Proceedings of the 17th International Congress of Histochemistry and Cytochemistry

August 27-30, 2025

PalaCongressi of Rimini, Italy

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The European Journal of Histochemistry was founded in 1954 by Maffo Vialli and published till 1979 under the title of Rivista di Istochimica Normale e Patologica, from 1980 to 1990 as Basic and Applied Histochemistry and in 1991 as European Journal of Basic and Applied Histochemistry. It is now published under the auspices of the University of Pavia, Italy.
The European Journal of Histochemistry is the official organ of the Italian Society of Histochemistry and a member of the journal subcommittee of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC), and has been an influential cytology journal for over 60 years, publishing research articles on functional cytology and histology in animals and plants.

The Journal publishes Original Papers, Technical Reports, Reviews, Brief Reports, Letters to the Editor, Views and Comments, and Book Reviews concerning investigations by histochemical and immunohistochemical methods, and performed with the aid of light, super-resolution and electron microscopy, cytometry and imaging techniques; attention is also given to articles on newly developed or originally applied histochemical and microscopical techniques.

Coverage extends to:
- functional cell and tissue biology in animals and plants;
- cell differentiation and death;
- cell-cell interaction and molecular trafficking;
- biology of cell development and senescence;
- nerve and muscle cell biology;
- cellular basis of diseases.

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PLENARY LECTURES OF AWARDEES

Robert Feulgen Lecture: *Roland Foisner (Austria)*

LAMINS IN NUCLEAR ORGANISATION AND CHROMATIN REGULATION

S. Ferraioli, F. Sarigöl, K. Georgiou, N. Naetar, R. Foisner

Max Perutz Labs, Medical University Vienna, Austria

Lamins form a filamentous network at the nuclear envelope that anchors heterochromatin and repressed genes to the nuclear periphery. Lamin A also exists as a non-filamentous pool in the nuclear interior, where it interacts with lamin-associated polypeptide 2 alpha (LAP2a). Both proteins associate with euchromatin containing active genes, but the functional significance for gene regulation is poorly understood. Using an *in vitro* myoblast differentiation cell culture model, we found that LAP2a relocates towards genomic regions containing myogenic genes in early stages of muscle differentiation, facilitating efficient myogenic gene expression, while lamins mostly associate with regions apart from these genes. Strikingly, upon depletion of LAP2a, A-type lamins spread across active chromatin and accumulate at regions of active H3K27ac and H3K4me3 histone marks in the vicinity of myogenic genes, leading to their impaired expression during differentiation. Reorganization of A-type lamins towards the active, gene rich chromatin regions is accompanied by depletion of the active chromatin mark H3K27ac and a significantly impaired myogenic differentiation. Thus, the interplay of LAP2a and A-type lamins is crucial for efficient expression of myogenic genes. While most of these genes are located in the nuclear interior, *MyoD1,* a master regulator of myogenesis is located at the nuclear periphery in proliferating myoblasts, but it is transcriptionally active. We developed a myoblast reporter cell line to detect the nuclear position of *MyoD1* in live cells, in order to identify mechanisms involved in anchoring *MyoD1* to the nuclear periphery. Knockout of lamin A caused detachment of heterochromatin from the nuclear periphery but did not affect the peripheral *MyoD1* localization. In contrast, depletion of the ERand nuclear envelope protein WFS1 released *MyoD1* into the nuclear interior. Genome wide chromatin interaction studies showed that WFS1 interacts with active *MyoD1* enhancer sequences. Thus, while peripheral lamins bind to repressed genes, WFS1 anchors active genes to the nuclear envelope allowing their efficient expression.

Funded by Austrian Science Fund (FWF P36503-B).

David Glick Award Lecture: *Christoph Cremer (Germany)*

SUPER RESOLUTION MICROSCOPY: NEW APPROACHES TO DISCOVER THE TOPOGRAPHICAL SECRETS OF GENE REGULATION

C. Cremer[1,2,3]

[1]Institute of Molecular Biology (IMB), Mainz, Germany; [2]Max Planck Institute for Polymer Research, and for Chemistry, Mainz, Germany; [3]Interdisciplinary Center for Scientific Computing (IWR), and Kirchhoff Institute for Physics, University Heidelberg, Heidelberg, Germany

The spatio-temporal folding pattern of the nuclear chromatin has emerged as a decisive key parameter for transcriptional control and hence for gene regulation[1]. A wealth of information on this subject has been obtained from a variety of biochemical approaches[2]. These methods allowed to measure relative contact frequencies between specific DNA sequences; relative distances and thus relative chromatin domain sizes; or relative DNA densities. Based on such data, even the calculation of probable 3D structures has become possible. However, the real processes of transcription regulation do not take place in the space of probability, but in real space (nm) and in real time (s);therefore an understanding of these mechanisms is only possible through knowledge of the actual processes in real space and in real time, measured in absolute units: Hence, complementary information is required on absolute distances (nm), absolute positions (x,y,z), absolute sizes (μm^3), absolute DNA densities (Mbp/μm^3), and really existing, not only probable structures in space and time (x,y,z,t) at the single cell level. Such topographical information may now be obtained by a variety of super-resolution methods[3,4], with perspectives down to the sub-nm resolution range[5].

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Paul Nakane Prize Lecture: *Pavel Hozák (Czech Republic)*

FROM NUCLEOLAR MORPHOLOGY TO MOLECULAR REGULATION OF GENE EXPRESSION BY PHASE SEPARATION

P. Hozák

Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

Processes such as gene expression or DNA repair are compartmentalized within eukaryotic nucleus, and nuclear environment contains dynamic membrane-less sub-compartments whose formation is prevalently driven by phase separation. It Apparently, formation of phase boundaries provides the surface for spatiotemporal control contributing to the high-rate kinetics of crucial processes such as transcription, ribosome maturation, splicing. I will briefly recapitulate the history of my research and devote a majority of my talk to recent findings from our Prague laboratory. findings. We discovered the Nuclear Lipid Islets (NLIs) –globular ~100 nm

structures containing PI (4,5) P2 (PIP2) at their periphery which associate with key transcription factors and showed that NLIs are crucial for efficient Polymerase II transcription. To decipher whether the NLIs surface recruits a transcription regulatory protein through PIP2 molecules in their surface, we employed a proteomic approach based on differential quantitative mass in combination with super-resolution microscopy. We identified more than 300 NLIs-associated proteins belonging to gene expression (53%) and pre-mRNA splicing (33%). Super resolution microscopy confirmed that some candidate proteins form foci in nucleoplasm and associate with sub-population of NLIs. Further, our bioinformatical analysis of putative NLIs proteins revealed that majority of them contain Intrinsically Disordered Regions (IDRs). IDRs are known features of proteins undergoing phase separation under *in vivo* and *in vitro* conditions. Moreover, we found that the vast majority of these proteins contain K/R rich motifs, which were previously shown as recognition sites for phosphoinositide (PIPs) binding. We hypothesize that NLIs may serve as a structural platform integrating RNA Polymerase II transcription and pre-mRNA splicing by attracting proteins which are prone to form liquid-like particles.

This study was supported by the Czech Academy of Sciences (JSPS-20-06); Grant Agency of the Czech Republic (19-05608S, 18-19714S); IMG ASCR, v. v. i. (RVO: 68378050); by the COST Action CA19105 and MEYS CR (COST Inter-excellence LTC19048), and Czech-BioImaging projects (LM2018129 and LM2023050, funded by MEYS CR).

PLENARY LECTURES OF KEYNOTE SPEAKERS

IMMUNOLOGICAL MECHANISMS IN REGENERATIVE MEDICINE: A LESSON FROM THE HUMAN PLACENTA

O. Parolini[1,2]

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A major goal in regenerative medicine is restoring damaged tissues through cell replacement or by activating endogenous repair pathways. Immune modulation is increasingly recognized as essential for creating a permissive environment for regeneration. Bioactive factors secreted by stem and stromal cells -either as soluble molecules or within extracellular vesicles (EVs)- can reshape immune responses, not just by reducing inflammation but by actively promoting pro-resolving, regenerative profiles. For nearly two decades, our research has focused on the therapeutic potential of human term placenta-derived cells, particularly mesenchymal stromal cells from the amniotic membrane (hAMSC). We have shown that hAMSC and their secretome (CM-hAMSC) exert potent immunomodulatory effects *in vitro*: suppressing T- and B-cell proliferation, downregulating proinflammatory Th1/Th17 phenotypes, enhancing regulatory T cells, and inhibiting naïve CD8 T cell polarization into memory subsets. They also reduce B-cell differentiation into antibody-secreting cells and steer monocytes away from dendritic cells and M1 macrophages toward anti-inflammatory M2 phenotypes. These effects have translated into therapeutic benefits in numerous preclinical models of inflammation-driven disease (including lung/liver fibrosis, sepsis, IBD, cardiac ischemia, autoimmune encephalomyelitis, rheumatoid arthritis, and traumatic brain injury) by tempering inflammation and promoting resolution. Ongoing studies aim to dissect the mechanisms driving these effects, especially whether they are mediated by soluble molecules, EVs, or both. A key area of interest is their ability to modulate the inflammasome pathway, a central driver of innate immunity linked to aging and degeneration. By regulating this pathway, placental factors may redirect chronic inflammation toward regeneration. Given inflammation's dual role as trigger and barrier in many degenerative conditions, placental cells -especially those from the amniotic membrane- emerge as a powerful paradigm. This lecture will explore how their unique immunological properties can inspire innovative regenerative strategies.

A TECHNOLOGY PLATFORM FOR TRANSLATION OF ATMPS (ADVANCED THERAPY MEDICINAL PRODUCTS): THE EXAMPLE OF HUMAN EYE AND SKIN

G. Pellegrini

Head of Cell Therapy Program Center for Regenerative Medicine Department of Life Sciences University of Modena e Reggio Emilia, Modena, Italy

Developing cellular therapies is not straightforward. This presentation summarizes the experience of academic stem cell investigators working in different clinical applications and aiming to share insight into what could be useful to consider, in light of the current

situation regarding advanced therapies. These include 1) choosing the stem cell type and assessing a platform of technologies possibly common to multiple products, 2) familiarity with GMP manufacturing, reagent validation, and supply chain management, 3) product delivery issues and the additional regulatory challenges, 4) the relationship between clinical trial design and preclinical studies, and 5) the market approval requirements, scalability, pathways, and partnerships needed.

ANTIBODY-TO-IMAGING PIPELINE TO MONITOR DRUG-TARGET ENGAGEMENT IN BREAST TUMORS

M. Barroso

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Monoclonal antibody-based therapies have become standard treatments for both hematological and solid malignancies. Trastuzumab (TZM), a monoclonal antibody targeting human epidermal growth factor receptor 2 (HER2), is a key therapy for HER2+ breast cancer. However, therapeutic efficacy is often compromised by tumor microenvironment (TME) features, such as dense extracellular matrix and poor vascularization, that limit antibody access and binding. Thus, there is a critical need for imaging approaches that can measure whether an antibody drug actually reaches and binds its target within heterogeneous tumors. We have developed a near-infrared (NIR) fluorescence lifetime (FLI) Förster Resonance Energy Transfer (FRET) optical imaging platform to address this challenge. This method directly reports drug-target engagement by measuring the fraction of donor labeled antibodies undergoing FRET when in proximity to acceptor-labeled antibodies upon receptor binding, using *in vitro* microscopy and *in vivo* macroscopy. We applied NIR-FLI-FRET to quantify HER2 binding in HER2+ breast cancer cells, tumor spheroids, and xenografts. To assess the influence of TME, we examined tumors with differing levels of collagen and vascularity. NIR-FLI-FRET imaging revealed that tumors with elevated collagen content and reduced vascularity showed decreased TZM- HER2 binding, despite robust HER2 expression, suggesting physical barriers to antibody penetration and reduced therapeutic efficacy. We further integrated site-specific labeling and antibody engineering *via* meditope- enabled TZM (MDT-TZM) to improve imaging precision and antibody performance. MDT-TZM exhibited deeper tumor penetration and more uniform binding *in vivo* compared to traditional NHS-labeled TZM. FRET signal maps correlated strongly with immunohistochemistry for HER2 and TZM, validating imaging results. Together, this antibody-to-imaging pipeline offers a powerful, non-invasive method to quantify drug-target engagement and understand how TME impacts antibody therapy. It represents a critical step toward precision imaging to guide antibody design and optimize treatment strategies in breast cancer.

SESSION: FRONTIERS IN STEM CELLS AND REGENERATIVE MEDICINE

HUMAN AMNIOTIC MEMBRANE POTENTIAL FOR BONE REPAIR: FROM THE LAB TO THE CLINIC - ROLE OF ITS IMMUNOGENICITY

F. Gindraux[1,2]

[1]CHU Besançon, Service de Chirurgie Maxillo-Faciale et Stomatologie, Besançon, France; [2]Université Marie et Louis Pasteur, SINERGIES UR 4662, Besançon, France

The human amniotic membrane (hAM) has low immunogenicity and offers anti-inflammatory, anti-fibrotic, antimicrobial, antiviral, and analgesic effects. It contains stem cells and growth factors that support tissue regeneration, with preservation methods including cryopreservation, lyophilization, and dehydration. We evaluated hAM in orthopedic and maxillofacial bone surgery, focusing on its potential to improve the induced membrane technique Our studies assessed cell survival, osteodifferentiation, and osteogenic potential both *in vitro* and *in vivo*, and tested various processing methods in collaboration with Inserm U1026 BioTis (Bordeaux)). Results indicated limited effectiveness of hAM for bone defect repair. Given these findings, we explored hAM for medication-related osteonecrosis of the jaw (MRONJ). In a 6-month prospective study of eight MRONJ patients treated with cryopreserved hAM, 80% showed complete or partial wound healing and all experienced pain relief and improved quality of life. Radiological follow-up confirmed lesion stability or new bone formation in most cases. A multicenter randomized clinical trial (NCT05664815) involving 57 patients is currently underway. In parallel, we investigated the humoral immune response following hAM transplantation. We prospectively followed 23 patients undergoing cryopreserved hAM transplantation between August 2022 and September 2023, collecting sera at transplantation, 1-, and 3-months post-transplant. Nine patients had prior immunizing events. Anti-HLA class I and II antibodies were assessed by Luminex, and anti-HLA-G by ELISA. No *de novo* immunization or change in anti-HLA antibody profiles was observed post-transplant, and no anti-HLA-G antibodies were detected. These results confirm the absence of humoral immunization after hAM transplantation, supporting its immunological safety and excellent clinical tolerance.

HUMAN AMNIOTIC EPITHELIAL CELLS FOR RETINAL REGENERATION: INVESTIGATING THEIR DIFFERENTIATIVE POTENTIAL *IN VITRO*

R. Pisu[1], S. Saponara[2], S. Angioni[2], E. Peiretti[2], S.G. Vitale[2], F. Marongiu[1]

[1]Department of Biomedical Sciences, University of Cagliari, Cagliari; [2]Department of Surgical Sciences, University of Cagliari, Cagliari, Italy

The human amniotic membrane (hAM) has long been used in ophthalmology for its regenerative and immunomodulatory properties. Its emerging application in retinal repair -particularly for

macular holes, retinal tears, and age-related macular degeneration (AMD)- typically focuses on structural or paracrine support, often overlooking its cellular components. Notably, human amniotic epithelial cells (hAECs), which form a natural monolayer on a basement membrane, resemble the architecture of the retinal pigment epithelium (RPE). This structural similarity suggests the potential to engineer RPE-like cell sheets directly on hAM for therapeutic use. Term placentas were obtained from uncomplicated cesarean deliveries with informed maternal consent. hAECs were either maintained on their native hAM scaffold or enzymatically isolated and cultured on plastic. Cell morphology was assessed under maintenance conditions. Expression of key RPE-specific genes and proteins was analyzed over time using qRT-PCR and immunofluorescence, following exposure to differentiation inducing factors involved in retinal development. hAECs cultured on native hAM retained superior epithelial integrity, exhibited reduced stress markers, and showed more stable intercellular organization compared to those cultured on plastic. Several RPE markers were present in freshly isolated hAM and remained more stably expressed in native cultures. Combinatorial use of specific differentiation cues further enhanced expression of characteristic RPE markers at both the transcript and protein levels. However, marker expression varied between placental samples, indicating biological variability that may influence differentiation outcomes. These preliminary findings suggest that hAECs possess the capacity to acquire RPE-like features *in vitro*, particularly when cultured on their native membrane. This highlights their promise as an ethically acceptable and readily available source for developing RPE-like grafts. Nonetheless, inter-donor variability underscores the need for standardized differentiation protocols and further investigation into *in vivo* integration and functional stability in AMD models.

PERICYTE INVOLVEMENT IN THE PATHOGENESIS OF COLVI-RELATED MYOPATHIES: INSIGHTS INTO NG2–COLVI AXIS

A. Fazio[1], C. Evangelisti[1], P. Sabatelli[2], A. Cappellini[1], A. Di Martino[3], C. Faldini[3], L. Manzoli[1], S. Ratti[1]

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Pericytes are multifunctional mesenchymal stem cells with myogenic potential, strategically located within the microvascular niche, where they contribute to vascular homeostasis and support skeletal muscle regeneration[1]. Their capacity to differentiate into myogenic lineages and coordinate repair processes positions them as key players in tissue regeneration. However, their involvement in collagen VI-related myopathies (COLVI-RMs) remains unexplored. This study investigates the contribution of pericytes to COLVI-RM pathogenesis, with a focus on the interaction between COLVI and neural/glial antigen 2 (NG2), a proteoglycan involved in COLVI anchoring at the cell membrane[2]. Morphological and ultrastructural analyses of muscle biopsies from COLVI-RM patients revealed altered pericyte distribution and thickening of the capillary basement membrane compared to healthy controls. *In vitro*, pericyte cultures from COLVI-RMs patients showed defective COLVI deposition in the extracellular matrix (ECM), with impaired anchoring to the cell surface, due to a disrupted interaction COLVI-NG2. Of note, COLVI-RM pericytes exhibited a shift toward a quiescent state, with reduced activation of proliferative pathways and upregulation of quiescent markers, consistent with an altered activation profile. In line with these findings, *in vitro* inhibition of COLVI–NG2 binding in control pericytes reproduced these alterations, confirming the functional relevance of this molecular axis. Collectively, our findings identify pericyte dysfunction as a potential contributor to the pathophysiology of COLVI-RMs, suggesting that targeting pericytes may offer a novel therapeutic strategy to enhance muscle regeneration in these diseases.

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THE AUTOPHAGY-INDEPENDENT ROLE OF ATG7 IN REGULATING NEUROGENESIS

Y. Shen, G. Wang

International Joint Laboratory for Embryonic Development & Prenatal Medicine, Division of Histology and Embryology, Medical College, Jinan University, Guangzhou, China

Spinal cord injury (SCI) is a widespread neurological disease that can cause long-term disability. Although there is currently no effective clinical treatment, stem cells have great potential for repairing SCI. Stem cells are a group of cells with unique selfrenewal and differentiation abilities. To achieve cell remodeling, stem cell renewal and differentiation require strict control of protein turnover in the stem cells. Autophagy, a highly conserved «gatekeeper» of cell homeostasis, can regulate cell remodeling by precisely controlling protein turnover in cells. Recently, it has been found that the expression of autophagy markers changes in animal models of SCI. Therefore, understanding whether autophagy can affect the fate of stem cells and promote SCI repair is of considerable clinical value. To explore the role of autophagy or its key genes in activating neuronal differentiation of endogenous neural stem cells for SCI repair, it is necessary to use the traumatic mouse spinal cord injury model. Based on the relationship between autophagy homeostasis control and stem cell function, we are exploring new strategies to help repair spinal cord injury.

SESSION: FROM HISTOCHEMISTRY AND HISTOPATHOLOGY TO CLINICAL TRANSLATION

UNRAVELING THE RELATIONSHIPS BETWEEN TUMOR CELLS AND THEIR MICROENVIRONMENT USING HIGH-RESOLUTION SPATIAL TRANSCRIPTOMICS

M. Cordenonsi

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During the last decade, single-cell RNA sequencing (scRNA-seq) methods paved the way for deep molecular analysis of normal and pathological tissues at unprecedented resolution. Single-cell molecular analyses allow to identify different cell types, including some never discovered before, and to have an insight to their ontogenetic relationships. Moreover, these data can also be used to infer the signals that different types of cells exchange with each other. Despite these advances, scRNA-seq data do not contain a crucial set of information, which is the spatial architecture of the analysed tissues and organs, as they are obtained through the dissociation of the original specimens at single-cell level. Without the knowledge of the spatial relationship between cells, it is difficult to get any insight on their functional interrelations and the workings of the entire cell ecosystem that compose a particular tissue. This problem is even more conspicuous for tumors, for which there is no prior knowledge on the structure of the tissue, as it varies from patient to patient. In recent years, new methods, generally referred as «spatial transcriptomics» have been developed to chart the high-detailed picture emerging from scRNA-seq analyses into tissue sections. Here I present a new study showing how high-resolution spatial transcriptomics helps to unravel the structure of tumor tissues, not only by charting the cell types identified by scRNA-seq on the tissue, but also allowing to make educated guesses on their functional relationships.

ROLE OF SCHWANN CELLS-CANCER CELLS CROSSTALK IN TUMOUR PROGRESSION

C. Giampietri[1], E. Pizzichini[1], F. Somma[1], S. Petrungaro[1], A. Facchiano[2], A. Filippini[1], C. Fabrizi[1], E. Gaudio[1]

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In the last years, relevant studies have shown that the peripheral nervous system is actively involved in the progression of cancer and some works have demonstrated the role of Schwann cells (SCs) in affecting cancer progression.[1,2] Our results (obtained on melanoma and hepatocarcinoma cells exposed to the conditioned medium of human SCs cultures) reveal that SCs may induce more aggressive features on tumour cell lines, including enhanced proliferation, migration, Matrigel invasion, changes in the protein levels of epithelial-to-mesenchymal transition markers (N-cadherin, E-cadherin, Vimentin), upregulation of key oncogenes and downregulation of tumour suppressors. Concurrently, paracrine signals from cancer cells may induce a chemotactic response in SCs, promoting processes such as proliferation, migration, Matrigel invasion and upregulation of repair-related markers (GFAP), thereby activating a programme resembling the one occurring at nerve injury sites. Our findings demonstrate a bidirectional interaction between SCs and cancer cells. This emphasizes the importance of developing a deeper understanding of the glial component in the tumour microenvironment, as this could significantly impact tumour progression. Human Cell cultures of melanoma (SK-MEL-28, A375), hepatocarcinoma (HepG2, Hep3B), primary SCs. Proliferation assays (cell counting, MTT, BrdU, clonogenic tests), migration/ invasion assays, WB and MS-based proteomic analyses.

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CAPTURE-CAPIVASERTIB AND PROSTATE CANCER TARGETING: UNRAVELING RESISTANCE ELEMENTS

M. Zavatti[1], V. Serafin[1], L. Antolini[1], S. Bresolin[2], L. Reggiani Bonetti[3], S. Marmiroli[1]

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Prostate cancer is the second most commonly diagnosed cancer in men and the third cause of cancer-related deaths in European men. Invariably, cancer secondary to genomic aberration is initially dependent on androgen receptor for growth. Therefore, castration by hormone therapies, such as androgen deprivation therapy combined to inhibitors of the androgen receptor, abiraterone or enzalutamide, remain the first therapeutic choice. Nevertheless, recurrence and progression to metastatic castration-resistant prostate cancer is frequent, and these patients have very short survival due to limited treatment options, with only one third surviving five years after diagnosis. Recently, several clinical studies (such as CAPItello 281) associated abiraterone to the firstin-class, ATP-competitive, pan AKT inhibitor capivasertib, with encouraging results. However, it is well known that adaptive signaling can cause attenuation of PI3K/AKT inhibitors efficacy, reducing clinical outcome. By means of SILAC-based proteome mapping, our study reveals that capivasertib alters the expression of several proteins involved in the endocytic pathway, such as Dynamin II, Rab5 as well as the transferrin receptor, helping cancer cells to evade apoptosis. Moreover, we demonstrate that inhibition of AKT prompts complete down-modulation of microRNA-145, a well-known anti-oncogenic and a major downregulated microRNA in prostate cancer. Remarkably, by the miRNet algorithm we found that several targets of miR-145 are downstream effectors or upstream activators of the PI3K/AKT pathway. Accordingly, we observed a dramatic Ras overexpression, as well as reactivation of signaling. As proof of principle, we have confirmed these findings in cell lines engineered to silence miR-145 and also *in vivo* in a xenograft model of prostate cancer. Altogether, these findings reveal the existence of a novel circuit of adaptive resistance to AKT inhibition in prostate cells. Mechanistically, our results indi-

cate that inhibition of AKT triggers downregulation of miR-145 which in turn upregulates RAS expression, leading to paradoxical reactivation of signaling that limits drug efficacy.

FILAMIN A IN BREAST CANCER: FROM LITERATURE TO FUNCTIONAL INSIGHTS

P. Zawadka[1], W. Arendt[1], M. Gagat[1,2], M. Izdebska[1]

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Breast cancer remains a major clinical challenge, particularly due to the poor prognosis associated with metastatic disease[1]. While early-stage tumors often respond to treatment, progression to metastasis significantly lowers survival rates, highlighting the need for novel therapeutic approaches[2]. Filamin A (FLNa), an actin binding protein, has been implicated in processes such as cell migration, adhesion, proliferation, and DNA repair. Its overexpression has been reported across various malignancies, including breast cancer, yet the precise role of FLNa in tumor progression remains incompletely understood[3,4]. In this study, we combined a comprehensive literature review with in silico analyses of available databases to evaluate FLNa expression patterns and their correlation with clinical outcomes. Furthermore, we conducted *in vitro* experiments using MCF-7 and MDA-MB-231 cell lines to investigate the functional role of FLNa in breast cancer-related processes. Our integrated approach aims to clarify FLNa's contribution to breast cancer progression and assess its viability as a therapeutic target.

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PLENARY SESSION: DEVELOPMENT, VALIDATION AND APPLICATION OF MULTIPLEX IMMUNOHISTOCHEMICAL ASSAYS

(Joint Session with the Histochemical Society)

FROM SEEING TO BELIEVING: A 43 YEARS LONG JOURNEY IN IMMUNOSTAINING

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In the last 43 years, thanks to fantastic collaborators, we used antibodies in immunostaining and published a few firsts: TP53 in breast cancer (1988), FFPE-proofs anti Ki-67 antibody MIB 1 and the citrate antigen retrieval buffer (1992), reticular cells in human bone marrow (1993), BCL6 in germinal centers (1995), PRDM1 in plasma cells (2005), the sub cellular localization and distribution of AID and the function of IRF4 (2006). From 2013 to 2021 we dissected the effects of tissue processing on antigenicity, a body of discoveries which led to a hyperplexed (>15 markers) multiplexing method, Multiple Iterative Labeling by Antibody Neodeposition (MILAN)[1,2]. The application of MILAN and the complexity of data obtained (~100 markers, millions of single cells) landed us in the rarefied world of dimensionality reduction algorithms, where math rules and statistical significance replaces "representative images". Because of these latest developments, we discovered that the human eye ability to discriminate shades of grey is very limited (less than 64/256)[3], thus low levels of staining are routinely missed and eye-guided assessment is unreliable. Bioinformatics tools we developed (BRAQUE)[4] provides highest sensitivity, granularity and robustness based on objective statistical parameters. Biologists and Pathologists need to believe (in math)[5] rather than see with their own flawed eyes.

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PATHOMICS – A NOVEL OMICS APPROACH FOR HISTOPATHOLOGY

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Artificial Intelligence (AI) and Deep Learning (DL) hold great promise to transform pathology practice. Currently, the majority of

commercially available products and AI research focuses on end-toend AI, *i.e.*, an approach in which the model learns all the steps between the initial input and the final output, providing qualitative or semiquantitative (class) data. A complementary or alternative approach to analyse histomorphology is using DL-based segmentation of relevant histological compartments and cells, followed by extraction of relevant quantitative data (features). If done on a large scale, it is termed pathomics, representing a novel -omics approach for morphology at the microscopical level. Pathomics complements molecular omics, like genomics or transcriptomics, or radiomics, which aims at quantifying radiology images at the macroscopic level. This lecture will explore the potential of pathomics and compare it with end-to-end models, focusing on kidney pathology.

ACCELERATING DISCOVERY TOGETHER: A COMMUNITY APPROACH TO ANTIBODY VALIDATION AND MULTIPLEXED IMAGING

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Leica Microsystems

Multiplexed imaging is a powerful approach for studying the spatial organization and cellular composition of intact tissues at single-cell resolution. The last decade has seen a rapid expansion in the development and commercialization of spatial biology techniques. These methods include technologies that probe RNA molecules using imaging-based approaches or spatial barcoding techniques. In addition, proteins may be targeted with antibodies applied to thin sections as well as thick tissue volumes using a variety of approaches. These methods vary in the optical resolution, tissue volume, and number and type of targets (RNA, protein, or both) that can be imaged in a specimen. These technologies have been foundational for the construction of single cell atlases and the study of naturally occurring cancers. Despite their promise, widespread adoption of these methods remains limited by high costs, specialized equipment, and the need for significant technical expertise in tissue processing, reagent validation, image acquisition, and data analysis. To address these barriers, community-driven initiatives led by the Human BioMolecular Atlas Program (HuBMAP) and IBEX Imaging Community are working to streamline and democratize multiplexed imaging. By sharing validated antibody panels, protocols, and best practices, these efforts are reducing the time, resources, and expertise required to generate high-quality spatial data— accelerating discovery through collaboration.

PLENARY WORKSHOP: FROM ANIMAL MODELS TO ANIMAL-FREE 3D CULTURES AND ORGANOIDS

ANIMAL MODELS FOR LINEAGE TRACING IN (CELL) TRANSDIFFERENTIATION

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The cell identity is defined by its anatomy and physiology[1]. Our and Other's data produced in the last decades seem to introduce a new concept in cell biology: the physiologic and reversible transdifferentiation[2]. When a cell is well differentiated the traditional widely accepted concept is that this cell type can only perform the physiologic role dictated by its anatomy. Thus, white adipocytes perform their vital role of storage and distribution of energetic molecules in the intervals between meals. We showed that under cold exposure (both in mice and humans) white adipocytes are able to convert into brown adipocytes changing their anatomy from cells with unilocular into multilocular cytoplasmic fat vacuoles and with a completely different type and number of mitochondria. This type of mitochondria allows new molecular pathways ending in heat production able to counteract the changed environmental conditions[3]. During pregnancy and lactation, the organism needs to develop structures able to synthesize and secrete milk to guarantee survival of pups. White adipocytes of mammary glands convert into epithelial alveolar glands producing milk. At the end of lactation, they convert back to adipocytes[4]. These extraordinary changes in anatomy and physiology of adipocytes can be followed with different techniques among which lineage tracing is one of the most important. X-Gal histochemistry for the expression of beta-galactosidase plays a key role in this technique.

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ANIMAL MODELS OF METASTATIC CANCER - ADVANCED IMAGE ANALYSIS FOR DISSECTION OF DRIVING SIGNALING PATHWAYS

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The identification of decisive drivers of metastasis is an urgent medical need. The characterization of cancer-specific signaling hubs may allow to identify molecular targets for tailored therapeutic interventions. Multi-layered analyses of Trop-2 molecular signaling were conducted using transcriptome profiling and proteomic analyses in colorectal cancer patients and in preclinical models of colon cancer. Dynamic confocal microscopy, FRET, FLIM, electron microscopy and image analysis were utilized to identify signaling complexes for high-dimensional signaling for cancer cell

growth. Recursive coalescence of signal transducers at cell membrane macroplatforms was shown to drive kinase, calcium and phosphoinositide signaling for cell growth. Macroscopic coalescence was shown for trans-membrane signal transducers (Trop-1, Trop-2, EGFR, CD9, CO-029, CD98), cytoplasmic effectors (Grb-2, PKCα, ERK, PI3K, Akt) and cytoskeletal components (βactin, ezrin, α-actinin). Trop-2 clustering was shown to lead to release of Ca^{2+} from internal stores, activation of Ca^{2+}-dependent PKCa by mTORC2 and PKCa-mediated phosphorylation of the Trop-2 cytoplasmic tail. This induced remodeling of the β-actin cytoskeleton, activation of Akt and ERK and phosphoinositide signaling, for enhanced cancer cell growth. Binding to Trop-2 was shown to cause cleavage of E-cadherin with release from the cytoskeleton, loss of cell-cell adhesion and activation of β-catenin. This induced cell migration, tissue invasion and resistance to apoptosis. wtTrop-2 increased the metastatic capacity of KM12SM colon cancer cells to 90% of tested xenotransplants with massive metastatic growth in the liver. This global, Trop-2/E-cadherin/β-catenin-driven pro-metastatic program was recapitulated in cancer patients, and was shown to impact on breast, colon, ovary, uterus cancer metastatic relapse and overall patient survival. A signaling axis triggered by Trop-2 defines key control hubs for cancer growth and metastatic diffusion, and identifies specific molecular targets for tailored therapeutic interventions

THE USE OF BIOMIMETIC 2D AND 3D UROTHELIAL *IN VITRO* MODELS WITH MOLECULAR, MORPHOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF NORMAL AND CANCEROUS BLADDER TISSUE AS AN ALTERNATIVE METHOD IN PRECLINICAL STUDIES

M.E. Kreft[1], L. Tratnjek[1], A. Janev[1], N. Resnik[1], U. Cerkvenik[1], U. D. Jerman[1], G. Markovič[1], T.Ž. Ramuta[1], T. Višnjar[1], Ž. Sardoč[1], A.Punčuh[1], S. Kralj[2], M. Čemažar[3], P. Veranič[1]

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Advanced *in vitro* models that mimic the architecture and physiology of human tissues provide a promising alternative to animal testing in preclinical research, in accordance with the 3Rs principle. We developed and validated biomimetic 2D and 3D urothelial models representing both normal and cancerous bladder tissue. These models are based on normal porcine urothelial (NPU) cells, normal human urothelial cells (SV-HUC1), and human bladder cancer cell lines (RT4, T24)[1–10]. To enhance biomimicry and explore therapeutic potential, we incorporated the human amniotic membrane (hAM) either as a biological scaffold or in the form of processed preparations with potential anticancer effects. The preparation of hAM followed the guidelines of COST Action CA17111.[11] Comprehensive structural and functional characterization was performed using live-cell imaging, immunocytochemistry, transmission and scanning electron microscopy, freeze-fracture replica immunolabeling and 3D reconstruction techniques[1–10]. These models allow for the assessment of cytotoxicity, epithelial barrier integrity, and treatment efficacy under various pathological conditions. Importantly, hAM selectively disrupted the cohesion and structure of cancer spheroids while sparing normal urothelium. These effects were linked to the downregulation of FAK and RhoGTPases, key molecular regulators of cell adhesion and motility. In conclusion, these biomimetic urothelial models closely replicate the molecular and morphological features of human bladder tissue. They offer a reliable, animal-free platform for testing novel therapeutic strategies, including hAM-based interventions, thereby improving translational relevance while adhering to ethical standards.

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LEVERAGING ORGANOID TECHNOLOGIES TO CHARACTERIZE DYNAMIC TUMOR CELL STATES DRIVING TREATMENT RESISTANCE IN PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by a pronounced inter- and intra-tumoral heterogeneity, which fuels chemoresistance and contributes to high mortality rates. Previously, we have developed a branched organoid system embedded in collagen matrices that robustly recapitulates the phenotypic heterogeneity seen in both murine and human PDAC[1,2]. These organoids display complex, self-organized branching morphogenesis and give rise to distinct, spatially ordered tumor cell populations that reflect their underlying molecular profiles and differentiation states. Importantly, we show that the observed heterogeneity is not random but governed by defined transcriptional programs, particularly epithelial-to-mesenchymal plasticity, that drive the emergence of discrete tumor-cell states. Using integrated phenotypic and transcriptomic profiling, we map this diversity to specific biological functions *in vivo*, demonstrating that each organoid phenotype corresponds to a tumor-cell state with unique metastatic potential, and therapeutic vulnerabilities. Moreover, we identify dynamic, treatment-induced phenotype reprogramming events that are targetable, paving the way for rational design of state-specific therapeutic interventions. Building on our findings, we are now focusing on elucidating intra-organoid heterogeneity-such as tip–trunk hierarchies at the single-cell level, to uncover the mechanisms of self-organization and to determine how distinct organoid phenotypes and subpopulations contribute to liver metastasis and treatment resistance. In summary, we have established a scalable and mechanistically informative organoid platform that enables *in vitro* modeling of PDAC heterogeneity. This system provides a framework for dissecting the tumor cell–intrinsic drivers of phenotypic plasticity and for developing phenotype-guided treatment strategies aimed at overcoming resistance and improving patient outcomes.

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SESSION: ADVANCED RESEARCH IN CELL DEATH, AGING AND DIFFERENTIATION

FROM BULK TO SELECTIVE: A JOURNEY THROUGH THE PHYSIOLOGICAL RELEVANCE OF AUTOPHAGY

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Macroautophagy (commonly referred to as autophagy) is an evolutionarily conserved catabolic process that degrades cytosolic components by sequestering them into double- membrane autophagic vesicles (AVs), which are subsequently broken down by lysosomes. The physiological and medical significance of autophagy is widely acknowledged, but we still lack important knowledge on the mechanism governing autophagy in response tissue specific needs. In this talk, I will discuss emerging pathways that regulate cargo selection in autophagy in response to various metabolic, developmental, and disease-related stimuli. Special attention will be given to new mechanisms controlling the degradation of the endoplasmic reticulum and ribosomes through autophagy. These findings challenge the long-standing view that starvation-induced autophagy is a bulk, non-selective process.

ROLE OF MITOCHONDRIAL METABOLISM AND CONTACT SITES IN CANCER CELL DEATH AND SURVIVAL

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Mitochondria are organelles not only involved in cellular respiration but also in several other pathways important for cell life and death. They are not isolated within the cells but are closely interconnected with other organelles, among which the Endoplasmic Reticulum (ER). Defective ER-mitochondria crosstalk and ER stress impacts on several cellular functions as well as on important intracellular pathways that promote the cancer development. Modulation of ER-mitochondria contacts have a role in cancer development and resistance to pharmacological therapy by impacting on cellular bioenergetics and metabolism. More recently, we showed that a reduction of mitochondria-ER contacts sites, by downregulation of tethers, can tune cancer cells intracellular signaling (*e.g.* Wnt signaling) both *in vitro* and *in vivo*, ultimately impacting on cancer cells proliferation. In addition, we have demonstrated that organelle contacts are mutually regulated in response to metabolism rewiring and to various diet and nutrients availability so affecting cancer formation/progression and cancer cells sensitivity to drugs. These findings reveal that affecting mitochondria-ER tethering may be beneficial against cancer by altering the cellular signaling, and in turn sensitizing tumor cells to chemotherapeutic treatment.

THE EFFECT OF MUTANT HUNTINGTIN ON CLEAVAGE OF CREST BY CALPAIN-2 AND ITS CYTOTOXITY

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Neuronal cell cycle re-entry is a pathological hallmark of Huntington's disease (HD) and other neurodegenerative disorders. Calcium-responsive transactivator(CREST) has been found to play a critical role in dendritic development through calcium-dependent transcriptional regulation and to form a functional complex with p300 and p53 to suppress retinoblastoma protein (Rb) phosphorylation and cyclin expression, thereby arresting the cell cycle and promoting neuronal differentiation. Here we demonstrated that mutant huntingtin (mHtt) significantly reduced full-length CREST levels while inducing the production of truncated CREST fragments. This process was blocked by inhibitors of calpain-1 and -2, suggesting calpain involvement. Using mass spectrometry, co-IP, and *in vitro* pull-down assays, we provided direct evidence that mHtt enhanced the interaction between calpain-2 and CREST, leading to increased CREST cleavage which generated a toxic C-terminal fragment that accumulated abnormally in the nucleus. Furthermore, we designed a TAT peptide targeting the calpain-2 cleavage site of CREST, which effectively prevented CREST degradation and significantly ameliorated mHtt-induced pathological phenotypes in N2a cells, including neuronal death, neurite retraction, and aberrant Rb phosphorylation. Collectively, our findings establish CREST as a specific substrate of calpain-2 and reveal a novel mechanism by which mHtt promotes neuronal cell cycle re-entry and neurotoxicity through calpain-2-mediated CREST degradation. The toxic C-terminal fragment of CREST exhibits distinct pathological properties. Importantly, our study identifies the calpain cleavage site on CREST as a potential therapeutic target for HD intervention. Targeting this site with specific inhibitors or peptide blockers may represent a promising strategy to prevent mHtt-induced neuronal dysfunction and cell death.

HACAT CELLS AS A 2D EXPERIMENTAL DIFFERENTIATING MODEL TO ANALYZE THE EARLY BIOLOGICAL EFFECTS INDUCED BY A PSORIATIC PROINFLAMMATORY MICROENVIRONMENT: CELL PROLIFERATION, DIFFERENTIATION AND FERROPTOSIS

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HaCaT cells are spontaneously transformed keratinocytes from human epidermis that are useful for studying epithelial homeostasis and related diseases due to their proliferative and differentiating abilities. We analyzed interactions between these cells and pro-inflammatory psoriatic cytokines (TNF-alpha and IL-17A), focusing on cell proliferation and differentiation in term of intercellular junctions, and the cytoskeleton. Additionally, we examined how psoriatic cytokines contribute to susceptibility to ferroptosis, a novel cell death mechanism recently recognised in psoriatic plaques. HaCaT cells were induced to differentiate using 1.8 mM $CaCl_2$. After four days, cells were exposed to a combination (MIX) of interleukins (IL-17A, IL-22, IL-23, and TNF-alpha) for 24 (T24) and 48 (T48) h. Proliferation was assessed through BrdU incorporation assay; ultrastructural morphology was also analyzed. Claudin 1 (CLDN-1), Zonula Occludens 1 (ZO-1), keratin (K) 10, and K14 were evaluated using immunofluorescence and western blot. We also induced ferroptosis for 24 and 48 h with 20uM erastin to evaluate cell susceptibility to MIX by analyzing the concentration of GSH (glutathione, an important antioxidant) and the ratio of GSH/GSSG (reduced glutathione/oxidized glutathione), examining membrane integrity, ATP concentration and cell availability. The biological effect induced by MIX on cell differentiation was evident after 48 h of incubation on all the considered markers. Cell proliferation progressively increased with time, but in T48 MIX-incubated samples, the proliferative activity was reduced ($p < 0.01$). The presence of both MIX and erastin after 48 h affected mitochondrial ultrastructure, suggesting an early involvement of psoriatic cytokines also in ferroptosis mechanisms. Our experimental conditions mimic the early (de)differentiation features of psoriatic keratinocytes, indicating this model can elucidate early cellular and molecular processes during the early pathogenetic phases of psoriatic plaque organization.

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SESSION: OMICS, MORPHOLOGY AND ARTIFICIAL INTELLIGENCE

FROM CLOUD TO BEDSIDE

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Our ability to treat diseases has long been based on the concept of treating the specific pathology. Therapeutic protocols have traditionally been defined using a purely statistical approach, measuring treatment effectiveness on large populations of patients ostensibly suffering from the same type of disease. However, the efficacy of a therapeutic protocol is rarely 100%, and varying percentages of patients suffering from a specific disease do not respond to 'standard of care' treatment, despite apparently carrying the same pathology as those who do respond. The recognition of the relevance of individual variability in prognostic prediction and therapeutic response, coupled with the awareness that disease and patient are inseparable, forms the basis of so-called precision or personalized medicine. The search for descriptive parameters of this uniqueness, *i.e.*, parameters able to best predict the characteristics of a given disease in a given patient, has consequently led to an impetuous increase in data, largely acquired or stored in digital format. The use of this enormous amount of data for predictive purposes (prognostic and/or therapeutic) increasingly requires sophisticated analysis techniques, and artificial intelligence (AI) can be the answer to this need. Leveraging a partnership with Microsoft, the San-Raffaele AI Center of Excellence (S-RACE) has engineered and implemented a secure, trustworthy, and responsible by-design interoperability platform on Azure cloud.[1] This platform, built on the FHIR protocol (the international standard for healthcare data processing), is designed to revolutionize how healthcare data is managed and analyzed. The platform's capabilities include retrieving, classifying (based on major medical ontologies), and analyzing multidimensional realworld data encompassing clinical reports, laboratory results, pathology, imaging, and omics. Crucially, integrated AutoML systems will enable the automatic generation of powerful black-box models for research, while parallel development of glass (white)box models will ensure transparency and explainability in decision making. This comprehensive process is fully traceable, reproducible, and strictly compliant with all major AI regulations (GDPR, ISO 42001, AI-Act, RMF). Currently 21 different projects are running on the platform to develop predictive models in the oncology, neurology, cardiovascular, diabetes areas.

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USE OF HIGH-DEFINITION IMAGING FLOW CYTOMETRY AND A.I. TO ENUMERATE AND CHARACTERISE CIRCULATING TUMOUR CELLS

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The last decade has shone a spotlight on the field of liquid biopsies, a minimally invasive, yet data-rich approach for cancer diagnosis and management. There are many types of liquid biopsies including cerebrospinal fluid, blood, urine, saliva or pleural effusion. Liquid biopsies appear to have certain advantages when compared to tissue biopsies, including shorter time of acquisition, lower costs of sample isolation, monitoring tumour evolution, or real-time drug response. Growing evidence suggests that circulating tumour cells (CTCs; cancer cells that detach from a primary tumour and enter the bloodstream) reflect molecular features of cells within tumour masses. To date, very few CTC based tests have received FDA approval. In our laboratory we have explored the clinical utility of CTCs as prognostic or diagnostic biomarkers, using Image Stream, a benchtop, multispectral, imaging flow cytometer designed for the rapid acquisition of millions of cells (CYTEK). This imaging flow cytometry platform discriminates CTCs *vs* white blood cells (WBCs) in flow, objectively and statistically based on their appearance and immunostaining without the need of further enrichment. In our clinical samples we have used pan-cytokeratin staining to differentiate CTCs from CD45 positive WBCs. We have also used brightfield imaging to capture the cell morphology, as well as DRAQ5™ a general nuclear stain. CTCs were identified and quantified using the IDEAs software. This is a promising approach that does not require prior enrichment of cells of epithelial origin using EpCAM antibodies. In the future the combination of imaging (*e.g.* ultrasound, H&E staining), clinicopathological features and use of AI algorithms, may be able to distinguish CTCs, independent of specific marker expression, and drive a better prognostic outcome by tailoring treatment strategies to individual patients, optimizing treatment efficacy and minimizing side effects.

SUMOYLATION AS AN EARLY ADAPTIVE MECHANISM IN THE PROTEOMIC RESPONSE OF HUMAN GERM CELLS TO SIMULATED MICROGRAVITY

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The growing accessibility of space travel highlights the need to deeper understand how altered gravitational environments affect

human physiology, and in this context reproductive health. This study analyses the effects of simulated microgravity (SμG) and hypogravity (ShG) on different pathways including SUMOylation (Small Ubiquitin-like Modifier) in human male germ cell line TCam-2, combining Reverse-Phase Protein microArrays (RPPA) and immunofluorescence. SUMO isoforms 1–4 are members of ubiquitin-related protein family that regulate transcription factors, DNA repair, cytoskeleton dynamics, stress, proliferation, and apoptosis responses[1-2. TCam-2 cells were subjected to SμG and ShG using a Random Positioning Machine for 3, 24, and 72 h. RPPA profiled alterations in key signaling pathways while immunofluorescence was subsequently performed to analyze the subcellular localization of SUMO2/3. RPPA analysis showed that 3h of SμG upregulated proteins related to cell cytoskeleton composition, proliferation, and apoptosis. Among them we focused on SUMO2/3. Prolonged exposure to SμG (24 h–72 h) did not alter SUMO2/3 expression, despite ongoing pathway alterations. Simulated ShG caused milder changes without affecting SUMO2/3. Immunofluorescence analysis after 3h of SμG revealed increased SUMO2/3 signal with enhanced nuclear localization. In line with previously reported data[3-4], we confirm TCam-2 sensitivity to microgravity. The early upregulation of SUMO2/3 and its increased nuclear localization in 3h SμG exposure led us to hypothesize that SUMOylation is an early response to the stress induced by microgravity. At 24 h to 72 h, SUMO2/3 protein levels remain comparable to those observed under unitary gravity (1g), highlighting the resilience and adaptive capacity of TCam-2. Further investigations are necessary to clarify the relationship between SUMO2/3 and the observed changes in proteins involved in cytoskeletal organization, proliferation, and apoptotic pathways.

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FROM PIXELS TO DIAGNOSIS: A DEEP LEARNING FRAMEWORK FOR HISTOPATHOLOGICAL IMAGE ANALYSIS IN CANINE TESTICULAR PATHOLOGY

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Canine testis pathology encompasses a range of disorders affecting the structure and function of the testes with significant implications for fertility, hormonal balance, and overall health. Common pathologies include testicular neoplasms, orchitis, testicular torsion, cryptorchidism, and degenerative changes. Histopathological definition of testicular pathologies may be prone to inter-observer variability, thus leading to an erroneous diagnosis or to the exclusion of differential concurrent alteration. We propose an artificial intelligence-based computational pathology approach to automate the discrimination of different testicular developmental, inflammatory or degenerative pathologies and the main testicular neoplasms (Seminoma, Sertolioma, Leydigoma). This computational approach is based on a deep learning pipeline that integrates convolutional neural networks, multi-class histological segmentation, and spatial attention *via* Grad-CAM maps to acquire and interpret complex morphological structures in haematoxylin-eosin-stained slides.[1] We created a histological whole-slide image dataset (N=400 slides) by collecting samples of healthy, pathological non-neoplastic and neoplastic testes from the archive of DIPSA laboratory of University of Naples Federico II. We fine-tuned the model on a proprietary dataset of testicular tissue sections, annotated by two pathologists. While quantitative performance metrics are still under systematic evaluation, preliminary findings indicate that the model exhibits highly promising behaviour in accurately distinguishing between nonneoplastic and neoplastic cases, as well as between tumour subtypes. The incorporation of saliency mapping has enhanced the consistency of predictions, offering a level of interpretability that supports pathological validation. Our preliminary results suggest that the automated system may have a remarkable potential to assist pathologists in the diagnostic process and to reduce interobserver variability, also allowing for better standardisation of the process.

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PLENARY SESSION: HISTOCHEMISTRY OF THE NUCLEUS

THE MYSTERY OF INTERMEDIATE FILAMENTS: STRUCTURAL PERSPECTIVE

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Intermediate filaments (IFs) are a diverse component of the cytoskeleton that exhibit remarkable cellular and tissue specificity. Their distinctive mechanical characteristics, including extraordinary elasticity, tensile strength, and distinctive stiffening behaviours under strain and compression, are pivotal for the mechanical resilience of cells and the integrity of tissues. The distinguishing characteristics of these elements are attributable to their intricate, hierarchical architecture, which differs fundamentally from the architectures of other cytoskeleton elements, such as actin and microtubules. The IFs family of proteins consists of over 70 distinct proteins, which are divided into six subtypes based on their sequence. Nevertheless, does this classification offer any insight into their assembly, structure, and functions? IFs possess a broad repertoire of cellular functions, including the provision of structural support to cells, as well as active roles in mechanical support and signaling pathways. Consequently, it has been demonstrated that defects in IFs are associated with more than 100 diseases. Our understanding of IF assembly, disassembly and the structure of the resulting mature filaments remains in its infancy and is mostly restricted to *in vitro* experiments. Recently, we have determined the first two structure of assembled IFs, from cellular assembled filaments. This work indicates fundamental structural differences between IFs but also the general plan of assembly.

DOMAINS AND SIGNALLING PATHWAYS IN THE NUCLEUS

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Since 1987 evidence from several laboratories has highlighted the presence of autonomous nuclear inositol lipid metabolism[1]. The evidence suggests that lipid signalling molecules are important components of signalling pathways operating within the nucleus. The findings are important given the fact that nuclear signalling activity controls cell growth and differentiation. Among the nuclear enzymes involved in this system, inositide-specific phospholipase C (PI-PLC) β1 has been one of the most extensively studied enzymes[2]. Besides the studies on its signalling activity in physiological conditions, clinically oriented ones have shown that PI-PLCβ1 gene is associated with several pathological conditions. Nuclear PI-PLCβ1 is involved in the early stages of hemopoiesis and, namely, in the control of cell-cycle progression in progenitor hemopoietic cells. In addition, nuclear PI-PLCβ1 plays a crucial role in the initiation of the genetic program responsible for muscle differentiation in that the enzyme activates the cyclin D3 promoter during the differentiation of myoblasts to myotubes. Down regulation of this enzyme is associated with progression of myelodysplastic syndromes (MDS) into acute myeloid leukaemia as well as with myotonic dystrophy or DM, both type 1 and type 2. Here we briefly highlight the most important evidence of the role of nuclear PI-PLCβ1 in these pathologies as well as the significance of subcellular localization of PI-PLCs. In addition, it is quite clear the potential role of PLCβ1 as biomarker in high-grade gliomas.

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LIPID DROPLETS AND NUCLEAR LIPID DYNAMICS

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Lipid droplets (LDs) are present in the nucleus, though much less abundant than cytoplasmic LDs. We investigated nuclear LDs generated in amino acid-deprived conditions, and found that diacylglycerol (DAG), produced by ATGL-mediated hydrolysis of triacylglycerol (TAG) in cytoplasmic LDs, flows into the inner nuclear membrane (INM) and gives rise to nuclear LDs. DAG in the INM is trafficked to PML nuclear bodies (PML NBs) *via* small vesicles, whose formation is abolished in PML knockout cells. These findings uncover a new class of vesicles in the nucleus that link PML NBs to TAG metabolism and prompt reconsideration of our understanding of the PML NB architecture.

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WORKSHOP: NEW HORIZONS IN FLOW CYTOMETRY: ADVANCES IN ANALYSES AND CHARACTERIZATION OF EXTRACELLULAR VESICLES

(Joint Session with GIC)

FLOW CYTOMETRY FOR THE ANALYSIS OF MICRO/NANOPARTICLES: CURRENT APPLICATIONS AND CHALLENGES

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The detection and quantification of micro- and nanoparticles in complex matrices is a growing challenge in environmental research. Among emerging contaminants, micro- and nanoplastics (MNPs) have raised particular concern due to their widespread release from synthetic textiles and their potential impacts on ecosystems and human health. A notable example is the massive production and uncontrolled dispersion of disposable face masks, extensively used during the COVID-19 pandemic. We conducted an experimental study simulating the environmental degradation of non-woven synthetic fabrics exposed to mechanical stress in aquatic conditions. The fragmentation process rapidly generated fibers, microplastics, and nanoplastics. Flow cytometry proved to be a promising tool for the direct detection, characterization, and quantification of MNPs, highlighting its potential in addressing key analytical challenges in nanoparticle research, such as distinguishing plastic particles in complex suspensions and assessing their environmental fate.

LABELING EXTRACELLULAR VESICLES FOR HIGHLY SENSITIVE FLOW CYTOMETRY ANALYSES

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Extracellular vesicles (EVs) are released by shedding during many different processes by all cell types. They cross all biological barriers and have been detected in all body fluids. For these reasons EVs are increasingly thought to be new potential dynamic biomarkers useful for liquid biopsy. However, the application of pre-analytical processing phases before any EV flow cytometry measurement is mandatory, even if their impact on results is not predictable. For this reason, the translation of basic research into clinical practice has been precluded. We have optimized a simple flow cytometry method, which, avoiding any pre-enrichment step, allows the identification, classification, enumeration, and separation, by fluorescence activated cell sorting, circulating EVs stemming from different parental cells. This protocol takes advantage of a lipophilic cationic dye (LCD) able to probe EVs, which has been combined with fluorescent phalloidin to exclude damaged elements and different mixes of specific monoclonal antibodies. The application of the newly optimized PFC protocol here described allowed the obtainment of repeatable EVs counts. The translation of this PFC protocol to fluorescence-activated cell sorting allowed us to separate EVs from fresh peripheral blood samples and many others biological fluids, as well as cell supernatants. Sorted EVs preparations resulted particularly suitable for proteomic analyses, which we applied to study their protein cargo, as well as for *in vitro* and *in vivo* functional assays. Therefore, LCD staining of EVs may open new routes for the EV clinical translation.

FLOW CYTOMETRY IN THE DEVELOPMENT AND CHARACTERIZATION OF PRECLINICAL TUMOR MODELS BASED ON THE ANALYSIS OF EXTRACELLULAR VESICLES AS CANDIDATE LIQUID BIOMARKERS

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The precise causes of most brain tumours in adults remain largely unknown. Glioblastoma (GBM) is characterized as the most aggressive form of brain tumour, with a 5-year survival rate from diagnosis of less than 5%. Brain metastases from different organ tumors account for more than one-half of all intracranial tumours in adults. Therefore, the identification of new biomarkers remains a major challenge to integrate prognosis and improve diagnosis for brain tumours. The molecular profiling of brain tumors has become an integral component of routine neuro-oncologic care. Currently, it is mainly achieved through invasive procedures such as tissue biopsy and surgical resection, often associated with complications and especially challenging. Due to these reasons, liquid biopsy is an attractive option providing the opportunity to capture the complex global heterogeneity of the whole brain tumour. Regardless of the tumour type, liquid biopsy is a minimally invasive technique performed on samples of blood or other human biological fluids to detect and quantify circulating tumor cells (CTCs) or other tumor-derived elements, such as cell-free tumor DNA (ctDNA) and, more recently, extracellular vesicles (EVs), which are released in the bloodstream or other fluids by cancer cells. Tumor cells produce large amounts of EVs and can select the EV cargo, which is constituted by proteins, nucleic acids and lipids, thus safeguarding them from degradation and conveying to nearby or distant cells. miRNAs packed into tumour-released EVs have been shown to contribute to tumour establishment and metastatic spread, suggesting not only diagnostic/prognostic but also therapeutic target potential. To identify miRNAs to be used as biomarkers for brain tumour diseases, we analysed the expression profiles of circulating small EV-enriched miRNAs in preclinical models of

intracranial human metastatic melanoma and GBM in nude mice. Thus, extracellular miRNAs have been studied in the small EVs (sEVs) purified from culture cell supernatants and from the plasma of mice bearing tumour to brain by next generation sequencing.

EXPRESSION OF PIEZO1 IN CLEAR CELL RENAL CELL CARCINOMA: INSIGHTS FROM BIOINFORMATIC AND IMMUNOHISTOCHEMICAL ANALYSES

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Clear cell renal cell carcinoma (ccRCC) represents the predominant histological subtype of kidney cancer and is frequently linked to unfavorable clinical outcomes and resistance to conventional therapies[1]. Due to the limited availability of validated prognostic markers, there is a pressing need to identify new molecular indicators that may support clinical stratification and treatment planning[2]. Piezo1, a mechanosensitive ion channel involved in cellular mechanotransduction, has been associated with tumor progression in several malignancies, though its relevance in ccRCC remains insufficiently characterized[3]. The present study investigated the prognostic significance of PIEZO1 by analyzing its expression at both transcript and protein levels in ccRCC tissues and matched non-tumorous samples. Analysis of transcriptomic data from The Cancer Genome Atlas (TCGA) revealed a significant upregulation of PIEZO1 mRNA in tumor specimens ($p<0.0001$). Moreover, elevated transcript levels were strongly correlated with reduced overall survival ($p<0.0001$), suggesting a relationship with more aggressive disease phenotypes. Immunohistochemical (IHC) evaluation showed that PIEZO1 protein expression was generally lower in tumor tissue compared to adjacent normal tissue ($p<0.0001$). Interestingly, within the tumor group, cases exhibiting higher intratumoral PIEZO1 protein levels were significantly associated with poorer survival outcomes ($p=0.0092$), indicating its potential as an independent adverse prognostic factor. The results indicate dual PIEZO1 regulation in ccRCC, with mRNA upregulation and variable protein levels suggesting tumor-specific mechanisms. PIEZO1 may serve as a prognostic marker worth further study in precision oncology.

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TRACKING HER2 TRAFFICKING AND ORGANELLE CROSSTALK IN TRASTUZUMAB-DERUXTECAN–TREATED BREAST CANCER CELLS USING MULTI-MODAL IMAGING

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Trastuzumab-deruxtecan (T-DXd) is a clinically effective antibody–drug conjugate (ADC) for HER2+ and HER2-low breast cancers, yet its intracellular mechanisms remain elusive. We used a multimodal imaging approach including confocal, immuno-EM, and biochemical assays to investigate T-DXd dynamics in HER2+ breast cancer cells. Cells were treated with T-DXd (10 µg/mL, 2–72 h) and compared to IgG controls. Early-phase responses (2–24 h) included HER2 phosphorylation, sustained ERK signaling despite total ERK reduction, and AKT downregulation. Imaging revealed active lysosomal remodeling, increased TFEB, and reduced LAMP1 and LC3, indicating modulation of autophagic flux. In late stages (48–72 h), T-DXd–HER2 complexes accumulated in lysosomes, forming previously undescribed lysosome–mitochondria–nucleus contact sites. These triads correlated with mitochondrial damage, γH2AX activation, nuclear envelope stress, and LaminB1 increase. Secreted cytokines (IL-6, IL-8, TNF-α) induced M2-like macrophage polarization. This study highlights the value of integrated imaging to dissect the morpho-functional responses induced by ADCs and proposes new cellular targets, such as lysosomal hubs or ERK signaling, for future therapeutic combinations.

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WORKSHOP: MULTILEVEL IMAGING: THE FUTURE OF CYTO/HISTOCHEMISTRY?

(Joint Session with SfH and TEMD)

SPATIO-TEMPORAL ORGANIZATION OF THE SUPER-ENHANCER STRUCTURE IN CANCER CELLS

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Our research investigates the spatiotemporal organization of nuclear architecture *via* biomolecular condensation, focusing on the role of liquid–liquid phase separation (LLPS) in gene regulation during cancer progression. We identify phosphatidylinositol 4,5 bisphosphate (PIP2) as a key nuclear lipid forming membrane less condensates with RNA, affecting transcriptional regulators and super-enhancer assembly. PIP2 shows RNA- dependent nuclear localization and interacts with RNA polymerases I and II, suggesting a structural role in transcriptionally active chromatin. Mass spectrometry revealed the RNA-dependent PIP2-associated (RDPA) nuclear proteome, enriched for intrinsically disordered regions and polybasic motifs, supporting LLPS. Among RDPA proteins, BRD4 undergoes PIP2- and RNA-dependent condensation. Using a system for inducible nuclear PIP2 depletion, we demonstrate its role in organizing transcriptional condensates and modulating super-enhancer hubs. These findings establish PIP2-driven phase separation as a key mechanism in nuclear domain organization and cancer-related gene regulation.

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ADVANCEMENTS AND EMERGING PERSPECTIVES IN MICROSCOPIC IMAGING

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Traditional imaging modalities are routinely complemented by advanced techniques including confocal, multiphoton, superresolution, and light-sheet microscopy. These platforms have been enhanced by the Airyscan detection, STED, PALM, and STORM, to overcome the diffraction limit and enable nanoscale imaging[1]. Specific applications for this presentation includes: (i) visualization of cytoskeletal and functional organization in human, mouse, and zebrafish embryos and gametes,and human stem cells; (ii) structural imaging of whole organs such as the liver and kidney; and (iii) whole-organism imaging in zebrafish larvae and mouse fetuses, with particular emphasis on the methodologies employed to prepare and visualize such samples. Imaging depth and signal fidelity have been improved through the implementation of adaptive optics and wavefront shaping. The integration of microscopic imaging with optogenetics, biosensors, and machine learning approaches has occurred[2]. High-throughput imaging workflows have been empowered by AIdriven image reconstruction. Label-free techniques as Raman spectroscopy and digital holographic microscopy have broadened imaging capabilities by offering minimally invasive contrast. Illustrative examples that will be presented would include the application of biosensors and optogenetic tools for functional imaging in neural tissues, the integration of deep learning algorithms with high-resolution imaging for analysis of murine organs, and the tracking of colon and breast cancer cells using label-free modalities that bridge histopathology and radiology, to improve diagnosis and therapy. Emerging perspectives -as the miniaturization of imaging platforms, hybridization with spectroscopy and nanotechnology, incorporation of cloud-based automated systems- will be introduced as novel approaches to shape the future of biomedical imaging.

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IMAGING THE LYSOSOME IN CELLULAR SYSTEMS FOR DRUG DISCOVERY AND SCREENING

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Lysosomal storage disorders (LSDs) are a group of rare diseases characterized by a genetic-derived lysosomal metabolism error. Within the complex spectrum of symptoms, the LSDs strongly affect the central nervous system (CNS). However, currently available treatments include enzymatic replacement therapies directed to peripherical symptoms, for the inability of the proteins to pass the blood-brain barrier. In the last years innovative solutions to deliver the drug to the CNS have been designed, highlighting the need of comprehensive reliable testing systems. We set up *in vitro* platforms using peripheral cells, *i.e.* primary human fibroblasts from patients affected by two model pathologies (alpha-mannosidosis, aMAN; Niemann-Pick A, NP-A) and healthy subjects, and neuronal cells using primary neuronal/astrocytes cultures from cerebral cortex of transgenic mice models of the two diseases. Lysosomal defects were analyzed by morphological and functional tools, and validation of the readouts was performed assessing the restoration of the lysosomal features by treatment with approved enzyme replacement therapies. LAMP1 staining was set up for the analysis by confocal microscopy. Using the voxel-based IMARIS image analysis software, we reconstructed the volume of each single lysosome in the cell, quantifying the average lysosome volume

per cell, also tracking the 3D intracellular distribution. We described how the mutations lead to an increase in number and volume of lysosomes, and the change in distribution, with the lysosomal net accumulating around the nucleus. We than implemented the morphological analysis with functional data using LysoTracker Red and DQ-BSA staining. The number of LysoTracker Red stained lysosomes significantly increased in mutated cells, describing an acidification of the lysosomal content. Moreover, the accumulation of DQ-BSA resulted increased as well, demonstrating that mutant lysosomes are not able to properly degrade the BSA. For each readout, scaling to High-Content Screening methodology was considered, to increase the statistical and translational power of the platform.

ASTROCYTE DYSFUNCTION IN AUTOSOMAL DOMINANT LEUKODYSTROPHY (ADLD): INSIGHTS FROM 2D AND 3D *IN VITRO* SYSTEMS AND *EX VIVO* CEREBROSPINAL FLUID METABOLOMICS

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Autosomal Dominant Leukodystrophy (ADLD) is a rare, fatal neurodegenerative disorder caused by *LMNB1* gene overexpression, leading to progressive central nervous system demyelination with no effective therapy. While oligodendrocytes are responsible for myelination, increasing evidence suggests that astrocytes play a crucial role in ADLD[1,2]. Astrocytes from ADLD patients and *LMNB1*-overexpressing cells exhibit nuclear alterations, inflammatory activation, and oxidative stress, absent in oligodendrocytes. This study investigated astrocyte dysfunction in ADLD through two complementary approaches. First, human astrocytes (HA) overexpressing *LMNB1* were analyzed for inflammatory cytokines and myelination support. Immunocytochemical analysis revealed nuclear localization of NFAT4 and NF-κB, suggesting astrocyte activation, while secretome analysis with proteome arrays confirmed elevated inflammatory cytokines. Transmission electron microscopy of the *LMNB1*-HA showed anomalous chromatin condensation in ADLD nuclei. When co-cultured with oligodendrocyte precursor cells (OPC) on a 3D microfiber scaffold, confocal microscopy revealed that *LMNB1*-HA impaired OPC myelin basic protein production, highlighting astrocytes' crucial role in supporting myelination. Second, patient-derived human induced pluripotent stem cells (hiPSC) were differentiated into astrocytes, revealing nuclear abnormalities. Additionally, astrocytes in ADLD cortical organoids exhibited increased nuclear abnormalities, further indicating astrocyte dysfunction. NMR metabolomics of cerebrospinal fluid from ADLD patients revealed the absence of glutamate and GABA, with glutamine present, along with altered alanine-lactate cycling and absent N-acetylaspartate, suggesting impaired neuronastrocyte interactions and metabolic impairments affecting myelination. These findings highlight astrocytes as key contributors to ADLD pathology, emphasizing their role in inflammation, metabolic dysfunction, and demyelination. Identifying specific biomarkers may enhance diagnostics and guide therapeutic strategies.

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SESSION: MOLECULAR TARGETS AND BARRIER DYNAMICS IN NEURODEGENERATION AND BRAIN FUNCTIONS
(Joint Session with GISN)

THE FACETED S100B PROTEIN: THE JEKYLL/HYDE SIDE

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S100B is a calcium-binding protein originally isolated in the nervous system, where it is concentrated in astrocytes. It has also been found in definite extra-neural cell types, although at a lower level, excepting adipocytes, which constitute an intriguing site of concentration for the protein. While many functions have been attributed to the protein, at present it appears to have a double concentration-dependent role. A trophic role at low (nM) physiological concentration (Jekyll side), and a toxic role at high (mM) pathological concentration (Hyde side). This latter aspect, understandably, mainly attracted the attention of the scientific community, which also individuated the protein levels in biological fluids as a biomarker, and an effector/therapeutic target of active disorders, mainly of the nervous system for review.[1] Thus, the trophic physiological role of the protein has been in fact disregarded, although it appears to deserve primary attention. In particular, after the indication that the protein is a natural constituent of milk, more recent discovery indicated that it is also a natural constituent of commonly used healthy aliments, such as vegetables, and its possible nutrient function is explained by its interaction with microbiota. In this respect, its presence in enteroglial cells, which essentially play a role similar to astrocytes in the enteric nervous system, deserves attention susceptible of interesting and unexpected developments (for review[2]).

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HOST/PATHOGEN INTERACTIONS AND THE ROLE OF THE BARRIERS: ON THE EDGE BETWEEN HEALTH AND MULTIFACTORIAL/COMPLEX DISEASES

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Microbial agents, whether pathogens, pathobionts, or commensals, have a multifaceted role in the pathogenesis of multifactorial diseases, with a particular emphasis on immune cell activation, antigen recognition, and trafficking through distinct tissues' barriers. The microbial colonization and immune education begin in utero, through another barrier able to influence immune cell phenotypes and shaping future responses to both infection and vaccination. The complexity of T cell responses, shaped by the cross-reactivity of T cell receptors to a diverse repertoire of microbial epitopes, challenges the classical infectious disease paradigm wherein a single pathogen is associated with a specific disease. Instead, sequential or concurrent microbial exposures may be required to elicit disease, either by priming T cells or modulating their trafficking properties through direct (cis) or indirect (trans) mechanisms. Genetics and epigenetics combined with microbial exposure are shown to influence immune cell migration to target organs and the permeabilization of the barriers, including the blood brain barrier and the central nervous system, thereby contributing to neurodegenerative disorders. Two models, cis- and trans-regulation, by which microbes can alter T cell trafficking and immune homeostasis, crossing tissue barriers and offering mechanistic insight into the microbial contribution to inflammatory disease flares. These findings open new avenues for therapeutic intervention, including targeted vaccination, microbiota modulation, and antimicrobial therapies. However, challenges remain in identifying causal microbial drivers, mitigating risks associated with immunomodulation, and preventing adverse outcomes such as antimicrobial resistance or unintended immune activation. Future challenges will be the integration of approaches combining immunogenetics, single-cell sequencing, and systems immunology to better define pathogen specific contributions to complex immune-mediated diseases.

ON THE WAY TO A NEW MODEL OF NEURODEGENERATION? FIRST EVIDENCE OF THE EXPRESSION OF GATA1 IN THE BRAIN: CORRELATIONS WITH AGING, MITOCHONDRIAL DAMAGE AND SYNUCLEINOPATHY

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GATA1 is a transcription factor belonging to GATA family and involved in the development and the maturation of the hematopoietic lineage, while there are no clear evidence about the expression of GATA1 in neuronal lineage[1]. A potential role for GATA1 in synucleinopathies has been suggested, since GATA1 regulates the transcription of SNCA gene, encoding for alphasynuclein (a-syn) in red blood cells. In the present study, we aimed at deciphering the effects of GATA1 hypoexpression on aging, myelination, mitochondrial damage and alpha-synuclein (a-syn) expression in the brain. The study was performed using control mice with CD1 background and a mice model of *GATA1low* (*STOCK GATA1tm2Sho/J*)[2]. Immunohistochemistry, immunofluorescence and RNAscope were used to analyze the expression of GATA1. Beta-glactosidase and mithocondrial specific staining were

employed for detecting neuronal aging. Stereological count for different neuronal subtypes was performed in the olfactory bulbs and in the midbrain. Finally, the expression levels of a-syn were analysed by ELISA and the morphological features of GATA1 expressing neurons were visualized by Transmission Electron Microscopy (T.E.M.). Myelin basic protein and Luxol fast blue staining were used for myelination analysis. The brains of CD1 mice were 21% larger in size than those of *GATA1low* mice. GATA1 is expressed predominantly in the olfactory bulbs. As expected, expression of GATA1 was reduced in *GATA1low* mice, where GATA1 expressing neurons appeared shrinker and with mitochondrial alterations. Stereological count and ELISA analysis demonstrate alteration in the number of DA neurons and the expression of a-syn in *GATA1low* mice. Similarly, myelination and beta-galactosidase staining were different in *GATA1low* mice on respect to CD1 littermate. Our results prove, for the first time, that GATA1 is expressed in the central nervous system. Regulation of SNCA gene and contribution to maturation and survival of neurons request detailed exploration to decipher the role of GATA1 in neurodegeneration.

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IMPAIRMENT OF HERP E3 LIGASE ACTIVITY BY MUTANT POLYGLUTAMINE PROTEINS: A NOVEL MECHANISM IN POLYGLUTAMINE DISEASE NEUROPATHOLOGY

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Herp (Homocysteine-induced endoplasmic reticulum protein) is an endoplasmic reticulum stress (ERS) response protein that regulates the clearance of misfolded proteins *via* the ER-associated degradation (ERAD) pathway. This study demonstrates for the first time that Herp possesses E3 ubiquitin ligase activity, catalyzing polyubiquitination of mutant huntingtin (mHtt) through K48- and K63-linked chains, thereby suppressing its aggregation and promoting proteasome-dependent degradation. Additionally, the ubiquitin-like (UBL) domain of Herp independently inhibits the aggregation of various polyglutamine (polyQ) proteins, suggesting its broad-spectrum anti-polyQ toxicity function. However, in polyQ diseases, caspase-7-mediated cleavage of Herp results in the loss of 33 C-terminal amino acids (Herp△33), converting its catalytic property from polyubiquitination to monoubiquitination and forming non-canonical K33-linked ubiquitin chains. This shift markedly enhances the stability of polyQ proteins, facilitating their aberrant accumulation. Herp△33 itself autonomously forms insoluble aggregates, impairs the complete activation of endoplasmic reticulum-phagy (ER-phagy) pathway, and exhibits strong cytotoxicity. Herp△33 knock-in mice display progressive cognitive, memory, and motor coordination deficits, accompanied by Herp△33 aggregation and prominent neurodegenerative pathology in the striatum. This study reveals the critical role of Herp's enzymatic switch and the aggregation toxicity of its cleavage product (Herp△33) in polyQ diseases, providing a crucial theoretical foundation for further investigation into Herp's novel functions and the pathogenesis of polyQ disorders.

SESSION: NUTRACEUTICALS: NEW PATHWAYS FOR CELLULAR HEALTH BEYOND FOODS
(Joint Session with SIBS)

THE CHEMISTRY OF NUTRACEUTICALS: DRIVING HEALTH INNOVATIONS AND SHAPING NEW PARADIGMS

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Longevity is a complex biological process influenced by genetic, environmental, and lifestyle factors. The whole organism undergoes this process, with all the organs proceeding with specific biological clocks[1]. Recent evidence demonstrates the ability of several secondary metabolites, such as polyphenols, to inhibit molecular processes underlying aging events, acting on a wild array of biological targets belonging to several molecular networks. In addition, these compounds are associated with a reduced incidence of age-related diseases, including cardiovascular, neurodegenerative, and metabolic disorders. Mechanistic studies have elucidated how these molecules modulate oxidative stress, inflammation, and cellular senescence pathways, contributing to healthy aging[2,3]. In this context, the amounts of dietary phytochemicals associated with a decreased incidence of neurodegenerative and cardiovascular pathologies gain high doses that are difficult to obtain with a normal Western diet. In the recent literature, the term "superfood" has frequently been used, meaning dietary matrices, rich in compounds able to exert a plethora of biological effects resulting in a decrease of specific parameters, such as cholesterol levels, glycemia, oxidative stress and low-grade inflammation. The mentioned activities seem to be due to the whole food constituents, however in some cases it was possible to ascribe to specific compounds the salutogenic properties, as reported for beta-glucans or hydroxytyrosol. Also, functional foods may be part of salutogenic and preventive strategies. Indeed, as matrices enriched with compounds endowed with biological effects, they may act in a clinical space before drugs, beyond foods, with the aim of delaying molecular events linked to aging and inflammatory conditions.

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NANOVESICLES IN THE GUT–BRAIN AXIS: INSIGHTS FROM PROBIOTIC SUPPLEMENTATION IN IRRITABLE BOWEL SYNDROME

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Dysbiosis, often caused by poor diet or chronic stress, disrupts gut

balance and is linked to various intestinal and systemic diseases[1]. Probiotics help restore microbiota stability and relieve irritable bowel syndrome (IBS) symptoms[2], including effects on the central nervous system (CNS). This study investigates the influence of probiotics on bowel-derived nanovesicles composition, particularly for what concerns the possibility of influencing the so-called "gut–brain axis" by acting on the tryptophan metabolic pathway, since the latter is pivotal in serotonin regulation and in modulating host neurophysiology and behavior[3]. Plasma-derived nanovesicles were isolated from individuals affected by IBS with diarrhoea, both before and after a 60-day supplementation with a multi-strain probiotic formulation. The levels of tryptophan 2,3-dioxygenase 2 (TDO2) within these vesicles were analyzed, revealing its increase following probiotic supplementation. To further explore the mechanistic effects of probiotics, HT29 intestinal epithelial cells were treated with the probiotic mix in the presence (or not) of hydrogen peroxide (H_2O_2) for inducing oxidative stress. The supplementation with the same probiotic mix used in the *in vivo* study exhibited a marked cytoprotective effect by attenuating the H_2O_2-induced stress response: *e.g.*, probiotic exposure led to a significant reduction of HSP60 protein levels and to a restoration of tight junction proteins, putatively contributing to preserving/remodeling the epithelial barrier integrity. Additionally, an upregulation of both TDO2 and serotonin receptor levels was observed in these cells. To confirm our in *vivo* data, we isolated nanovesicles from HT29 cells treated with the probiotics mix and, remarkably, TDO2 levels were higher in nanovesicles from treated cells compared to the controls[4]. Our findings suggest that probiotic-induced nanovesicles may influence CNS function through the gut–brain axis and help maintain gut homeostasis under stress.

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EXPLORING THE CYTOTOXIC AND APOPTOTIC ACTIVITIES OF PRUNUS SPINOSA ECOTYPE EXTRACT TRIGNO ON 3D AND 2D MODELS OF HUMAN CANCER CELLS

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Plant-based therapies have played a key role in cancer treatment. Among them, *Prunus spinosa L.* (blackthorn), rich in phenolic acids, flavonoids, and anthocyanins, has shown anticancer potential.[1] Prunus spinosa Trigno ecotype drupe extract (PsT, a plant indigenous to Molise) complexed with a nutraceutical activator complex (NAC), consisting of amino acids, vitamins and mineral salt mixtures, was patented by us in the formulation Trigno M (PsT + NAC)®.[2] We have shown that it is cytotoxic to several tumour cell lines but not to normal cells. Trigno M reduced the viability of colorectal cancer (CRC) HCT116 and SW480 cells, by inducing apoptosis. It inhibited colony formation compared to 5-fluorouracil (5-FU) and was effective on 3D models. Trigno M significantly delayed tumour growth in CRC xenografts without toxicity.[3,4] Furthermore, Trigno M combined with 5-FU inhibited autophagy and enhanced apoptosis in CRC spheroids, suggesting that it could improve efficacy of chemotherapy and reduce side effects.[5] Given these promising results, we extended our study to melanoma. Trigno M reduced the viability of WM115, WM266-4, and A375 melanoma cells in a dose- and time-dependent manner, especially on metastatic WM266-4 cells. It induced irreversible morphological changes, cell cycle arrest, and apoptosis *via* a caspase-dependent mechanism. In conclusion, the *Prunus spinosa* Trigno ecotype extract could serve as an adjuvant therapy for CRC and melanoma, improving tumour responsiveness to conventional chemotherapy and modulating side effects by reducing cytotoxic drug doses.

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BIOLOGICAL RESPONSE OF TREATMENT WITH SAFFRON PETAL EXTRACT ON CYTOKYNE-INDUCED OXIDATIVE STRESS AND INFLAMMATION IN THE CACO-2/THP1 COCOLTURE 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[1]. 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[2,3]. 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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POSTER SESSION

P01

PUTATIVE EFFECTS OF THE HYDROGEL-STEM CELL-MELATONIN COMBINATION WITH OR WITHOUT A SCAFFOLD TO TREAT THIOACETAMIDE-INDUCED LIVER DEGENERATION IN RAT

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This study investigates the effects of Wharton's Jelly-Mesenchymal Stem Cells and type I collagen-based hydrogel solution containing melatonin on liver regeneration in experimental liver degeneration model induced by thioacetamide (TAA) in Wistar albino male rats. Control and MSC group were given saline 3/w. MSC group was administered MSCs *via* i.p. injection at 6th week. Injury group was given 200 mg/kg TAA i.p. 3/w for 6 weeks. At week 6, one group of rats received an i.p.injection of hydrogel-WJ-MSC-melatonin combination (injection group), while another group received hydrogel-WJ-MSC-melatonin combination carried by a fibrous scaffold, directly applied to the liver surface (implantation group). After 21 days of treatment period, all rats were sacrificed. Blood serum samples and liver tissues were examined by histological, ultrastructural and biochemical analyses (Bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in serum). Malondialdehyde (MDA), glutathione (GSH), myeloperoxidase (MPO) and superoxide dismutase (SOD) were measured. TAA administration led to significant increase in hepatocellular vacuolization, leukocyte infiltration and vasocongestion. Injection and implantation treatments significantly reduced the hepatocellular vacuolization and vasocongestion. Leukocyte infiltration remained significantly higher compared to the control group. An increased collagen accumulation with TAA and a decreased collagen in the implantation group were detected. In injury group, serum levels of ALT, AST, and TB were significantly increased compared to the control and MSC groups. However, ALT and AST levels were significantly decreased in the injection and implantation groups compared to the injury group. MDA, MPO and SOD were significantly increased in injury group compared to control group, while MDA and SOD were significantly reduced in the implantation group. These results suggest that the fibrous scaffold with stem cells and melatonin-loaded hydrogel may offer a promising approach for the treatment of liver degeneration.

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P02

THE C-C CHEMOKINE-RECEPTOR 2 (CCR2)/CCL2 AXIS PARTICIPATE IN PLATELET FUNCTION IN MYELOFIBROSIS

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Myelofibrosis (MF) is the most severe myeloproliferative neoplasm driven by mutations of the MPL/JAK2 axis induced by megakaryocytes (MKs) with reduced GATA1 content[1]. The proinflammatory milieu in the bone marrow (BM) microenvironment favors the disease evolution, by sustaining BM fibrosis, hematopoietic failure and extramedullary hematopoiesis[2]. The CC Chemokine 2 (CCL2) is an immune-modulatory cytokine highly expressed in MF, exerting its biological function by engaging the CCR2 receptor, by regulating the MAPK/ERK, PI3K/Akt, and JAK/ STAT pathways. The CCR2 increased expression was detected on CD34+ hematopoietic stem cells (HSC) isolated from MF patients. The percentage of CD34+CCR2+ cells parallels the degree of BM reticular deposition in overt-MF *vs* Pre-MF, suggesting that CCR2 expression can be used to track the fibrotic changes in MPN disease progression. The GATA1 modulatory function in the transcription of CCR2 was confirmed by the correlation between the GATA1 hypo-expression and the CCR2 overexpression in the HSC compartment isolated from MF patients. In addition, BM samples from patients with MF showed that GATA1 negative MKs had an increased expression of CCR2. The morphological observation on BM samples from MF patients highlighted the increased CCR2 expression in the cytoplasm of MKs, mostly arranged in aggregate-like structures. Both P-selectin staining and TEM observations confirmed the platelet aggregation in the MF patients, suggesting the CCR2 involvement in the residual platelet function in MF. In line with the abnormal MK phenotype, recent proof indicates that myeloproliferative disorders are associated with a high incidence of arterial and venous thrombotic events[3]. Finally, Ruxolitinib rescue the MKs maturation, by down regulating the CCR2 expression and restoring the platelet production. In conclusion, our result showed that CCR2 is highly expressed in MKs in MF, and the CCR2/CCL2 axis might exert several biological functions in MF progression and contribute to secondary complications.

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P03

THE ROLE OF FILAMIN A IN REGULATING BREAST CANCER CELL BEHAVIOR AND TUMOR MICROENVIRONMENT INTERACTIONS

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Filamin A (FLNa) is a multifunctional actin-binding protein involved in maintaining cell architecture, transducing mechanical signals, and regulating cytoskeletal dynamics. Its role in cancer is complex and context-dependent, particularly in breast cancer, where FLNa has been reported to act either as a tumor suppressor or a promoter of malignancy. In this study, we investigated the contribution of FLNa to key processes driving breast cancer progression, including proliferation, migration, invasion, and adhesion. FLNa expression was modulated in two breast cancer cell lines: the highly invasive triple-negative MDA-MB-231 and the luminal, less aggressive MCF-7. Efficient downregulation of FLNa was confirmed by Western blotting and quantitative fluorescence analysis. A series of functional assays -including wound healing, open-field migration, modified Boyden chamber, and adhesion assays- were conducted to assess the phenotypic consequences of FLNa depletion. Control groups consisted of cells with unaltered FLNa expression. Our findings show that reduced FLNa expression significantly alters the migratory, invasive, and adhesive properties of breast cancer cells. These results underscore the role of FLNa as a critical regulator of tumor cell behavior and support its relevance as a potential therapeutic target. However, due to the cell line-specific effects and conflicting reports in the literature, further research is required to clarify the context-dependent functions of FLNa within diverse tumor microenvironments.

P04

CYTOCHEMICAL AND BIOLOGICAL EFFECT OF BIORESONANCE IN IRRITABLE BOWEL SYNDROME

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Technological advances in the field of instrumental therapies have allowed for progress in the creation of equipment capable of treating multiple pathologies through a bio-quantum approach[1,2]. The opportunity of exploiting electromagnetic physical energies opens new scenarios in relation to the possibilities of conditioning in a curative way the structure and functionality of specific tissues and systems of the human body[1,3]. In this context we can find the approach of Bioresonance Therapy (BT), which leverages the specificity of the electromagnetic signal of each biological tissue, that could in fact be the objective and means of calibrated stimulations aimed at inducing an inversion of pathobiological processes[1,3]. A pathology characterized by altered quality and function of tissues involved is Irritable Bowel Syndrome (IBS)[1,4]. Therefore, we made a study on 20 patients (12 women and 8 men, mean age of 51 years) to verify the ability of BT to influence IBS through a direct action of its low-intensity magnetic fields on the biological tissues of the affected organs[1]. To monitor the health of patients, we observed the values recorded, before and after the execution of a cycle of 8 BT sessions, for the Short-Form Health Survey 36, the Bristol Stool Form Scale and, above all, the Fecal Calprotectin. The results of our study suggest that BT is associated with a significant improvement in all the parameters observed and to an inversely proportional correlation between the Calprotectin values and the quality of life experienced by the patients in relation to the perceived IBS symptomatology[1]. Therefore, it is possible to highlight both the efficacy of the proposed treatment and the important role of the collaboration between clinical medicine and the physical and histochemical sciences in defining the validity and the possibilities of improvement attributable to innovative therapeutic techniques such as BT.

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P05

ENTERIC NERVOUS SYSTEM NEUROPLASTICITY IN COLON OF ANIMAL MODELS OF OBESITY

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An altered functional innervation of the intestine by the enteric nervous system (ENS) has been demonstrated in different diseases. Pathological changes in histological features confirm neuronal plasticity in metabolic syndrome and related obesity conditions. Changes in functionality and composition of gut microbiota and intestinal dysbiosis seem closely linked to the degeneration of myenteric plexus in the ENS[1]. Animal models of genetic obesity (Obese Zucker's rat), Diet-induced obesity (DIO rats) and Western diet-induced obesity (Cafeteria rats)[2] were studied to appreciate the progression of changes in glial cells and neurons along the innervation of the gut, particularly at the level of colon. Using different markers through immunohistochemical and immunochemical techniques, the morphological and functional modulations of the heterogeneous neuronal population of the gut wall were evaluated and the histological damage, neurodegeneration, and pro-inflammatory cytokines expression were detected on the colon. Myenteric neurons showed a reduction in neuronal markers expression, suggesting a degeneration associated with obesity. An altered immunoreactivity of enteric glial cells (EGCs) was found in the obese rats, which pointed out a suffering condition of nervous tissue suggesting a degeneration related to lipotoxicity. Also, the cholinergic and nitrergic networks appear affected by dysmetabolic conditions. In conclusion, the obesity establishment

seems to induce myenteric neurodegeneration. Also, gut dysbiosis promoted by an increment of intestinal permeability and related pro-inflammatory microenvironment makes an alteration of EGCs and neurons along the innervation of the gut in animal models of dysmetabolic conditions. However, further studies are needed to understand if the use a possible supplementation, such as prebiotic, probiotic or specific food are helpful in the management of intestinal disorders and resulting in a modulation of the ENS in the obese condition.

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P06

MICROGRAVITY-INDUCED CHANGES IN CYTOSKELETON STRUCTURE OF TCAM-2 CELLS

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Cytoskeleton, a mechano-sensing apparatus of the cells, is sensitive to gravity conditions[1]. In our previous study, we investigated the effects of simulated microgravity (SM) on the cytoskeleton of human male germ cells (TCam-2). We observed that SM for 24 h altered microtubule and actin organization[2]. To determine whether cytoskeletal changes occur during the early stages of SM exposure, we analysed the effects after a short 3 h period. Quantitative analyses were performed using Reverse Phase Protein Array (RPPA). The distribution pattern of cytoskeletal filaments was studied by indirect immunofluorescence (IIF) and Confocal microscopy, using maximum projections obtained from 1µm optical sections. RPPA analyses showed an increase in total cofilin and tubulin as early as 3h of exposure. Cellular cofilin exists in two forms: cofilin and phospho-cofilin (the latter represents the inactive form), both regulating actin polymerization and dynamics in the cytoplasm. However, both forms are also present in the nucleus, where they transiently influence gene regulation in response to specific stimuli. This dual localization highlights the dynamic regulatory role of cofilin phosphorylation in cellular processes[3]. Indirect IIF showed that, in SM samples, total cofilin levels increased while nuclear phospho-cofilin levels decreased. Consistent with RPPA data, tubulin levels also increased according to IIF. These observations suggest a possible perturbation of actin cytoskeleton caused by SM exposure, which in turn also affects tubulin polymerization and organization confirming the already known interaction between microtubules and microfilaments[4]. Future studies are necessary to better understand the biological significance of these modifications. Nonetheless, these results indicate that cytoskeleton remodelling occurs in TCam-2 cells as early as 3 h after SM exposure, providing further insight into how TCam-2 cells adapt to SM condition.

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P07

DISRUPTED GUT-VASCULAR BARRIER AND INFLAMMATORY REMODELING IN MNGIE: A SPATIAL TRANSCRIPTOMIC AND HISTOLOGICAL STUDY

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Mitochondrial Neurogastrointestinal Encephalomyopathy (MNGIE) is a rare genetic disorder caused by loss-of-function mutations in the enzyme thymidine phosphorylase (TP). TP deficiency disrupts nucleoside metabolism, leading to progressive and fatal mitochondrial dysfunction. Although liver transplantation restores TP activity and improves survival, it does not reverse gastrointestinal damage, which includes fibrosis, hypoxia, muscle layer disorganization, and sometimes life-threatening haemorrhage. TP plays a critical role in early blood vessel development, and its absence in MNGIE is associated with impaired vascularization, marked by the preponderance of very small (<50 µm) and more fragile vessels. To study vascular alterations, we applied Visium CytAssist spatial transcriptomics to full-thickness jejunal samples from two MNGIE patients and two controls. Despite limited tissue availability, this approach revealed distinct layer-specific transcriptional profiles, highlighting signatures of aberrant vascular remodelling. Among all the analysed tissue layers, considering specifically the vascular compartment, 68 differentially expressed genes (DEGs) were identified with a $p<0.001$. Among these, we observed increased expression of genes involved in inflammatory and bacterial responses, including IGHA1, DEFA6, REG3A, DEFA5, JCHAIN, OLFM4, IGKC, REG1A, LYZ, DMBT1, and APOC3. This observation is consistent with the quantification of mast cells in full-thickness jejunal tissue. Compared to controls, MNGIE samples showed a 68% increase in mast cell count in the mucosa ($p=0.0115$), an 82% increase in the submucosa ($p=0.0076$), and a 98% increase in the neuromuscular layer ($p=0.0017$). The submucosa itself contained 48 DEGs ($p<0.001$), mirroring the vascular signature of increased inflammatory and bacterial response. Collectively, these data, together with jejunal histology, suggest impaired gut-vascular barrier integrity and increased permeability. Ongoing analyses of circulating biomarkers and dedicated permeability assays are underway to validate the molecular evidence presented in this preliminary study.

P08

COMBINED EFFECTS OF NONYLPHENOL AND STEROID HORMONES ON HUMAN PROSTATE CELL LINE

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Nonylphenol (NP) is a compound classified as an Endocrine Disrupting Chemical due to its estrogenic activity[1]. Used in detergents, pesticides, and plastics, NP tends to be accumulated in aquatic environments due to poor degradability[2,3]. This study assessed NP effects on the non-tumoral human prostate cell line PNT1A, alone and combined with natural hormones 17β-oestradiol (E2) and testosterone (T), to explore potential synergistic or antagonistic interactions. NP alone increased cell viability, confirming its xenoestrogen role likely through Androgen/oestrogen-receptor α (AR/ERα) activation. NP+T and NP+T+E2 further increased viability, suggesting co-activation of ERα and AR pathways. Conversely, NP+E2 reduced proliferation and AR protein levels, indicating antagonism and a potential antiandrogenic effect. All treatments caused a delayed translocation of ERα and AR from cytoplasm to nucleus, possibly due to NP lower receptor affinity versus natural hormones. Migration assays revealed E2 and T promoted migration, while NP and its combinations inhibited it. NP also transiently increased ERα levels, affecting cell's hormonal sensitivity. To Sum up, NP altered prostate cell homeostasis by disrupting hormone receptor function, influencing viability, migration, and receptor localization, especially when combined with endogenous hormones.

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P09

EFFECTS OF NUTLIN-3A ACTIVITY ON 2D AND 3D RETINOBLASTOMA MODELS

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Retinoblastoma is the predominant paediatric tumor impacting the retina[1] To effectively mimic the *in vivo* primary tumor, novel therapeutic approaches must be examined in increasingly complex models. The primary aim of this study was to evaluate the anticancer efficacy of MDM2 inhibitors in the treatment of retinoblastoma using preclinical 2D and 3D bio-printed innovative models. As retinoblastoma exhibits a p53 wild-type phenotype, the use of Nutlin-3a, a molecule known to free p53 from its inhibitor MDM2, can activate the p53 apoptotic pathway limiting retinoblastoma growth and survival.[2] We first assessed Nutlin-3a in 2D models, using the retinoblastoma cell lines Y79 and Weri-Rb1, both expressing $p53^{wt}$, demonstrating a concentration dependent response as early as 24 h. We observed significant cell viability reduction, cell cycle blockade in the G2/M phase, apoptosis increases and p53 pathway activation, by cytofluorometric and western blotting analysis. Next, we used the same cell lines to create and characterize innovative 3D bioprinted models, using 2% alginate and 5% gelatine bioink and printing $10x10^6$/mL Y79 and $20x10^6$/mL Weri Rb1 cells; crosslinking with 50 mM $CaCl_2$ stabilized the model. Two days after bioprinting, the 3D structures were treated with Nutlin-3a and assessed for viability using MTT 72 h after. In parallel, the 3D structures were fixed and embedded to paraffin and subjected to histological and immunohistochemical investigation. Haematoxylin and eosin staining revealed a 3D cellular architecture similar to the primary retinoblastoma tissue, with cells having a typically rosette distribution. Interestingly, in the 3D tissues the number and dimension of rosettes were significantly reduced after Nutlin-3a treatment, associated with a decrease expression of Ki-67. Our successful creation of a 3D model that closely resembles tumor tissue might potentially be used as a platform for future co-culture of tumor and healthy cells to assess innovative drugs and/or to study the administration of Nutlin-3a *via* nanoparticles.

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P10

ELECTRON MICROSCOPY-ADAPTED PHTHALOCYANINE AND VON KOSSA HISTOCHEMISTRY: A POWERFUL TOOL TO STUDY AORTIC VALVE CALCIFICATION IN *AD HOC* MODELS AND ACTUAL DISEASE

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Valve calcification is a multifactorial disorder whose etiopathogenesis remains largely unclear. In our laboratory, the use of copper phthalocyanine Cuprolinic Blue (CB) for histochemical reactions under electron microscopy has provided deeper insights into the pro-calcific degeneration involving aortic valve interstitial cells (VICs) in *ad hoc in vivo* and *in vitro* calcification models[1-4]. Specifically, CB dissolved in acidulated buffer, gently solubilizing hydroxyapatite (HA) crystals, promotes simultaneous unmasking and staining of acidic lipid material (PPM) derived from organelle and plasma membrane breakdown in VICs, alongside nuclear chromatin and ribosomal derivatives. A major pro-calcific role is clearly exhibited by PPM-derived CB-positive layers (PPLs) forming at VIC edges, as revealed by the selective overlapping of nonremoved HA crystals. Blebbing of PPLs produces PPL-lined

cell byproducts, including final *calcospherulae*, which mediate calcification spreading throughout the extracellular spaces. The ability of PPLs to act as major HA nucleators is further confirmed by superimposition of metallic silver particles after postembedding von Kossa reaction on semithin sections and their reembedding. This VIC degeneration is also paralleled by overexpression of cytosolic phospholipase A2α (cPLA2α)[5]. Importantly, these findings are now being demonstrated to occur also in true calcific aortic valve disease (CAVD), challenging the currently prevailing paradigm that attributes valve calcification primarily to osteogenic processes. In conclusion, the visualization protocols applied both in suitable pro-calcific models, including future specifically designed co-cultures, and in actual CAVD, along with further investigations into cPLA2α involvement, will contribute valuable insights into ectopic calcification.

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P11

DOMINO TRANSPLANTATION IN MNGIE: IS THE LIVER A SUITABLE GRAFT?

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Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is a rare, fatal disorder caused by *TYMP* mutations leading to toxic nucleoside accumulation and mitochondrial DNA (mtDNA) damage. Liver transplantation, which restores thymidine phosphorylase activity, is a life-saving treatment. As the liver has not been considered a primary target of the disease, explanted MNGIE livers have been proposed for domino liver transplantation. To fully characterize the liver in MNGIE patients at different disease stages and assess its suitability for domino donation. Liver biopsies from 9 MNGIE patients and 7 controls (age and sex matched) were analysed. FFPE sections were used to evaluate tissue architecture, fibrosis, hypoxia, immune activation, and mtDNA depletion. Snap-frozen tissues underwent metabolomic (NMR), proteomic (mass spectrometry), and transcriptomic (RNAseq) profiling. Spatial transcriptomic on gut tissue was used to test gut liver axis. COX-SDH staining and HIF-1α expression revealed a hypoxic liver environment in MNGIE. Glucose and glycogen metabolism were upregulated. Lipid metabolism was reprogrammed, with steatosis correlating with ATP citrate synthase upregulation. Fibrosis and septa formation involved activation of NFκB, SERPINE family, CXCL8 and MYC pathways. Vascular and biliary alterations were linked to increased VEGF expression. Microdissection showed progressive mtDNA depletion in hepatocytes and portal areas. Immune infiltration was detected in the tissue and activation were in line with multi-omics analyses. Cytoarchitectural and molecular findings reveal a stepwise progression that parallels disease staging, supporting a model of gradual and cumulative organ deterioration. Additional spatial transcriptomics in matched gut tissues suggested impaired gut–liver axis and possible bacterial translocation. Our findings demonstrate that the liver is a progressively affected target organ in MNGIE. This raises critical concerns about the use of explanted MNGIE livers in domino transplantation and warrants careful ethical and clinical consideration.

P12

VISUALIZING NUCLEAR ARCHITECTURE DURING MAMMAL ERYTHROPOIESIS

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The spatial organization of the genome is a highly dynamic phenomenon responsive to multiple endogenous and exogenous stimuli[1]. Mammalian erythropoiesis is characterized by profound chromatin reorganization prior to nuclear extrusion[2], representing a suitable model to investigate nuclear architecture and its impact on gene expression regulation. This study applies transmission electron microscopy combined with cytochemical and immunocytochemical methods for the visualization of key players involved in nuclear remodeling. Morphological analyses revealed major cellular alterations, gradual clearance of cytoplasmic organelles, and spread of heterochromatic regions. The progressive chromatin compaction was assessed through osmium ammine, which enables selective[3] DNA visualization and standardized thresholding. Trimethylated H3K9, an epigenetic marker of chromatin condensation, was targeted *via* immunogold labelling, revealing a stage-dependent increase and relocalization to the perichromatin region. Nuclear complexity was further assessed by RNA visualization, performed with terbium citrate staining, which enables its ultrastructural localization at the single fibril level[4], and electron microscopy *in situ* hybridization selectively labelling pre-mRNAs. Moreover, EDTA regressive staining enabled the visualization of ribonucleoproteins, highlighting their preferential localization in relation to the progressive increase of chromatin condensation. Altogether, this combination of methodologies delineates the coordinated remodelling of the nucleoplasm during erythropoiesis and enables deep investigation of genome features at the nanoscale applicable to further systems.

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P13

MORPHO-FUNCTIONAL DIFFERENCES IN THE GUT OF AGED MICE WITH THE POSSIBLE EFFECTS OF ANTI-INFLAMMATORY TREATMENTS

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The gastrointestinal (GI) barrier represents one of the most important interfaces between organism and the external environment with a continue exposure to external substances[1]. For these reasons, preserving the structural and functional integrity of the GI barrier is fundamental to control and prevent inflammation, which contributes to age-related diseases[2]. Evidence suggests associations between the inflammation/ senescence and the presence of chronic disease in the elderly, thus the aim of the study has been to evaluate the morpho-functional differences in the intestinal wall of aged mice with or without an anti-inflammatory treatment. We used C57BL/6J mice intranasal treated with vehicle (control) or IL4-EVs (extracellular vesicles released by anti-inflammatory microglia stimulated with IL-4) to reduce the inflammatory phenotype of microglia[3]. We evaluated the gut transit and the inflammatory factors in plasma, such as IL16, CCL2 and TIMP1. Then we performed hematoxylin & eosin (H&E) and periodic acid-Schiff (PAS) staining to describe the differences in the morphological aspects of the intestinal wall. In the end, we investigated the changes in permeability through occludin expression and the presence of specific nervous markers such as Glial Fibrillary Acidic Protein (GFAP) and α-synuclein (αsyn) to better characterize the possible connections between the enteric nervous system (ENS) and the aging.[4] IL-4- EVs increase the gut transit and reduce inflammatory factors in plasma. Upon the treatment, we found changes in: (i) villus's dimension, (ii) quantity of goblet cells, (iii) architecture of occludin, and (iv) the co-expression of GFAP/α-syn. Since IL-4-EVs are capable to modify inflammatory profile in late adult mice, they could play a role in the gut-brain axis in aged mice and in the inflammatory process acting in the tight relationship between permeability of the intestinal barrier and the enteric glial cells.

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P14

THE ROLE OF CHROMATIN REORGANIZATION IN HYPOXIA ADAPTATION

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The spatial organization of the cell nucleus bears functional relevance in both maintaining cellular homeostasis and facilitating pathological transformations. In response to external cues, it undergoes dynamic reorganization of chromatin architecture, causing the spatial relocalization of nucleic acids and specific proteins into defined nuclear clusters, whose stimulus-driven compartmentalization remains largely elusive. Among the various environmental stimuli capable of triggering such nuclear reorganization, oxygen availability plays a pivotal role. Recent studies have highlighted the role of hypoxia in reshaping the epigenetic landscape, suggesting that HIF-1α upregulation may drive chromatin remodelling events governing the expression of key genes[1,2]. Nevertheless, the intricate dynamics of nuclear reorganization elicited by hypoxic stress remain poorly understood. Therefore, this work is aimed at investigating chromatin reorganization in mouse hepatocytes exposed to hypoxia mimicking conditions and during a subsequent reoxygenation phase. Our analyses suggest that cells adapt to hypoxic stress by reshaping the epigenetic landscape, resulting in a more accessible chromatin state that may support enhanced transcriptional activity, as indicated by the increased density of perichromatin granules (PGs). However, the highly decondensed environment may render the genome more susceptible to DSBs, potentially activating cell cycle checkpoints, as evidenced by the accumulation of hypoxic cells in G2/M phase. Notably, this arrest coincides with the clustering of subnuclear components, including PGs and RNP assemblies, which may serve as reservoirs for mature RNA and RNA-processing factors, respectively. In this context, we explored the role of the lncRNA MALAT1, which may contribute to the spatial organization of these functional nuclear assemblies. Further investigation could offer valuable insights into the intricate mechanisms of cellular adaptation to hypoxia, with significant implications for understanding tumour progression within hypoxic microenvironments.

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P15

PHB/JAK/H3Y41PH AXIS REGULATES MEIOTIC RECOMBINATION IN SPERMATOGENESIS

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Previously, we have shown that human sperm Prohibitin (PHB) expression is significantly negatively correlated with mitochondrial ROS levels but positively correlated with mitochondrial membrane potential and motility. However, the possible role of PHB in mammalian spermatogenesis has not been investigated. Here we document the presence of PHB in spermatocytes and its functional roles in meiosis by generating the first male germ cell-specific PhbcKO mouse. Loss of PHB in spermatocytes resulted in complete male infertility, associated with not only meiotic pachytene arrest, but also apoptosis resulting from mitochondrial morphology and function impairment. Our mechanistic studies show that PHB in spermatocytes regulates the expression of STAG3, a key component of the meiotic cohesin complex, *via* a non-canonical JAK/STAT pathway, and consequently promotes meiotic DSB repair and homologous recombination. Furthermore, the PHB/JAK2 axis was found as a novel mechanism in the maintenance of stabilization of meiotic STAG3 cohesin complex and the modulation of heterochromatin formation in spermatocytes during meiosis. The observed JAK2-mediated epigenetic changes in histone modifications, reflected in a reduction of histone 3 tyrosine 41 phosphorylation (H3Y41ph) and a retention of H3K9me3 at the Stag3 locus, could be responsible for Stag3 dysregulation in spermatocytes with the loss of PHB.

P16

A 3D *IN VITRO* MODEL TO STUDY CAF PLASTICITY AND TUMOR-STROMA INTERACTIONS IN OVARIAN CANCER

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The tumor microenvironment (TME) plays a pivotal role in cancer progression and therapy resistance. Among its components, cancer-associated fibroblasts (CAF) are key players in remodeling the extracellular matrix, promoting tumor invasion, and modulating immune responses. In this context we developed a robust 3D *in vitro* model of ovarian cancer, integrating cancer cells and stromal components to recreate key structural and functional aspects of the human TME. Using heterotypic tumor spheroids composed of ovarian cancer cell lines and stromal cells, we recreated a relevant microenvironment to investigate CAF-like cell behavior. Human dermal fibroblasts were exposed to tumor cell-conditioned media, with or without TGF-β, to induce CAF-like phenotypes. Our findings demonstrate that distinct microenvironmental stimuli drive the emergence of heterogeneous CAF-like populations, which in turn influence spheroid properties. This 3D model recapitulates key features of the reactive stroma and enables functional analysis of tumor-stroma crosstalk under controlled conditions. It provides a robust platform for mechanistic studies and therapeutic investigation, offering new opportunities to dissect the dynamic interactions within the ovarian tumor microenvironment.

P17

INTERMITTENT FASTING AND FUCOIDAN ADMINISTRATION AMELIORATE THE EFFECT OF TYPE 2 DIABETES ON ENTERIC NERVOUS SYSTEM IN RATS

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Type 2 diabetes mellitus (T2DM) is a chronic inflammatory disorder that affects glucose metabolism, the enteric nervous system (ENS), and the gut-brain axis 1. The study investigates the effects of intermittent fasting (IF) and fucoidan (F), a sulfated polysaccharide with antioxidant properties, on oxidative stress (OS) and neuroinflammation in a streptozotocin (STZ)/nicotinamide (NA)-induced rat T2DM model. The study involved 40 adult male Sprague-Dawley rats divided into six groups: Control, T2DM, T2DM+IF (16-h fasting for 30 days), T2DM+F (100 mg/kg/day, oral), and T2DM+IF+F. The results showed that hyperglycemia significantly decreased in all treatment groups, while diabetes reduced body weight. Neuronal density, goblet cell count, and villus height were reduced in diabetic rats, while iNOS and occludin expression levels were suppressed. Elevated levels of luminol, lucigenin, MDA, and MPO, and reduced GSH in diabetic rats were significantly reversed in treated groups. These findings suggest that IF and fucoidan synergistically mitigate OS, enhance mucosal integrity, and reduce neuroinflammation, supporting their potential as adjunctive strategies for T2DM management.

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P18

NESTIN EXPRESSION IN THE MYOCARDIUM OF SPONTANEOUSLY HYPERTENSIVE RATS AND CONPLASTIC SHR-MTBN STRAIN, AND CO-EXPRESSION WITH CONNEXIN 43

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A unique intermediate filament protein nestin is present in some stem, progenitor and developing cells and re-expressed in the adult tissues during processes recapitulating the developmental phases such as regeneration, *e.g.* of the skeletal muscle. In the myocardium nestin is shortly identified early prenatally in rodents and for as yet unexplained reasons it occurs in the adult diseased heart in humans

as well. Previously we detected nestin in rare desmin+ cardiomyocytes, some vimentin+ interstitial cells and endothelia in the hypertrophic heart of the aging spontaneously hypertensive rats (SHR). In this work we immunohistochemically detected nestin in the hearts of the SHR-mt^{BN} conplastic rat strain to reveal the impact of the mitochondrial genome of the normotensive BN rats on nestin expression in SHR myocardium. Then, we performed double immunofluorescence of nestin and connexin 43 to unveil any differences in the intercalated discs gap junction pattern between nestin+ and nestin- cardiomyocytes in the SHR myocardium. Nestin expression was only mildly more abundant in the SHR than in the SHR-mt^{BN} 5-month-old rats. In the 15-month-old SHR rats, nestin was detected in higher number of cardiac cells evenly located in wall of the left ventricle and the interventricular septum, but in the SHR-mt^{BN} rats of the same age this protein was expressed more in groups of cells in certain regions. The distribution of connexin 43 did not substantially differ between nestin+ and nestin- cardiomyocytes in the 5- and 15month-old SHR rats. In the aged hypertrophic myocardium gap junction arrangement was more irregular and connexin 43 expression was also situated at lateral plasmalemma. In conclusion, nestin occurrence in the heart of SHR rats depends on age and mitochondrial genome. Less frequent nestin re-expression reflects a lower intensity of cytoskeletal remodelling of myocardial cells more resistant to hypoxia in the SHR-mt^{BN} rats. In the SHR cardiomyocytes nestin expression seems to be unrelated to impulse propagation by connexin 43. Nestin re-expression is apparently involved in structural remodelling in myocardial hypertrophy in SHR rats during aging.

P19

LOQANT: IMAGEJ SCRIPT FOR ANALYSING NUCLEOCYTOPLASMIC PROTEIN TRANSLOCATION

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Nucleocytoplasmic shuttling is a key regulatory mechanism in cellular signalling. Many proteins, including nuclear receptors, transit between the cytoplasm and the nucleus in order to control gene expression and other vital processes. Monitoring their subcellular localisation using immunostaining provides valuable insights into cellular responses, disease mechanisms and therapeutic strategies. However, manual assessment is very time consuming, introduces a considerable degree of subjectivity and bias, and is unsuitable for high-throughput analysis. Despite its importance, there is currently a lack of standardised, user-friendly tools that can quantitatively assess nucleocytoplasmic translocation efficiently and reproducibly. To evaluate the variability of manual scoring when assessing nuclear receptor translocation, 20 micrographs of SH-SY5Y cells immunostained for PPARα were analysed independently by six observers with varying levels of expertise. As expected, manual grading showed considerable variability and poor interobserver reliability, as determined by intraclass correlation coefficient analysis. As a solution, we developed LoQANT (Localisation and Quantification of Antigen Nuclear Translocation), an open-source, Python-based ImageJ/Fiji script. It reliably and efficiently quantifies nucleocytoplasmic shuttling in both DAB- and fluorescence-stained specimens. This single-cell-based approach specifically assesses the presence and intensity of the nuclear signal, independently of cytoplasmic staining, thus avoiding confounding background interference. Depending on the detection method, it can perform both semiquantitative and quantitative analyses.

P20

RNASEQ AND HISTOCHEMICAL STAININGS ASSESS BIOCHEMICAL AND STRUCTURAL CHANGES IN THE MOUSE INTERVERTEBRAL DISC ACROSS THE LIFESPAN

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The intervertebral disc (IVD) is the largest avascular and aneural tissue in the body. IVDs form the joint in the spine, helping to provide flexibility and resist compressive forces in young and healthy individuals. However, following injury, or with natural aging, the IVD undergoes natural structural and molecular changes leading to its degeneration, and is a leading cause of disability, affecting over 70% of adults, but with no cure. Each IVD has a proteoglycan-rich core made up of the nucleus pulposus (NP). NP is laterally surrounded by collagen-rich annulus fibrosus (AF). The extracellular matrix (ECM) is crucial for IVD hydration, structure, and function. Here, using the mouse as a model system, we analyzed the molecular changes in NP and AF cells from one week to one year of age using bulk RNA sequencing. Results showed that ECM content and turnover were impacted by age in both NP and AF cells. Next, using an array of histochemical staining methods, we analyzed the changes in the lumbar IVD structure and biochemical composition across the lifespan, from newborn to over two years of age. Due to the high collagen and proteoglycan content of the IVD, safranin-O and fast green, or picrosirius red, are generally used to determine biochemical changes in the IVD. Here, using additional staining methods, including Masson's trichrome and Movat pentachrome, we observed that, in addition to changes in collagen and proteoglycan content with age, the aging mouse IVDs were vascularized and had a high amount of endogenous fibrin forming fibrinoids. Next, we determined the clinical relevance of these findings using clinical samples obtained under an IRB-approved study, following informed consent, and validated the presence of fibrinoids in degenerated human IVDs. To our knowledge, this is the first study to describe the presence of fibrinoid in the IVDs, which was made possible by the histochemical staining approach.

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EARLY BIOLOGICAL EFFECTS ON CELL PROLIFERATION AND DIFFERENTIATION INDUCED BY EXTRACELLULAR MICROVESICLES (EVS) DERIVED FROM HUMAN KERATINOCYTES IN PSORIATIC MICROENVIRONMENT

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Extracellular vesicles (EVs), a small bound-membrane particles involved in cell communication, influence skin processes like wound healing and proliferation, even in psoriasis. Spontaneously

immortalized keratinocytes (HaCaT cell line) were differentiated for 4 days with CaCl2 1.8 mM and exposed for 24 h and 48 h to a proinflammatory psoriatic microenvironment, a cytokine combination (MIX) of interleukin (IL)-17A (10 ng/mL), IL-22 (20 ng/mL), IL-23 (10 ng/mL) and Tumor Necrosis Factor α (20 ng/mL). Untreated HaCaT cells differentiated for 6 days as controls (CTR). At each time, the culture medium was collected from CTR and MIX samples, to isolate EVs by standard centrifugation steps. After determination of total EV-associated proteins, differentiated HaCaT cells were incubated for 24 h and 48 h with medium containing 10 μg/mL of EVs. Proliferation and differentiation, in terms of tight junctions proteins (CLDN-1, ZO-1) and keratins (CK10, CK14) were evaluated by immunofluorescence and Western blot. MIX-EVstreated *vs* CTR cells showed an increased proliferation and higher expression of CK14 and CLDN-1. Results suggest a role in crosstalk between epithelial cells, cytokines and EVs causing the modification of cell differentiation, so future studies will apply to clarify their early biological effects on psoriatic lesion formation, playing a promising diagnostic and therapeutic role.

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P22

EXPLORING THE UNEXPECTED DUAL ROLE OF GARLIC DERIVATIVES IN REGULATING INVASIVENESS IN BREAST CANCER SUBTYPES

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Breast cancer includes tumor subtypes with distinct morphological, molecular, and clinical profiles. The intrinsic heterogeneity, combined with the toxicity of chemotherapeutics, often impacts therapeutic efficacy and contributes to the development of resistance[1]. Therefore, there is growing interest in identifying adjuvant strategies to enhance the effectiveness of conventional treatments. Among these, natural compounds are gaining attention due to their broad biological activities and relatively low toxicity. Garlic (*Allium sativum*) has been extensively studied for its anticancer properties, with several reports indicating its ability to counteract breast tumor aggressiveness[2]. However, studies fail to account for the high heterogeneity within triple-negative breast cancer (TNBC), and no data are currently available about its effects on HER2$^+$ tumors, which, despite recent therapeutic advances, frequently develop resistance. To address this gap, we investigated the effects of garlic-derivatives on PDX-derived TNBC and HER2$^+$ breast cancer cells. Interestingly, in TNBC cells we found a selective molecular subtype-dependent decrease of the invasive potential[3], while a dual effect was shown in HER2+ cells, with reduced invasiveness after a short-term exposure, and a paradoxically enhanced invasive potential after prolonged treatment. In both TNBC and HER2$^+$ cell models, modulation of invasiveness was associated with activation of the Akt/GSK3β/βcatenin signaling axis, culminating in nuclear accumulation of βcatenin, a key regulator of genes involved in tumor progression and malignancy[4]. Although *in vivo* validation is required, our findings highlight the importance of understanding the precise molecular mechanisms triggered by natural compounds in specific tumor contexts. These results also raise concerns about the uncritical use of natural substances in oncology, as their effects may vary depending on tumor subtype and treatment conditions.

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P23

CALRETININ IMMUNOREACTIVE NON-TRADITIONAL LARGE NEURONS IN THE GRANULAR LAYER OF THE HUMAN CEREBELLAR CORTEX

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Calretinin (CR) is a calcium binding protein of the EF-hand family. CR and Calbindin D28k present 58% of identical amino acids residues. CR is widely expressed in the neuronal cell bodies and processes and form asymmetrical and symmetrical synaptic specializations. CR is a modulator neuronal excitability. Although several studies suggest a role of the CR and of the cerebellum in neurological and psychiatric disorders (*e.g.* Alzheimer's disease, Parkinson's disease, schizophrenia, spinocerebellar ataxias). In the cerebellar cortex, few studies suggest CR expression in the granules and in two non-traditional large neuron types (unipolar brush neuron, Lugaro neuron). Currently, in the granular layer of the human cerebellum does not exist a detailed morphofunctional description of CR immunoreactive non-traditional large neuron types. Therefore, the aim of this study is to carry out, using an immunohistochemical approach, a detailed analysis of CR in the non-traditional large neurons of the human cerebellar granular layer[1-3]. The study was carried out on postmortem fragments of human cerebellar cortex fixed in neutral buffered formalin, embedded in paraffin, cut into 5 μm sections and subjected to light microscopic immunohistochemistry with mouse polyclonal antibody for CR. In the granular layer of the cerebellar cortex the CR immunoreactivity (ir) in cell bodies and processes of different non-traditional large neuron types (*e.g.* perivascular neurons, synarmotic neurons) and in the sites of cerebellar glomeruli have been detected. These results indicate in the cerebellar granular layer a

widely presence of CR-ir in different non-traditional large neuron types and suggest in these neurons a role of CR in calcium homeostasis and in neurotransmission mechanisms. Although, further research is needed, CR dysregulation and non-traditional large neuron types may be impaired in brain psychiatric and neurologic diseases in which the cerebellum is involved, and in specific cerebellar diseases.

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P24

DEVELOPMENT OF HEAD AND NECK SQUAMOUS CELL CARCINOMA PATIENT-DERIVED XENOGRAFTS (PDXS) AND ORGANOIDS (PDXOS) FOR MORPHOLOGICAL ANALYSIS AND DRUG SCREENING

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Head and neck squamous cell carcinoma (HNSCC), arising from the oral cavity, larynx, and pharynx, represents the most prevalent tumour of the head and neck region. The epidermal growth factor receptor (EGFR) is often mutated or overexpressed in HNSCC, representing a key therapeutic target. The anti-EGFR monoclonal antibody cetuximab (CTX) was approved as a radiation sensitiser for recurrent or metastatic HNSCCs, but patients often develop resistance[1]. Overall, therapeutic options for HNSCC remain limited and reliable models to study the disease are still lacking. Our research aims to develop reliable 3D models to study HNSCC and to provide a therapeutic alternative for patients resistant to the currently approved anti-EGFR therapy[2–4]. Here, we established a set of Patient-Derived Xenografts (PDXs) by collecting tumour samples during surgical procedures and implanting them into immunocompromised mice (NODCB17Prkdcscid IL2rgtm1/Bc gen). The PDXs were subsequently expanded for drug testing and molecular analysis. Moreover, some of the tumours were processed to generate PDX Organoids (PDXOs) for further *in vitro* experiments. PDXOs were propagated *in vitro* in Matrigel, and the architecture ranged from solid dense spheres to hollow structures with a lumen, resembling glandular or cyst-like architecture. Most importantly, immunohistochemical characterisation of the PDXs and PDXOs confirmed the preservation of the patient-specific morphological and functional traits. In addition, immunohistochemical and molecular analysis revealed that EGFR expression varied across the samples, suggesting a heterogeneous response to EGFR inhibition. These results confirmed that PDXs and PDXOs represent a robust model to study HNSCC and constitute a fundamental step towards developing more effective and personalised therapeutic strategies.

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P25

RESPONSE TO HEDGEHOG AND PI3K/AKT/MTOR PATHWAY INHIBITORS IN PREB-ALL: ANALYSIS OF THE INTRACELLULAR DISTRIBUTION OF PATHWAY ENZYMES AND OF THE AUTOPHAGY PROCESS

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PreB-Acute Lymphoblastic Leukemia (ALL) is characterized by the uncontrolled proliferation of lymphoid precursors and the dysregulation of key intracellular signaling pathways. Among these, the PI3K/AKT/mTOR and Hedgehog (Hh) pathways play critical roles in promoting leukemic cell growth, survival, and resistance to therapy[1,2]. While the PI3K/AKT/ mTOR axis has been extensively studied, the contribution of GLI1 a key downstream transcriptional effector of the Hh pathway remains largely unexplored in hematologic malignancies. To identify the role of these signaling pathways in development, maintenance and progression of preBALL lines. To investigate the effect of single or combined inhibition of the PI3K/AKT/mTOR and Hh/GLI1 pathways, in preB-ALL cell lines (NALM-6, KOPN8 and SEM) were treated with the GLI1 inhibitor GANT-61 and/or the AKT inhibitor MK-2206. Cell viability was assessed using the CCK-8 assay. Apoptosis, cell cycle distribution, and autophagy were analyzed by flow cytometry. Protein expression and localization of Gli1 and Akt was evaluated by Western blotting and immunofluorescence, respectively. Dual pathway inhibition significantly decreased cell viability when compared with single treatments in ALL cell lines, underscoring a synergistic cytotoxic effect. The combined treatment induced G0/G1 cell cycle arrest, increased apoptotic cell death and significantly triggered autophagy, as evidenced by elevated LC3B expression. Western blot analyses revealed a relevant downregulation of the total form GLI1 and of phosphorylated AKT (p-AKT), GSK3β (p-GSK3β), p70S6K (pp70S6K). Before treatments immunofluorescence analysis showed a nuclear localization of GLI1 whereas p-Akt was mainly in the cytoplasm. After treatments the fluorescent signals reduced their intensity. Co-inhibition of the PI3K/AKT/mTOR and Hh/GLI1 pathways showed a synergistic cytotoxic effect in preB-ALL models, indicating a potential strategy to overcome therapeutic resistance in leukemia.

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HUMAN CYTOMEGALOVIRUS-DRIVEN ADAPTIVE NK CELLS AND MATRIX METALLOPROTEINASES EXPRESSION AS KEY ELEMENTS IN ATHEROSCLEROTIC PLAQUE DESTABILIZATION

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Human Cytomegalovirus (HCMV) infects multiple cell types and can persist in latency post-primary infection. Recent evidence links HCMV with atherosclerosis, as its DNA has been found in atherosclerotic plaques (pq). In some patients (pts), HCMV shapes the Natural Killer (NK) cell compartment toward an adaptive profile, marked by long-term persistence, enhanced antibody dependent cytotoxicity (ADCC), and increased cytokine production, notably IFN-γ. These adaptive NK cells have been associated with pq progression, though their role in pq destabilization remains to be fully understood. This study investigates HCMV's role in plaque (pq) instability and its impact on NK cell behavior in atherosclerotic microenvironments. We analyzed 64 pts with carotid stenosis, stratified into high-risk (HR) and low-risk (LR) groups. HR pts underwent endarterectomy; blood and plaque samples were analyzed *via* flow cytometry and immunohistochemistry. Histochemical analysis revealed infiltration of NKp46+ NK cells and $CD68^+$ macrophages in the plaques of HCMV+ HR pts, with HCMV detected within $CD68^+$ cells. Flow cytometry showed enrichment of adaptive $Fc\varepsilon R1\gamma^-$ NK cells expressing the activating receptor NKG2D in HR $HCMV^+$ pts. These cells increased in blood during pq destabilization and accumulated in plaques. NK cells from HR HCMV+ pts showed elevated IFN-γ production *via* ADCC, which correlated with $Fc\varepsilon R1\gamma^-$ NK cell frequency. Blocking NKG2D reduced IFN-γ release, implying a role in activation. Symptomatic HR $HCMV^+$ pts exhibited strong expression of MMP-9 within $CD68^+$ cells in plaques. MMP-9, involved in extracellular matrix degradation, correlated with $Fc\varepsilon R1\gamma^-$ NK cell frequency, suggesting a link between MMP-9, this NK cell subset, and plaque instability. These results support the hypothesis that the HCMV–NK cell– MMP-9 axis contributes to plaque destabilization and could serve as a predictive marker for pts at high risk of atherosclerotic complications.

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HISTOCHEMICAL APPROACHES FOR EVALUATING THE REPRODUCTIVE HEALTH OF MUSSEL MYTILUS GALLOPROVINCIALIS EXPOSED TO ACUTE THERMAL STRESS

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The variation of chemical-physical parameters in response to the human impact on natural environments has led to a global increase in temperature (T) that can put at risk the balance of the most exposed ecosystems, like the aquatic-coastal ones[1]. Hence, it is needed to find specific biomarkers that can be rapidly used to evaluate the potential cytotoxicological risk of warming on biota. Therefore, to understand the biological responses dependent on climate change in a tissue fundamental to the survival of the species, such as the reproductive system, a thermal priming experiment was performed in which male and female organisms of the edible mussel *Mytilus galloprovincialis*, previously maintained at 17±1°C, were subjected to 28, 30, or 32°C for 2 h and returned to 17±1°C for 24 h, to then be exposed to a thermal shock at 36°C for 2 h. A negative control group (no T variation) and a positive one (directly exposed to 36 °C without priming) were considered. By histochemical techniques like dPAS/PAS (to highlight glycogen), May Grünwald-Giemsa (to discern haemocyte classes), and Schmorl (to evaluate the innate immune response relative to phenoloxidase), the obtained results validated the reliability of applied methods, confirming how a pre-treatment can influence adaptation to subsequent thermal stress, and how the selected biomarkers are adequate to describe the impact on the reproductive health of an engineer of intertidal hierarchies. Moreover, these data underline the strict interdependence between human actions and the consequences on biodiversity of marine ecosystems, providing new insights on the resistance of biota to this ecological stress factor.

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EXPLORING THE POTENTIAL LINK BETWEEN MITOCHONDRIAL DYSFUNCTION AND MITO-EVS IN PANCREATIC DUCTAL ADENOCARCINOMA (PDAC)

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The trafficking of mitochondrial cargoes in the extracellular space has been associated with tumor survival and chemoresistance in several human cancers[1]. Furthermore, a significant enrichment of mitochondria-derived extracellular vesicles (mito-EVs) carrying specific mutations in (mt)DNA has been recently reported in the serum of patients with pancreatic ductal adenocarcinoma (PDAC)[2]. Since we had previously observed exocytosis of fragmented mitochondria by a specific PDAC aggressive histotype[3], we sought to correlate the metabolic reprogramming of PDAC cells, involving mitochondria dysfunction, with the intercellular shuttle of mitochondrial cargoes mediated by EVs. First, we evaluated *via* cytofluorimetric analysis the presence of the translocase of outer mitochondrial membrane, (TOMM) 20 marker, in cell-conditioned media derived from 2D-PDAC *in vitro* cell cultures, finding that, compared to non-malignant cells (HPDE), cancer cells (PANC-1, BXPC3) release a higher amount of EVs of mitochondrial origin (TOMM20+). RT- PCR performed on EVs, isolated by ultracentrifugation, confirmed that tumor-derived EVs also contained more mtDNA than those derived from control cells. Interestingly, PANC-1 and BXPC3 exhibited a different potential in secreting mitochondrial cargoes, that might be function of their metabolic phenotypes and KRAS-signatures. Thus, to better explore this possible link, we went to assess the quantity and the quality of EVs release by patient-derived organoids (PDOs).[4] In this way, we would add strong evidence to support mito-EVs as a new parameter for stratifying PDAC sub-populations.

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GLUTAMATERGIC MODULATION OF HYPOTHALAMIC RSPONDIN3 NEURONS UNDER PHYSIOLOGICAL CONDITIONS

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Food intake is a complex behavior regulated by neuropeptides released from the central nervous system. R-spondin (Rspo) peptides are expressed at the mRNA level in hypothalamic regions involved in feeding behavior and play a role in appetite regulation[1]. The glutamatergic system, as a major excitatory neurotransmitter in the hypothalamus, regulates many endocrine and peptidergic systems[2]. However, there is limited information about neurotransmitter control of R-spondin neurons, and no data exist regarding glutamatergic modulation.This study investigated whether Rspo3-expressing hypothalamic neurons are activated after refeeding or glucose administration following 48 h of fasting, and whether this activation can be suppressed by glutamatergic antagonists. c-Fos was used as a neuronal activation marker. Sprague Dawley rats (5 males and 5 females per group) were divided into seven groups receiving combinations of fasting, refeeding, glucose, CNQX (2 mg/kg), or MK-801 (0.05 or 1 mg/kg). Coronal brain sections (40 μm) were processed for Rspo3/c-Fos immunoperoxidase labeling. Double-labeled neurons were quantified in SON, PVN, and ARC. Plasma Rspo3 levels were measured by ELISA. The percentage of activated Rspo3 neurons in SON, PVN, and ARC was respectively: fasting (♂1.53%, 6.54%, 7.95; ♀6.90%, 13.01%, 27.26), refeeding (♂93.67%, 75.72%, 1.11; ♀82.15%, 45.84%, 19.76), and CNQX+refeeding (♂9.24%, 9.34%, 2.74; ♀17.02%, 5.69%, 11.31). Glucose injection increased Rspo3 activation, which was suppressed by antagonist pretreatment ($p<0.001$). ELISA confirmed that refeeding elevated Rspo3 levels, which were reduced by CNQX to control-like values ($p<0.05$). Glucose protocols revealed significant group differences in both sexes ($p<0.05$). These findings show that refeeding or glucose activates Rspo3 neurons through glutamatergic mechanisms involving c-Fos, supported by peripheral ELISA data.

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P30

NEW INSIGHT IN THE *IN VITRO*-GENERATION OF INSULIN-PRODUCING CELLS: VAV1 AS A REGULATOR OF PDX1 AND MIR-375 LEVELS DURING DIFFERENTIATION OF HIPSCS INTO B-CELLS

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Type 1 diabetes (T1D) is a chronic autoimmune disorder marked by the destruction of pancreatic β-cells, leading to impaired insulin production[1]. Transplantation of insulin-producing cells (IPCs) derived from human induced pluripotent stem cells (hiPSCs) represents a promising therapeutic strategy to restore insulin levels in T1D patients. However, differentiation remains heterogeneous, and despite ongoing advances, the efficient production of fully functional β-cells continues to pose a significant challenge[2]. Among the signaling molecules implicated in IPCs generation, Vav1 has recently gained attention due to its expression in adult human β-cells and its essential role in generation of IPCs from biliary tree stem/progenitor cells (hBTSCs) and the transdifferentiation of pancreatic adenocarcinoma (PDAC)derived cells[3]. Based on these findings, this study investigated the role of Vav1 in the differentiation of hiPSCs into IPCs, focusing on its involvement in modulating key molecules associated with βcell development and function. We found that Vav1 exhibited dynamic expression during differentiation, peaking at the endocrine progenitor (EP) stage and declining in terminally differentiated β-like cells. Silencing Vav1 in endocrine progenitors led to reduced levels of insulin, PDX1, and miR-375, whereas Vav1 overexpression, prior to its natural decline, enhanced their expression. Additionally, in differentiating EP cells, Vav1 negatively regulated Akt, a key factor in β-cell survival, proliferation, apoptosis, and insulin secretion, known to be downregulated by miR-375[4]. These findings identify Vav1 as a pivotal regulator in the transition from pancreatic progenitors to mature IPCs, revealing a potential Vav1/PDX1/miR-375/Akt axis involved in insulin biosynthesis. This regulatory network may offer new opportunities to enhance the efficiency of *in vitro* β-cell generation for diabetes therapy in T1D patients.

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MORPHOLOGICAL AND ULTRASTRUCTURAL MARKERS OF RADIATION-INDUCED ADIPOSE TISSUE DAMAGE

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Radiotherapy (RT) is utilized in approximately 50% of all cancer cases as curative treatment. High-energy Ionizing radiation causes direct DNA damage as well as indirect damage to cellular structures through the formation of reactive oxygen species, resulting in impaired metabolism, and ultimately cell death. Anticancer efficacy of RT relies on enhanced radiosensitivity of cancer cells, due to their extremely high proliferation rate. However, RT is associated with both acute and late toxicities. Among the latter, an increased risk of developing metabolic syndrome (MS) has been observed in childhood cancer survivors, possibly caused by long term effects on white adipose tissue (WAT)[1]. WAT morphology is tightly related to the function of the adipose organ, particularly in relation to MS[2]. Hence, we applied morphological and ultrastructural analyses to a murine preclinical model of RT exposure to detect early markers of RT-induced WAT dysfunctions. Corresponding molecular profiling was also obtained by means of bulk RNA sequencing. Ten-week-old C57BL/6J male mice were irradiated to the left hind limb with a total dose of 35 Gy. Animals in the control group did not receive any treatment. After 4 months animals were sacrificed and tissues removed and processed for transmission electron microscopy or RNA-seq analyses. In treated animals as compared to untreated controls both dermal and subcutaneous white adipocytes showed morphological alterations and signs of delipidation, including presence of intra-cytoplasmic clear vacuoles and electrontransparent areas. Characteristic signs of adipocyte death were also observed as peri-adipocyte "crown-like"structures[3]. Consistent with this, transcriptomics data showed marked dysregulation of key pathways involved in lipid metabolism. Our observations indicate that RT induces durable and extensive morpho-functional damage to the adipose organ, with a possible involvement in MS onset.

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P32

BLOCKING CANCER CELL MOVEMENT: THE ROLE OF PAXILLIN AND EZRIN IN CERVICAL TUMOR

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Cervical cancer remains one of the most common malignancies affecting women worldwide, with metastasis being a key factor contributing to poor prognosis and treatment failure. Understanding the molecular mechanisms underlying cancer cell migration and invasion is essential for identifying new therapeutic targets. In this context, cytoskeleton-associated proteins such as paxillin (PXN) and ezrin (EZR) have gained increasing attention due to their role in linking actin microfilaments to the cellular environment and modulating cell motility. This study aimed to investigate the influence of PXN and EZR on the metastatic potential of cervical cancer cells. Using two cell lines -SiHa (derived from a primary cervical lesion) and HT-3 (from lymph node metastasis)- we examined how silencing the expression of these proteins affects key cellular processes. siRNA was employed to reduce PXN and EZR levels, followed by assays to evaluate changes in adhesion, migration, invasion, and response to cisplatin, a commonly used chemotherapeutic agent. The results demonstrated that reduced expression of both PXN and EZR significantly impairs the adhesive and migratory capabilities of cervical cancer cells. Notably, disruption of EZR expression led to a pronounced reorganization of the actin cytoskeleton and the appearance of cells with a mitotic catastrophe-like phenotype. Furthermore, knockdown of either protein enhanced cellular sensitivity to cisplatin, suggesting an additional benefit in the context of chemotherapy. In conclusion, PXN and EZR play a crucial role in regulating cervical cancer cells' invasive and metastatic potential. Their inhibition limits cell motility and increases susceptibility to cytotoxic treatment. These findings support the potential of PXN and EZR as targets for novel anti-metastatic strategies in cervical cancer therapy.

P33

IMPACT OF CYCLIN C EXPRESSION ON THE AGGRESSIVENESS OF A549 NON-SMALL CELL LUNG CANCER CELLS

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Non-small cell lung cancer (NSCLC), accounting for the majority of lung cancer cases, is a malignancy with complex molecular background and high treatment resistance, complicating effective therapy. Identifying new molecular therapeutic targets remains a significant research challenge. Cyclin C (CCNC) is a cell cycle regulatory protein that, in complex with CDK3, controls the transition from G_0 to G_1 phase, and together with CDK8/CDK19 participates in gene transcription regulation. Depending on cancer type, Cyclin C may act as both tumor suppressor and oncogene. Its role in NSCLC remains poorly understood. This study aimed to assess the impact of Cyclin C expression on the aggressiveness of A549 cells. shRNA-mediated transduction was used to downregulate CCNC expression. Verification of knockdown efficacy by immunofluorescence and western blot revealed significant phenotypic changes. Cyclin C silencing in A549 cells causes proliferation arrest by prolonging the cell cycle in the G_2/M phase. Reduced Cyclin C expression leads to decreased cell viability, suggesting an important role of Cyclin C in maintaining proliferation and survival of cancer cells.

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ADJUDIN-INDUCED MODEL IN RATS REVEALS HIPPO SIGNALING PATHWAY ACTIVITY IN THE TESTIS

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In the seminiferous tubule of mammalian testis, there are various stages of development of germ cells and Sertoli cells. Mitosis, meiosis, proliferation, and differentiation occur in this epithelium. It is known that cytoskeletons and actin-containing proteins are effective during sperm secretion[1]. The Hippo signaling pathway has been demonstrated to be crucial in many stages where the fate of cells is determined, such as cell division, death or proliferation[2]. Studies have also shown that the Hippo pathway is highly influenced by actin proteins and cytoskeletons. However, its effect on the testis has not been fully elucidated. For this reason, we created an experimental rat model in which the actin cytoskeleton and actin-associated proteins are affected by adjudin to perform the sperm secretion model. The control(untreated), sham control (methylcellulose-treated only), and experimental (methylcellulose and adjudin-treated) groups were established. At 0, 6, 12,24 and 96 h, the testis samples were collected and evaluated in terms of the Hippo signaling pathway *via* immunohistochemistry, Western blotting, and RT-qPCR methods. At the same time, actin cytoskeleton (labeled with FITC phalloidin) and actin-associated proteins (Arp3, EPS8) were also evaluated. The control and sham control groups showed similar characteristics in each hour period. However, differences began to be observed in the experimental group at the 6th hour. Especially at the 96th hour, it was clear that actin distribution and Hippo pathway components yes-associated protein (YAP) and phosphoYAP had declined in the experimental group ($p<0.05$). In conclusion, Hippo signaling appears to play a key role in the timedependent changes of the actin cytoskeleton in the seminiferous tubules under the influence of adjudin.

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EARLY LIFE STRESS CAN CAUSE CHANGES IN THE HIPPO SIGNALING PATHWAY IN RAT OVARIES

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Hippo signaling pathway has been reported that it participates in many events such as cell division, proliferation, differentiation, and programmed cell death. YAP (Yes-Associated Protein) is one of the key components of this pathway[1]. It has been reported that early life stress causes oxidative stress in the mouse ovary which can affect follicle development[2]. However, the molecular mechanisms underlying these problems caused by early life stress have not yet been elucidated. 180-min maternal deprivation and isolation model was applied to the experimental group every day between the 1st and 14th postnatal day[3]. In total of 6 newborn Wistar albino female rats were used as control (n=3) and experimental (n=3) groups. Tail blood was collected to determine the serum corticosterone level before and after stress conditions on 14th postnatal day whether early life stress had developed. The groups were sacrificed on the 55th postnatal day. The YAP expression level was evaluated for immunohistochemical analyses. When compared to the control group, the serum corticosterone levels were high ($p<0.05$) and YAP expressions increased in granulosa cells and corpus luteum of the experimental group ($p<0.05$). This study suggests that stress created in the early period may cause various errors as the ovaries enter the adulthood period. It is also thought that the Hippo pathway plays an active role in this process.

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P36

BIOLOGICAL ROLE OF DNA G-QUADRUPLEX DURING LIVER REGENERATION AFTER PARTIAL HEPATECTOMY

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Guanine-rich DNA sequences can form four-stranded secondary structures known as G-quadruplexes (G4s). G4s exist in chromatin DNA and have linked G4 formation with the key processes from transcription and translation to genome instability[1]. However, the biological roles of G4s *in vivo* remain unclear. In this study, we investigated the localization in G4s during liver regeneration by immunohistochemistry in wild-type (WT) and a high-mobility group box 2 (HMGB2) knockout (KO) mice, where HMGB2, a chromatin associated protein, has been implicated as a G4 binding protein[2,3]. Liver tissues were sampled at 0, 24, 36, 48, 72, 120, 168 h after 70% partial hepatectomy (PHx). In WT mice, G4 positive cells were not detected at 0 hr after PHx, but were increased at 24 h and peaked at 36 h. Subsequently, G4 positive cells were gradually decreased. G4 positive cells were mainly localized in the nuclei of hepatocytes in the peripheral region between periportal (zone 1) and midzonal (zone 2) regions, but not in the pericentral (zone 3) region. To investigate the G4 formation during cell cycle progression, we evaluated the expression of cell cycle markers, including Cyclin D1, proliferating cell nuclear antigen (PCNA), 5ethynyl-2'-deoxyuridine (EdU), Cyclin A2 and phosphorylated H3S10 (pH3S10). G4 positive cells were colocalized with EdU and Cyclin A2 at 36 and 48 hr after PHx, but no colocalization with Cyclin D1, PCNA, or pH3S10. In HMGB2-KO mice, the number of G4 positive cells was significantly reduced compared with WT mice, although the temporal peak at 36 hr after PHx was preserved. In conclusion, DNA G4s may play a direct and crucial role in the liver regeneration after PHx for the cell cycle regulation, particularly with DNA synthesis.

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P37

THE ROLE OF ACTIN-BINDING PROTEINS IN ENDOMETRIOSIS

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Endometriosis is a chronic inflammatory disease with serious effects on reproductive and general health, affecting up to 10% (190 million) of women of reproductive age worldwide. It is marked by endometrial-like cells growing outside the uterus, most often on the ovaries, fallopian tubes, pelvic peritoneum, and uterosacral ligaments. Less frequently, lesions appear in the gastrointestinal and urinary tracts, soft tissues, and beyond the pelvic region[1]. Several theories explain its origin, including retrograde menstruation, metaplasia, stem cell involvement, vascular spread, and embryonic cell remnants[2]. A crucial role in lesion formation is attributed to epithelial-mesenchymal transition (EMT), driven by factors like TGF-β and EGF through Wnt and Notch pathways. EMT leads to E-cadherin loss and increased markers such as N-cadherin and vimentin. This process is supported by evidence of decreased adhesion molecules and elevated mesenchymal markers in endometriotic tissues. Enhanced matrix metalloproteinase activity further promotes migration and adhesion. Multiple signaling pathways, including Wnt/β-catenin, NF-κB, MAPK, PI3K/Akt/mTOR, and Rho/ROCK, contribute to lesion development *via* proliferation, invasion, angiogenesis, oxidative stress, and inflammation[3,4]. These processes rely on dynamic cytoskeletal remodeling, controlled by actin-binding proteins (ABPs), which govern cell migration, interaction, and structure[5]. Changes in ABP expression have been observed in endometriosis. Despite progress, the mechanisms remain unclear, limiting treatment to symptom management. This review explores ABPs' roles in endometriosis and potential diagnostic and therapeutic directions.

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THE ROLE OF SPEXIN IN COLORECTAL CANCER

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Spexin (SPX), a recently discovered member of the galaninergic system, acts through the receptors: GALR2 and GALR3. SPX is involved in the regulation of colonic functions; however, its role in colorectal cancer (CRC) has not been comprehensively studied. Given that our previous research implicated galanin receptors, but not galanin, in the pathogenesis of CRC, we aimed to investigate the potential contribution of SPX, an alternative GALR agonist, to this disease mechanism. SPX immunolocalization was evaluated in sections of noncancerous large intestine and CRC tumors derived from 5 patients using immunohistochemistry method (IHC-P). To quantify SPX levels, ELISA was performed on tissue homogenates obtained from 16 CRC patients. Tissue samples were carefully dissected and collected from five distinct regions: CRC, peritumoral mucosa with submucosa (pM+SM) and muscularis externa (pME), and matched samples from the distant, noncancerous part of the large intestine (dM+SM, dME). SPX concentrations were also measured in the sera collected from 53 CRC patients and 22 healthy controls. IHC-P revealed immunoexpression of SPX within the cytoplasm of CRC cells, enterocytes, goblet cells, stromal/immune cells, as well as in the submucosal and myenteric plexuses. Serum SPX levels did not differ significantly between CRC patients and healthy controls. Similarly, SPX concentrations in tissue homogenates collected from various regions of the large intestine and CRC tumors showed no significant variation. However, CRC patients without lymph node metastases (N0) exhibited significantly higher SPX levels in the pM+SM compared to patients with regional lymph node involvement (N1+N2). Interestingly, this relationship was not observed in dM+SM samples. High SPX levels may contribute to the protection of mucosal and submucosal stromal cells, potentially indicating a regulatory role of SPX in colonic function under pathological conditions. The mechanisms underlying SPX action in CRC and peritumoral tissues warrant further investigation.

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NEW PERSPECTIVES FOR THE EXPLOITATION OF FEMALE REPRODUCTIVE POTENTIAL IN MAMMALS: GENERATION OF GRANULOSA-LIKE CELLS FROM HUMAN ADIPOSE MESENCHYMAL STEM CELLS

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The aim of this innovative study is to generate human granulosa like cells (hGLCs) from human adipose mesenchymal stem cells derived induced pluripotent stem cells (hASC-iPSCs) or directly from hASCs whose embryonic origin is identical to granulosa cells (GCs). Previous work from our group has demonstrated that hASCiPSCs, and less efficiently hASCs, can generate human primordial germ cell-like cells (hPGCLCs)[1]. In the present work, by using a modification of a published protocol[2], we generated hGLCs from hASC-iPSCs with a significant increase of the markers of mature GCs (FSHR, CYP19A1, AMH), whereas from hASCs, were upregulated transcripts of pre- or early GCs (FOXL2, FSHR, AMH). To verify their functional state, hiPSC-GLCs and hASC-GLCs were aggregated with 12.5 dpc mouse primordial germ cells (mPGCs) to have organoids in which, after 4 weeks of culture, significant number of germ cells, likely growing oocytes, were found, but with low efficiency. For this reason, two recent protocols[3,4] developed in mouse were employed, with some modifications to adapt to our cells. According to the first study[3], by using the appropriate cocktail of growth factors, hiPSCs should differentiate into GLCs by passing through a multistep protocol that includes epiblast, intermediate mesoderm, and coelomic epithelium, recapitulating the embryonic developmental stages. We successfully performed the first step and obtained epiblast-like cells confirmed by Brachyury's expression. Based on the second study[4], hASCiPSCs were cultured for 10 days with vitamin C and AM580 to induce directly GLCs differentiation. In this case, we observed evident morphological changes that suggest GC phenotype gaining. Experiments are in progress to complete both protocols, also starting from hASCs, and then proceed with mPGC/hGLC and hPGCLC/hGLC aggregate formation to generate primordial follicles.

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P40

VOLUME ELECTRON MICROSCOPY REVEALS SPECIFIC FEATURES OF MITOTIC CELLS IN THE PINEAL ORGAN OF THE DOMESTIC TURKEY

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The avian pineal organ originates as an evagination of the third ventricle. This evagination elongates to form the pineal canal, which often extends to the apical region of the pineal organ and remains connected to the third ventricle during embryonic and, in some cases, early postembryonic development. The pineal canal serves as the primary source of cells for the formation of parenchymal structures such as follicles and rosettes. In this study, we examined the 3D ultrastructure of the pineal canal in 18-dayold domestic turkey embryos to characterize mitotic cells. Imaging was performed using a 3View 2XP system (Gatan) integrated with an EF-SEM Gemini 450 (Carl Zeiss). Two cell types were identified: rudimentary receptor pinealocytes (RRP) and ependymal-like supporting cells (ELSC), both exhibiting polarized and highly organized cytoplasmic architecture. RRP formed single, elongated basal processes, which created prominent flattened segments that lay within invaginations of ELSC. Fine extensions of RRP occasionally reached the basement membrane. ELSC had broad basal portions resting on the basement membrane. Mitotic cells were located between RRP and ELSC and consistently maintained contact with the canal lumen. These cells exhibited numerous fine, long projections, resembling horse-tail hairs extending toward the basement membrane. In prophase, Golgi dictyosomes appeared small and dispersed, mitochondria were present on both apical and basal sides of the nucleus, and endoplasmic reticulum (ER) was localized mainly peripherally. During metaphase, ER was restricted to the cell periphery and mitochondria were sparse. In telophase and cytokinesis, the hair-like projections were asymmetrically distributed between daughter cells. The daughter cells were connected by a fine cytoplasmic bridge near the canal lumen, which disappeared as differentiation progressed. Our findings show that mitotic cells in the pineal canal share some morphological characteristics with those in the developing neural tube. Further research is required to clarify the differentiation of RRP and ELSC.

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INVOLVEMENT OF THE DOLPHIN'S MELON IN IMMUNE FUNCTION

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The striped dolphin (*Stenella coeruleoalba*) is a small pelagic dolphin. The melon, present in most odontocetes, is a rounded structure located in the center of the forehead, between the blowhole and the tip of the head. The dolphin's melon, as well as the blubber, consists of a lipid component similar to that of adipose tissue, with large, rounded cells closely related to each other and a muscular component. The melon primarily plays a role in echolocation, but this organ can also be involved in immune function. Numerous studies demonstrate the accumulation of contaminants such as heavy metals in the fatty tissues of cetaceans (melon and blubber), altering the immune system and stimulating an inflammatory response. This study aims to describe the involvement of *S. coeruleoalba* melon in immune function. Histological samples of the melon of *S. coeruleoalba*, preserved in the histology archive of our laboratory, were used in this study. To describe the morphology of this organ, the sections of the tissue, were stained with Giemsa, Masson, and Mallory staining techniques. Immunoperoxidase and immunofluorescence techniques using specific antibodies such as MHCII, Langerin/CD 207, TLR2, and CD14 were used to characterize, for the first time, melon immune cells. The results showed scattered immune cells among melon adipocytes immunoreactive to the tested antibodies; macrophages are the principal immune cells infiltrating adipose tissue, and their function is to secrete proinflammatory mediators. Furthermore, the adipocytes themselves appear to be labelled with the antibodies used. This study demonstrates the involvement of the dolphin melon in immune function and helps us better understand the immune system of cetaceans, still little known. Furthermore, it could also demonstrate that non-invasive monitoring of blubbers from highly protected species can provide valuable information regarding their health status and exposure to contaminants.

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PANCREATIC CANCER STEM CELLS AND FUNCTIONAL CHARACTERIZATION OF MIR-216A-5P

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Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related death. Its poor prognosis is closely related to late-stage diagnosis, which results from both nonspecific symptoms and the absence of biomarkers for early diagnosis[1]. MicroRNAs (miRNAs) exert a regulatory role in numerous biological processes, and their aberrant expression has been found in a broad spectrum of diseases, including cancer[2]. Cancer stem cells (CSCs) represent a driving force for PDAC initiation, progression, and metastatic spread. Our previous research highlighted the interesting behavior of miR-216a-5p and miR-125a-5p related to PDAC progression and the CSC phenotype. Our study aimed to evaluate the effect of miR-216a-5p on the acquisition or suppression of pancreatic CSC traits. BxPC-3, AsPC-1 cell lines, and their CSC-like models were transfected with miR-216a-5p mimics and inhibitors. We evaluated their impact on the expression of CSC surface markers (CD44/CD24/CxCR4), ALDH1 activity, pluripotency- and EMT-related gene expression, and clonogenic potential. Our results show that miR-216a-5p enhances the expression of

CD44/CD24/CxCR4 while negatively affecting the activity of ALDH1 and the expression of EMT genes. Comprehensively, our results provide further knowledge on the role of miRNAs in pancreatic CSCs. Moreover, they corroborate our previous findings about miR-216a-5p's potential dual role and miR-125a-5p's promotive function in PDAC.

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NEW INSIGHTS IN THE ACTIVATION OF FERROPTOSIS PROCESS DURING LIVER FIBROSIS

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Ferroptosis is an iron-dependent regulated cell death characterized by iron accumulation, lipid peroxidation, and production of ROS[1]. The liver represents the primary site of iron-overload injury for its critical role in iron metabolism[2]. Patients with chronic liver disease may exhibit hepatic and splenic iron overloading[3]. The involvement of ferroptosis in liver fibrosis was recently investigated at the level of Kupffer cells[4], hepatocytes and hepatic stellate cells (HSCs)[5], however its role in biliary epithelium is yet unknown. We investigated the possible role of ferroptosis in the bile duct ligated (BDL) rat and we highlighted the effects of ferroptosis inducers and inhibitors in primary murine cholangiocytes. *In vivo*, we evaluated the iron deposits through Perls Prussian blue and the presence of iron regulator proteins, such as Ferritin, glutathione peroxidase-4 (GPX4) and acyl-CoA synthetase long-chain family member 4 (ACLS4) through immunohistochemstry. *In vitro*, for each inducer and inhibitor we evaluated the toxicity, the effects in cellular morphology and the cell cycle perturbation. We found a decrease in the expression of both Ferritin forms (heavy and light chain). Moreover, ACLS4, a protein regulator of lipid peroxidation, increased in BDL model, whereas GPX4, that plays a crucial role against membrane lipid peroxidation, was reduced in the cholestatic model compared the control. We confirmed that erastin is a potent and specific inducer of ferroptosis also in our primary cholangiocytes. Whereas, we provided evidence that sorafenib and ellagic acid fails to trigger ferroptosis. In the other side, we found that ferrostatin-1 (Fer-1) is unable to inhibit ferroptosis, while β-Mercaptoethanol (βME) could protect cholangiocytes in ferroptosis-induced cell death. All these findings could be important to understand the role of ferroptotic modulators in treating liver fibrosis.

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CROSSTALK AMONG NEURONS, MUSCLE AND BONE: HUMAN NEUROMUSCULAR JUNCTION ON A CHIP

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Aging is associated with gradual degeneration, in mass and function, of the neuro-musculo-skeletal system. Neuromuscular junctions (NMJs) are specialized synapses, which are crucial for the communication between motor neurons and skeletal muscle and become vulnerable in muscle atrophy, where the impairment of NMJs results in muscle weakness. During aging this muscle wasting condition occurs and is called sarcopenia. Indeed, osteoporosis and sarcopenia - osteosarcopenia (OS) - are twinaging diseases. With the purpose to study the perturbation in NMJs occurring in OS, beside bone side defects, an ideal model would contain MNs, myotubes and osteoblasts to better recapitulate the human disease pathology. Therefore, we developed a personalized homemade microfluidic device to study compartmentalized human NMJs in an *in vitro* muscle model connected to the bone side. A selective pretreatment in bone compartment with dexamethasone (Dexa) was used to induce osteoporosis: in this way, we investigated if this pathological condition could be the driver to have an OS linked to NMJ perturbations. A soft master in SU-8 was fabricated through a photolithographic process tailored for high-aspect-ratio microstructures, enabling the creation of mold structures with micrometric resolution from which a replica in polydimethylsiloxane was obtained. This device is composed by three different compartments: a left-neuronal, a central-muscular and a right-bone one. The first two are connected by around 100 microgrooves. The 1μM diameter allows only neurites to pass through and take contact with the myotubes forming NMJs. The last compartment containing osteoblasts should be in an open or closed condition, to treat osteoblasts with Dexa separately from myotubes, but to allow afterwards the sharing of the medium. This innovative experimental model allowed us to perform immunofluorescence analysis on fixed samples or by live imaging. Collectively, this method will be useful to understand if the modifications induced in osteoblasts during bone disorders have a cascade in the muscle and neuron parts, such as in osteoporosis arising in females after menopause that leads to muscle weakness.

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TARGETING PRELAMIN A PROCESSING TO SENSITIZE GLIOBLASTOMA CELLS TO OXIDATIVE STRESS

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Glioblastoma is the most common and aggressive tumor of the Central Nervous System, with limited therapeutic options and poor prognosis. Current standard treatments (surgical resection, radiotherapy, and chemotherapy) offer only marginal improvements in patient survival, underscoring the urgency of developing novel therapeutic strategies. In this study, it was explored an innovative approach that interferes with the processing of lamin A precursor, prelamin A, in glioblastoma cells. Specifically, farnesyltransferase activity was inhibited using SCH66336 (Lonafarnib), leading to intracellular accumulation of prelamin A. This accumulation mimics a «laminopathic» phenotype -similar to that observed in systemic progeroid syndromes caused by defects in lamin A processing[1]- selectively induced in glioblastoma cells, which, unlike healthy brain tissue, express high levels of lamin A[2]. The resulting nuclear dysfunction sensitizes glioblastoma cells to oxidative stress triggered by Menadione, while having minimal effects on normal human astrocytes. The combined treatment with SCH66336 and Menadione significantly reduced proliferation, altered the expression of key stemness markers, and compromised the viability of glioblastoma stem cells (GSCs) derived from patients - cells known to drive tumor progression and resistance to therapy. These results suggest that targeting prelamin A processing could represent a promising adjunctive strategy to attenuate glioblastoma aggressiveness, particularly by impairing therapy-resistant GSC populations[3]. This dual-hit approach may pave the way for more effective combination therapies in the clinical management of glioblastoma.

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P46

ANALYSIS OF AQUAPORIN-2 MUTANT MICE

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Aquaporin-2 (AQP2), a membrane water channel protein in kidney collecting duct cells, is necessary for urine concentration regulated by vasopressin (VP). AQP2 traffics to the apical membrane from intracellular vesicles, which is regulated by the phosphorylation and dephosphorylation of Ser256, Ser261, Ser264, and Ser269 of AQP2 caused by VP. Among them, Ser256 is critical for VP-induced urine concentration because S256L mice exhibit severe polyuria[1]. We focused on Ser269 and demonstrated that it is rapidly phosphorylated upon VP injection in rats[2]. To investigate the significance of Ser269 in AQP2 trafficking and urine concentration, we generated mutant mice using genome editing. In the process, we unexpectedly obtained AQP2.p.R252_V257del; S269D mutant mice. Despite lacking S256, these mice did not become polyuric. We analyzed the detailed phenotypes of this mutant. The osmolality of urine collected at any time in the morning, the 24-hour urine volume, and the 24-h water intake were not significantly different from those in wild-type mice. Then, we analyzed the immunolocalization of mutated AQP2 in the control state and after the administration of VP receptor agonist dDAVP or its antagonist OPC-31260. In wild-type mice, AQP2 localizes to the intracellular vesicles both in control state and after OPC-31260 administration, and it highly accumulates on the apical membrane after dDAVP administration. In mutant mice, mutated AQP2 localizes to some extent at the apical membrane, even in control. Neither dDAVPnor OPC-31260-administration changed the extent of apical localization of mutated AQP2. We then analyzed urine osmolality after oral water gavage and found that the mutant mice were unable to dilute their urine. These results suggest that the S269D mutation enables the accumulation of AQP2 on the apical membrane and rescues mice from polyuria, likely caused by the S256 deletion. On the other hand, uncontrolled accumulation of the mutated AQP2 on the apical membrane hampers urine dilution, even in cases of overhydration.

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ASSESSMENT OF THE ANTI-INFLAMMATORY AND ANTIFIBROTIC EFFECTS OF HUMAN AMNIOTIC EPITHELIAL CELLS AFTER THEIR INTRATRACHEAL ADMINISTRATION IN A MOUSE MODEL OF BLEOMYCIN-INDUCED PULMONARY FIBROSIS

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Lung fibrosis (LF) is a natural healing process of lung tissue under physiological conditions. However, in pathological states, it can progress uncontrollably, leading to irreversible scarring and structural remodeling. Current pharmacological treatment of progressive LF primarily aims to slow down the progression of the disease, without reversing existing damage. Stem cells and their derivatives, such as conditioning media or exosomes, have become promising supplements or alternatives to traditional drug therapies. Human amniotic epithelial cells (hAECs) are known for their immunomodulatory and anti-fibrotic properties, making them a promising population of stem cells for clinical applications[1,2]. In this study, the therapeutic potential of hAECs administered *via* intratracheal injection was evaluated in a mouse model of bleomycin-induced LF. hAECs showed high expression of epithelial

(CK 95%) and pluripotency markers (SSEA4 88%), as well as HLAG (79%), with a low proportion of the mesenchymal marker CD105 (7%). In 10-week-old male C57BL/6 mice, a dose of 10 mg/kg intratracheal injection of bleomycin caused persistent inflammation up to day 7 and significant fibrosis by day 21, achieving 6/8 points on the Ashcroft scale[3]. A single administration of 1 million hAECs into the trachea caused a significant reduction in inflammatory areas after 7 days. The degree of reduction of fibrous areas compared to daily treatment with Pirfenidone has also been assessed. The results support further research on hAECs as a potential cell therapy in fibrotic lung diseases.

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NEW STRATEGIES TO IMPROVE THE RESPONSE OF AML CELLS TO TARGETED THERAPIES BY OVERCOMING THE PROTECTIVE EFFECT OF THE BONE MARROW MESENCHYMAL STEM CELLS

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Drug resistance and relapses are significant issues for the success of targeted therapies for acute myeloid leukemia (AML), rendering the rate of cure unsatisfying. This is primarily due to clonal selection and the protective effect of the bone marrow microenvironment (BM)[1]. Accordingly, we demonstrated that BM mesenchymal stem cells (MSCs) protect AML cells from the Azacytidine + Venetoclax (AV) treatment (an established standard of care for AML patients[2]) as well as from a strategy that we previously developed, inducing proteotoxic and oxidative stress using Retinoic acid, Bortezomib and Arsenic trioxide (RBA)[3]. Thus, we added the Bcl-2 inhibitor Venetoclax to the combination RBA (RV) to treat AML cells in mono or co-culture with MSCs in 2D and 3D models. We assessed that the combination RV overcomes the MSCs protection and significantly prolongs the life span of a murine model of human AML. To study the mechanisms underlying the crosstalk between AML and MSCs, we analyzed the morphology of the MSCs after treatment of co-cultures with AV and RV by immunofluorescence analysis and live-cell imaging. Furthermore, we performed proteomic analysis, revealing the involvement of molecular pathways related to extracellular matrix reorganization, highlighting a possible role of the actin cytoskeleton in this protective crosstalk.

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DEVELOPMENT OF AN INJECTABLE AND SELFASSEMBLING SCAFFOLD FOR THE DIRECT DELIVERY OF CARDIAC EXTRACELLULAR MATRIX AND CARDIAC PROGENITOR CELLS

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Injectable biomaterials offer an attractive alternative for cardiac tissue engineering due to the ability to polymerize at 37°C and the possibility of being administrated directly *in situ, via* minimally invasive procedures. As a physiological component of the blood clot, which implies biocompatibility, biodegradability and ability to incorporate cells and bioactive molecules, fibrin has been exploited as an injectable biomaterial. The aim of the present study was to develop a bioconstruct for myocardial regeneration based on a biological, injectable and self-assembling scaffold loaded with cardiac decellularized extracellular matrix (d-ECM) and cardiac progenitor cells (CPCs). Human cardiac samples were obtained from waste material of cardiac transplants. To isolate CPCs, samples were minced and enzymatically digested, while cardiac dECM was obtained by decellularization in a solution of SDS and Triton. Successful decellularization was demonstrated by the absence of nuclei in hematoxylin and eosin staining and a residual dsDNA content of 3.5±0.5 ng/mg of dry tissue. To prepare bioconstructs thrombin was added to a mixture of fibrinogen, CPCs ($3x10^6$/100 µL) and lyophilized and solubilized d-ECM, with a fibrin to matrix ratio of 1:1. After 3 days of culture, bioconstructs were analyzed to evaluate 3D architecture and cell viability. Histological analyses including hematoxylin and eosin, Mallory's, PAS and Sirius Red stainings, and immunofluorescence for fibronectin, laminin, tenascin and collagen revealed the uniform d-ECM incorporation and homogenous cell distribution within bioconstructs. Trypan Blue exclusion and CellTiter-Glo luminescence assays proved that over 90% of CPCs were viable while gene expression analyses for cardiac myocyte markers (ACTC1, CX43, CX40, NKX2.5, MYH7, MEF2C, TBX3, TBX5, ITGB1) revealed that they also remained functionally active. Overall, our results support the suitability of fibrin as biomaterial for direct delivery of cells and physiological cardiac signals in a self-assembling bioconstruct to stimulate cardiac repair and regeneration.

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OPTIMIZATION OF A RAPID AND EFFICIENT DECELLULARIZATION PROTOCOL FOR COMPOSITE VASCULARIZED FACIAL GRAFT FABRICATION

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Reconstruction of complex facial defects remains a significant challenge in reconstructive surgery. Current solutions, including autografts, allografts, and synthetic implants, are complicated by incomplete aesthetic, morphological, and functional recovery, lifelong immunosuppression, and limited graft survival. Decellularized cadaveric facial grafts, obtained by removing cellular components while preserving the composition and architecture of the extracellular matrix (ECM) and the vascular network, offer a promising alternative. The aim of the present study was to develop a protocol for the complete decellularization of composite facial grafts. To this end, full-thickness rat faces, including both ears, were obtained with vascular pedicles formed by the external carotid artery (ECA) and the external jugular vein (EJV), from animals sacrificed following other experimental procedures (n=4). The specimens were decellularized using a combination of SDS, Triton and antibiotics, under constant agitation. After seven days of decellularization, the blanching of the facial grafts became evident while the overall macroscopic architecture remained unchanged. Samples were then collected from each specimen and processed for histological or molecular analysis. The absence of nuclei in histological slides stained with hematoxylin and eosin and a residual dsDNA content of 12.13±2.94 ng/mg of dry tissue demonstrated the efficacy of decellularization. Furthermore, staining with Sirius Red, Masson and Mallory, immunostaining for fibronectin, laminin and tenascin, and specific quantitative assays for GAGs, collagen or elastin, demonstrated the preservation of the ECM structure and composition after decellularization. Finally, the uniform distribution of the Patent Blue V dye upon antegrade injection through the ECA and retrograde injection through the EJV demonstrated the patency of the vascular network. Overall, our results support the efficacy of the proposed decellularization protocol in producing a well-preserved, completely acellular, and vascularized facial graft in an experimental model of whole face composite graft.

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DECELLULARIZATION OF HUMAN LUNG SCAFFOLD FOR TUMOR MICROENVIRONMENT MODELLING STUDY

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Decellularized extracellular matrix (d-ECM) is a promising biological scaffold for *in vitro* cancer research, due to its ability to replicate the native microenvironment and maintain key biomechanical properties[1]. An effective decellularization process must remove all cellular material while preserving the ECM's structure and composition[2]. Human tissue-derived d-ECM offers relevant biochemical and structural cues, making it valuable for disease modelling[3]. In this study, we developed an *in vitro* model to investigate the pulmonary metastatic microenvironment induced by breast cancer. Human lung biopsies from pathological donors were snap-frozen, sectioned at 100 µm, and decellularized using 1% SDS and 1% Triton X-100 for 24 h. To prevent contamination, decellularized scaffolds were treated with an antibiotic-antimycotic solution and sterilized with UV light. H&E staining confirmed successful cell removal while maintaining ECM integrity. The lung-derived d-ECM was reseeded with normal human lung fibroblasts, recreating a native-like microenvironment. Human lung fibroblasts were then stimulated with breast cancer conditioned medium to study how stromal cells remodel the ECM and support tumor invasion. This model offers a reliable platform to explore cell–matrix interactions and the stromal role in cancer progression, providing new insights for preclinical oncology research.

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MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATION OF THE ANTI-INFLAMMATORY AND ANTIOXIDANT PROPERTIES OF HEMP EXTRACTS ON THE LIVER OF RATS INVOLVED IN INFLAMMATION CAUSED BY LPS

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The liver of mice has a lobular arrangement based on the distributions of portal regions and central venules, like the general histological structure of other mammalian species[1]. Bacterial endotoxin lipopolysaccharide (LPS) influences inflammatory mediator levels and contributes to developing a systemic inflammatory response, which can lead to liver damage. *Cannabis sativa* L. is an aromatic annual herb belonging to the Cannabaceae family, and it is widely cultivated around the world. Several studies show different pharmacological properties, such as antioxidant, anti-inflammatory, antimicrobial, neuroprotective, anxiolytic, and anticonvulsant activity of hemp[2,3]. This study aims to characterise mouse liver immune cells involved in inflammation caused by lipopolysaccha-

ride (LPS) and how inflammation responds to treatment with aqueous extracts from two by-products of the hemp-seed oil process, namely hemp seed cake flour and hemp seed protein concentrate, as well as their enzymatic hydrolysates. In this study, immune hepatic cells have been characterized with the following antibodies: Toll-like receptor 4 (TLR4), TNFα, and IL-6[4]. Immunoistochemical results showed a significant difference between control, LPS-infected, and hemptreated samples. Macrophages were positive for these antibodies in the samples obtained from the inflamed mouse liver, especially in the region between the portal space and the central vein. In control samples and samples treated with aqueous hemp extracts, instead, there is an extremely small number of antibody-positive cells, which means that the extracts contributed to a reduction in inflammation. In conclusion, this preliminary study shows the antiinflammatory and antioxidant effects of industrial hemp.

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CIRCADIAN DEPENDENT MOTILITY: THE ROLE FOR THE PERINUCLEAR ACTIN CAP

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The circadian rhythm is responsible for the regulation of the daynight cycle by release of several factors, including the master regulator glucocorticoids (GCs). Previous studies reveal that the GCs dampen the transcriptional response to EGFR activation, by leading to the hypothesis of a nocturnal activation of the pathway[1]. In line, a recent study proved that the metastatic dissemination occurs preferentially during the rest phase and is controlled also by GCs[2]. In this work, we aim to analyze the circadian modulation of the *quasi*-normal epithelial cell line (MCF10A), upon dexamethasone (DEX) synchronization. We confirmed the oscillation of clock-related genes such as CLOCK and PER1 by mRNA analysis and BMAL1 by protein analysis. Interestingly, we observed a rhythmic production of EGFR, with an asynchronous upregulation of its negative feedback regulator ERRFI1. Notably, this interaction leads to decreased phosphorylation of EGFR, along with downstream signaling. The actin cap architecture has been described as a driver of cell dissemination[3]. Thus, we aim to mechanistically link nighttime spreading to circadian modulation of the actin cap *via* SUN1 regulation. Notably, we detected rhythmic oscillation of the *SUN1* gene, which encodes a component of the LINC complex essential for anchoring the perinuclear actin cap[4]. So far, our data suggest that a circadian regulation of EGFR may influence *SUN1* expression. Indeed, by employing MCF10A HER2+ cells, which lack EGFR oscillation, we observed a marked disruption of both clock-related genes and SUN1 expression compared to normal cells.

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FATTY INFILTRATION AND POTENTIAL ADIPOGENIC SHIFT OF SKELETAL MUSCLE CELLS: MULTIPARAMETRIC HISTOLOGICAL ANALYSIS OF MYOSTEATOSIS

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Myosteatosis, defined as an ectopic fat accumulation within skeletal muscle, has gained increasing clinical relevance as it fits within the context of metabolic disorders and aging. Elderly, sedentary, obese, type 2 diabetic and dyslipidemic patients are more prone, and it worsens the prognosis in both malignant and non-malignant diseases[1]. Currently, no effective therapeutic interventions exist; prevention through lifestyle modifications remains the only option. The prevailing theory suggests that ectopic fat originates from interstitial fibro-adipogenic precursors (FAPs), which tend to follow an adipogenic fate under chronic proinflammatory stimuli[2]. Since histological analysis is the gold standard for assessing myosteatosis - allowing the evaluation of both morphological changes in muscle cells and lipid accumulation, - we performed a multiparametric histological investigation to highlight pathological alterations in skeletal muscle and to explore the potential adipogenic shift (transdifferentiation) of muscle fibers due to lipid accumulation. Biopsy samples from the *Vastus lateralis*muscle were collected from patients undergoing arthroplasty surgery (M:F = 1:2; mean age ± SD = 71.9±7.1) and processed according to FFPE ("Formalin-fixed Paraffin Embedding"), cryo-embedding and resin embedding protocols. FFPE sections were stained with hematoxylin-eosin, Masson's trichrome and indirect immunohistochemistry for perilipin-1 and a-sarcomeric actin. Cryosections were stained with lipophilic dyes Sudan Black B and Oil Red-O. Our analysis confirms pathological changes of muscle cells[3] like atrophy, necrosis, fragmentation, vacuolization and significant intramyocellular lipids and intramuscolar fat deposition. Most importantly, our study suggests that massive fat accumulation in skeletal muscle is not solely due to ectopic infiltration but also related to a subpopolation of muscle cells that may lose its contractile phenotype, acquiring adipocyte-like morphological features.

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ENDOTHELIAL THYMIDINE PHOSPHORYLASE DEFICIENCY TRIGGERS MORPHO-FUNCTIONAL DEGENERATION OF ENTERIC NEURONS AND REDUCES INTESTINAL STEM CELL PROLIFERATION

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The enteric nervous system (ENS) operates within a complex neurovascular niche where endothelial thymidine phosphorylase (TP) regulates vascular integrity. In mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), TP is genetically absent, resulting in enteric neuronal loss and microvascular abnormalities[1]. Of note, MNGIE leads to a severe dysmotility disorder called chronic intestinal pseudo-obstruction (CIPO)[2]. Remarkably, in a cohort of idiopathic CIPO patients, we revealed through histochemical analysis a scenario similar to MINGIE, with a 50% reduction in jejunal TP correlating with vascular alterations and partial denervation. Moreover, we observed that TP downregulation leads to impaired angiogenesis. Under this light, we investigated whether endothelial TP deficiency directly affects enteric neurons and intestinal epithelial stem cells. Primary murine myenteric neurons and intestinal epithelial organoids (enteroids) were exposed to conditioned medium from 50% TP-silenced endothelial cells. Immunofluorescence and live imaging revealed neurite fragmentation and increased glial and fibroblast proliferation, while neuronal counts remained stable. Stress features included neuronal swelling and elevated nNOS expression. Electrophysiological analysis showed a 200% spike rate increase after acute exposure, followed by complete activity loss within 24h, with no recovery after acetylcholine stimulation. Interestingly, in enteroids, Ki-67 expression decreased by 47%, indicating impaired epithelial proliferation. These results demonstrate that TP deficiency in endothelial cells alters both ENS and epithelial compartment integrity, suggesting TP as a histochemically traceable factor crucial for gut neuroepithelial homeostasis. Finally, this work supports the hypothesis of a vascular-driven mechanism contributing to neuronal and stem cell dysfunction in both idiopathic and genetic forms of CIPO.

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HISTOLOGICAL PROFILE OF INACTIVE UNILATERAL CONDYLAR HYPERPLASIA: EVIDENCE OF BORDERLINE TISSUE ACTIVITY

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Unilateral condylar hyperplasia (UCH) is a rare condition affecting mandibular symmetry and temporomandibular joint function[1]. While "active" UCH is marked by ongoing growth and histological changes, the "inactive" form is traditionally considered stable and without evident metabolic activity[2]. However, few studies have addressed its microscopic features. In this study, we analysed 15 condylar specimens from patients diagnosed with inactive UCH *via* SPECT/CT, using quantitative criteria[3]. Samples were examined through hematoxylin and eosin, Masson's Trichrome staining, and immunofluorescence for Collagen I/II, MMP-2, MMP-9, RANK, and Osteocalcin. Histologically, we observed thickened hypertrophic cartilage layers and irregular cartilage–bone interfaces, including downward expansions of chondrocytes into bone tissue. Immunofluorescence showed moderate to intense expression of collagen type II, MMP-2, and osteocalcin, as well as RANK-positive osteoclasts near the cartilage–bone border. These findings are consistent with prior microstructural characterizations of condylar tissue remodeling. Our results suggest that, despite being classified as inactive, residual biological activity persists in condylar tissues. This "borderline" state may predispose to reactivation or indicate a slow-progressing pathology.

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SELONSERTIB (ASK1 INHIBITOR) IN SKIN TISSUE REPAIR OF OBESE MICE

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Obesity can impair skin wound healing due to an increase in the oxidative stress that activates apoptosis signal-regulating kinase 1 (ASK1), an enzyme involved in cell death pathways. This study aimed to investigate the effects of ASK1 inhibition on skin tissue repair of obese mice. Adult male C57BL/6 mice were fed with a high-fat and high-sucrose diet for eight weeks to induce obesity. After six weeks, two full-thickness excisional wounds were created on the dorsum of each animal. Wounds were treated with selonsertib, an ASK1 inhibitor, and tissue samples were collected eight days post-wounding. The study was approved by the Animal Ethics Committee (CEUA No. 038/2022). Obese animals showed

increased body mass and epididymal fat, but the topical treatment with selonsertib did not alter those parameters. In addition, obese animals presented impaired wound healing compared to controls. Selonsertib treatment did not significantly alter wound closure rate. However, histological analysis revealed a higher granulation tissue formation in obese animals and a lower collagen fiber deposition, which was partially attenuated by selonsertib. Total and differential quantification of macrophages in the wound area was performed. Regarding total macrophages marked with F4/80, obese group showed a lower number of macrophages. However, topical treatment with selonsertib led to an increase in total macrophages in the obese animals eight days after wounding. In the analysis of M2 macrophages positive for arginase-1, obese group treated with selonsertib exhibited a higher number of antiinflammatory macrophages. Western blot showed an increase of ASK1 in obese animals, but the obese group treated with selonsertib showed a reduction in the parameter. These findings suggest that although ASK1 inhibitor does not improve obesogenic parameters and wound closure in obese mice, it can improve specific aspects of tissue repair, such as collagen deposition, inflammatory response and oxidative damage.

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VITAMIN D ATTENUATES LIVER INJURY BY REDUCING APOTOSIS, PYROPTOSIS, FERROPTOSIS AND PROMOTING REGENERATION IN TYPE 2 DIABETES

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High-fructose diets are strongly implicated in the pathogenesis of type 2 diabetes mellitus (T2DM) and associated liver injury[1]. Vitamin D (VitD) deficiency has been linked to increased insulin resistance and progression of liver fibrosis[2]. This study aimed to evaluate the regenerative and protective effects of VitD in a modified T2DM rat model[3]. Sprague-Dawley rats were divided into four groups: Diabetic, VitD-treated Diabetic (170 IU/week orally from week 5), VitD-only, and Control. T2DM was induced using 10% fructose in drinking water and a single STZ dose (40 mg/kg). Metabolic parameters (glucose, weight, calorie intake) were recorded. Liver tissues were analyzed histologically (hematoxylin-eosin, Van Gieson, PAS, Prussian blue) for fibrosis, glycogen and iron deposition, and bile duct regeneration. Morphometric analysis quantified bile ducts in periportal regions. Oxidative stress (GSH/GSSG), inflammation, and cell death pathways (pyroptosis, ferroptosis, apoptosis) were examined using immunohistochemistry for NLRP3, GSDMD, GPX4, TGFβ1, Caspase-3, αSMA, VDR, Ki67, YAP/TAZ, SIRT1, OV6, and TUNEL assays. Diabetic rats showed persistent hyperglycemia, hepatocellular vacuolization, iron overload, fibrosis, and elevated TGFβ1, αSMA, NLRP3, GSDMD, and apoptotic markers, with decreased GPX4 and GSH/GSSG ratio, indicating ferroptosis. VitD treatment ameliorated hyperglycemia, hepatic fibrosis, oxidative stress, inflammation, and regulated cell death in diabetics. It restored antioxidant status and increased Ki67, VDR, YAP/TAZ, SIRT1, and OV6 expression, indicating enhanced regeneration and progenitor activation. These findings support the therapeutic potential of VitD in managing fructoseinduced diabetic liver injury by modulating oxidative stress, inflammation, and cell death, while promoting hepatic repair.

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P59

IMMUNOHISTOCHEMISTRY ANALYSIS TO DETECT A MOLECULAR SIGNATURE IN INTERVERTEBRAL DISC DEGENERATION

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Intervertebral disc degeneration (IDD) is known as a primary contributor to low back pain, a debilitating condition which is the leading cause of disability worldwide. Traditionally, its evaluation is based exclusively on clinical parameters including MRI. Evidence to date shows that patients with similar radiological findings may have significantly different prognoses, suggesting that additional factors influence the outcome of IDD. These factors may include genetic predisposition, lifestyle, comorbidities[1], and the role of "protein sensors" whose expression and activity can promote cell protection. To try to resolve this discrepancy and understand the variability in disease progression and response to treatment, research is focusing on patient-specific biomarkers and risk factors. With this in mind, we conducted a study on IVD biopsies from 40 patients with mild IVD degeneration (Pfirrmann grade III) undergoing discectomy, in order to identify a molecular signature correlating with clinical parameters. Immunohisto-chemistry focused on the expression of specific proteins belonging to different potentially informative signaling: the transcription factors FOXO3a, HIF1α, BRY, the enzyme superoxide dismutase-2, and the glucose transporter GLUT1. Cross-sectional analysis showed that all these proteins may be considered stable biomarkers of IDD. However, no significant differences in their expression were found based on sex, age, smoking status, BMI, duration of symptoms prior to surgery, inflammatory cell density or surgical site. Post-surgical follow-up (n=24) showed healing in 75% of cases, although 62.5% had inflammation and 25% relapses: this means that the IVD cannot be said to be fully healed from a cellular and molecular point of view, due to associated pathobiological changes, causing recurrent symptoms. Consistently, no statistically significant differences were observed in the expression of the proteins under investigation. Our approach to biomarker discovery is just beginning and requires examining other pathways and enlarging the group of patients also comparing different grades of disease.

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P60

UNDERSTANDING HUMAN INTERVERTEBRAL DISC HOMEOSTASIS: 3D EXPERIMENTAL MODELS

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In recent years, there has been an increasing focus on the creation of *in vitro* experimental models that, by mimicking the physiopathological conditions of a given tissue, allow to obtain increasingly informative data, overcoming the use of often inadequate animal models. This is the case of the human musculoskeletal system, which, with regard to the anatomical and biomechanical properties associated with posture, cannot be faithfully replicated in animals. The attempt to create a model that mimics the human intervertebral disc (IVD) led us to develop the models reported below mainly to satisfy two reasons. The first concerns the creation of a cellular platform useful for studying the mechanisms underlying the maintenance of IVD homeostasis and the cellular response to physical/chemical treatments. The second concerns the creation of tissue-engineered constructs that can be implanted or injected into the site of damage, where the IVD has undergone degeneration/inflammation. Physiologically, IVD cells reside in hypoxic condition, devoid of vascularization and constantly exposed to mechanical stress (compression, tension and shear) which regulate their metabolism. IVD cells can degenerate due to aging, obesity, environment and genetics and cause neck and lower back pain, which are a major cause of disabling diseases. We have developed 3D systems based on multifunctional hydrogels (WJMs, 3D millicylinders), composed of various percentages of alginate, gelatin, and human decellularized Wharton's jelly (DWJ) that together constitute an optimal scaffold for IVD cells from biopsies of IDD patients undergoing discectomy[1]. We have demonstrated that WJMs are particularly effective in: a) supporting the viability and functional recovery of degenerated cells that resume their healthy chondrogenic-like phenotype (expressing SOX9, Integrin β1, Brachyury previously recognized as critical regulators of IVD homeostasis), thanks to the anabolic factors present in the DWJ; b) transmitting mechanical load to the cells and resisting permanent deformation when subjected to FlexCell FX-4000C pressure apparatus.

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IMMUNOPROFILE OF MERKEL CELLS IN THE HUMAN VAGINAL EPITHELIUM

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The role of Merkel cells (MCs) as mechanoreceptors in areas sensitive to touch in human skin has been well-established for some time[1]. Their function in the epithelium of the human vagina remains to be elucidated. Consequently, an attempt was made to ascertain their immunoprofile, a process which could potentially reveal their presumed role in this unexplored localization. The immunohistochemical experiment was conducted on samples of the vaginal wall, obtained from nine subjects during surgery. The specific epithelial, and neuroendocrine markers for MCs were used to ascertain their immunoprofile. The present study has confirmed the presence of 100% CK20+, 78% NSE+, 22% SYN+, 89% CHGA+, 22% VIP+ and CGRP and PIEZO2 negative MCs in the epithelium of the anterior wall of the human vagina. In the absence of positivity of MCs for PIEZO2 and CGRP and positivity for CK20, NSE, SYN and CHRA, it can be concluded that these cells are not connected to the nerve fibre. The presence of non-innervated MCs has been demonstrated in non-keratinized epithelia[2,3]. They are maybe responsible for paracrine function and are involved in the renewal of the epithelium[4]. Immunostaining of MCs revealed that their function in the human vagina is different from their function in the skin. It is hoped that the results of this study will contribute to a deeper understanding of the presence of these species in this area.

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FFAR4/GPR120 ACTIVATION COUNTERACTS FIBROGENIC PATHWAYS IN A CHOLESTATIC LIVER INJURY MODEL

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Free fatty acids (FFAs) are non-esterified fatty acids that act as natural ligands for a group of G protein-coupled receptors known as FFARs (free fatty acid receptors or G-protein coupled receptors, GPRs), including FFAR4 (GPR120)[1]. Unsaturated FFAs may play a direct role in improving liver diseases, including cholestatic con-

ditions, which are characterized by reduced bile flow caused by dysfunction of hepatocytes and cholangiocytes[2]. This can progress to extensive fibrosis and functional impairment of the organ, treatable only by transplantation. Based on this evidence, the project aims to evaluate the effect of a selective GPR120 agonist (GprA) on liver fibrosis in a murine model of cholestasis induced surgically through bile duct ligation (BDL), and to compare its effects with those of obeticholic acid (OCA), a drug used in the treatment of primary biliary cirrhosis[3]. GprA was well tolerated by the treated animals, which showed significantly higher survival than the vehicle-treated BDL group. Further evaluations demonstrated that GprA did not show positive effects in modulating the main inflammatory interleukins 1α, -1β, -4, -5, -10, and -6. However, the agonist reduced the expression of key pro-fibrotic markers, including collagens I and III, connective tissue growth factor (CTGF), and α-SMA. Finally, GprA interacts with enzymes involved in extracellular matrix remodeling, leading to a noticeable reduction in the fibrogenic process. Based on the obtained results, the test of the efficacy of GprA in other experimental models of fibrosis could represent an important objective in the effort to identify effective molecules for the treatment of various unresolved liver diseases.

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ROLE OF ION CYCLOTRON RESONANCE ON BODY ELECTRICAL IMPEDANCE IN CHRONIC PAIN: CLINICAL EFFECTS AND FUTURE PERSPECTIVES

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The rising field of quantum medicine defines matter, even in biological systems, as an element in constant electromagnetic resonance based on the interaction of ultra-weak electromagnetic fields[1]. The electromagnetic activity that characterizes each element, including biological tissues and individual cells, could play a fundamental role in the advancement of diagnostic and therapeutic techniques[2]. A therapeutic approach that falls into this field is Ion Cyclotron Resonance (ICR), which exploits targeted ultra-weak electromagnetic fields to restore impaired enzymatic activities in the body by moving ions and restoring molecular exchanges between cell membranes[3]. However, given the novelty of the approach, more studies are needed to assess the mechanisms of action and the actual efficacy of the treatment. Therefore, we conducted a pilot observational study involving 7 men and 7 women (average age 57.4±8.4 and 68.7±14.7 years respectively) to verify the efficacy and safety of a 6 sessions cycle of Ion Cyclotron Resonance therapy in reducing chronic musculoskeletal pain, detected by the Numeric Pain Rating Scale, and restoring a correct whole-body hydroelectrolytic balance monitored through the bioelectrical impedance analysis. The results of our study associate the ICR treatment with a significant improvement in all observed parameters between the beginning and the end of the treatment cycle. No treatment-related adverse effects were observed during the observation. Our study may lay the foundation for new and more in-depth research on the topic. New studies will require the cooperation of multiple disciplines, from physics to histochemistry through the various health specialties, in order to frame the biochemical and clinical effects of this therapeutic approach in a perspective of multidisciplinary collaboration and translational medicine[4].

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EX VIVO EXTRACTION OF GLIOMA SECRETOME: A NOVEL MICROENVIRONMENT-BASED PROTOCOL FOR METABOLIC AND MOLECULAR PROFILING

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Gliomas represent the most frequent primary brain tumors in adults and are marked by considerable molecular and clinical heterogeneity. The 2021 WHO classification of central nervous system tumors identifies three major adult-type diffuse glioma subtypes: astrocytoma IDH-mutant, oligodendroglioma IDHmutant with 1p/19q-codeletion, and glioblastoma IDH-wildtype.[1] Despite ongoing advances, treatment resistance and poor prognosis remain major challenges, largely due to intratumoral heterogeneity and the complexity of the tumor microenvironment.[2] A promising yet underexplored avenue for patient-centered glioma profiling is the tumor secretome, which includes soluble factors, extracellular vesicles, and metabolites released by both neoplastic and stromal cells.[3] Existing studies rely on *in vitro* models that fail to recapitulate the complexity of tumor-host interactions. To bridge this gap, we established an innovative, standardized ex vivo protocol for secretome isolation directly from glioma tissue. The protocol preserves microenvironmental cues from the operating room to laboratory analysis, ensuring spontaneous secretome release under controlled conditions. Feasibility and performance were assessed in a preliminary cohort of seven adult diffuse glioma patients. Transmission electron microscopy confirmed tissue structural integrity. Proteomic profiling identified 84 cancer-associated proteins within the secretome. Additionally, metabolomic analysis revealed a distinctive signature of small molecules. Notably, glioblastoma IDH-wildtype samples exhibited increased lactate levels, consistent with hypoxia-driven metabolism, and decreased N-acetylaspartate, indicating neuronal loss. Overall, this *ex vivo* secretome protocol offers a robust and physiologically relevant platform for glioma characterization, with potential applications in biomarker discovery, patient stratification, and the development of personalized therapeutic strategies.

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(YS) INVESTIGATING THE EFFECTS OF THE SECRETOME FROM HUMAN AMNIOTIC MESENCHYMAL STROMAL CELLS ON INFLAMMASOME ACTIVATION

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Macrophages play a pivotal role in inflammation and, upon exposure to stimuli such as lipopolysaccharides (LPS), they activate the NLRP3 inflammasome – a multiprotein complex of the NOD-like receptor family. This activation drives macrophage polarization toward the pro-inflammatory M1 phenotype through metabolic reprogramming, reactive oxygen species (ROS) production, and the release of pro-inflammatory cytokines[1]. The canonical activation pathway of NLRP3 involves the oligomerization of ASC (apoptosis-associated speck-like protein containing a CARD), forming cytosolic aggregates known as ASC specks, and triggers caspase-1 activation and IL-1β maturation[2]. We previously demonstrated that the secretome from human amniotic mesenchymal stromal cells (hAMSC), also known as conditioned medium (CM-hAMSC), affects macrophage polarization by promoting a shift from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype[3]. This study investigates whether CM-hAMSC can modulate inflammasome activation in M1 macrophages. Monocytes were differentiated into M1 macrophages using GM-CSF for 5 days and then stimulated with LPS and ATP in presence or absence of CM-hAMSC. After 5 h, inflammasome activation was evaluated as IL-1β release, ASC speck formation and mitochondrial ROS levels through ELISA, fluorescence microscopy and flow cytometry analyses. CM-hAMSC significantly reduced IL-1β secretion and ROS accumulation, with a slight decrease in ASC speck formation. These findings support the anti-inflammatory role of CM-hAMSC and highlights the need for more detailed mechanistic studies.

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FROM INNERVATION TO STEROIDOGENIC DEFECT: IMPLICATIONS OF CGRP IN THE PATHOPHYSIOLOGY OF POLYCYSTIC OVARY SYNDROME

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Polycystic ovary syndrome (PCOS) is a multifactorial endocrine disorder affecting up to 20% of women of reproductive age. It is characterized by hyperandrogenism, menstrual irregularities, anovulation, and metabolic disturbances, including insulin resistance and increased cardiometabolic risk[1]. Although its pathogenesis remains unclear, recent studies suggest a potential role for neuropeptides in modulating ovarian function[2]. This study aims to investigate the presence of calcitonin gene related peptide (CGRP), a 37-amino-acid neuropeptide involved in vasodilation, nociceptive signaling, and modulation of the inflammatory response[3], within the ovary and follicular fluid of women with PCOS. CGRP levels were quantified in follicular fluid from PCOS (n=27) and non-PCOS (n=27) women, showing significantly higher concentrations in the PCOS group ($p<0.05$). Immunohistochemistry analyses revealed intense CGRP immunoreactivity surrounding preantral follicles in PCOS ovaries, particularly around the theca cell layer. In contrast, non-PCOS samples exhibited weaker CGRP expression. This differential pattern suggests a possible involvement of CGRP in early follicular development. The altered follicular microenvironment in PCOS may permit the accumulation of small neuropeptides like CGRP, which could exert autocrine/paracrine effects on follicular cells, influencing both development and steroidogenesis[4]. To further investigate this hypothesis, we assessed the effects of CGRP on cell viability, mitochondrial and steroidogenic activity in primary h-GCs. These findings suggest that CGRP may contribute to the dysregulation of follicular development observed in PCOS. A better understanding of its role could offer new insights for the development of targeted therapies aimed at restoring ovarian function in affected women.

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EMERGING ROLE OF NUCLEAR PHOSPHOLIPASE C BETA 1 AS POTENTIAL BIOMARKER IN LUNG CANCER

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Lung Cancer (LC) is the most common neoplasm in both females and males, representing the 11.6% of all new cancer cases and accounting for the 18.4% of all cancer-related deaths. In spite of innovative approaches in LC treatment, the prognosis remains poor, due to late-stage diagnosis, resistance to therapies and metastases[1]. Hence, there is a compelling need to identify new biomarkers for the early diagnosis, prognosis and targeted therapy. Recently, the potential role of Phospholipase C (PLC) enzymes in tumor proliferation and metastasis has been proposed[2]. Our study examined the involvement of PLCs in three LC cellular models: A549, H460 and H358. First, mRNA expression of all PLC isoforms was estimated by real-time qPCR, and protein levels were observed for the most promising enzymes. PLCβ1 displayed favorable results, therefore its expression was evaluated through immunohistochemistry on a commercial tissue microarray. Notably, the array revealed a consistent nuclear overexpression of the enzyme in tumoral tissue of patients. In addition, PLCβ1 was found to be localized in the nucleus through confocal laser scanner microscopy. Finally, we transfected H358 cells with a novel construct for omomyc peptide[3], whose induction was able to reduce PLCβ1 expression. In this experimental model we observed changes in cell cycle, viability and migration, suggesting the implication of PLCβ1 in cellular mechanisms supporting cancer. These findings demonstrate the involvement of PLC enzymes, particularly of PLCβ1, in LC, suggesting their emerging role as potential biomarkers.

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EARLY EFFECTS OF MECHANOACUSTIC FOCUSED VIBRATION (MFV) ON PRIMARY HUMAN SATELLITE CELLS ISOLATED FROM YOUNG AND OLDER INDIVIDUALS

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Skeletal muscle is composed of elongated, multinucleated, and contractile units known as muscle fibers, which can regenerate and repair tissue damage *via* resident stem cells, called satellite cells (SCs)[1,2]. Impaired regeneration and progressive muscle atrophy are hallmarks of sarcopenia, a condition with significant clinical impact, contributing to frailty, functional decline, and comorbidities in the elderly[3]. Current therapeutic strategies primarily involve physical activity and nutritional support, occasionally combined with rehabilitation approach such as mechanoacoustic stimulation. MFV activates type III and IV mechanoreceptors, including Pacinian and Meissner corpuscles, Golgi tendon organs, and muscle spindle afferents. This stimulation induces the vibratory tonic reflex (VTR), resulting in involuntary muscle contractions *via* α-motor neuron activation and subsequent recruitment of otherwise inactive fibers[4]. However, the direct effect of vibration on satellite cells remains to explore. In this study, we examined, after 72 h, the effects of MFV stimulation with increasing time intervals (10, 20, and 30 min) at a constant intensity (100 mbar) and frequency (300 Hz) on primary human myogenic precursor cells isolated from Vastus Lateralis muscle biopsies of 3 young (23±5) and 3 older (72±9) healthy donors and cultured *in vitro*. MFV treatment reduced satellite cells apoptosis, increased cell size, alignment and mitotic activity and raised the proportion of activated cells in both agegroups. These effects were more pronounced in aged cells after 20-30 min treatment. All in all, our *in vitro* results indicate for the first time the presence of direct effects of 2 MFV on human SCs. Interestingly, these effects seem to be age-dependent, with a mainly proliferative response of cells from young subjects and a mainly differentiative response of cells from aged subjects.

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MICROPLASTICS AND ATHEROSCLEROSIS: EVIDENCE OF MICROPLASTICS IN CAROTID ATHEROSCLEROTIC PLAQUES

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A very recent study reported for the first time the presence of four types of microplastics in human arteries, including coronary, carotid and aortic arteries. The coronary and carotid arteries with atherosclerotic plaques had significantly higher concentrations than aortic arteries without atherosclerotic plaques, which could imply a link between microplastics and atherosclerosis[1]. We analysed 27 atherosclerotic plaques taken from patients with carotid atherosclerosis who had undergone carotid endarterectomy in order to determine the distribution of microplastics within the plaques. Microplastics were detected by pyrolysi-gass chromatography/mass spectrometry an average concentration of 13.99±3.00 µg/mg of plaque. Four types of microplastics were detected: PET (36.5%), PA-66 (38.6%), PVC (15.5%), and PE (9.22%). Secondary endpoint included levels of tissue biomarkers CD68 and CD3 measured by immunohistochemistry in plaques where at least one type of microplastic was identified versus those without. Scanning electron microscopy images showed small particles of

low-reflection material surrounded by a thin line of high-reflection material identified outside in the amorphous material of the plaque. These findings are consistent with a multicenter observational study conducted in patients undergoing carotid endarterectomy for asymptomatic carotid artery disease[2], suggesting that microplastics may be associated with human cardiovascular health.

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TRICHOSANTHIN TARGETS WNT/NF-KB PATHWAYS TO ATTENUATE PSORIATIC INFLAMMATION AND KERATINOCYTE HYPERPROLIFERATION

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Psoriasis is a chronic immune-mediated inflammatory disorder characterized by accelerated keratinocyte proliferation and immune dysfunction[1]. Trichosanthin (TCS) demonstrates potent induction of apoptosis and regulates T-cell activities[2]. This was the first study to investigate the therapeutic effects of TCS topical application in the treatment of psoriasis. After a series of *in vitro* and *in vivo* experiments, we found that TCS significantly alleviated psoriatic lesions in mice, reducing PASI scores comparable to those achieved with tacrolimus. TCS also decreased the protein expression levels of Ki-67, KRT14, and IL-17, as well as reduced T cell infiltration in psoriatic skin tissue, and inhibited the expression of Ki-67, β-catenin, Wnt5a, STAT3, KRT14, and KRT17 in HaCat cells. Furthermore, TCS suppressed the protein levels of TRAF6, IKKα/β, and NF-κB, along with its phosphorylated form, and downregulated the mRNA expression of inflammatory cytokines including IL-17, IL-12, TNF-α, and IFN-γ. In summary, TCS effectively suppresses epithelial hyperproliferation, keratinization, and skin inflammation by modulating Wnt and NF- κB signaling pathways, underscoring its potential as a novel therapeutic agent for psoriasis.

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THE EXTRACELLULAR VESICLE POPULATIONS UNDERWENT ALTERATIONS SUBSEQUENT TO THE PRETREATMENT OF ADIPOSE-DERIVED STEM CELLS WITH MELATONIN RECEPTOR AGONIST

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Melatonin, a neurohormone primarily involved in the regulation of circadian rhythms, exerts its physiological effects through MT1 and MT2 receptors expressed in various tissues, including adipose derived stem cells (ADSCs). Beyond its role in sleep-wake regulation, melatonin possesses anti-inflammatory, antioxidative, and potential anticancer properties, prompting growing interest in its influence on stem cell behavior. ADSCs are known to exert therapeutic effects largely *via* paracrine signaling, particularly through the secretion of extracellular vesicles (EVs) -membrane bound carriers that transport proteins, lipids, and nucleic acids to target cells. Recent attention has focused on modulating EV release and composition to enhance their therapeutic utility. While melatonin's effect on ADSC viability and differentiation has been explored, its impact on EV production remains poorly defined. In this context, the use of tasimelteon, a selective melatonin receptor agonist, offers a novel approach to modulating melatonin receptor mediated pathways in ADSCs. This study investigates how pretreatment with tasimelteon alters the characteristics of EV populations secreted by ADSCs, providing new insight into the potential application of melatonin receptor modulation in optimizing cell-free regenerative therapies. ADSC were evaluated by trilineage differentiation assay and flow cytometry. The cell culture was conducted in DMEM with 10% of exosome-depleted FBS and 1% of antibiotics. ADSC-EVs were isolated from freshly collected conditioned medium from ADSC culture at logarithmic phase of growth, after 48 h of incubation by differential centrifugation or ion exchange chromatography. Cells were treated with melatonin, tesimelteon, a melatonin receptor antagonist – luzindole or combined. The control groups were untreated cells. The EV characteristics were assessed with nanoparticle tracking analysis, bead-based cytometry, Western blot, scanning electron microscopy, transmission electron microscopy and qPCR. Results suggest that melatonin receptor-mediated pathways in ADSCs might play a crucial role in EV development.

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A SWAB NEGATIVE PATIENT AFTER SARS-COV-2 INFECTION DISPLAYS INCREASE OF VEGF AND FIBRONECTIN ASSOCIATED WITH ALTERATIONS OF INTERCELLULAR JUNCTIONS

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SARS-CoV-2 infection was responsible for the pandemic COrona VIrus Disease (COVID-19) and caused a range of symptoms, including gastrointestinal disorders and abdominal pain, making COVID-19 a multi-organ dysfunction. It is known that the host receptor for SARS-CoV-2, ACE2, is expressed also in the gut, where SARS-CoV-2 could induce vascular damage and immune system dysregulation, similarly to what has been widely described in the lung[1]. This case report concerns a middle-aged man admitted to the Emergency Department of the University Hospital of Ferrara (Italy) and tested negative to the nose-oropharyngeal swab for SARS-CoV-2 four weeks after recovering from the infection. Due to thrombotic and hemorragic gut conditions, accompanied by severe ulceration, the patient underwent resection of a segment of ileum. Immunohistochemical analyses showed the presence of SARS-CoV-2 nucleocapsid protein and an increased human leukocyte antigen (HLA-G) in the ulcerated ileum portion when compared to the non-ulcerated one[2]. Moreover, expression and co-expression of Vascular Endothelial Growth Factor (VEGF) and Fibronectin (FN) were analyzed. VEGF expression was increased in the ulcerated ileum portion when compared with control and non-ulcerated ileum. FN staining was progressively increasing in non-ulcerated and ulcerated ileum. By electron microscopy the microvilli alteration of brush border and of intercellular junctions were detected in the ulcerated area but not in other areas of patient's ileum or in the control sample. The salient aspect of this study is that although the patient was declared negative for SARS-CoV-2 at the respiratory level, SPIKE and SARS-CoV-2 nucleocapsid proteins were still present in the ileum, concentrated in the ulcerated area and parallelled by an increased expression of vascular markers. The ultrastructural analysis gave insights on the morpho-functional effects of SARS-Cov-2 infection on the gut epithelium, indicating that the damage is not limited to vascular structures.

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ANALYSIS OF SAPK/JNK PATHWAY IN DOXORUBICIN INDUCED ADIPOSE-DERIVED STEM CELL CYTOTOXICITY

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Doxorubicin (DOX) is a member of the anthracycline group of chemotherapy drugs, which have a broad spectrum of activity. Its mechanism is based on intercalation into cancer cell DNA. However, DOX also exerts harmful effects on normal tissues, contributing to adverse outcomes such as cardiotoxicity. Furthermore, it has been demonstrated that DOX can lead to damage stem cell populations, thereby impairing their regenerative capacity[1]. Despite extensive research, the mechanisms by which DOX acts on mesenchymal stem cells (MSCs) and the potential strategies to protect them remain to be fully elucidated. It is likely that the SAPK/ JNK (Stress-activated protein kinases/Jun amino-terminal kinases) pathway plays a crucial role in malignancy and resistance of cancer cells[2]. The study's objective was to evaluate the cytotoxic effect of DOX on adipose-derived stem cells (ADSCs), and to analyze whether SAPK/ JNK expression is associated with this process. The viability of ADSCs was evaluated at concentrations of DOX, ranging from 0.1 μM to 100 μM after 24-h incubation period. Three concentrations were selected for further experiments. The cells were then analyzed for gene and protein expression related to the SAPK/JNK pathway. It was observed that there was a decrease in cell viability in a dose-dependent manner following treatment with DOX at concentrations of 5 μM and above. Furthermore, DOX has been shown to modify the expression of SAPK/JNK-related genes and to protein levels. The study concluded that SAPK/JNK pathway undergoes alterations in response to DOX treatment and it can play a role in activating the stress response to DOX exposure.

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EXPRESSION OF PISCIDIN, TLR-4, ERK2 AND P-P38 MAPK IN THE ZEBRAFISH GUT EXPOSED TO PCB-126 AND POSSIBLE PROTECTIVE EFFECTS OF GINESTEIN

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Polychlorinated biphenyls (PCBs) represent one of the main categories of persistent organic pollutants, capable of altering intestinal function and immune response. PCB-126 is a potent, immunotoxic, and bioaccumulative dioxin-like agent[1]. Zebrafish (*Danio rerio*) is a model organism in the study of intestinal inflammation. The aim of our study was to evaluate the damage to the intestinal epithelium of Zebrafish (*D. rerio*), following exposure to PCB 126. To characterise inflammatory state, some antibodies as well as: Piscidin, TLR-4, ERK2 and p-p38 MAPK were used. Piscidin is an antimicrobial peptide found in teleost fish, active against pathogens[2]. TLR4 plays a crucial role in intestinal inflammatory responses induced by PCB 126[3]. ERK2 and p-p38 are mitogen-activated protein kinases (MAPKs) involved in the innate immune response that can be activated by pro-inflammatory cytokines and stress[4]. Genistein is an isoflavone first isolated from the brooming plant Dyer's *Genista tinctoria* L.[5]. Our results showed a strong expression of the tested antibodies, in the intestinal epithelium of PCB-126-exposed fish, indicating inflammation. Minor immunoreactivity has been found in treated fish with genistein and PCB-126, suggesting that genistein attenuates PCB-126-induced inflammation and preserves intestinal integrity. The preliminary

results of this study demonstrate the usefulness of zebrafish as an animal model to deepen the signaling pathways involved in the intestinal inflammation caused by exposures to PCBs, moreover, it would be interesting to deepent the effects of genistein that are not well established yet[6].

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MICROBIOTA-GUT-BRAIN AXIS IN AN AUTISM SPECTRUM DISORDER ANIMAL MODEL

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Autism spectrum disorder (ASD) primarily affects the brain, but it can also promote gastrointestinal damage and gut microbiota alterations (leaky gut). There is increasing evidence that supports the interaction between gut microbiota and brain development and function, contributing to define the concept of the "microbiota-gutbrain axis"[1-3]. The absence of gut microbiota in germ-free rodents is associated with structural alterations of tight junctions (TJs) in the blood-brain barrier, characterized by decreased expression of TJ proteins. Alterations in the TJs also result in increased permeability compared to mice with healthy microbiota[4-6]. This study aimed to evaluate the morphology of the intestinal barrier and the mechanisms contributing to leaky gut in BTBR T+Itpr3tf/J mice, an animal model of ASD, treated orally with 10 mg/kg/day of melatonin (MLT) for 16 weeks. MLT is found in various fruits and vegetables at different concentrations, and its presence alongside other polyphenols may contribute to improved global health[7]. Together with morphological analyses, we evaluated the expression of TJ proteins in the small intestine using immunohistochemistry. Morphological analysis showed that the mucosal tunica of BTBR mice presented longer intestinal villi, which altered intestinal permeability and microbiota composition. MLT significantly reduced the villi length in BTBR mice and appeared to modulate TJs expression, potentially decreasing leaky gut. These findings suggest an involvement of the microbiota-gut-brain axis in ASD and support a simil-therapeutic potential of MLT in limiting ASD symptoms through its multitasking properties[8,9].

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PERICAPILLARY SPACES IN THE SHEEP PINEAL GLAND – 2D AND 3D ANALYSIS

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The structural organization of pericapillary spaces in the mammalian pineal gland varies significantly among species. In rats - the most widely used in pineal research - fenestrated capillaries are surrounded by wide pericapillary spaces rich in pinealocyte processes. Notably, the rat pineal gland is among the few brain structures that lack a blood-brain barrier. In contrast, species such as squirrels, rabbits and pigs exhibit non-fenestrated capillaries with relatively narrow pericapillary spaces. The sheep pineal gland is considered a more suitable model for human studies than the rat pineal gland. This study aimed to provide a detailed ultrastructural analysis of the pericapillary spaces in the pineal gland of 9-monthold sheep using both 2D and 3D electron microscopy. Ultrathin sections were mounted on silicon wafers and imaged with a SenseBSD detector in a Gemini 450 SEM (Zeiss). Additionally, serial block-face imaging (SBFI) was performed using the 3View 2XP system (Gatan) in the same microscope. The analysis revealed that the capillaries consisted of a continuous layer of non-fenestrated endothelial cells resting on the basement membrane. Numerous pericytes were present and enclosed by their own basal membranes. The capillaries were surrounded by a thick layer of collagen fibers, itself completely enclosed by the basement membrane. The outer surface of this membrane was lined with flattened astrocytic processes ("leaflets") that adhered tightly to each other, forming a barrier that fully separated the pericapillary space from the pineal parenchyma. SBFI showed that these astrocytic leaflets originated as terminal expansions or flattened segments along astrocytic processes. The astrocytic processes forming leaflets were extremely long and often extended beyond the imaged volume (72×72×50 μm), with no visible connections to their cell bodies. Notably, no direct contact was observed between pinealocyte processes and the basement membrane surrounding the collagen layer. Our findings indicate that the sheep pineal gland possesses a unique blood - pineal barrier.

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MEMBRANE SKELETAL PROTEIN, MEMBRANE PALMITOYLATED PROTEIN 6 (MPP6), IN MOUSE CEREBRAL SYNAPSES

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A membrane skeletal protein, membrane palmitoylated protein 6 (MPP6) that interacts with protein 4.1G in PNS, is also expressed in the CNS. In this study, we investigated the localization of MPP6 and its associated protein complexes in the mouse cerebrum, as well as its effects on behaviour using MPP6 protein-deficient (KO) mice. MPP6 was detected in mouse cerebral lysates and synaptic membrane fractions, where it formed protein complexes with other MPP family members, including MPP1, MPP2, and a signal protein calcium/calmodulin-dependent serine protein kinase (CASK). However, the amounts of these complexes did not differ between KO and wild-type (WT) mice. Immunohistochemistry revealed that MPP6 was localized at synapses throughout the cerebrum, particularly in the postsynaptic regions. Ultrastructural analysis showed that synaptic cleft distances and postsynaptic density thickness were slightly reduced in KO mice compared to WT mice. In the elevated plus-maze test, a KO mouse exhibited unusual behaviour not observed in WT mice, although there was no statistically significant difference in the time spent in the open and closed arms between the two groups. Locomotor activity measurements revealed that KO mice were more active at midnight and less active from morning to noon than WT mice, implying alterations in sleep-wake regulation. These findings suggest that MPP6 plays a role in synaptic function by forming protein complexes with other MPP family members and signal proteins.

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THE ROLE OF EXTRACELLULAR VESICLES DERIVED FROM MESENCHYMAL STROMAL CELLS IN MYOFIBER REGENERATION

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Traumatic injuries can severely impair skeletal muscle, leading to limited regeneration, muscle loss, fibrosis, and compromised function. Despite the exploration of various therapeutic approaches, no fully effective treatment has yet been established. Mesenchymal stem cells (MSCs) have emerged as a promising strategy for muscle repair, largely due to their paracrine signaling and immunomodulatory properties mediated by extracellular vesicles (EVs). Although the cellular and molecular processes involved in skeletal muscle regeneration are well understood, the specific role of EVs in mediating intercellular communication during myofiber repair remains under active investigation. This study aims to clarify the contribution of MSC-derived EVs to muscle repair and regeneration. EVs were isolated from murine myoblasts using a polyethylene glycol (PEG)-based liquid exchange precipitation protocol. EVs from control myoblasts, differentiated myoblasts, and MSCs were characterized in terms of size, surface markers, and myokine content using electron microscopy, western blotting, and ProQuantum immunoassays. These EVs were subsequently added to cultures of damaged, differentiated myoblasts to assess their regenerative potential. Myotube formation was evaluated *via* inverted light microscopy, while the expression of key myogenic markers was assessed through western blot analysis. The results confirmed the presence of EVs in both myoblasts and MSCs. The expression of EV markers varied depending on the experimental conditions used to simulate muscle damage. Injured myoblasts released EVs containing inflammatory factors, whereas MSC-derived EVs attenuated the inflammatory environment, enhanced myogenic repair, and promoted muscle regeneration. In conclusion, our findings highlight the critical role of MSC-derived extracellular vesicles in regulating myogenic differentiation and supporting the regeneration of skeletal muscle following injury.

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AN INNOVATIVE FUNCTIONAL COOKIE MODULATES THE MUCOSA AND MYENTERIC PLEXUS IN THE GUT OF 3xTg-AD MOUSE MODEL OF ALZHEIMER'S DISEASE

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Neurodegenerative diseases are characterized by the gradual degeneration of the neuronal populations, leading to Alzheimer's disease (AD) with the progressive onset of dementia, making this pathology one of the costliest and deadliest diseases of the current century. Patients with AD frequently exhibit reduced gut microbial diversity, suggesting a significant involvement of gut microbiota in influencing the pathogenesis and progression of AD. It has been proposed that gut dysbiosis, dysfunction of the intestinal epithelial barrier, and vascular deposition of amyloid-beta (Aβ) in the gut may precede the cerebral deposition of Aβ in a transgenic mouse

model of AD.[1] A hypocaloric cookie with prebiotic-rich ingredients (red lentils) coated with a multi-strain probiotic (SLAB51®) enriched chocolate[2] was tested on 3xTg-AD mice, a murine model of AD, to evaluate the protective effects on the mucosa and ENS of the ileum and colon. 8-week-old 3xTg-AD gender-balanced mice were divided into five groups and organized for the supplementation of functional cookies. The morphology of the ileum and colonic wall, the mucus secretion, the intestinal barrier integrity, and neurodegeneration of the myenteric plexus were assessed with histochemical and immunochemical approaches. All the experimental animals showed a well-conserved morphology of the intestinal wall without fibrosis. The SLAB51®, administered alone or with the functional prebiotic enriched cookies, red lentils based appeared to be useful to reduce mucus secretion. The intestinal barrier integrity seems to be enhanced by the cookie supplementation, related to a decrease in inflammatory pathways. Moreover, in the ENS, gliosis was decreased in functional cookies supplemented mice without a clear modulation of enteric cholinergic and nitrergic neurons. This innovative symbiotic cookie could represent an innovative nutritional approach to prevent the onset of AD-related gut alterations and promote healthy aging.

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MITOCHONDRIA-LYSOSOME INTERACTIONS: EMERGING PLAYERS IN GLIOMA

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Despite research efforts, the results obtained in the therapy against glioma have improved but only slightly. For this reason, identifying new targets remains an important and necessary goal. The role of mitochondria, as potential target, has attracted considerable attention in recent times. Mitochondria and lysosomes communicate with each other through specialized membrane contact sites to regulate cellular metabolism and signaling pathways, impacting tumor cell survival and response to therapy[1]. Mitochondria dynamics play a crucial role in glioma pathophysiology, contributing to metabolic reprogramming, fast proliferation, invasion and drug resistance. It has been recently demonstrated that the mitochondrial structure and functions are regulated by the TRPML1 lysosomal channel by enabling the calcium flux from lysosomes to mitochondria[2]. To better investigate mitochondria in glioma progression, a potent TRPML1 agonist, ML-SA5 was used to stimulate Ca^{2+} influx into mitochondria in glioma cells. The activation of the lysosomal channel TRPML1 induces the production of mitochondrial reactive oxygen species, hyperpolarization of the mitochondrial membrane and cellular redistribution of these organelles. These changes are also associated with the activation of the mitochondrial enzyme P5Cs, the enhancement in collagen synthesis and increase of glioma migrating abilities. Overall, these preliminary results suggest that the interaction between lysosomes and mitochondria *via* TRPML1 represents a promising avenue to target mitochondrial dynamics for glioma therapeutic strategies.

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AN *IN VITRO* CYTOTOXIC, PROAPOPTOTIC AND ANTITUMOR ACTIVITY ASSESSMENT FOR CERAMIDASE INHIBITORS ON HUMAN LARYNGEAL CANCER CELLS

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Laryngeal cancer is diagnosed approximately 20% of head and neck cancers worldwide[1]. Among the potential sphingolipid-based treatment methods for the treatment of these fatal cancers[2] acid ceramidase inhibition is indicated as a beneficial treatment option[3]. In this study, the cytotoxic, proapoptotic and antitumor effects of recently developed human acid ceramidase inhibitors ceranib-2 and carmofur were investigated in human laryngeal cancer cells using MTT, Annexin V and colony formation assay. It was found that ceranib-2 and carmofur caused cytotoxicity in monotherapy and in combination at low doses. Proapoptotic effect was detected both in monotherapy and in combination therapy at the IC_{50} concentrations (54.18, 51.94 and 27.15 μM for carmofur, ceranib-2 and their combination, respectively) after 24 h of exposure. The highest apoptosis rate was detected after the combination treatment. Inhibition of colony formation, which indicates antitumor effect, was most clearly seen in the combination group, and a significant decrease in colony number was also recorded in the monotherapy groups.

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PROINFLAMMATORY BONE MARROW NICHES AND NEUTROPHIL ACTIVATION ARE ASSOCIATED WITH TIGIT EXPRESSION IN MULTIPLE MYELOMA

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Multiple myeloma (MM) is a hematological disorder due to the abnormal proliferation of clonal Plasma Cells (PCs) characterized by the increase of monoclonal immunoglobulins[1]. The interaction between MM cells and the bone marrow (BM) microenvironment is crucial to the progression and treatment of the disease. The PCs interaction with the hematopoietic cells induce the secretion of cytokines and growth factors, by establishing circuits that support PCs activation. In this context the malignant TIGIT positive PCs are able to interact with the T lymphocytes, a process that fuel the immune evasion, typical of the MM[2]. Our knowledge about the BM myeloma microenvironment is still poorly defined as well as the cellular interaction between PCs. We exploited BM biopsies of 22 MM patients. TIGIT was expressed in 86% (19/22) of evaluable samples. Among patients with PCs % >60% or FLC ratio >100, 92% were TIGIT+. Morphological differences were evident between groups: TIGIT-negative PCs were larger and altered, while TIGIT-positive PCs showed polarized nuclei and proximity to neutrophils. TIGIT-positive samples displayed increased neutrophils undergoing NETosis, as confirmed by neutrophil elastase and Ly6b co-expression (p=0.0067). Elevated IL-6 (p=0.0003) and IL-8 (p=0.004) in TIGIT-positive samples suggest a proinflammatory microenvironment promoting neutrophil recruitment and NETosis. In MM, the Neutrophil to lymphocyte ratio combines a marker of inflammation and the reduced cell turnover, reflecting alteration in the immune system. TIGIT expression in the bone marrow of MM patients is associated with more aggressive disease features and an inflammatory microenvironment enriched with NET-forming neutrophils. These findings support TIGIT as a potential biomarker of disease severity and a therapeutic target in MM.

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INFLAMMATORY PROFILE IN SKIN, LUNG, AND KIDNEY OF A HOCL-INDUCED MURINE MODEL OF SYSTEMIC SCLEROSIS: AN IMMUNOHISTOCHEMICAL STUDY

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Systemic sclerosis (SSc) is a complex autoimmune disease characterized by widespread microvascular damage, inflammation, and fibrosis[1,2]. While inflammation is a recognized early event in disease pathogenesis, its distribution and intensity across different organs remain incompletely understood. This study investigates the inflammatory signature in skin, lung, and kidney of a murine model of SSc induced by daily subcutaneous injections of hypochlorous acid (HOCl) for six weeks. Tissue samples were collected and processed for histology (Hematoxylin and Eosin) and immunohistochemistry. We focused on macrophage infiltration (CD68, F4/80) and activation of the inflammasome pathway (NLRP3, Caspase-1, IL-1β). The skin displayed intense perivascular and interstitial infiltration by macrophages, along with marked NLRP3 and IL-1β expression. In the lung, inflammatory cells accumulated around bronchi and vessels, with strong upregulation of inflammasome markers. The kidney showed both glomerular and interstitial immune activation, suggesting early involvement despite minimal fibrotic remodeling.Our findings demonstrate a widespread activation of innate immune responses, particularly the NLRP3 inflammasome, in cutaneous and visceral tissues of SSc mice. These results highlight the key role of inflammation in systemic sclerosis and reinforce the importance of organ-specific histochemical profiling in understanding disease mechanisms and guiding therapeutic strategies.

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OMOMYC PEPTIDE LOCALIZES IN THE NUCLEUS AND DISPLAYS ANTIPROLIFERATIVE EFFECTS ON CELLULAR MODELS OF HUMAN OSTEOSARCOMA

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Osteosarcoma (OS) is the most common primary bone tumor affecting children and adolescents. It is characterized by irregular bone growth and common metastases, which determine its aggressiveness and poor outcomes[1]. Standard therapy includes a combination of chemotherapy and surgical resection; however, the overall survival rate has remained almost identical over the past thirty years. Therefore, there is a strong need to find novel approaches to the treatment of OS. Recently, c-Myc oncogene was proposed as potential therapeutic target, since it is frequently deregulated in cancer and it is commonly amplified in OS as well. The oncogene works as a pleiotropic transcription factor and it modulates cell growth, proliferation, apoptosis, and immune suppression[2]. Omomyc is a novel c-Myc inhibitor that acts as a dominant negative for c-Myc transcriptional activity[2]. In cellular models of human OS, we observed that Omomyc localizes exclusively in the nucleus, where it counteracts c-Myc activity by decreasing cell viability and modulating cellular signaling. In fact, our results

demonstrate the involvement of signaling cascades related to cell proliferation and survival, including β-catenin and Akt-mTOR pathways. The outcomes of this study suggest that the inhibition of c-Myc exerted by Omomyc peptide reduces significantly cell growth and migration. Therefore, it represents a promising strategy in OS treatment.

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ALTERATION OF SUBMUCOSAL AND MYENTERIC GANGLIA IN SEVERE GUT DYSMOTILITY: A QUANTITATIVE MORPHOMETRIC ANALYSIS

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Severe gut dysmotility (SD) is marked by impaired gut propulsion and distinct histopathological features, including fewer myenteric and submucosal neurons per ganglion and increased interganglionic distance. However, neurons counting remains complex and time-consuming, even with simplified protocols. The aim of this study is therefore to assess whether the number of ganglia in the submucosal and myenteric plexuses may serve as practical and discriminative indices for distinguishing patients from controls and supporting the diagnosis. The study included 39 patients and 8 controls. Patients were analyzed as a group or by histochemical phenotype: apparently normal (AN), inflammatory neuromyopathy (INF), and degenerative neuromyopathy (DEG). Formalin-fixed, paraffin embedded jejunal sections were immunolabelled for Neuron Specific Enolase (NSE) as pan neuronal marker. Following the neuromuscular ridge from right to left, all consecutive fields containing the myenteric plexus (≥8 fields) were analyzed and normalized to the plexus length (in mm), while all fields including the submucosal plexus were analyzed and normalized to the area of submucosa examined. The analysis of myenteric ganglia revealed a significant reduction in the number of ganglia per mm of myenteric ridge in SD patients (p = 0.0002), with 87% of patients falling below the threshold defined by the lowest value observed in controls. This reduction remained consistent across histopathological subgroups (AN: p = 0.0032; INF: p =0.0145; and DEG: p =0.0004). Although the analysis of the submucosal plexus revealed a significant reduction in the number of ganglia per mm² of submucosa in SD patients (p = 0.0410), only 26% of cases fell below the threshold defined by the lowest value observed in controls. This reduction remained consistent only in the AN and DEG subgroups. While requiring further validation, this study provides proof of concept for a new diagnostic algorithm for SD, based on the stepwise assessment of morphometric indices, with a focus on the myenteric plexus. Future analyses will consider ganglion volume, sex-related differences, and will expand the investigation from the jejunum to the colon.

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THE EFFECTS OF NICOTINIC ACETYLCHOLINE RECEPTOR INHIBITION ON THE EXPRESSION OF BRAIN DERIVED NEUROTROPHIC FACTOR IN THE OLFACTORY BULB ORGANOTYPIC SLICE CULTURES

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Olfactory bulb (OB) exhibits high levels of nicotinic acetylcholine receptor (nAChR) expression and receive strong cholinergic input from the basal forebrain. Acetylcholine is important modulator in olfactory related memory. It has been suggested that Ach and nicotinic acetylcholine receptor may have an important role in odor discrimination and modulation of smell memory. Demonstration of the role of BDNF signaling in olfactory bulb neurogenesis provided the first link between disruptions in neurotrophin signaling and olfactory behavioural performance. Impaired olfactory function appears to be one of the earliest detectable functional alterations in Alzheimer disease[1,2]. Methods In the study; organotypic olfactory bulb slice cultures, which allows to obtain results closest to *in vivo* models by protecting tissue architecture and microenvironment were generated. Specific nAChR a7 antagonist methylcaconitine (MLA) was applied to the organotypic slices. Effects of these treatments on Brain derived neurotrophic factor (BDNF) and nAChR a7 receptor expressions, were determined at protein level by Western blot method. Regional localizations of these proteins in the olfactory bulb were also examined by immunofluorescence method. Results it was shown that the application of MLA, caused a decrease in nAChR α7 expression in the olfactory bulb. Low-dose MLA application in olfactory bulb, caused a decrease in BDNF protein levels at the early period. Our findings indicate that Acetylcholine through its receptor could have direct or indirect outcome on synaptic plasticity by regulating protein expression levels of nAChR a7 and BDNF by a dose and time dependent manner. Our immunohistochemical results showed that nAChR α7 is concentrated in the glomerular layer, outer plexiform and inner plexiform layers in the olfactory bulb. BDNF is located in the olfactory neuron layer and glomerular layer. Conclusion: Our results indicated that cholinergic activity through nAChR α7 receptor may contribute to the maintenance of synaptic plasticity in the olfactory bulb.

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EVALUATION OF SPEXIN AND KISSPEPTIN EXPRESSIONS IN ADIPOSE TISSUES OF WISTAR ALBINO RATS DURING PUBERTY

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Spexin (SPX) and Kisspeptin (KISS) are peptides implicated in regulating metabolism and reproduction. White adipose tissue (WAT) and brown adipose tissue (BAT) play distinct roles in lipid metabolism, with BAT activity potentially increasing during adolescence. This study investigates the relationship between pubertal status and SPX/KISS expression in female rat adipose tissues. To evaluate SPX and KISS expression levels in serum, WAT, and BAT of female Wistar Albino rats in relation to pubertal status. Serum and adipose tissue samples were collected from prepubertal (n=10, postnatal day 28) and pubertal (n=10, postnatal day 42) female Wistar rats. Serum was analyzed for SPX, KISS, glucose and insulin. Homeostasis-model assessment-insulin resistance (HOMA-IR) was calculated. SPX and KISS protein expression in WAT and BAT was assessed using immunohistochemistry and quantified with ImageJ. SPX and KISS1 mRNA expression in WAT and BAT were analyzed using qRT-PCR. Statistical significance was defined as $p<0.05$. The weight-to-birth weight ratio and HOMA-IR were significantly higher in pubertal rats ($p<0.001$ and $p=0.018$, respectively). Serum SPX levels were significantly lower in pubertal rats ($p=0.029$), while KISS1 showed a decreasing trend ($p=0.07$). SPX staining intensity was significantly reduced in WAT ($p=0.019$) and significantly increased in BAT ($p<0.001$) of pubertal female rats. SPX mRNA expression was lower in WAT ($p=0.047$) of pubertal rats. KISS1 mRNA expression also decreased, though not significantly ($p=0.06$). Puberty in female rats is associated with decreased serum SPX, decreased SPX expression in WAT, and increased SPX expression in BAT. Increased BAT SPX expression during puberty may relate to reported changes in BAT volume during this developmental stage.

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REGENERATIVE MEDICINE, REHABILITATION AND CYTO-HISTOCHEMISTRY: RECENT EVALUATIONS OF INTEGRATED TREATMENTS

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Regenerative medicine is a rapidly evolving field that focuses on repairing, replacing, or regenerating damaged tissues and organs. Recent advancements in this field have shown promising results in various areas, including tissue engineering and rehabilitation. The Italian Academy of Bio-Regenerative Medicine (BIORI) is a group of health Italian operators, about a hundred all over Italy, involved in developing all the new science regenerative technologies. We also are involved in using and promoting many less-invasive, surgical approaches or non-surgical regenerative orthopedics. The current experiences and guidelines, published on the best international papers, are currently applied in our daily work following the Italian rules and laws. What is interventional non-surgical regenerative orthopedics? It represents an innovative, natural, and highly successful alternative to common medical approaches to conditions that would otherwise require surgery. This exciting field opens opportunities to treat orthopedic or musculoskeletal injuries, arthritis, and other degenerative conditions by using a tissue preservation strategy that uses the body's own healing cells to help restore more normal function to the human body. Regenerative orthopedic procedures encourage your body to heal and strengthen the damaged tissue. Advanced treatment protocols concentrate your own platelets, growth factors, and stem cells and deliver them precisely (under image guidance) into the site of your injury to support and promote natural healing.

Applications in Rehabilitation involved:

- nerve Regeneration: researchers are exploring the use of biomaterials and tissue engineering strategies to promote nerve regeneration. Studies have shown promising results in using nerve conduits and scaffolds to support nerve growth and repair.
- muscle Regeneration: decellularized extracellular matrices (dECM) are being used to promote muscle regeneration. Researchers have shown that dECM can support muscle cell growth and differentiation, making it a promising tool for muscle tissue engineering.
- bone Regeneration: biomaterials like hydroxyapatite and bioactive glasses are being used to create scaffolds for bone regeneration. These scaffolds can promote bone growth and osteointegration, making them suitable for orthopedic applications.

In the field of tissue engineering cyto-histochemistry plays a crucial role in tissue engineering, allowing researchers to analyze tissue structure and cell behavior. This knowledge can be used to develop more effective tissue engineering strategies. Moreover, understanding cellular response to biomaterials and tissue engineering strategies is essential for developing effective regenerative medicine treatments. Researchers are using cyto-histochemistry to study cellular behavior and optimize treatment outcomes. Overall, regenerative medicine, rehabilitation, and cyto-histochemistry are interconnected fields that hold great promise for improving human health. Ongoing research and advancements in these areas are likely to lead to innovative treatments and therapies for a range of diseases and conditions.