 1 (CB1) and type 2 (CB2) cannabinoid receptors, both expressed in testis. Interestingly, a decreasing CB2 and 2-AG metabolizing enzyme gradient from spermatogonia (SPGs) -to- spermatocytes (SPCs) and spermatids (SPTs) suggested a polarized 2-AG tone at the basal compartment of seminiferous epithelium involved in the correct temporal progression of spermatogenesis. Thus, any pathophysiological condition promoting adverse effects on ECS components conceivably impairs germ cell activities.

Here, using wild type (WT) vs CB1 knock out ($CB1^{-/-}$) we characterized CB1-loss dependent effects on testis ECS and germ cell progression. Our results show that CB1 deletion affects the expression levels of some ECS components, namely the 2-AG hydrolysing monoacylglycerol-lipase (MAGL) enzyme. In agreement, gene expression analysis of germ-cell specific markers revealed that CB1 deletion decreased SPG, while increasing SPC and SPT content. Male mice exposed to i) bisphenol-A (BPA) and ii) high-fat diet (HFD), here chosen as poor-quality spermatogenesis experimental models, were used to corroborate the central role of ECS in modulating germ cell progression. Conversely to $CB1^{-/-}$ model, BPA and HFD exposure significantly decreased the expression of MAGL, while CB1 and CB2 protein increased. Accordingly, a significant increase of SPG content, associated to SPC and SPT down-regulation, was observed.

Our results define a mutual regulation among the ECS components in the testis, providing a new intriguing role for the ECS in the signaling pathway involved in the control of male germ cell progression.

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MC1R-LY6G6D INTERACTION IN MELANOMA: GENETIC INSIGHT AND FUNCTIONAL IMPLICATIONS

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Cutaneous melanoma (CM) is an aggressive type of tumor of the skin, with poor prognosis for patients with metastatic disease. While UVR-induced DNA damage is the major CM driver, including the profound change in methylation profile, genetic factors also play a role. MC1R, a G-coupled receptor (GPCR) with high affinity for α-melanocyte stimulating hormone (α-MSH), regulates pigmentation and non-pigmentary functions, including DNA repair. Polymorphisms in MC1R (R, r) are among the most well-established genetic risk factors for CM. Analysis of RNA-seq data from sun-exposed (SE) and non-exposed (NE) skin revealed UVR significantly reduces LY6G6D expression in $MC1R^{wt/wt}$ compared to NE (p=0.0004). However, among SE individuals, those $MC1R^{wt/p.D294H}$ (melanoma associated R- allele) exhibit a significant upregulation of LY6G6D expression compared to $MC1R^{wt/wt}$ (p=3.2e-5), indicating the p.D294H variant disrupts LY6G6D regulation by UVR. LY6G6D, a LY6/uPAR superfamily membrane protein, implicated in inflammation, is still under investigation. To further explore the functional implications of LY6G6D expression, we investigated its relationship with T cells (GEPIA2-database). In NE skin, naive T cells showed a positive correlation with LY6G6D expression (p=2.7e-10; R=0.4). This correlation was weaker in SE (p=0.00026; R=0.2), indicating that UVR disrupts this association. Analysis of LY6G6D expression in CM and normal tissues revealed a trend of downregulation in CM. However, when examining LY6G6D expression across different CM-stages (I-IV), an upregulation trend was observed, especially in stages II-III. These findings collectively suggest a potential protective role for MC1R mediated through the LY6G6D-axis. Therefore the compromised skin response to sunlight, driven by the disrupted MC1R-LY6G6D methylation profile, would represent the mechanism contributing to CM-development.

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UNVEILING THE ROLE OF EXTRACELLULAR VESICLE MIRNAS IN HCC PATHOGENESIS AND THERAPY

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Hepatocellular cancer (HCC) is the sixth most common tumor worldwide. The main etiologies include Hepatitis virus B (HBV), Hepatitis virus C (HCV), metabolic disfunction associated fatty liver disease (MASH) and excessive alcohol consumption. Although several biomarkers have already been proposed for the diagnosis of hepatocellular carcinoma (HCC), the search for novel biomarkers is a priority. In this context, circulating extracellular vesicles (EVs) are now recognized as critical mediators of intercellular communication. Biomarkers such as microRNAs (miRNAs) represent a promising tool.

EVs were isolated from the serum of cirrhotic patients with and without HCC. Patients in each category were further stratified into four groups based on their underlying etiology: HBV, HCV, MASH, and alcohol-related (ALC). EVs were analyzed via nanoparticle tracking analysis and characterized by immunoblotting. A multiplex bead-based approach coupled with flow cytometry was then used to assess the immunological profile of serum-derived EVs. Next, EV-miRNAs were extracted and sequenced to identify differentially expressed miRNAs between HCC and non-HCC samples. Bioinformatic analyses were performed to identify key dysregulated miRNAs. Candidate EV-miRNAs were validated by digital PCR (dPCR).

Our results revealed a distinct EV-miRNA signature differentiating HCC patients from non-HCC individuals. Functional studies demonstrated that altered miRNAs influence key oncogenic pathways, including proliferation, invasion, and inflammatory response. Notably, specific EV-miRNAs were associated with critical molecular mechanisms underlying HCC pathogenesis, reinforcing their potential as both diagnostic biomarkers and therapeutic targets. This study highlights the potential

of EV-derived miRNAs as non-invasive biomarkers for HCC diagnosis and monitoring, as well as promising therapeutic targets.

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ALPHA-1 ANTITRYPSIN PROMOTES EPITHELIAL MESENCHYMAL TRANSITION AND APOPTOTIC RESISTANCE IN PLEURAL MESOTHELIOMA CELLS

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Alpha-1 antitrypsin (AAT) is a secreted protein encoded by the SERPINA1 gene that plays a role in cell survival and tissue protection by counteracting programmed cell death in different physiological contexts. However, AAT uptake in cancer cells leads to apoptosis resistance and to enhance migration. In our previous study we found that pleural mesothelioma (PM) cell lines of different histotypes, such as epithelioid, biphasic and sarcomatoid, over-expressing AAT increased vitality and proliferation compared to PM cells expressing AAT at low level, suggesting a potential AAT involvement in PM tumorigenesis. In this study we evaluated if AAT could affect Epithelial Mesenchymal Transition (EMT) and apoptosis resistance of PM cells. To this purpose, PM cells (i) overexpressing or not AAT were subjected to Real-time PCR to analyze the expression of EMT-related genes, or (ii) treated with exogenous AAT to assay apoptosis by flow cytometry using Annexin V and propidium iodide staining. A decreased expression of the epithelial genes E-cadherin and desmoplakin, and an increased expression of the mesenchymal genes N-cadherin, vimentin, fibronectin-1, and matrix metalloprotease-9 were observed in epithelioid PM cells overexpressing AAT, while the opposite effect occurred in silenced-AAT PM cells, suggesting AAT a player of epithelial mesenchymal transition. PM cells co-treated with exogenous AAT and the pro-apoptotic agent staurosporine showed a lower percentage of apoptotic cells compared to PM cells treated with staurosporine alone, or to untreated PM cells. Overall, our data indicate AAT involvement in epithelial-mesenchymal transition process and apoptotic resistance of PM cells, suggesting this protein may play a role in PM onset/progression.

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IDENTIFICATION AND CHARACTERIZATION OF A NOVEL MOLECULAR MECHANISM ASSOCIATED WITH GASTRIC CANCER PROGRESSION.

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Gastric cancer (GC) is a predominant malignant neoplasia with an increase in incidence and mortality over the next years. Conventional treatments for this malignancy consist of surgery in association with the administration of platinum compounds or 5-FU, capecitabine. The identification of novel biomarkers related to the GC progression is a key point in improving the pharmacological treatment to get a more efficient patient response. In this study, we reported the role of Hormonally Upregulated Neu tumor-associated Kinase **(**HUNK) in promoting gastric cancer progression. Particularly, we demonstrated that HUNK increases gastric cancer cell proliferation through the binding and phosphorylation of p38 MAP kinase and that its depletion is associated with a reduction of survival both in gastric cancer cells and patient-derived organoids. Further, we reported that HUNK positively regulates the expression of MUC16/CA-125. Our findings describe a novel molecular mechanism regulated by HUNK in gastric cancer cells, making this kinase a promising candidate for novel therapeutic strategies

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SECRETOME RELEASE DURING IN VITRO BONE MARROW-DERIVED MESENCHYMAL STEM CELL DIFFERENTIATION INDUCED BY BIO-OSS® COLLAGEN MATERIAL

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Bone diseases represent a growing healthcare challenge due to population aging and lifestyle changes. Although bone has a natural regenerative capacity, approximately 10% of fractures fail to heal properly, requiring advanced therapeutic approaches. Bone tissue engineering (BTE) has advanced the use of osteoinductive and osteoconductive biomaterials to support bone regeneration. Among them, Bio-Oss/ Collagen, a composite of bovine hydroxyapatite and collagen, has shown excellent biocompatibility and bioactivity properties. Mesenchymal stem cells (MSCs) have immunomodulatory properties that support bone repair by interacting with immune cells to influence both innate and adaptive immune responses. Numerous regulatory molecules, such as cytokines, chemokines, receptors, and transcription factors, link the immune and skeletal systems. This study analyzes the effect of Bio-Oss/Collagen material in human bone marrow-derived mesenchymal stem cells (hBMSCs), assessing its osteoinductive and immunomodulatory potential. After 7 days of culture, the biomaterial modulated the expression of key genes involved in osteogenesis and chondrogenesis, which are known for their role in bone formation and maturation including COL1A1, COMP, ITGA1, BMP1, TWIST1, IGF1R and IGF-1. Higher expression of osteocalcin and osteopontin protein were present in hBMSCs grown on Bio-Oss/ Collagen. Secretome analysis revealed a controlled release of pro-regenerative cytokines, suggesting a role of the biomaterial in modulating inflammation to promote bone regeneration; RANTES and VEGF proteins were significantly upregulated while IL-6 was down regulated in hBMSCs grown on Bio-Oss/Collagen scaffold. These findings indicate that Bio-Oss/Collagen scaffold supports osteogenesis and modulates the immune response, creating a microenvironment favorable for bone regeneration.

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SWIMMING IN PLASTAMINATION: NANOPLASTICS AND SPERM FUNCTIONS

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The increasing demand of plastic-goods and the accumulation of plastic-waste in the environment make *plast*ic cont*amination* (PLASTAMINATION) one of the main troubles of XXI century. Recently, attention has been focused on microplastics (MPs, 5mm-1µm) and nanoplastics (NPs, <1µm) that can be intentionally produced or originate from the degradation of larger plastic-waste in marine and terrestrial environments. MNPs enter the food chain, bypass the biological barriers, and enter cells exerting toxic/inflammatory effects. The presence of MNPs has been demonstrated in the tissues of aquatic and terrestrial organisms, included human, and also in biological fluids like blood, breastmilk, follicular fluid, and semen. Recent studies linked MNPs exposure to higher rate of chronic diseases, miscarriage or poor semen production. Nevertheless, the use of biodegradable polymers like the the poly-lactic acid (PLA) does not guarantee they are safe for health.

Hence, we investigated the ability of PLA-NPs to enter cells (i.e., C6, HT29, Caco-2, hPBMCs) and mammalian spermatozoa (SPZ). Focusing on SPZ, rat SPZ collected from caput/cauda epididymis and seminal material from bulls were incubated with increasing concentrations of Rhodamine B conjugated-PLA-NPs (0-300 µg/ml); PLA-NPs internalization was investigated by immunofluorescence (IFL) analysis. In bovine, the effects on sperm kinetics was evaluated using a computerized semen analyzer. Additionally, flow cytometry was used to determine if the PLA-NPs could increase cytoplasmic membrane permeability, alter membrane organization, and whether PLA-NPs affects mitochondrial function by assessing the bioenergetic state. PLA-NPs entry in SPZ was investigated by IFL and further confirmed by co-localization with α-tubulin, used as a marker for the sperm flagellum. We demonstrate that PLA-NPs were effectively internalized by various cell types, but their internalization, uptake and biological effects were strictly dependent on cell type.

In conclusion, PLASTAMINATION warrants consideration. The development of strategies to mitigate PLASTAMINATION and further studies on the biological effects of biodegradable plastics are recommended.

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SERTOLI CELL SURVIVAL GUIDED BY CIRCULAR RNAS IN AGING

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The physiological aging leads to male fertility decline, mainly affecting spermatogenesis and sperm production. In the field of male reproduction, circular RNAs (circRNAs), consisting of covalently closed RNA molecules produced by the backsplicing mechanism, are acquiring a prominent role in sperm quality setting. We previously characterized the reproductive phenotype of the aged male mouse experimental model and identified several age-related defects including i) spermatogenesis anomalies and ii) the production of spermatozoa (SPZ) with reduced motility and abnormal morphology. Interestingly, a de-regulated expression of selective circRNAs was highlighted in Aged- vs Young- SPZ. Among the profiled de-circRNAs in Aged-SPZ, in the current study we focused on circAbcb9 as a spermatic circRNA potentially working as a predictive marker of Sertoli cell (SC) senescence. Bioinformatic approaches were used to build its circRNA/miRNA/mRNA network (ceRNET) active in the modulation of SC senescence. A significant reduction of circAbcb9-ceRNET targets (SOX8 and NOTCH2) occurred in Aged testis. *In vitro* experimental strategies, performed in immortalized SC line TM4 by using siRNA and mimic-miRNA molecules, validated and confirmed the involvement of circAbcb9 in SC senescence pathway. In addition, new insights for the sertolian circRNA biogenesis, dependent on the fused protein in sarcoma (FUS), was demonstrated. Collectively, our study highlights new findings on the involvement of circRNAs as molecular actors modulating Sertoli cell survivor.

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A COMPARATIVE STUDY OF MIGRATION IN HEALTHY OSTEOBLASTS AND OSTEOSARCOMA CELLS

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Background: Cell migration plays a crucial role in bone remodeling and cancer metastasis, yet the migratory behavioral shift of healthy osteoblasts when nearby osteosarcoma cells remain largely unexplored.

Aim: To investigate the influence of osteosarcoma cells on the migratory behavior of healthy osteoblasts, in order to better understand the mechanisms underlying bone cancer progression and identify potential therapeutic targets.

Material and methods: In this study, we investigated the migration dynamics of primary osteoblasts and multiple osteosarcoma cell lines using wound healing assays in co-cultured models. Cells were differentially stained to enable precise tracking of each population, and results were compared to controls obtained from single-line cultures.

Results: Our preliminary results show that in the same 24-hour period, monocultured osteoblasts performed close to no migration (0% RMD, Relative Migration Distance), while osteosarcoma cells reached up to 19% RMD. In the same period, co-cultured cells reached a staggering aggregated 52% RMD, with osteoblasts greatly changing their behavior and showing much higher migration capabilities.

Conclusions: This finding suggests that the aggressive nature of osteosarcoma may partly be attributed to mechanisms such as chemotactic factors release or extracellular matrix remodeling. Understanding this shift in behavior may provide useful insights into bone cancer progression and mobilization, while offering new perspectives into innovative therapeutic strategies targeting osteosarcoma cells.

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MOLECULAR INSIGHTS INTO OBESITY-LINKED MALE INFERTILITY: THE EMERGING ROLE OF CIRCRNAS

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Obesity, a pathophysiological condition characterized by excessive body fat accumulation, is strongly associated with the onset of male infertility. Indeed, the accumulation of adipose tissue promotes a chronic inflammatory state that negatively affects spermatogenesis by disrupting steroidogenesis and, in turn, promoting spermatozoa apoptosis. As a result, sperm morpho-functional parameters such as morphology, concentration, viability and motility, may be significantly impaired. In this context, the identification of new molecular markers to assess sperm quality and fertilizing potential represents a topic of great interest in the field of reproductive medicine. Circular RNAs (circRNAs), a class of covalently closed non-coding RNAs produced by backsplicing, have recently emerged as important regulators of sperm quality and function, as differentially expressed circRNAs have been identified in human spermatozoa in relation to quality parameters, subcellular localization, and pathological conditions. With this background, we performed a microarray analysis to explore the circRNA expression profile in the high-quality spermatozoa isolated from obese men, aiming to investigate a possible link between spermatic circRNAs and obesity-induced fertility impairment. The validation of selected differentially expressed circRNAs led to the identification of specific circRNAs potentially involved in regulatory networks related to apoptotic pathways and embryo development. These findings provide new insights into the molecular mechanisms underlying obesity-related male infertility and highlight sperm circRNAs as promising biomarkers to assess reproductive health.

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PIEZO1 FUNCTIONAL MODULATION ALTERS THE EXPRESSION OF SEVERAL NEURODEGENERATION-RELATED GENES IN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

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PIEZO1 is a trimeric Ca^{2+} cation channel involved in the conversion of mechanical stimuli into biochemical signals. By sensing membrane tension and shear stress, it dynamically transitions between closed (non-patent) and open (patent) conformations. Emerging evidence highlights a significant link between PIEZO1 activity and neurodegeneration-related proteinopathies. In the central nervous system (CNS), the accumulation of mutant proteins induces tissue stiffness, which is detected by membrane-bound PIEZO1. Recent studies demonstrated that Amyloid β1-40 (Aβ1-40) activates PIEZO1 in brain capillary endothelial cells, an effect abrogated by PIEZO1 deletion or the application of a superoxide dismutase/catalase mimetic. Furthermore, Aβ1-40 fragments enhance PIEZO1 mechanosensitivity by reducing its activation threshold, suggesting a contributory role of brain endothelial cells in neurodegeneration.

Based on this evidence, we conducted a transcriptomic analysis on Human Cerebral Microvascular Endothelial Cells (hCMECs) treated with Yoda1 (a PIEZO1 agonist) and GSMTx4 (a PIEZO1 antagonist). Our preliminary findings indicate that PIEZO1 activation triggers neuroinflammatory responses in hCMECs, modulating the expression of genes associated with endothelial-glial crosstalk. Understanding these pathways could elucidate the vascular component's role in neurodegenerative processes, highlighting PIEZO1 as a potential therapeutic target.

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VITAMIN E-DERIVATIVE TRIGGERS APOPTOSIS IN HUMAN HEPATOCARCINOMA VIA AUTOPHAGY/MITOPHAGY ACTIVATION

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Hepatocellular carcinoma (HCC) is the predominant form of primary liver cancer. Surgical resection, tumor ablation, and liver transplantation are curative treatments indicated for early-stage HCC. The management of intermediate and advanced stages of pathology is based on the use of systemic therapies which often show important side effects. Vitamin E-derivative tocotrienols (TTs) play antitumoral properties in different tumors. Here we analyzed the activity of delta-TT on HCC human cell lines (HepG2 and HuH7 cells). We analyzed the ability of delta-TT to trigger apoptosis, to induce oxidative stress, autophagy and mitophagy in HCC cell lines. We evaluated the correlation between the activation of autophagy/mitophagy with the ability of delta-TT to induce cell death. The data obtained demonstrate that delta-TT exerts a cytototoxic and proapoptotic effect in HCC cells. Furthermore delta-TT induces the release of mitochondrial ROS and causes a structural and functional alteration of the mitochondria compatible with a fission process. Finally, delta-TT triggers selective autophagy process (mitophagy) removing dysfunctional mitochondria and the inhibition of mitophagy reversed the cytotoxic action of delta-TT. Our results demonstrate that delta-TT through the activation of autophagy/mitophagy could represent a potential new approach in the treatment of advanced HCC.

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AUTOPHAGY IS INFLUENCED BY VITAMIN D3 LEVEL IN PEOPLE WITH HIV-1

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Abstract

Autophagy is the primary catabolic process responsible for degrading intracellular components and potentially harmful cytosolic entities by delivering them to lysosomes. Notably, this mechanism is crucial for controlling intracellular pathogens, with significant implications for both innate and adaptive immunity. In the context of HIV-1 infection, emerging evidence suggests that autophagy contributes to immune responses against the virus. Various compounds can modulate autophagy, among which vitamin D_3 is particularly effective due to its ability to prevent inflammation and slow HIV-1 disease progression.

In this study, we investigated the relationship between serum vitamin D_3 levels and the expression of autophagy markers in peripheral blood mononuclear cells from different categories of people with HIV (PWH) under antiretroviral therapy (ART) with either normal vitamin D_3 levels or hypovitaminosis, and treatment-naïve PWH with either normal vitamin D_3 levels or hypovitaminosis.

The results obtained so far indicate that low serum vitamin D_3 levels are associated with reduced expression of key autophagy-related factors, particularly in treatment-naïve PWH. These findings suggest that maintaining adequate vitamin D_3 levels may play a crucial role in supporting autophagy in individuals with HIV. The observed differences in the expression of autophagy-related proteins between ART-treated and untreated patients underscore the complex interplay between vitamin D_3 status, antiretroviral therapy, and the regulation of autophagy.

We are currently conducting *ex vivo* experiments to determine whether the hormonally active form of vitamin D3 (1,25(OH)2D), that is able to induce autophagy in human HIV-infected PBMC, can effectively reduce viral replication.

A deeper understanding of these mechanisms could support the development of innovative therapeutic strategies aimed at mitigating immune depletion and chronic inflammation, ultimately improving clinical outcomes for individuals living with HIV.

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CD44 MODULATES IRON-INDUCED CYTOTOXICITY IN HEY AND SKOV3 OVARIAN CANCER STEM CELLS

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CD44, a well established stemness marker, also functions as an iron importer, thereby linking the maintenance of the cancer stem cell (CSC) pool to iron homeostasis. In this study, we explored the role of CD44 in modulating iron handling capacity in HEY and SKOV3 ovarian cancer (OVCA) cell lines cultured in 3D conditions to enrich for the CSC pool. qPCR and Western Blot analyses revealed that 3D HEY cells exhibited a downregulation of CD44, along with key stemness-associated transcription factors OCT4, NANOG, and SOX2. These cells also showed reduced expression of CD71, a primary iron importer, and decreased labile iron pool (LIP) measured by flow cytometry using the iron-sensitive fluorescent probe FerroOrange. 3D-cultured SKOV3 cells, instead, displayed a distinct phenotype characterized by upregulation of CD44, CD71, and FtH1 (the main iron storage protein), along with downregulation of the iron importer ferroportin. Consistently, these cells exhibited an increased LIP, suggesting a heightened iron-dependent metabolic state. To further interrogate the iron-handling capacity of these two OVCA cell lines, we induced iron overload by using Ferlixit and ferric ammonium citrate (FAC). In HEY, iron administration led to a pronounced upregulation of CD44, confirming a positive feedback loop between iron accumulation and CD44 expression, and an iron-induced cytotoxicity (PI^+ cells: 48%). 3D SKOV3 cells remained, instead, unaffected. Notably, CD44 knockdown in HEY cells significantly decreased the LIP and reduced iron-induced cytotoxicity by aproximately 50%, further implicating CD44 in iron uptake and sensitivity. Overall, these findings highlight a cell type-specific role for CD44 in regulating iron metabolism in ovarian cancer cells and underscore its potential as a therapeutic target to disrupt iron homeostasis in ovarian CSCs.

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STUDYING THE BIOLOGICAL EFFECTS OF NANOPLASTICS ON CELLULAR BEHAVIOURS AND TRANSCRIPTOMIC SIGNATURES OF GNRH NEURONS BY IN VITRO MODELS

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Pollution from nanoplastics (NPs) poses an emerging threat to environmental and human health and recent evidence highlights their potential effects on neurodevelopmental processes. Yet, the impact of NPs on the development and function of hypothalamic gonadotropin-releasing hormone (GnRH) neurons, the master regulator of reproduction in mammals, is poorly known. Here, we investigated the effects of polystyrene NPs (PS-NPs) on GnRH neuron biology using two *in vitro* models of peptide-secreting (GT1-7) and migrating (GN11) GnRH neurons. Specifically, we demonstrated that PS-NPs are internalized via non-classical endocytosis and do not affect cell viability; further, they impaired GnRH peptide secretion in mature GT1-7 cells and cell migration of immature GN11 cells. Finally, transcriptomic analyses of NP-exposed GN11 cells revealed differential expression of genes key for GnRH neuron migration and differentiation and of genes enriching pathways related to cell adhesion and migration. Moreover, by matching our data with exome sequencing data from patients affected by GnRH deficiency, an inherited form of infertility whose genetics is still only partially understood, we revealed a rare variant in *NPAS2* gene. Overall, these findings suggest that PS-NPs impact key biological functions of GnRH neurons, thus representing an environmental threat to reproductive health, and provide novel insights into molecular mechanisms underlying GD.

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ENDOSOMAL TRAFFICKING AND SYNAPTOJANIN 1 (SYNJ1): A DANGEROUS LIAISON

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The endolysosomal system is a critical hub for neuronal viability and functions. Synaptojanin 1 (Synj1; 21q22.11 locus) is a ubiquitous inositol-phosphatase acting on various phosphoinositides, whose loss-of-function mutations are causative of the hereditary form of Parkinson's disease PARK20, while its excessive expression was observed in the post-mortem brains of individuals with Down syndrome (DS).

Combining quantitative confocal microscopy and Western blot analysis, we show that the structure, dynamics and activity of early endosomes (EEs) are altered upon Synj1 knockdown or overexpression, thus indicating its critical role for the homeostasis and functions of these compartments. Remarkably, these alterations were also found in PARK20 and foetal DS fibroblasts, highlighting that the dysfunction of this critical cellular hub may be a pathological mechanism of the diseases. Moreover, by taking the advantage of techniques to differentiate induced-pluripotent stem cells (iPSCs) into neural precursors (NPCs), we have highlighted that the homeostasis and dynamics of EEs is already perturbed at the undifferentiated state and these alterations are exacerbated during neural differentiation with a compromising of EE functions. Furthermore, transcriptomic analysis revealed that *SYNJ1* knockdown or its overexpression alters the expression levels of hundreds of genes, including important regulators of synaptic activity, neurogenesis, and axon guidance in SH-SY5Y cells.

Overall, our data pointed out that proper levels of Synj1 are critical for neuronal physiology. They also highlight a functional cross-talk among endolysosomal

trafficking, Synj1 and neuronal homeostasis, emphasizing a dangerous liaison with brain disorders.

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STING REGULATES INNATE IMMUNITY BY MODULATING MACROPHAGE POLARIZATION

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STING is a transmembrane ER resident protein that was initially described as a regulator of innate immune response triggered by viral DNA and later found to be involved in a broader range of immune processes, in particular in the innate immunity. To assess whether STING is involved in the MHC-I-dependent antigen presentation, in a previous study from our team, we generated a STING KO murine macrophage cell line and treated it with ovoalbumin (OVA). Those cells showed an impaired internalization, processing, and presentation of the OVA-specific epitope SIINFEKL, which did not depend neither on the entry nor on the proteolysis of OVA exogenous protein. Given that the levels of MHC-I on the plasma membrane were decreased, we further analyzed the heavy and light chains of the complexes and hypothesized that this was probably due to a lower expression of β2m since there were no relevant alteration of the peptide-loading complex (TAPs). Moreover, following OVA and LPS treatments the JAK-STAT signaling resulted impaired in the STING KO cells, thus suggesting a hampered activation of the immune response. Given all these data we asked whether the lack of STING could interfere with macrophages polarization toward M1 or M2 phenotype in vitro. Therefore, we generated THP1 (Macrophages derived from human monocytic leukemia) STING KO cells and induced the polarization using a proper cocktail of cytokines. The analysis of specific markers, in particular pSTAT1 for M1 and pSTAT6 for M2, revealed a tendency toward the M2 phenotype in absence of STING. Consistent with these results, a phagocytosis assay revealed an increased activity in the STING KO M2 macrophages compared to the CTRL M2. Ongoing studies using human iPSC-derived macrophages aim to further elucidate the molecular mechanisms involved, with additional investigations into STING's potential role in macrophage differentiation.

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BIOLOGICAL RESPONSE OF TREATMENT WITH SAFFRON PETAL EXTRACT ON CYTOKYNE-INDUCED OXIDATIVE STRESS AND INFLAMMATION IN THE CACO-2/THP1 CO-COLTURE MODEL.

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Inflammatory bowel disease (IBD) is a chronic disorder that affects the ileum, rectum and colon. It includes ulcerative colitis and Crohn's disease. The global burden of IBD remains a persistent health problem due to the high costs of treatments that are not able to definitively cure the disease. The pathogenesis of IBD involves complex mechanisms, including immune dysregulation, gut microbiota imbalances, oxidative stress, and defects in the gastrointestinal mucosal barrier. Although the progression of IBD therapy is controlled with chemical drugs and biological therapies, healing results cannot yet be achieved, along with the inevitable side effects. As a result, a variety of research have focused on exploring novel therapies and found that natural products with anti-inflammatory and antioxidant could be used for IBD management. There is increasing interest in exploring food industry waste as a source of bioactive molecules with healthcare applications. In this study, a co-culture system of Caco-2 cells and PMA-differentiated THP-1 macrophages was used to simulate the human intestinal microenvironment. Inflammation was induced using TNF-α and IFN-γ, followed by treatment with Saffron Petal Extract (SPE). The results demonstrated that SPE significantly attenuated oxidative stress and inflammation by downregulating the expression of pro-inflammatory mediators such as iNOS, COX-2, IL-1β, and IL-6 via modulation of the Fbw7/NF-κB pathway, a key regulator of macrophage-driven inflammation. Furthermore, the results of our model suggest that SPE treatment restores the functionality of the intestinal barrier by reducing the destruction of tight junctions induced by the inflammatory stimulus. Our findings suggest that SPE could represent a complementary option to conventional drugs for those patients who develop resistance or intolerance to standard therapies.

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INDOLEAMINE 2,3 DIOXYGENASE /GLYOXALASE 1/MG-H1 AXIS AS A POTENTIAL NOVEL MECHANISM DRIVING OXIDATIVE STRESS IN CYSTIC FIBROSIS

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Glyoxalase 1 (Glo1) is the major enzyme that metabolizes methylglyoxal (MG), a potent precursor of pro-oxidative advanced glycation end products (AGEs). Preferentially, MG reacts with arginine residues of proteins generating the AGE, named MG-H1.

Cystic fibrosis (CF) is a genetic disease where the alteration of the respiratory function represents the main cause of morbidity and mortality. One of the key aspects involved in the pathogenesis of CF is oxidative stress, which contributes to chronic inflammation and tissue damage. We recently demonstrated in CF mice models upon infection with *Aspergillus fumigatus*, a common fungal pathogen of CF, that Glo1 is down-regulated and this is paralleled by MG-H1 accumulation, and tissue damage. Indoleamine 2,3 dioxygenase (IDO1) is a crucial modulator of oxidative stress and it is down-regulated in CF. More importantly, in IDO1 knock-out mice, Glo1 is down-regulated, and in CF models, anakinra, the recombinant form of IL-1 receptor antagonist, is able to rescue both IDO1 and Glo1 functionality.

Given these premises we investigated whether IDO1/Glo1/MG-H1 axis was involved as a potential novel mechanism driving oxidative stress and the associated tissue damage in CF.

By using mice homozygous for the prevalent mutation in the CFTR gene (the deletion of Phe at position 508) and wild type mice we indeed found that the downregulation of Glo1 and the consequent accumulation of MG-H1 were associated with increased levels of oxidative stress, measured by oxygen peroxide (H_2O_2) levels, and with IDO1 downregulation. Notably, anakinra induced the rescue of IDO1, Glo1/MG-H1 and H_2O_2, improving lung health, thus supporting the hypothesized mechanism and pointing out a novel potential therapeutic target to mitigate CF lung damage.

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MATERNAL EXPOSURE TO A MIXTURE OF ENDOCRINE DISRUPTORS: INVESTIGATING MOLECULAR MECHANISMS AFFECTING REPRODUCTIVE AND NEURO-BEHAVIORAL DEVELOPMENT

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Endocrine disruptors (EDs) are environmental chemicals that can interfere with endogenous hormone balance leading to a variety of health outcomes. The Life MILCH project (*Mother and Infant dyads: Lowering the impact of endocrine disrupting chemicals in milk for a Healthy Life)* investigated ED exposure in 689 mother-infant dyads and its impact on infant neurodevelopment during the first year of life. We identified a mixture of bisphenols, phthalates and parabens (LM-Mix) in over 50% of pregnant women in the Life MILCH cohort, thus representing real-life exposure. In this study we aimed to reproduce the maternal exposure to LM-Mix in a mouse model to investigate the effects on neurodevelopment, reproductive function and the underlying molecular mechanisms. CD1 female mice were exposed to LM-Mix at 1X, 10X, 100X levels detected in the pregnant women or vehicle from gestational day 12 to postnatal day 12. Maternal reproductive endpoints (pregnancy length, litter size, sex ratio) were assessed and maternal behavior was observed during the first postnatal week. Pups were evaluated for body weight, sensorimotor development, ultrasonic vocalizations and reproductive physiology (anogenital distance – AGD, pubertal indexes). Preliminary results showed no effects on maternal reproductive endpoints but increased pup-directed behaviors in 10X groups compared to controls. LM-Mix did not alter pups' body weight, AGD and sensorimotor development although 10X pups showed greater grasping reflex and strength. Ongoing analyses are focusing on pubertal markers (vaginal opening, estrous cycle, testis descent and sex hormones dosage) and brain analysis for gene expression. These findings suggest that perinatal LM-Mix exposure may alter maternal behavior and early neurodevelopment potentially leading to long-term consequences on offspring brain and behavioral function.

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HUMAN AMNIOTIC FLUID STROMAL CELLS AS MODULATORS OF EXTRACELLULAR MATRIX DEPOSITION ON AN IN VITRO GLAUCOMA MODEL

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Glaucoma, a leading cause of blindness due to elevated intraocular pressure (IOP), is characterized by excessive extracellular matrix (ECM) deposition in the trabecular meshwork (TM), which drains aqueous humor in the eye. However, the mechanisms driving to TM dysfunction are not fully understood and no therapies are currently available to restore or improve TM function. In this context, mesenchymal stromal cells (MSCs) and their secreted factors offer promising strategies for restoring TM homeostasis. Among these, human amniotic fluid SCs (hAFSCs) are notable for secreting anti-fibrotic factors that may reduce ECM deposition. Therefore, this study aimed to investigate the mechanisms underlying ECM deposition in an *in vitro* glaucoma model and to evaluate the hAFSCs effects in reducing fibrosis.

To this end, human TM cells (HTMCs) were treated with transforming growth factor beta 2 (TGF-β2) and subsequently co-cultured with hAFSCs (c-kit+) using a transwell system. Additionally, to mimic TM three-dimensional (3D) architecture, HTMCs 3D spheroids were developed and then co-cultured with hAFSCs.

After 4 days of treatment, gene and protein expression results showed that TGF-β2 increased ECM deposition *via* SMAD and MAPK pathways. While the co-culture with hAFSCs significantly reduced ECM markers and decreased SMAD/MAPK signaling, confirming the involvement of these pathways in glaucoma fibrosis. Of note, also in the 3D model, hAFSCs similarly downregulated fibrotic markers, further confirming their anti-fibrotic potential in a more physiologically relevant context.

Our findings show that hAFSCs effectively attenuate ECM deposition induced by TGF-β2 in HTMCs, mainly through modulating SMAD and MAPK pathways.

Hence, by reducing fibrosis, hAFSCs emerge as promising therapeutic for glaucoma, paving the way for novel cell-based strategies targeting glaucoma-related fibrosis.

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PCB-INDUCED DISRUPTION OF IRON HOMEOSTASIS AND DEVELOPMENTAL PROGRAMS IN DICTYOSTELIUM DISCOIDEUM: A MODEL FOR CONSERVED TOXICITY MECHANISMS

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Persistent Organic Pollutants (POPs) are toxic chemicals that bioaccumulate and interfere with endocrine, immune, reproductive and cardiovascular systems. Among them, Polychlorinated Biphenyls (PCBs) pose significant risks to human health. In this study, we adopted a multi-model approach using Dictyostelium discoideum and human THP-1 cells to investigate PCB 138 and PCB 153 cellular effects. In Dictyostelium, PCBs impaired growth and development, reducing proliferation and inducing a small-fruiting-body phenotype. In THP-1 cells, PCB exposure reduced viability and selectively downregulated hepcidin, indicating disrupted iron homeostasis. Consistently, PCB 138 altered iron-related gene expression in Dictyostelium, upregulating abcB7 and ferroportin, while downregulating ferritin. Calcein assays confirmed reduced intracellular iron. Both PCBs increased ROS levels and downregulated key superoxide dismutase genes. Moreover, confocal microscopy revealed mitochondrial network fragmentation. Despite evolutionary distance between the two models, results suggest that PCBs promote mitochondrial/cytoplasmic iron efflux, disrupting iron balance and thereby increasing oxidative stress. Under starvation, PCB-treated Dictyostelium cells upregulated genes involved in aggregation and differentiation, including those in cAMP pathway. Proteomic analysis confirmed differential expression of proteins linked to cell adhesion, oxidative stress, mRNA processing, amino acid metabolism and development. Moreover, GtaC (GATA-3) overexpression may mediate the link between developmental genes upregulation and PCBs mechanism of action. By linking iron dysregulation, oxidative stress and altered developmental signalling, our study reveals shared toxic responses to PCBs across models, supporting Dictyostelium as a cheap tool in human-relevant toxicological research.

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CONSERVED AND UNIQUE FEATURES OF CHROMATIN PROFILE IN A MARINE MOLLUSK: INSIGHTS FROM MULTI-OMICS ANALYSIS OF RUDITAPES PHILIPPINARUM

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The functional identity of tissues in a multicellular organism is thought to be maintained by tissue-specific epigenetic profiles running tissue-specific gene expression programs. Several epigenome atlases, describing the epigenetic landscape of various tissues, have been published so far. However, with the exception of the fruit fly, almost exclusively model vertebrate species have been analysed. Here we present epigenetic data for a marine invertebrate species of ecological and economic importance, the Japanese clam *Ruditapes philippinarum.* Chromatin accessibility was assessed using ATAC-seq and four key histone modifications (H3K4me3, H3K27ac, H3K4me, H3k27me3) via ChIP-seq. In parallel, transcriptome profiles were obtained using RNA-seq. Four tissues (digestive gland, gill, foot, and mantle), in four individuals were evaluated. Using the Uniform Manifold Approximation and Projection (UMAP) technique for dimension reduction on ChIP-and ATAC-seq data we showed that mechanisms underlying tissue-specific gene regulation are conserved in this species, as running UMAP considering only the combination of active enhancers and promoters results in samples clustering by tissue, as reported in model species. RNA-seq data also showed the expected clustering by tissue type. However, for other genomic regions with different epigenetic profiles such as actively repressed and open chromatin elements, samples clustered by individual rather than by tissue type, at variance with what reported from vertebrate model species. Such puzzling evidence of great inter-individual variability in chromatin accessibly and actively repressed chromatin status requires further investigations, although it might be linked to the extreme level of genomic sequence polymorphism (one single-nucletide polymorphism every 10-15 nucleotides) that is observed in this as well as other mollusc species.

between tobacco exposure, metal detoxification pathways, and ferroptosis susceptibility in oral cancer.

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PYRROLOQUINOLINE QUINONE (PQQ) ATTENUATES HYDROGEN PEROXIDE-INDUCED INJURY THROUGH THE ENHANCEMENT OF MITOCHONDRIAL FUNCTION IN HUMAN TRABECULAR CELLS

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Mitochondrial metabolism in the trabecular meshwork (TM) plays a critical role in maintaining intraocular pressure homeostasis by supporting the energy-demanding processes involved in aqueous humour outflow. Consequently, oxidative stress-induced mitochondrial dysfunction contributes to increased oxidative damage, cellular senescence, and impaired TM function, key factors in the pathogenesis of primary open-angle glaucoma. Therefore, understanding and targeting mitochondrial metabolism in TM cells could represent a promising therapeutic strategy to prevent or mitigate glaucomatous damage. Pyrroloquinoline quinone (PQQ) is a redox cofactor with several physiological functions, including free radical scavenging, reduction of oxidative stress, and enhancement of mitochondrial health and function. However, its effects on human trabecular meshwork (HTM) cells remain largely unexplored. The aim of this study was to investigate the *in vitro* cytoprotective effects of PQQ against H_2O_2-induced oxidative and bioenergetic stress in HTM cells and to elucidate the underlying molecular mechanisms. Our findings demonstrate that PQQ alone enhances mitochondrial respiratory capacity and ATP production in HTMs. Moreover, PQQ mitigates H_2O_2-induced cellular damage and preserves mitochondrial function by normalizing proton leak, increasing ATP levels, partially alleviating structural damage, restoring mitochondrial network morphology, and finally reducing cell death. Although these protective effects seem not to be mediated by changes in mitochondrial content or activation of the SIRT1/PGC-1α pathway, they may involve SIRT3 upregulation, a key factor of mitochondrial metabolism and homeostasis. Taken together, these results suggest that PQQ may represent a promising candidate for restoring mitochondrial function and reversing damage in HTM cells associated with glaucomatous pathology.

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THERAPEUTIC POTENTIAL OF HDAC8 INHIBITION AND SIRT1 ACTIVATION IN DUCHENNE MUSCULAR DYSTROPHY

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Duchenne Muscular Dystrophy (DMD) is a severe genetic disease causing progressive muscle degeneration, inflammation, and fibrosis. While gene and cell therapies face limitations, pharmacological approaches are showing promise. Modulating histone deacetylases (HDACs) has emerged as a key strategy. Specifically, inhibiting HDAC8 with PCI-34051 improves muscle function by stabilizing the cytoskeleton, while activating SIRT1 with SRT2104 boosts energy metabolism and muscle regeneration. In our study, we evaluated a novel combination therapy involving HDAC8 inhibition and SIRT1 activation. In *dmd* zebrafish embryos, the co-administration of PCI-34051 and SRT2104 showed a greater effect in reducing muscle loss and inflammation, demonstrating the synergy of this approach. Importantly, the combination therapy allowed for lower doses of each compound, maximizing therapeutic effects while minimizing potential side effects. Indeed, as evidenced by our behavioral analysis, toxic effects, including those linked to the nervous system, are mitigated by reducing the doses. These findings highlight the potential of this synergistic strategy as an innovative and effective approach for DMD therapy.

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HDAC8 AND HDAC6 COMBINED INHIBITION: A NEW FRONTIER IN GLIOBLASTOMA TREATMENT

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GBM is the most aggressive cancer form in adults, with an extremely poor prognosis. Despite the current treatments, combining surgery, radiotherapy, and chemotherapy, most patients fail to respond with a higher mortality rate. Multiple molecular mechanisms contribute to GBM development and drug resistance such as signaling through the receptor tyrosine kinase/Ras/phosphoinositide 3-kinase, p53-ARF-MDM2, retinoblastoma, and overexpression of the histone deacetylases (*i.e.* HDAC1,4,6,7,8,9,10). The specific inhibition of HDAC6 and HDAC8 reduces GBM progression and their combined inhibition could be even more effective for GBM treatment. Indeed, they display a high degree of similarity in their active site, share common cytoplasmatic substrates and converge on the same molecular mechanisms, especially on those contributing to the development of tumor resistance.

In this project we compared the effects of single HDAC6 or HDAC8 inhibitors with a combination of the two. We performed *in vivo* analyses, using the GBM zebrafish model zic:RAS generated through the expression of different oncogenes in neural cells during development; and *in vitro* analyses in human GBM cell lines. Moreover, we tested the single or combined effects of gold standard GBM drugs (*i.e.* Temozolomide) with selective HDAC inhibition and we developed a computational system for the development of new dual HDAC8/HDAC6 inhibitors testing their ability to cross the human or zebrafish blood-brain barrier.

Further goals of this project are to unravel the HDAC6-HDAC8-regulated mechanisms underlining GBM development and to discover new inhibitors to use in pharmacological treatments. Interestingly, our findings can be translated to other tumors overexpressing HDAC6 and HDAC8 such as acute myeloid leukemia and colon cancers.

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ALTERATION OF CELL ENERGY METABOLISM MAY INFLUENCE PHENOTYPE AND FUNCTION OF CD8+ T CELLS IN CHRONIC ANTIBODY MEDIATED REJECTION

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Chronic antibody mediated rejection (CAMR) is the leading cause of graft loss. Metabolic reprogramming is involved in the differentiation, proliferation and function of immune cells. We investigated the molecular mechanisms underlying CD8+ T lymphocytes metabolic phenotype and function in CAMR.

Gene expression profiles of CD8+ T cells showed impaired lipid metabolism in CAMR patients with the triglyceride biosynthesis pathway (p=2.69E-3) as the most altered pathway. CD8+ Oxygen Consumption Rate (OCR) from CAMR patients was mostly dependent on mitochondrial respiration, since it was deeply reduced by rotenone/antimycin A. Interestingly, in CD8+ cells from CAMR patients OCR was not affected by oligomycin treatment, while OCR was noticeably stimulated by FCCP, suggesting that mitochondrial oxygen uptake was not ATP linked but rather associated to increased uncoupling. We also observed an increased concentration of NADH in CAMR CD8+ T cells, compared to CD8- T cells thus suggesting that NADH might not be involved in mitochondrial oxidative phosphorylation. Moreover, under low glucose conditions we observed a statistically significant reduction in PBMC viability in CAMR patients compared to healthy controls. These data suggest that CD8+ T cells from CAMR patients are activated effector T cells primarily utilizing aerobic glycolysis, as further confirmed by flow cytometry. We also observed an increased expression of HIF1a and a reduced expression of CD36, a fatty acid transporter, in CD8+T cells from CAMR patients compared to controls ($p<0.05$), confirming the T effector phenotype.

Our data suggest that CD8+ T cell from CAMR patients showed permanent dependency on glycolysis leading to altered immune phenotype and function. These

results indicate novel opportunities to modulate immune cells with metabolic inhibitors in kidney transplanted patients and reduce graft rejection.

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THBS1 AND THBS2 EMERGE AS KEY DRIVERS OF THE ICCA PROGRESSION AND PROMISING TARGETS FOR THERAPY

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Cancer cells display an adhesion strength to the extracellular matrix (ECM) up to threefold higher than normal cells. This augmented adhesive capacity facilitates the acquisition of a more malignant phenotype by enabling resistance to mechanical stresses and promoting cellular survival and metastatic dissemination. Over the past four years, we have discovered that the matricellular proteins thrombospondin 1 (THBS1) and 2 (THBS2) are highly expressed in the tumour microenvironment of intrahepatic cholangiocarcinoma (iCCA) by both the cancer-associated fibroblasts (CAFs) and tumor cells. We recently demonstrated that these proteins are key drivers of the iCCA progression by promoting cell adhesion and motility. However, the specific cancer cell surface receptors engaged by THBS1 and THBS2 remain unidentified.

Here, we present data indicating that recombinant human THBS1 and THBS2 enhance iCCA cell malignancy by interacting with integrins α3β1 and α6β1 on the cell surface of epithelial cancer cells, as well as integrin α6β1 and CD36 on the surface of the mesenchymal cancer cells. Functional inhibition using blocking antibodies against these receptors effectively counteracts the adhesion of iCCA cells induced by thrombospondins. Furthermore, CRISPR/Cas9-mediated knockout of the THBS1 gene in the epithelial iCCA cell line HuCCT-1, which expresses THBS1, resulted in the loss of autocrine signaling that activates integrins α3β1 and α6β1. This disruption led to a marked reduction in cancer cell adhesion and invasive capabilities. In addition, THBS1 depletion impaired the formation of filopodial extensions, and significantly inhibited 3D spheroid formation. Our data suggest that targeting integrins β1- and CD36-mediated seeding of iCCA cells at any stage of the disease may represent a valuable therapeutic strategy.

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A METHOD FOR THE ANALYSIS OF THE OLIGOMERIZATION PROFILE OF THE HD-ASSOCIATED, AGGREGATION-PRONE MUTANT HUNTINGTIN BY ISOPYCNIC ULTRACENTRIFUGATION

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Conformational diseases, such as Alzheimer's, Parkinson's and Huntington' s diseases as well as ataxias and fronto-temporal disorders, are part of common class of neurological disorders characterised by the aggregation and progressive accumulation of mutant proteins which display aberrant conformation. In particular, Huntington's disease (HD) is caused by mutations leading to an abnormal expansion in the polyglutamine (poly-Q) tract of the huntingtin protein (HTT), leading to the formation of inclusion bodies in neurons of affected patients. Furthermore, recent experimental evidence is challenging the conventional view of the disease by revealing the ability of mutant HTT to be transferred between cells by means of extracellular vesicles (EVs), allowing the mutant protein to seed oligomers involving both the mutant and wild type forms of the protein. There is still no successful strategy to treat HD. In addition, the current understanding of the biological processes leading to the oligomerization and aggregation of proteins bearing the poly-Q tract has been derived from studies conducted on isolated poly-Q monomers and oligomers, whose structural properties are still unclear and often inconsistent. Here we describe a standardised biochemical approach to analyse by isopycnic ultracentrifugation the oligomerization of the N-terminal fragment of mutant HTT. The dynamic range of our method allows one to detect large and heterogeneous HTT complexes. Hence, it could be harnessed for the identification of novel molecular determinants responsible for the aggregation and the prion-like spreading properties of HTT in the context of HD. Equally, it provides a tool to test novel small molecules or bioactive compounds designed to inhibit the aggregation of mutant HTT.

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EXERCISE-INDUCED MUSCLE-DERIVED EXTRACELLULAR VESICLES IMPACT ON MUSCLE AND NEURONAL CELL SURVIVAL IN SOD-G93A MICE

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by motor neuron loss and muscle atrophy. Evidence suggests that metabolic dysfunctions contribute to ALS onset and progression, with skeletal muscle exhibiting metabolic alterations before symptom onset.

Given the impact of physical activity (PA) on muscle and whole-body energy metabolism, it has been hypothesized its possible role in disease onset and progression. Depending in its intensity, PA can either exacerbate energy imbalance or offer neuroprotection by improving locomotor function. We previously observed that high-intensity interval training (HIIT) is beneficial and protective, improving muscle strength and performance in SOD1-G93A mice. Building on these findings, we hypothesize that the observed beneficial effects are mediated, at least in part, by muscle-derived extracellular vesicles (EVs) specifically induced by the exercise protocol, contributing to muscle–motor neuron signaling and potentially influencing neuronal survival and function.

To test whether EVs contribute to the positive effects of HIIT, we isolated EVs from the gastrocnemius of sedentary and HIIT-trained wild-type (WT) and SOD1-G93A mice. NanoParticle Tracking Assay revealed changes in the size of HIIT-derived EVs in SOD1-G93A mice, suggesting molecular and functional differences. Furthermore, sedentary and HIIT-trained EVs were isolated and administered to muscle and motor neuron cell lines (C2C12 and NSC34). Notably, we found that EVs from HIIT SOD1-G93A muscle improved cell viability and mitochondrial functions compared to sedentary-derived EVs.

These findings suggest that HIIT-induced beneficial effect were mediated, at least in part, by muscle-derived EVs, which may protect muscle cells and motor neurons, potentially preventing denervation. Identifying the signalling pathways involved in this crosstalk could open the path to new promising therapeutic approach.

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DOXORUBICIN-INDUCED CELLULAR SENESCENCE IN OVARIAN CELLS: A FOCUS ON THE PROTECTIVE ROLE OF FISETIN

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Ovarian aging is one of the earliest signs of aging in the human body, significantly impacting both overall health and fertility in women. This aging process is primarily driven by cellular senescence, a condition characterized by irreversible cell cycle arrest and specific molecular and phenotypic alterations. As aging progresses, the accumulation of senescent cells plays a critical role in the development of age-related diseases. However, the exact mechanisms underlying cellular senescence in ovarian aging remain only partially understood. This study investigates the induction of senescence in the human ovarian granulosa tumor cell line (KGN cells) through doxorubicin treatment and explores the potential of senolytic treatments. In particular, the study examines the role of Fisetin in alleviating doxorubicin-induced senescence in KGN cells. The results demonstrate that doxorubicin treatment induced senescence in KGN cells, as evidenced by increased biomarkers of senescence such as β-galactosidase activity (SA-β-gal), elevated expression of CDKN1A/p21, and changes in the gene expression profiles of senescence-associated secretory phenotype (SASP) factors. Fisetin treatment exhibited senomorphic effects by suppressing specific SASP factors associated with senescent cells. Transmission electron microscopy (TEM) analysis revealed reduced cytoplasmic vacuolization. Furthermore, Fisetin modulated DNA repair mechanisms and cell cycle progression, reducing apoptosis and extracellular vesicle release. In conclusion, understanding the mechanisms of doxorubicin-induced senescence in KGN cells and investigating the therapeutic potential of senolytic and senomorphic agents such as Fisetin could offer valuable insights into strategies to mitigate the adverse effects of chemotherapy on reproductive health.

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CELLULAR MICROENVIRONMENT AND IMMUNE SYSTEM: EXPLORING THE ECM'S ROLE IN MACROPHAGE RESPONSE TO NANOMATERIALS

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The extracellular matrix (ECM) modulates the behaviour of immune cells in healthy and diseased tissues and play a crucial role in the cellular response to nanomaterials (NMs). In most *in vitro* studies, cells are exposed to NMs suspended in culture medium, however in human tissues NMs are not only diffusible in biological fluids but can also be trapped or adsorbed by ECM components. This interaction could alter the ECM properties and how cells interact with NMs.

In this study, funded by the PRIN 2022 PNRR program, we aim to explore the role of the microenvironment in influencing macrophage response to nanomaterials embedded in the ECM. We exposed macrophage-like cells from the human monocytic THP-1 cell line to ECM-like substrates pre-loaded with polystyrene nanoparticles (NPs). These substrates, resembling the structural, chemical, and physical properties of native ECM, were made from polymer and gelatin and hyaluronic acid (HA) using electrospinning. Preliminary experiments showed macrophages adhered to all substrates, particularly those with gelatin, without affecting cell viability up to 48 hours after seeding. Furthermore, gelatin matrices adsorbed more NPs compared to controls without gelatin. By using fluorescence microscopy, it was confirmed that the NPs embedded in these ECM-like substrates are bioavailable and can be internalized by macrophages.

This research highlights the need for further studies to fully understand the cellular and molecular mechanisms of immune cell response to nanomaterials and the role of ECM in these processes.

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INVESTIGATION OF GLYOXALASE 2 LOCALIZATION AND ITS ROLE AS SURVIVAL FACTOR IN CANCER CELLS

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This study concerning the glyoxalase 2 and additional roles in breast cancer and non-cancer cell lines. Glyoxalase 2 (Glo2) is an enzyme of the glyoxalase system, critical for detoxification of methylglyoxal (MGO) and active in parallel with glycolysis, using glutathione (GSH) as a cofactor. Encoded by the HAGH gene, Glo2 is present in both mitochondria and cytoplasm and plays an important function in numerous species and tissues, both prokaryotic and eukaryotic. This study delves into new aspects of Glo2 function in breast carcinoma cells (MCF7) compared with normal cells (HDF), exploring its nuclear localization and its role in cell proliferation and chemotherapy resistance. The results indicate overexpression of Glo2 in cancer cells, with levels increasing during the proliferative (S and G2/M) phases of the cell cycle, suggesting direct involvement in tumor growth. In addition, Glo2 has been observed to participate in S-glutathionylation, an enhanced post-translational modification (PTM) in cancer cells in both the cytoplasm and nucleus. Inhibition of Glo2 by the inhibitor P-nitrocarbobenzoxyglutathione (p-NCBG) increased the sensitivity of cancer cells to doxorubicin, suggesting that Glo2 may contribute to tumor resistance by modulating oxidative stress. These findings identify Glo2 as a potential therapeutic target for improving the efficacy of cancer treatments, and suggest a broader role of Glo2 in cellular regulatory mechanisms and pathologies associated with oxidative stress.

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HSA-MIR-34A-5P INHIBITS MERKEL CELL CARCINOMA CELL GROWTH AND MIGRATION BY REGULATING CELL CYCLE AND EPITHELIAL-TO-MESENCHYMAL TRANSITION PATHWAYS

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Hsa-miR-34a-5p is a multifaceted microRNA known to play a role in various biological processes, including cell proliferation, migration and apoptosis. Although recent evidence suggests a tumor-suppressor activity for hsa-miR-34a-5p, its role in Merkel cell carcinoma (MCC), a skin tumor caused by either Merkel cell polyomavirus (MCPyV) oncogenic activity or ultraviolet radiation exposure, is unknown. This study aimed to functionally investigate the role of hsa-miR-34a-5p on the malignant phenotype of virus-negative MCC (VN-MCC) and elucidate the potential underlying mechanisms. Hsa-miR-34a-5p was markedly downexpressed in VN-MCC cells and tissues compared to their respective MCPyV-positive counterparts, as well as to control fibroblast and epithelial cells. Mechanistically, ectopic hsa-miR-34a-5p expression in VN-MCC MCC13 cells significantly inhibited proliferation, colony formation, and migration abilities, while promoted apoptosis. Hsa-miR-34a-5p silencing in epithelial control HaCaT cells increased colony formation and partially enhanced migration. Protein–protein interaction network and enrichment analyses revealed that ectopic hsa-miR-34a-5p expression in MCC13 cells negatively regulated key target genes and pathways involved in both G1/S transition of the cell cycle (CDK4, CDK6, CCNE2) and epithelial-to-mesenchymal transition (MET, NOTCH1, JAG1, along with Snail protein), leading to anti-proliferative and anti-migratory effects. Moreover, ectopic hsa-miR-34a-5p expression strongly inhibited MCC13 spheroid formation, whereas miRNA inhibition yielded the opposite effect in HaCaT spheroids from intermediate time points onward. This study provides the first mechanistic evidence of the pleiotropic tumor suppressor role of hsa-miR-34a-5p in VN-MCC.

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THE MOLECULAR FINGERPRINT OF MOUSE EMBRYONIC STEM CELL-DERIVED EXTRACELLULAR VESICLES

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Embryonic stem cells (ESCs) are highly specialized pluripotent cells that originate from the inner cell mass of blastocyst-stage embryos, which possess the ability to differentiate into any tissue. Recently, extracellular vesicles (EVs) have emerged as critical mediators of intercellular communication, influencing a variety of biological processes. Given their significance, we aim to characterize the EVs derived from mouse embryonic stem cells (mESC) by analyzing their cargo after enhancing the ESC ZSCAN4 "metastate" through retinoic acid treatment. The ZSCAN4 condition is recognized for its high stem cell potency and presence of 2-cell-stage preimplantation embryos molecular signature. To this end, we genetically modified mES cells with the *LNGFR* (low-affinity nerve growth factor receptor gene) expressing system under Zscan4 promoter control. This system did enable the collection and purification of EV subpopulations (LNGFR -/+). Here, we characterized the bulk EV population derived from retinoic acid-treated mES cells, revealing a typical size range of particles and, at the morphological level, a cup-shaped morphology. The bulk population of EVs was extensively analyzed at both the protein and RNA levels. Specifically, these EVs expressed a ZSCAN4-related gene signature, indicating a strong link to the characteristics of the embryonic stem cells from which they derived. The enriched LNGFR+ subset exhibits an augmented activation of ZSCAN-related genes, which is indicative of a pronounced ZSCAN4 activation and its positive selection. Our findings suggest the phenotypic similarity of EVs to their mES cells of origin and open new avenues in stem cell biology.

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DISSECTING THE ROLE OF MEOX2 IN MODULATING CHROMATIN ACCESSIBILITY LANDSCAPES IN GLIOBLASTOMA STEM CELLS

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Glioblastoma (GBM) is a brain tumor with poor prognosis, thought to arise from glioblastoma stem cells (GSCs), driving tumor growth, therapy resistance, and recurrence. MEOX2, a homeobox transcription factor, has emerged as an oncogenic regulator in GBM. Its expression is elevated in gliomas compared to normal brain tissue, correlates with poor patient survival, and is particularly enriched in GSCs. Our transcriptomic analysis of patient-derived GSC lines confirms that MEOX2 is significantly overexpressed in GSCs relative to non-stem glioblastoma cells. MEOX2 is essential for GSC self-renewal and viability, as siRNA knockdown impairs these traits and induces apoptosis and gene expression changes. However, the mechanisms underlying MEOX2 function remain unclear. We hypothesized that MEOX2 targets distinct genomic regions in GSCs, influencing chromatin accessibility and gene regulation. We performed ATAC-Seq on patient-derived GSC lines transduced with either a control or MEOX2-targeting shRNA. DNA was sequenced and analyzed using standard bioinformatics tools within the nf-core/atacseq pipeline. Peak calling and differential accessibility analysis identified significantly altered chromatin regions (FDR < 0.05, $\log_2 FC \geq 0.7$). ATAC-Seq revealed that MEOX2 knockdown alters chromatin accessibility at regulatory regions of genes previously identified by RNA-Seq. Among repressed genes, several (e.g., LOXL2, NEAT1, SDC1) showed reduced accessibility within known super-enhancers, suggesting MEOX2 maintains accessibility at key oncogenic loci. Conversely, knockdown increased accessibility at genes like PCDH10, ENPP2, and MMP2—implicating potential anti-tumor responses and compensatory mechanisms. These results highlight MEOX2 as a key regulator of the chromatin landscape in GSCs and suggest that targeting its activity may provide novel therapeutic opportunities in GBM.

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DEVELOPMENT OF TUMOR-SPECIFIC EV-MIRNA SIGNATURES TO IMPROVE LUNG CANCER EARLY DETECTION (EV-MIR-TEST)

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Lung cancer (LC) is the deadliest cancer worldwide, causing ~2 million deaths annually. The two primary subtypes are non-small-cell lung cancer (NSCLC, ~85%) and small-cell lung cancer (SCLC, ~15%). Due to late-stage diagnoses and the lack of effective early screening, LC has a low 5-year survival rate of less than 15%. Liquid biopsies have emerged as a promising, non-invasive alternative to tissue biopsies, analyzing circulating tumor cells (CTCs), cell-free DNA (cfDNA), and extracellular vesicles (EVs) in bodily fluids. EVs, abundant and stable carriers of molecular cargos like microRNAs (miRNAs), are critical for understanding cancer biology as they regulate cancer development, progression, and resistance to therapies. Additionally, cell-free miRNAs (cf-miRNAs) are highly stable and tissue-specific, making them promising candidates for LC diagnosis and prognosis. To identify a tumor-specific EV-miRNA signature, a global miRNA profiling was performed on plasma from early-stage NSCLC patients versus healthy controls. EVs were isolated by ultracentrifugation and immunoaffinity beads, characterized via Nanoparticle Tracking Analysis and Immunoblotting. EpCAM+ epithelial EVs (EP-EVs) were enriched using EpCAM antibodies to distinguish epithelial-derived miRNAs (EP-EV-miRNAs) from inflammatory ones (TME-EV-miRNAs). This EV-miRNA signature was validated in calibration, validation, and clinical groups. while functional assays investigated EV-miRNAs roles in NSCLC cell proliferation, migration and invasion. A new EV-miRNA signature was identified, distinguishing LC patients from healthy individuals and clarifying the origin of LC-derived EV-miRNAs. These findings suggest EV-miRNAs are valuable non-invasive biomarkers for monitoring LC progression and early detection, with potential to improve diagnosis, treatment, and survival.

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MODULATION OF ENDOSOMAL TRAFFICKING AND AMYLOIDOGENESIS IN FAMILIAL ALZHEIMER'S DISEASE

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The "amyloid cascade hypothesis" for Alzheimer's disease (AD) pathogenesis, highlights the accumulation of amyloid-β (Aβ) as a crucial trigger for the pathology. However, AD is an extremely complex disease influenced by multiple pathophysiological processes, making it impossible to attribute its onset to a single hypothesis. The endocytic pathway, where the amyloidogenic processing of APP occurs, has emerged as a pathogenic "hub" for AD. In this study, we found altered homeostasis and dynamics of endolysosomal compartments in fibroblasts from patients affected by a genetic form of AD (APP V717I mutation). These alterations corresponded to an abnormal trafficking of APP along the endolysosomal pathway, favouring its amyloidogenic processing. The identification of APP interactors involved in its trafficking and processing, and finding molecules able to interfere with these interactions, represents a promising therapeutic approach. However, the role of endosomal pathway and the possibility of modulating APP processing through it remains elusive. Among the proteins participating to APP metabolism, the RPSA receptor and its inhibitor molecule NSC47924 have been identified. In this study, we found that the inhibitor, likely by displacing APP from interaction with its receptor, reduced APP accumulation in EEs in AD cells, finally restoring both endosomal dynamics and APP distribution to those of unaffected cells. We also demonstrated that RPSA inhibition affected the aberrant APP cleavage, as it reduced the production of both APP-βCTF (C-Terminal Fragment) and Aβ in AD fibroblasts. These results highlight significant differences in endolysosomal compartments and APP processing in AD-affected cells, refining our understanding of APP/RPSA intersection.

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IMPACT OF HSV-1 INFECTION ON ENDOMETRIAL INTEGRITY: DISRUPTION OF CELLULAR HOMEOSTASIS AND MORPHOLOGICAL CHANGES IN ORGANOTYPIC MODELS

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Endometrial infections are implicated in implantation failure and pregnancy outcomes; however, the molecular mechanisms by which they disrupt endometrial function remain poorly explored. Here we investigated the cellular, molecular and functional effects of HSV-1 infection on endometrial integrity using patient-derived endometrial organoids (PDOs) as an innovative *in vitro* model.

Endometrial biopsies were obtained from 4 patients undergoing hysteroscopy, and PDOs were generated by isolating and culturing epithelial cells in a Matrigel-based 3D system. In HSV-1 (un)infected (0.001 MOI) mature organoids (300–400 μm) viral replication, membrane integrity (ZO-1, ZO-2, ZO-3, Claudins, and E-cadherin MMP3), microvilli formation, molecular pathways and cytokine release were assessed by digital droplet PCR (ddPCR), RT-PCR, Western-Blot (WB), ICH, Multiplex ELISA, 24 and 48 hours post infection.

Differentiated PDO, as assessed by ICH detection of CK7, PAX8, and E-cadherin expression, were permissive to HSV-1 infection and supported viral replication (ddPCR, ICH). Moreover, HSV-1 infection altered nuclear morphology, increased microvilli formation, disrupted cell-cell adhesion and was accompanied by an increased expression of HSV-1 receptors (MYH10, SDC, PVRL1) on endometrial epithelium (ICH, RT-PCR, WB). In particular, increased MMP3 expression correlated with a downregulation of key junctional proteins such as ZO-1, ZO-2, ZO-3, Claudins, and E-cadherin (RT-PCR, WB). This, in turn, increased permeability, facilitating viral spread and secretion of cytokines such as IL-1β, IL-18, and TNF-α, (RT-PCR, Multiplex ELISA), which further target and degrade junctional proteins, amplifying disassembly of tight junctions.

The combined effects of junctional breakdown, increased microvilli formation, and cytokine-induced tissue deterioration may critically undermine the endometrium's

receptivity, heightening the risk of implantation failure and adverse pregnancy outcomes.

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DECODING EXTRACELLULAR VESICLE MICRORNA PROFILES AS EMERGING BIOMARKERS AND THERAPEUTIC AVENUES IN SARCOPENIC HEPATOCELLULAR CARCINOMA

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Introduction:
Hepatocellular carcinoma (HCC) is the most common type of liver cancer and carries a high mortality rate, which is often further aggravated by sarcopenia — a severe form of muscle wasting. This condition negatively impacts both prognosis and the quality of life of patients, yet the molecular basis behind this association remains inadequately understood. Recent findings indicate that microRNAs (miRNAs) contained in extracellular vesicles (EVs) may play significant roles in cancer development and cellular communication. This research seeks to uncover and confirm new EV-derived miRNA profiles linked to sarcopenic HCC, with the ultimate goal of enhancing diagnostic accuracy, prognosis, and therapeutic approaches.

Materials and Methods:
A comprehensive strategy combining molecular techniques, next-generation sequencing, and computational analyses has been employed. Blood samples will be taken from individuals diagnosed with HCC (both with and without sarcopenia) as well as from healthy individuals. EVs will be isolated and analyzed through nanoparticle tracking analysis (NTA), western blotting, and transmission electron microscopy. The miRNA content within these EVs will be sequenced, followed by bioinformatics evaluation to determine miRNAs that are differentially expressed in sarcopenic HCC. Selected miRNAs will undergo further validation via droplet digital PCR (ddPCR) using a separate patient cohort. Functional tests will also be performed by introducing HCC-derived EVs to muscle cells to investigate their involvement in metabolic imbalance and muscle degradation.

Results and Discussion:
Initial findings indicate that certain EV-miRNAs show unique expression profiles in sarcopenic HCC patients compared to both non-sarcopenic individuals and healthy controls. These miRNAs are potentially involved in regulating processes such as

muscle metabolism, inflammation, and cancer development. Further experiments will explore how EVs influence skeletal muscle cells and elucidate molecular pathways responsible for sarcopenia. Additionally, AI-driven meta-analysis will be used to improve the predictive accuracy of these biomarkers. The collective insights from this research may lead to the development of RNA-based treatments aimed at reducing muscle wasting in HCC patients.

Conclusion:
This study marks an important advancement in deciphering the role of EV-derived miRNAs in the context of sarcopenic HCC. Through the identification of specific miRNA profiles, the research aspires to facilitate earlier diagnosis, improve prognostic tools, and drive the creation of novel treatment options. These discoveries could significantly change the way HCC is managed, introducing non-invasive, liquid biopsy techniques for tracking disease progression and treatment responses. Ultimately, the project supports the goals of precision medicine by aiming to optimize patient care through individualized treatment strategies targeting both liver cancer and related metabolic disorders.

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CHOLESTEROL DYNAMICS AS A THERAPEUTIC TARGET FOR NEUROINFLAMMATION: EFFECTS OF URTICA DIOICA PHYTOSTEROLS-RICH PHYTOCOMPLEXES IN HUMAN MICROGLIA

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Intracellular cholesterol dynamics are often ignored among other processes, yet they show to be fundamental in the neuroinflammatory response and cell's vitality. Many studies highlight how cholesterol dyshomeostasis is characteristic in various diseases with chronic neuroinflammation, such as neurodegenerative ones. We aim to delve into this context by administering to human microglia, which are the primary innate immune cells of the brain, extract of *Urtica Dioica's* roots, well known in literature to be rich in phytosterols. These natural compounds are notoriously integrated into mammalian steroidogenesis, a fundamental steroid hormone producing process directly involved in cholesterol metabolism. Our experiments have been conducted in resting conditions and neuroinflammatory ones too, to evaluate the potential of this treatment in the autocrine control of cholesterol trafficking.

We tested various phytosterols-rich phytocomplex (PPs) concentrations and obtained an efficient stimulation of the *de novo* neurosteroidogenesis, which has determined beneficial effects in moderating microglia-mediated neuroinflammation. On the front of genes and proteins responsible for cholesterol trafficking, we achieved downregulation of HMGCR and CEH genes expression, and upregulation of CYP27A1, LXRalpha, LXRbeta and SOAT1 genes expression. Moreover, we registered an increase in cholesterol membrane efflux and reduction of cholesterol accumulated in lipid droplets. To dispose of excess cholesterol, PPs enhanced the mechanisms of reverse cholesterol transport favoring its adequate clearance.

Our findings suggest that targeting cholesterol system with *Urtica dioica* roots-derived PPs may represent an effective approach to preserve cholesterol homeostasis and attenuate microglia-mediated neuroinflammation in patients suffering from neurodegenerative diseases.

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REDOX IMBALANCE AND IONIC PUMP DYSFUNCTIONS IN THE BLOOD OF CHILDREN WITH AUTISM SPECTRUM DISORDER

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Oxidative stress (OS), resulting from an imbalance between pro-oxidant molecules and antioxidant defenses, can damage lipids, proteins, and DNA, ultimately, leading to cell death. OS can arise from several physiological and pathological conditions and is a common etiological factor in several neurological diseases, including Autism Spectrum Disorder (ASD). ASD is a highly heterogeneous neurodevelopmental condition with a complex and multifactorial biological background. It is characterized by deficits in social interaction and communication, and by repetitive and restrictive behaviours. Additionally, ASD is associated with a wide range of comorbidities, including intellectual disability, gastrointestinal disturbances, and immune system dysregulation. This study is part of a broader research project and aims to evaluate a panel of OS parameters and the activity of two key ionic pumps, Na^+/K^+-ATPase and Ca^{2+}-ATPase, in the blood of 49 male children with idiopathic autism and in 50 neurotypically developing (NT) children, matched for gender and age (3 to 8 years). Plasma levels of reactive oxygen metabolites measured using dROMs test, plasma antioxidant capacity assessed by Bap test, plasma GSH/GSSG ratio and urinary isoprostane concentration indicated a significant redox imbalance in ASD children compared to NT. Consistent with our previous findings, a marked reduction in Na^+/K^+-ATPase activity in red blood cells (RBC) was observed. Notably, a significant decrease in RBC Ca^{2+}-ATPase activity was identified for the first time in ASD children. These data were further analyzed to explore correlations between redox status and ion pump dysfunctions. These findings reinforce our previous observations and highlight the role of redox imbalance in disrupting cellular regulatory mechanisms, thereby contributing to the neurophysiopathology of ASD.

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FUNCTIONAL PROTEOMICS AND BIOLOGICAL SCREENING OF A PROTEIN MISFOLDING CORRECTOR UNDER ER STRESS CONDITIONS

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Endoplasmic reticulum (ER) stress has been found to play a key role in many human diseases. Several intra- and extra- cellular factors may interfere with protein folding, leading to protein misfolding diseases (PMDs), which include genetic, cardiovascular, neurodegenerative conditions, and cancer. Cystic fibrosis (CF) is one such disease and its pharmacological treatment is based on the use of modulators combinations (correctors and potentiator) that target the underlying defect in the transmembrane conductance regulator. Recent studies conducted in our laboratory strongly suggest that modulators for CFTR, commonly used in therapy, seem to have potential for off-label applications. Since only in vitro binding studies have been performed and no direct interaction between these small molecules and CFTR has been proven in the cellular environment, their exact mechanism of action is unclear. As scientific data on off-target effects are increasing, our study aims to identify possible intracellular protein partners through expression proteomics and investigate the main pathways involved. These findings may help to better understand the cellular targets of CFTR modulators and open new perspectives for drug repositioning strategies.

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BIOMOLECULES OF EXERCISE-DERIVED EVS AS A THERAPEUTIC STRATEGY TO ENHANCE ANTICANCER TREATMENT EFFICACY IN BREAST CANCER PATIENTS

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Regular physical activity (PA) is a well-established protective factor against breast cancer (BC) and enhances therapeutic outcomes. These effects could be partly mediated by the exercise-induced secretome, which includes proteins and non-coding RNAs (ncRNAs) within extracellular vesicles (EVs). As key mediators of intercellular signaling, EVs regulate tumor behavior and treatment response, although their mechanistic role remains incompletely understood. This study investigated the effects of exercise-derived EVs on BC cell biology. Forty newly diagnosed BC patients (aged 50–65) were randomized into a control group (CG, n=20) receiving standard care and an exercise group (EG, n=20) completing a 16-week structured PA program alongside treatment. EVs were isolated from plasma collected pre- (PRE) and post-intervention (POST), characterized, and applied to non-tumorigenic mammary epithelial cells (MCF10A) and BC cell lines (MCF7, MDA-MB-231). Functional assays assessed EV uptake, proliferation, migration, invasion, and morphology. EVs were internalized by all cell types examined. Following uptake, EVs from the exercise group (EG POST; $1\times10^9/cm^2$) reduced cell viability by 23.25% in MCF7 and 19.07% in MDA-MB-231, with no effect on MCF10A. BC cells treated with EG POST EVs showed morphological changes, reduced proliferation, and motility. At 72 hours, cell migration decreased to 38.55% in MDA-MB-231 and 23.28% in MCF7, compared to 99.02% and 74.41% with PRE EVs, respectively.

Invasion in MDA-MB-231 was reduced by 41.61%. Furthermore, EG POST EVs enhanced doxorubicin (DOX) sensitivity, improving drug response by 8.6% in MDA-MB-231 and 10.49% in MCF7, with reduction in IC50. These findings suggest that PA modulates EV cargo to suppress tumor aggressiveness and enhance chemotherapy efficacy, supporting its role as a non-invasive adjunct to BC treatment.

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SPERM-DERIVED LIPIDS AS EPIGENETIC DRIVERS OF INTERGENERATIONAL INHERITANCE: SHAPING EARLY EMBRYONIC DEVELOPMENT AND DISEASE SUSCEPTIBILITY

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Paternal contribution to embryo development and offspring phenotypic variability (including intergenerational transmission of metabolic traits and disease susceptibility) has so far been ascribed exclusively to paternally inherited allelic variants. Nowadays, many epigenetic signals have been shown to contribute to paternal inheritance. Epigenetic signals so far identified include methyl groups covalently added to cytosines of the parental genome, post-translational modifications of histones that remain associated with sperm chromosomes and are delivered to the oocyte, and regulatory RNA molecules released from sperm into the oocyte at fertilization. Along with nucleic acids, the sperm also contributes metabolomic components to the zygote, such as lipids, carbohydrates, and amino acids. These metabolites, particularly those endowed with modulatory activities, could influence zygotic gene expression and embryonic development and thus might act as epigenetic signals. Here, we propose that the sperm lipidome might act as an additional carrier of epigenetic information, potentially affecting the embryo's development. Lean or obese male mice are isotopically labeled by drinking $^{2}H_2O$- or $U^{13}C$-glucose-enriched water to allow integration of the isotopes into sperm metabolites. Labeled males are then mated with unlabeled lean females, and preimplantation embryos (zygotes, morulae, blastocysts) are collected. Sperm and embryos are analyzed by mass spectrometry to detect isotopically labeled paternal metabolites transferred into the oocyte and assess the impact of paternal diet on the composition and profile of the transferred lipid species. Our findings reveal a previously underappreciated contribution of the paternal sperm lipidome to early embryonic development and shed light on a new molecular vehicle involved in intergenerational transmission of metabolic traits.

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AN HDAC1-MEDIATED WT1/SP1 SWITCH PROMOTES INVASION IN PERITONEAL MESOTHELIAL CELLS BY INDUCTION OF LNCRNA H19 EXPRESSION

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Abdominal adhesions, peritoneal metastases, and peritoneal dialysis (PD) are among the factors that can cause peritoneal fibrosis, a pathological change of the peritoneal membrane. This process is characterized by mesothelial cells' (MCs) acquisition of invasive/pro-fibrotic capabilities through the induction of mesothelial to mesenchymal transition (MMT), a cell-specific form of EMT.
Long noncoding (lnc) RNAs are important components of the cell's physiological regulatory systems. One of the earliest lncRNAs to be discovered, LncRNA-H19 (H19), has garnered special attention because of its involvement in cancer progression.

The purpose of this study was to clarify the epigenetic mechanisms governing H19 expression and explore the functions of the three main isoforms in primary fibrotic MCs from PD patients.

Genetic silencing/ectopic expression experiments showed that H19 downregulated the epithelial marker E-Cadherin, favored MC invasion on collagen matrix, and increased the expression of MMT markers SNAIL, TGFBRI, SMAD3, and PAI-1. When the three primary H19 isoforms were silenced, a mesenchymal phenotype was synergistically induced. H19 was downregulated by HDAC1 genetic silencing and upon treatment with MS-275, an HDAC1-3 selective inhibitor that was previously shown to favour MMT reversal. Wilm's Tumor Protein 1 (WT1), the master gene of mesothelial differentiation, was found, according to bioinformatic analysis, to bind to the H19 promoter at a region with several acetylation peaks that partially overlapped the binding site of Specificity protein 1 (Sp1), another transcription factor controlling cellular plasticity. Chromatin Immunoprecipitation (ChIP) and genetic silencing studies showed that favors an inhibitory effect of WT1 on H19 expression. Mechanistically, HDAC1 inhibition promoted the reversal towards an epithelial-like phenotype by favoring a switch between WT1 and Sp1 in H19 promoter occupancy.

Overall, we identified an HDAC1-WT1/Sp1-H19 axis that may be important for developing novel treatments meant to reverse peritoneal fibrosis.

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BONE MOLECULAR AND FUNCTIONAL ADAPTATIONS TO 3G HYPERGRAVITY IN A MULTIDISCIPLINARY MOUSE STUDY

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Exposure to hypergravity profoundly affects biological systems, including bone tissue. We investigated murine adaptation to 3g hypergravity using the Mice Drawer System (MDS) in a 27-day study combining behavioral, hematological, transcriptomic, and bone structural analyses. While initial stress responses were evidenced by body weight loss and water intake reduction, mice showed rapid behavioral adaptation. Femoral diaphysis RNA-seq revealed 429 differentially expressed genes, 93% of which were upregulated under 3g. Enriched pathways included extracellular matrix organization and collagen biosynthesis, suggesting enhanced molecular activity in bone formation. Canonical pathway analysis confirmed activation of osteogenesis-related functions, with positive regulation of osteoblast differentiation and mineralization, and suppression of osteopenia. qPCR validated upregulation of Bglap and Col1a1. Moreover, LCN2 expression was significantly reduced, with a decreased RANKL/OPG ratio, indicating a shift toward bone anabolism. MicroCT confirmed improved trabecular bone volume and architecture. Overall, our findings reveal a multifaceted bone adaptation to hypergravity, highlighting the MDS-3g model as a powerful tool to study musculoskeletal plasticity under altered gravity.

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COMMON AND NOVEL MOLECULAR MECHANISMS OF FRAMESHIFT MUTATIONS CAUSING HSPB8 PATHOLOGY

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The small heat shock protein B8 (HSPB8) is an essential chaperone in maintaining proteostasis in striatal muscle and neuronal cells. By interacting with the cochaperone BAG3, HSPA family members, and the E3-ubiquitin ligase STUB1, HSPB8 forms the chaperone-assisted selective autophagy (CASA) complex, which promotes the removal of misfolded proteins. Both others and we have reported that frameshift (fs) mutations in the last exon of the HSPB8 gene cause myopathies, with or without neurogenic involvement and cardiomyopathy. These HSPB8 fs mutations can lead to a +1 or +2 nucleotide shift in the open reading frame, resulting in the expression of HSPB8 proteins with approximately 20 or 50 amino acid-long C-terminal extensions. Here, we demonstrate that cell models expressing fs+1 or fs+2 mutants exhibit comparable behavior. Indeed, our analyses reveal that the expression of HSPB8 fs results in aggregation and sequestration of the HSPB8 wildtype along with the CASA members. Consequently, misfolded and ubiquitinated substrates get entrapped in HSPB8 mutant aggregates with autophagy receptors. Thus, through a mechanism based on the gain of toxic function and a dominant negative effect on HSPB8 WT, HSPB8 fs mutants cause a general impairment of proteostasis. To gain insight into the mechanisms regulating HSPB8 fs mutant turnover and degradation, we investigate the crosstalk between HSPB8 and Valosin-containing protein (VCP), a multifunctional member of the proteostasis network. Using an HSPB8 fs+1 as a reference mutant, we observed that the HSPB8 mutant interacts with and sequesters VCP, and that VCP silencing exacerbates HSPB8 aggregation, likely contributing to the pathogenesis of (neuro)myopathies caused by HSPB8. In summary, our results

define pathogenic mechanisms shared among different HSPB8 fs mutations and reveal a novel player in HSPB8 pathology.

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MODELING BRAIN TUMORS WITH HUMAN ORGANOIDS

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Brain tumors are a large and heterogeneous group of neoplasms that affect the central nervous system and include some of the deadliest cancers. Almost all the conventional and new treatments fail to hinder tumoral growth of the most malignant brain tumors. This is due to multiple factors, such as intra-tumor heterogeneity, the microenvironmental properties of the human brain, and the lack of reliable models to test new therapies. Therefore, creating faithful models for each tumor and discovering tailored treatments pose great challenges in the fight against brain cancer. We have recently developed human IPSCs-derived organoid models of pediatric medulloblastoma and pediatric high-grade glioma. These models overcome the limitations of using animal models and pave the way to gaining unprecedented new knowledge into the development of brain cancer in the human system. Nevertheless, to fill the gap of the brain cancer field it will be crucial to use patient-derived tissues. To this aim, we developed patient-derived organoids (PDO) for pediatric brain tumors. These organoids recapitulate several features of primary human tumors, such as intra-tumor heterogeneity and genomic alternation. The production of PDOs will allow us to generate important new knowledge, discover of potential drug targets, and small molecules that could be exploited in early-phase clinical trials, and hopefully, develop and validate new treatments. Interestingly, the possibility of using organoids directly generated from pediatric tumors will also pave the bases for personalized therapies.

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INFLAMMATION-ASSOCIATED MICRORNAS ARE DYSREGULATED IN PLEURAL MESOTHELIOMA CELL LINES.

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Pleural Mesothelioma (PM) is an aggressive tumor of the lung mesothelium with poor therapeutic
options. Chronic inflammation is considered the main cell-transforming driver of mesothelial cells,
leading to genetic and epigenetics alterations. However, many inflammation-induced alterations
are currently unknown in PM, hampering novel cancer treatments. In this study, we focused on
dysregulations of microRNAs (miRNA) involved in PM inflammatory response. RT² Profiler PCR
Array Human Inflammatory Response (n=84 miRNAs) was employed in primary PM cell lines of
different histotypes, such as epithelioid (n=2), biphasic (n=2) and sarcomatoid (n=2), and control,
normal human mesothelial cell lines (n=2). A total of 10/84 (12%) miRNAs tested differentially
expressed in PM cells compared to normal cells. Specifically, miRNAs tested either upregulated
(6/10; 60%) or downregulated (4/10; 40%). The miRSYSTEM algorithm analysis predicted, for the
4 downregulated miRNAs, 2,201 target genes. Gene Ontology (GO)/Kyoto Encyclopedia of Genes
and Genomes (KEGG) enrichment analyses showed that cell cycle transcription regulation and
G1/S phase transition were the most enriched biological processes, while activin receptor activity,
protein serine/threonine kinase activity, and DNA binding were the most enriched molecular
functions in PM cell lines. In addition, serine/threonine kinase complex, activin receptor activity,
nucleus, and heterochromatin were the most enriched cellular components. Our results indicate
that inflammation-related miRNAs are dysregulated in PM cells, highlighting their potential as
therapeutic targets. Current investigations are validating these miRNAs in a larger number of cell

lines and PM tissues. Further studies are needed to assess functional roles in PM pathogenesis of
identified miRNAs.

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SINGLE-CELL SEQUENCING ANALYSIS HIGHLIGHTS THE TRANSDIFFERENTIATION POTENTIAL OF CANCER CELLS IN A NEUROBLASTOMA CELL LINE

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Introduction

Neuroblastoma is the most frequent extracranial tumor in infants and originates from neural crest cells during embryonic development. Our laboratory identified a region within the first intron of the *ASCL3* gene that encodes a long non-coding RNA (lncRNA), named 29A, which plays a role in neuroblastoma progression. Studies have shown that elevated expression of 29A promotes neuronal differentiation in tumor cells, resulting in reduced malignancy. Clones overexpressing 29A were generated from the SK-N-BE(2) neuroblastoma cell line. High 29A expression enhanced neuronal features, confirmed by specific markers and the generation of action potentials. Interestingly, molecular and microscopic analyses revealed various cell types, despite the clonal origin of the cultures, suggesting ongoing transdifferentiation processes.

Materials and Methods

Single-cell transcriptome analysis identified nine distinct cell populations. Membrane markers allowed separation of these subpopulations using Fluorescence-Activated Cell Sorting (FACS) for targeted analysis.

Results and Discussion

We investigated the gene expression of pathways involved in tumorigenesis. Notably, we found subpopulations producing VEGF and others expressing its receptor, indicating a possible communication loop regulating angiogenesis. Additional analyses included genes related to DNA repair, cell cycle, invasion, metastasis, and epithelial-mesenchymal transition.

Conclusion

Single-cell approaches are essential for understanding tumor heterogeneity and may guide the development of targeted therapies against specific cellular subtypes within tumors. Using antibody panels to detect distinct subpopulations could improve the precision of future therapeutic strategies.

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DIGENIC AND PSEUDO-DIGENIC INHERITANCE IN NOONAN SYNDROME: ROLE OF HYPOMORPHIC VARIANTS IN RAS PATHWAY FUNCTIONAL ACTIVATION

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Noonan syndrome (NS) is an autosomal dominant multisystem disorder classified among RASopathies, caused by mutations in a subset of genes encoding components of the RAS/MAPK signaling pathway, resulting in its hyperactivation. However, the high inter- and intra-familial variable expressivity and the lack of a molecular diagnosis in 20–30% of patients suggest the involvement of additional genes and/or mechanisms in the NS pathogenesis. We recently proposed digenic and pseudo-digenic inheritance of more than one hypomorphic RAS pathway gene variants, as an alternative NS pathogenic model.

We previously described a patient with a severe NS phenotype—facial dysmorphisms, cardiac defects, and ectodermal abnormalities—carrying a de novo pathogenic *RAF1* mutation (c.781C>T, p.P261S), already associated with NS, and a likely benign *SOS1* variant (c.1490G>A, p.R497Q), inherited from his father and grandfather. As these relatives exhibited ectodermal features and mild facial dysmorphisms shared with the patient, a possible contribution of *SOS1* variant to the clinical phenotype has been hypothesized, according to the pseudo-digenic inheritance model.

Recently, we generated iPSCs from the patient's PBMCs ($RAF1^{+/-}$ and $SOS1^{+/-}$) and isogenic *SOS1*-corrected iPSCs ($RAF1^{+/-}$ and $SOS1^{+/+}$) via HDR-mediated CRISPR/Cas9 knock-in. To dissect cellular and molecular phenotypes according to the presence of one/two or no variants, we plan to differentiate the patient isogenic and *SOS1*-mutated iPSCs, along with iPSCs from healthy donors, into keratinocytes and cardiomyocytes, followed by comprehensive morphological and multi-omics characterization.

This study could provide new insights on the additive effect of RAS pathway hypomorphic variants causing signal transduction activation and their contribution to phenotypic severity, addressing in perspectives applications in precision medicine.

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ALTERATIONS IN AUTOPHAGY, B-CATENIN PATHWAY, AND PROTEASOME ACTIVITY IN OSTEOSARCOMA CELL LINES

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Osteosarcoma is the most common primary malignant bone tumor in adolescents and young adults with an incidence in Italy of around 350 new cases per year. The altered molecular mechanisms in osteosarcoma cells remain incompletely understood. In this study, we investigated the expression of key regulators of protein homeostasis in two osteosarcoma cell lines (MG63 and U2OS) compared to normal human osteoblasts (HOB). Autophagic activity was markedly reduced in the tumor lines, as shown by decreased levels of LC3B and Beclin-1, suggesting impaired autophagosome formation or turnover.

In parallel, we observed a significant increase in β-catenin expression in osteosarcoma cells, both in its total form and in phosphorylated forms at Ser675 and Ser33/37/Thr41, indicating aberrant activation of the Wnt/β-catenin signaling pathway. Notably, this dysregulation may be associated with altered proteostasis, contributing to the intracellular accumulation of misfolded or damaged proteins.

Consistent with this hypothesis, proteasome activity assays revealed a marked reduction in proteasome efficiency in MG63 and U2OS cells compared to HOB controls. The combined impairment of autophagy and proteasome function suggests a compromised protein degradation system in osteosarcoma, which could facilitate tumor cell survival under stress conditions and promote oncogenic signaling pathways.

Together, these findings highlight the disruption of protein homeostasis as a hallmark of osteosarcoma and point to the autophagy-proteasome axis and Wnt/β-catenin signaling as potential targets for therapeutic intervention.

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ESTABLISHMENT OF PATIENT-DERIVED BREAST TUMOR ORGANOIDS FOR TREATMENT EVALUATION

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Mammary gland cells express hormone receptors, specifically estrogen, progestin and androgen receptors (ER, PR and AR). These receptors are one of the drivers for tumor growth as well as a target for therapy. Some breast tumor subtypes have a specific therapeutic iter but the co-presence of receptors may interfere with it. We aim to establish and treat patient-derived organoids from different breast tumors subtypes to identify patient-specific targets toward a personalized medicine and to better understand the role of the receptors in the different phenotypic contexts of breast neoplasia. In particular, we will perform a genomic and epigenomic characterization of the collected tumor subtypes to identify specific molecular characteristics; develop organoids from tumor tissues that will be subjected to new and repurposed drugs and evaluate their effectiveness at the functional level. Normal tissue adjacent to the tumor will be obtained by pathologists from macroscopically healthy portions beyond the tumor edges. All samples are classified based on their positivity to ER, PR, AR and HER2 by immunohistochemistry. We have currently started sample collection, DNA/RNA storage and organoid development. Fresh samples are processed for breast organoid formation following published protocols and they are cryopreserved at low passages. Organoid growth is monitored using Incucyte, an innovative microscope inside the incubator allowing cell analysis in their appropriate environment. Treatment efficacy will be evaluated by the morphology/size of the organoids, functional assays including proliferation and apoptosis, and electrophysiological recordings.

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MELATONIN TO THE RESCUE: SAFEGUARDING TESTICULAR FUNCTION FROM ZEARALENONE

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The steady decline in male fertility is a growing concern, with environmental pollutants increasingly recognized as key contributors. Among emerging contaminants, endocrine-disrupting compounds like mycotoxins pose serious risks to reproductive health. Given the high sensitivity of the testis—where exposure can alter testicular activity—clarifying the underlying cellular and molecular mechanisms is essential to identify effective protective strategies. This study investigated the impact of zearalenone (ZEN), a mycotoxin produced by Fusarium species, on testicular function in adult Wistar rats, and evaluated the protective role of melatonin (MLT), a pineal hormone with antioxidant and antiapoptotic properties. Animals were divided into four groups and treated for 30 days: control, ZEN-treated (100 µg/kg/day, i.p.), MLT-treated (4 mg/L in drinking water), and ZEN+MLT co-administered. ZEN exposure led to pronounced endocrine disruption, evidenced by decreased serum testosterone and elevated estradiol levels, along with downregulation of 3β-HSD and upregulation of aromatase, as demonstrated by western blot and immunofluorescence analyses. Spermatogenesis was impaired, with a significant reduction in PCNA-positive germ cells. Oxidative stress intensified, as evidenced by increased TBARS levels and enhanced 4-HNE immunoreactivity. In parallel, apoptotic activity also escalated, marked by a higher Bax/Bcl-2 ratio, caspase-3 upregulation, and an increased number of TUNEL-positive cells. Co-administration of MLT effectively mitigated these deleterious effects by restoring steroidogenic enzyme expression, preserving germ cell proliferation, reducing lipid peroxidation, and limiting apoptosis. These findings highlight the vulnerability of the testis to environmental disruptors like ZEN and support MLT as a promising protective agent for male reproductive function.

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SIZE-DEPENDENT EFFECTS OF NANO-MICROPLASTICS ON MOUSE OOCYTE MATURATION AND REDOX HOMEOSTASIS

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Recently, nano- and microplastics (NMPs) have received significant attention due to their widespread presence and potential implications for human health. Although some evidence suggests that NMPs can accumulate in reproductive tissues, few studies have investigated their effects on female germ cells. In this context, our study aimed to determine whether NMPs can penetrate oocytes and to assess their effects on meiotic resumption and redox homeostasis. Cumulus-oocyte complexes (COCs) were collected from PMSG primed-young CD1 mice and exposed to 5-50 µg/ml NMPs of two different sizes (40 nm or 200 nm) during 16 h-*invitro* maturation (IVM). NMP uptake was assessed under confocal microscopy by using fluorescent NMPs. After IVM, oocytes were isolated from cumulus cells (CCs). Number of oocytes reaching metaphase II (MII) stage was evaluated. MII oocytes and CC were processed for real-time Taqman PCR. MII oocytes were also stained for the analysis of mitochondrial ROS production by MitoSOX staining and subjected to immunofluorescence for the analysis of MII apparatus (spindle and chromosomes) and SIRT1 level. Our results showed that NMPs were internalized by CCs, but only 40 nm NMP entered the oocyte. After IVM a lower percentage of MII oocytes were observed in 40 nm NMPs compared to the control and 200 nm NMP groups. Accordingly, 40 nm NMPs induced altered spindle assembly. Mitochondrial ROS were increased in the 200 nm NMP oocytes. Sirt1 and Sod2 transcripts decreased in oocytes and CCs exposed to 40 nm NMP during IVM. Higher SIRT1 protein level was found in the oocytes from 40 nm NMP group. These results provide the first evidence that 40 nm NMPs enter the mammalian oocyte, impair meiosis and MII spindle assembly. Moreover, our data indicate that the oocyte activates an adaptive response mediated by SIRT1-signalling in order to cope with NMP-induced oxidative stress.

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EPIGENETICALLY MODIFIED ENDOTHELIAL CELLS BY HYPERGLYCEMIA AS A USEFUL IN VITRO MODEL TO STUDY THE ANTI-INFLAMMATORY POTENTIAL OF OLIVE LEAF EXTRACTS

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We previously showed that chronic hyperglycemia during Gestational Diabetes (GD) induces epigenetic alterations that contribute to a pro-inflammatory and pro-oxidant phenotype in human umbilical vein endothelial cells (HUVECs). Considering the increasing interest in olive leaf extracts (OLEs) for their potential anti-inflammatory and antioxidant properties, and since the epigenetic changes are modifiable by environmental factors such as diet, this study aimed to evaluate the anti-inflammatory effects of OLEs on endothelial cells derived from control women (C-HUVECs) and women with gestational diabetes (GD-HUVECs).

First, the antioxidant phenolic profile of OLE from Changlot Real and Picual cultivars were analyzed using ultrasound-assisted extraction and by HPLC-ESI-TOF-MS. Both OLEs were tested by assessing cellular vitality (MTT assay). C- and GD-HUVECs were then pre-treated with OLEs (0.1, 10, and 50 μg/mL) for 24 hours before exposure to low levels of the inflammatory cytokine TNF-α (10 ng/mL) for 16 hours. NFKb-p65 and Monocyte Chemoattractant Protein (MCP-1) gene expression was assessed by RT-qPCR. Protein expression of Vascular Cell Adhesion Molecules (VCAM-1), pNFKb-p65/NFKb-p65 ratio (flow cytometry), monocyte-endothelium adhesion were also evaluated.

The data show that Changlot Real and Picual OLE contained 14.7 and 8.7 mg/g of phenolic compounds, respectively. Pre-treatment with both OLEs reduce NFKb-p65 and MCP-1 gene expression ($p<0.05$), pNFKb-p65/NFKb-p65 ratio ($p<0.05$), VCAM-1 protein expression ($p<0.05$), and monocyte adhesion ($p<0.001$), demonstrating their potential anti-inflammatory activity.

Thus, our epigenetically modified *in vitro* endothelial cell model has been highly effective in exploring mechanisms potentially involved *in vivo* in the reduction of endothelial inflammation and dysfunction following OLEs consumption.

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ONE-CARBON PATHWAY IMBALANCE IN TRISOMY 21

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The one-carbon pathway is involved in critical human cellular functions such as cell proliferation, mitochondrial respiration, and epigenetic regulation, and its alteration impairs proper human neurodevelopment.

Trisomy 21 (T21) is the genetic cause of Down syndrome (DS). The additional copy of chromosome 21 (Hsa21) causes transcriptomic and metabolic alterations. The genetic cause of intellectual disability in T21 is unknown, and anomalies of the one-carbon cycle may play an essential role. It was recently demonstrated that some metabolites of this pathway are altered in the plasma of subjects with T21 compared with euploid controls (N).

We decided to test by liquid chromatography if metabolite alterations are also identifiable in urine by comparing 58 samples from DS subjects and 48 N, focusing on two molecules of the homocysteine-methionine cycle involved in cellular methylation. Moreover, the plasma concentration of methylcobalamin (MeCbl) in 10 T21 subjects and 7 N was analyzed. Finally, we examined the expression of 42 genes involved in the one-carbon cycle in blood samples from 10 T21 subjects and 10 N.

The results showed that S-adenosyl-homocysteine is slightly more excreted in the urine of T21 subjects, while MeCbl is slightly reduced in the plasma of the T21 group. Moreover, 13 genes out of 42 are differentially expressed in blood samples, and among these, 4 overexpressed genes are located on Hsa21 (*GART*, *PRMT2*, *SETD4*, *SLC19A1*). Statistical analyses revealed significant correlations between

gene expression and metabolic data and highlighted possible new gene interconnections.

In conclusion, we can state that the presence of three copies of Hsa21 results in dysregulation of the expression of genes involved in the one-carbon cycle, located on Hsa21 and other chromosomes. This results in metabolic alterations visible in the plasma and urine of T21 subjects.

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SUBPOPULATIONS OF CRANIAL SUTURE NICHE MESENCHYMAL STROMAL CELLS IN CRANIOSYNOSTOSIS: MOLECULAR AND FUNCTIONAL INSIGHTS

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Skull flat bones and sutures house multipotent calvarial mesenchymal stromal cells (CMSCs) contributing to cranial development. Disruption between proliferation and differentiation in this niche accelerates osteogenesis, leading to premature suture fusion (craniosynostosis, CS). Recent evidence suggests CS may result from imbalance between CTSK$^+$ CMSCs, promoting ossification, and DDR2$^+$ CMSCs, preserving suture patency. This study investigates molecular and functional profiles of CMSCs and osteoclast precursors from CS patients to clarify mechanisms underlying suture niche dysfunction. CMSCs were isolated from cranial suture samples of CS patients and controls (protocol #4876), from fused and unfused sutures. Proliferation, osteogenic differentiation, and subpopulation markers were assessed by live-cell imaging, qPCR, and Alizarin Red S staining; differentiation potential of osteoclast precursors was analyzed by TRAP staining. Shotgun proteomics was used for expression profiles of patient-derived CMSCs. CMSCs showed reduced proliferation by live imaging and lower expression of cell cycle markers (Ki67, CCNB1) compared to controls; while osteogenic genes (RUNX2, COL1A1, COL1A2, BGLAP, ALP) were upregulated. Proteomics revealed activation of key osteogenic pathways including FGFR2, BMP2, WNT/β-catenin, and ECM-remodeling cascades in patient-CMSCs. Osteoclast precursors from CS patients showed impaired maturation, with more bi-nucleated cells and fewer mature multinucleated cells (>3 nuclei), suggesting defective fusion and reduced bone resorption. These findings showed a pathological shift in cranial suture niche homeostasis in CS, characterized by enhanced osteogenic differentiation of CMSCs and impaired osteoclast maturation. The identified molecular profiles provide new insights into CS pathogenesis and may inform strategies to restore physiological suture dynamics.

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CHAGA MUSHROOM EXTRACT INHIBITS DIHYDROFOLATE REDUCTASE AND ACTS SYNERGISTICALLY WITH CONVENTIONAL THERAPIES IN BREAST CANCER

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Inonotus obliquus (Chaga) is a medicinal mushroom that is used as a tea in traditional Chinese medicine. In this study, Chaga water extract was digested *in vitro* to mimic the natural processing and absorption of its biocomponents when it is consumed as functional beverage, and its anticancer activities were evaluated in breast cancer (BC) cell lines. After chemical characterization by liquid chromatography/mass spectrometry (HR-QTOF) analysis, four highly bioactive triterpenoids (inotodiol, trametenolic acid, 3-hydroxy-lanosta-8,24-dien-21-al, and betulin) were identified as the main Chaga components. Digested Chaga extract decreased SK-BR-3 (HER2-positive) and MDA-MB-231 (triple negative) cell viability in a time- and dose-dependent manner, induced a downregulation of cyclin D1, CDK4, and cyclin E and consequently reduced retinoblastoma protein phosphorylation (Ser 780), leading to cell cycle arrest in G0/G1, as assessed by FACS analysis. Moreover, Chaga exerted a synergistic action with cisplatin and with trastuzumab in BC cells by inhibiting both HER2 and HER1 activation and displayed an immunomodulatory effect. Due to the central role of the enzyme dihydrofolate reductase (DHFR) in regulating cell viability and proliferation, the effects of Chaga on DHFR activity were also investigated. The enzymatic activity of DHFR was significantly inhibited by Chaga in BC cells. In addition, we observed that chaga water extract was able to reduce BC growth in Δ16HER2 mice (BC preclinical model) in the absence of systemic toxicity.

In conclusion, *Inonotus obliquus* represents a source of triterpenoids that are effective against aggressive BC subtypes and display properties of targeted drugs. It can also represent a remedy that, in combination with conventional drugs, may increase their effectiveness or reduce their dosage.

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VALORISING GRAPE POMACE: A SUSTAINABLE SOURCE OF ANTIOXIDANT AND ANTI-INFLAMMATORY COMPOUNDS FOR PSORIASIS TREATMENT

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Psoriasis is a chronic inflammatory skin disease characterized by keratinocyte hyperproliferation and immune dysregulation. This study explores the potential of an aqueous extract from Rosé winemaking grape pomace as a sustainable, natural topical treatment. UHPLC analysis revealed a rich phytochemical composition, particularly malvidin-3-O-glucoside, catechins, and flavonols. In vitro assays demonstrated strong antioxidant activity (DPPH, ABTS, FRAP) and anti-inflammatory effects, including nitric oxide inhibition. In human BJ fibroblasts and HaCaT keratinocytes, the extract protected cells from ROS-induced damage and significantly reduced LPS-induced IL-6 expression. In HaCaT cells, it also downregulated VEGF-A levels. Moreover, conditioned media from HaCaT cells treated with the extract prevented tube formation in endothelial cells, which was otherwise promoted by LPS-conditioned media. Notably, the extract upregulated miR-30c-5p, a microRNA involved in immune and inflammatory regulation. Bioinformatic analyses (GO, KEGG, Reactome) and protein-protein interaction (PPI) network mapping highlighted its role in key pathways, including TGF-β and MAPK signaling. These findings support Rosé grape pomace as a promising eco-friendly ingredient for the development of topical therapies targeting inflammatory skin conditions such as psoriasis. A topical cream formulated with the extract's "active water" showed no cytotoxic or sensitizing effects on artificial skin and could be used for further in vivo experiments on patients.

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