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XXXIX Congress of the Italian Society of Histochemistry

14-16 June 2023

Hotel Therasia Resort
Vulcano Island, Italy

President of the Italian Society of Histochemistry
Roberta Di Pietro

President of the Congress
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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.

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- biology of cell development and senescence;
- nerve and muscle cell biology;
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KEYNOTE SPEAKERS

CHIMERIC ANTIGEN RECEPTOR (CAR) POSITIVE EXTRACELLULAR VESICLES AS *IN VIVO* BIOMARKERS OF CAR-T CELL ACTIVITY

M. Bonafè[1,2], G. Storci[1], F. De Felice[1,2], F. Ricci[1], S. Santi[3,4], N. Laprovitera[1], M. Dicataldo[1], N. S. Bertuccio[1], D. Messelodi[1], L. Rossini[2], S. De Matteis[1], B. Casadei[1,2], F. Vaglio[1], M. Ursi[1,2], F. Barbato[1,2], M. Arpinati[1], E. Maffini[1], E. Tomassini[1], M. Naddeo[2], P. Tazzari[1], E. Dan[1], B. Sinigaglia[1], P. Garagnani[1,2], K.M. Kwiatkowska[1], M. Ferracin[1,2], P. L. Zinzani[1,2], F. Bonifazi[1]

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Chimeric antigen receptor (CAR) T cell therapy has profoundly changed the treatment of therapy-resistant B-cell neoplasms.[1] The pharmacokinetic assessment of such 'living drugs' represents a conundrum. Currently, CAR-T cells activity is monitored by non-specific plasma biomarkers (*e.g.*, cytokines) or by imaging (*e.g.* CT/PET scans) that falls short to reach resolution limits suitable for detecting the few millions of CAR-T cells that can actually reach the target tissue. Based on these premises, and on the fact that in the clinical practice, tissues other than plasma are unlikely to be routinely available, we reasoned that the measurement of extracellular CAR protein in the biofluids may be a suitable *in vivo* biomarker for CAR-T cells activity.[3] Following this reasoning, we assessed whether a compartment within the CAR-T cells extracellular vesicles (EVs) milieu carries the CAR protein. Using various methodologies (ultracentrifugation, size exclusion chromatography, antigen-specific pull down) we purified a population of EVs that tested positive for the CAR antigen by multicolour flow cytometry and by high resolution imaging by Stochastic Optical Reconstruction Microscopy. We then measured CAR+EV in CAR-T patients' biofluids and we observed that CAR+EV kinetics is a novel biomarker for monitoring *in vivo* CAR-T activity.

References

1. Maude S et al. Engl J Med 2014;371:1507-17.
2. Pasqui DM et al. Eur J Haematol 2022;109:601-18.
3. Fu W et al. Nat Commun 2019;10:4355.

CELLULAR INTERACTIONS IN THE CNS HISTOLOGICAL MICRODOMAINS: TOWARD A DYNAMIC 3D NEUROANATOMY

L. Calzà[1], V.A. Baldassarro[2], M. Moretti[2], L. Zanella[2], C. Quadalti[1], L. Lorenzini[2], L. Giardino[2]

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Molecular neuroanatomy defined new histological subdomains in the central nervous system, as the neurovascular/gliovascular unit (NVU) and the stem cell "niche". The NVU is the structural interface for the neurovascular functional coupling; the stem cell "niches" are histological subdomains where endogenous stem/progenitor cells reside in the adult brain. Both structures have a capillary vessel around which several cell types are organized and dynamically regulated by microenvironmental factors. Our laboratory is involved in the study of these histological domains in pathological condition, to try to establish if structural alterations are causal to or a consequence of diseases. We used a mouse model of Alzheimer disease (Tg2576, Sw-hAPP transgene) in which amyloid plaque deposition starts at 7-8 months of age, but cognitive defects appear at 3-5 months. We described age-dependent structural, cellular, and molecular alterations of the NVU and the SVZ niche, occurring before the appearance of amyloid plaques, that could explain the early learning and memory impairment.[1] In particular: (i) gene expression analysis of neural stem cells derived from the SVZ revealed that genes associated to the neurovascular coupling are differentially expressed in Tg2576 compared to age-matching WT mice.[2] (ii) In hypoxia, APPSwe neurons display higher cell death and mitochondrial depolarization.[3] (iii) The age-related histological structure and molecular phenotype of the NVU in the cerebral cortex differs in Tg2576 and age-matched wild-type (WT) mice, by capillary density and capillary/astrocyte interface.[4] Moreover, age-related expression levels of hypoxia-related genes is impaired in Tg2576. These results support a causal role of NVU structural and molecular alteration in shifting presymptomatic to symptomatic AD in Tg2576 mice. We then moved to 3D light sheet microscopy on tissue cleared brain, to map region- and age-specific capillary net alterations.

References

1. Baldassarro VA et al. The neuroscience of Dementia, Elsevier Inc 2020:407-22.
2. Baldassarro VA et al. WJSC 2013;5:229-37.
3. Baldassarro VA et al. DMM 2017;10:671-8.
4. Giuliani A et al. JNEN 2019;78:101-12.

HISTOCHEMISTRY AND ELECTRON MICROSCOPY OF THE TRANSDIFFERENTIATION OF THE ADIPOSE ORGAN

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Obesity is classified among the top ten causes of death. This disease is widespread in the world and in Italy it affects about 25% of adults and about 12% of children.[1] The affected organ in this disease is the adipose organ.[2] The tissues composing this organ are white (WAT) and brown (BAT) adipose tissues. The gross anatomy in mice and humans shows that the adipose organ is dissectible and composed by subcutaneous and visceral parts coloured white in WAT areas and brown in BAT areas. WAT stores fat to allow survival in the intervals between meals. BAT burns fat for thermogenesis to allow survival in environments below 37°C.[3] WAT can convert to BAT when chronic cold exposure requires more thermogenesis. BAT can convert into WAT when there is a chronic positive energy balance. In females, during pregnancy subcutaneous WAT and BAT convert into epithelial cells able to produce milk to allow pups survival through lactation. The term adipocyte defines a cell rich in fat. The epithelial cells producing milk are rich in fat, thus we call them pink adipocytes because the colour of the adipose organ during pregnancy is pink.[4] Thus, in the adipose organ reversible trans differentiation occurs among white, brown, and pink adipocytes.

During chronic positive energy balance white adipocytes become hypertrophic and altered organelles activate the inflammasome system inducing death of adipocytes by pyroptosis. Debris of dead adipocytes is gigantic and requires chronic activity of macrophages. During their activity this cell type produces citokines with secondary negative effects on the activity of insulin receptors with a series of consequences ultimately inducing type2 diabetes, Alzheimer and cancer.[5]

Electron microscopy and histochemistry played a key role in all the above-described discoveries.

References

1. World Health Organization 2019
2. Cinti S Rev Endocr Metab Disord 2022;23(1):1-4.
3. Cannon B et al. Physiol Rev. 2004;84(1):277-359.
4. Cinti S Trends Endocrinol Metab 2018;29(9):651-66.
5. Giordano A et al. J Lipid Res 2013;54(9):2423-36.

NUCLEAR PHOSPHOINOSITIDES - NEW PLAYERS IN REGULATION OF GENE EXPRESSION

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Processes such as gene expression or DNA repair are compartmentalised within eukaryotic nucleus, and the nuclear environment contains dynamic membrane-less sub-compartments whose formation is prevalently driven by phase separation. Formation of phase boundaries provides the surface for spatiotemporal control contributing to the high-rate kinetics of crucial processes such as transcription, ribosome maturation, and splicing. Our laboratory discovered 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 transcription regulatory proteins 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 bioinformatic analysis of putative NLIs proteins revealed that the most 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 most of these proteins contain K/R rich motifs, which were previously shown as recognition sites for phosphoinositide (PIPs) binding. We hypothesise 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.

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EXPLORING AND EXPLOITING THE TUMOR MICROENVIRONMENT TO REINVIGORATE ANTITUMOR IMMUNITY

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Tumor cells stimulate molecular, cellular, and physical changes within their host tissues creating a tumor microenvironment (TME), a complex and continuously evolving structure which supports tumor growth and progression. Its composition varies between tumor types, but hallmark features include accumulation of immune cells, stromal cells, blood vessels, and extracellular matrix. Antitumor immunity is inhibited or eluded by tumor-secreted factors that reprogram infiltrating myeloid cells and create the immunosuppressive TME. Advancements of single-cell techniques provide powerful means to systematically profile the multiple-omic status of the TME at a single-cell resolution, revealing the phenotypes and functionalities of disease-specific cell populations. We employed single-cell RNA and protein sequencing (CITEseq) and spatial transcriptomics to identify the functional diversity and localization of immune cells into the TME of experimental malignant brain tumors. Infiltrating monocytes were found to be proinflammatory and express interferon signature but after reaching TME they transformed into pro tumor, immunosuppressive macrophages. This transition was coupled with a phenotypic switch from the IFN-related to antigen-presentation and tumor-supportive gene expression. Monocyte-derived dendritic cells did not mature in the TME. Computational analyses revealed interactions between tumor, myeloid cells and lymphocytes and pointed to some factors responsible for tumor-induced reprogramming of immune cells. The emerging pathways have been blocked with innovative peptides or siRNA and the effects of therapeutic interventions on TME reprogramming and antitumor immunity have been assessed. The results shed light on the pathogenic mechanisms and dysfunctions of antitumor immunity in brain tumors at unprecedented resolution. Reversing the immunosuppressive TME reprogramming is an effective way to push immunotherapy into action.

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ORGANOIDS AS MODELS OF HUMAN DISEASES

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To explore new models that can recapitulate the architecture and function of human organs more and more accurately, the past decade has seen an explosion in the field of *in vitro* disease modeling, in particular the development of organoids. In our lab, we explored the possibility to replace severely damaged organs, such as the pancreas in type 1 diabetes patients, using human pancreas organoids (hPO), as well as using lung organoids (hLO) as a model to investigate the biological effects associated with microplastic exposure. Starting from discarded pancreatic tissues, we developed a large-scale process for obtaining clinically relevant quantities of undifferentiated organoids. Successively, we characterized hPO, observing consistent cell growth, with genetic stability, expression of pancreatic markers, and epithelial organization. Next, we identified hPO cell types using single-cell RNA sequencing and we demonstrated the univocal ductal nature of hPO. Regarding the lung project, we generated hLO from human lung tissue-resident adult stem cells as a respiratory model. They were organized in a polarized pseudostratified epithelium with a central hollow lumen, resulting positive for basal (KRT5), ciliated (AC-TUB), goblet (Mucin), alveolar epithelial type I (SFTPB) and club (SCGB1A1) cell markers. Confocal 3D reconstruction confirmed that the hLO contained well differentiated cells, showing the presence of ciliated cells and non-ciliated cells, including club cells (identified by the presence of microvilli). After an extensive characterization, hLO were exposed to fibers from synthetic clothes and fabrics as a major source of airborne microplastics, that were released by the filter of a household dryer. While the presence of microplastics did not inhibit organoid growth, we observed a significant reduction of SCGB1A1 gene expression. SCGB1A1 has frequently been used as a biomarker to monitor lung injury caused by various diseases or environmental exposures and its decrease has been consistently observed to be associated with airway anti-inflammatory diseases. In summary, we proposed two innovative 3D models as novel platforms for future studies of pancreas and lung health and disease, and for more effective development of new drugs.

THE TICKING CLOCK: CIRCADIAN RHYTHM IN PREGNANCY AND DEVELOPMENT

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The circadian rhythm plays an essential role in regulating various physiological processes, including sleep-wake cycles, hormonal secretion, and metabolism. Our modern society operates 24/7, with many individuals experiencing circadian rhythm disruptions due to shift work or travel across time zones. These disruptions can have negative effects on pregnant women, resulting in maternal circadian rhythm disruption (MCRD) during gestation, which has been shown to cause intrauterine growth restriction (IUGR) in 3-7% of all births. Emerging evidence suggests that IUGR babies are more likely to develop neuropsychiatric and cardiovascular disorders in adulthood. Furthermore, it has been observed that the foetal circadian clock starts to develop as early as 8 weeks post-conception and is influenced by the mother's circadian rhythms. In recent years, there has been increasing interest in understanding the impact of circadian rhythms on pregnancy and foetal development. Despite the challenges in differentiating the effects of circadian rhythm on pregnancy outcomes in clinical practice, such distinctions remain crucial in many medical settings, as the identification and targeting of the underlying pathophysiology is a primary objective. Therefore, research efforts should prioritise controlled *in vivo* animal studies and human placenta organoid studies that integrate gene editing technologies and high-throughput analysis to address gaps in understanding the impact of MCRD on placental and fetal development. The knowledge gained from such research can then be translated into clinical practice.

References

1. Nakamura TJ, Takasu NN J Obstet Gynaecol Res 2018;44:12-9.
2. Voiculescu SE et al. Chronobiology Int 2020;1:1-20.
3. Cai C et al. Am J Obstet Gynecol 2019;6:563-76.
4. Ono M et al. Int J Mol Sci 2023; 24:1545.
5. Mark PJ et al. J Biol Rhythms 2017;32:534-49.

ARTIFICIAL INTELLIGENCE IN HEALTHCARE: FROM CLOUD TO BEDSIDE

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The recognition of the relevance of individual variabilities in prognostic prediction and therapeutic response, and the awareness that disease and patient are a single inseparable unit, are the basis of the so-called precision or personalized medicine. Despite the enormous amount of data available and the possibility of analyzing them using artificial intelligence (AI), the AI models currently available for daily clinical practice are still very few and largely linked to diagnostic imaging. Based on these needs a strategic agreement between UniSR, HSR, and Microsoft (MSFT), was established for the development of an interoperability platform in the field of oncology (AI-HOPE, Artificial - Intelligence Oncology Patients Suite), based on cloud, able to progressively integrate all the health data generated in HSR, to develop predictive AI models, in order to guide the personalization of care in oncology. and in perspective also towards other areas (cardiovascular, neurological, metabolic). Here we will describe the basis of the AI applied to healthcare, the strategy and the IT structure behind the development of the interoperability platform, and the first results obtained on three use-cases, *i.e.* Covid-19, Non Small Cell Lung Cancer (PD-L1 positive), and Kidney cancer.

ADIPOCYTE-MYOCYTE CROSSTALK AND MUSCLE QUALITY IN SARCOPENIC OBESITY

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The prevalence of sarcopenic obesity (SO), defined as the co-existence of excess adiposity and low muscle mass and function increases with age and weight gain. The pathogenesis of SO is complex and multifactorial. Gain in white adipose tissue (WAT) may represent an independent determinant for the development of loss and dysfunction of muscle mass. In addition, a decline in muscle mass may facilitate fat accumulation. SO is more frequently present in older adults, particularly because of age-related changes in body composition (*i.e.*, decline in muscle mass, WAT redistribution and ectopic fat deposition). Ectopic fat deposition in muscle, liver, pancreas, and heart, may contribute to the dysfunction of these organs. With aging WAT also becomes dysfunctional, showing an increased profile of proinflammatory adipokines produced by adipose cells, greater infiltration of inflammatory cells, and preadipocytes and adipocyte incompetence, all together strongly related to muscle mass quality and function decline. The crosstalk between age-related dysfunctional WAT and muscle cells is one of the mechanisms leading to SO. However, prevention strategies aimed to reduce the occurrence of SO by correcting crosstalk between muscle and adipose cells are needed because SO has consistently been demonstrated to be a strong and independent risk factor for frailty, metabolic disorders, hospitalization, and mortality in the older population.

References

1. Cappellari G et al. Clin Nutr 2023;42(5):687-99.
2. Zamboni M et al. Cells 2022;25;11(21):3361.
3. Correa-De-Araujo R et al. Front Physiol 2022;4;13:847883.
4. Zamboni M, et al. Exp Gerontol 2021;143:111162.

INVITED SPEAKERS

MICROPLASTICS AND ASSOCIATED CONTAMINANTS: POTENTIAL IMPACT ON CELLS AND DEVELOPING ORGANISMS

P. Bovolin[1], A. Saraceni[1,2], E. Cottone[1], B. Mognetti[1], M.P. Gallo[1], G. Merlo[2], F. Dal Bello[2]

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Differently from macroplastics, the effects of microplastics (MPs) on living organisms are still largely uncharacterized. It has been proposed that MPs can interact with multiple chemicals in the environment, many of which may act as Endocrine Disrupting Chemicals (EDCs), with deleterious consequences especially on developing organisms. Previous studies in our laboratory investigated the biological impact of MPs collected from seawater.[1] However, experimental studies with environmental MPs pose technical challenges mainly due to plastics heterogeneity. To overcome this issue, in the present work we employed polystyrene MPs of specific sizes (5-0.5 µm), surface chemistry (virgin and –COOH functionalized, the latter to mimic environmentally aged MPs) and toxicant absorption (pristine and Bisphenol A-sorbed). We tested the impact of MPs exposure on early stages of developing zebrafish (zf) larvae and on several mammalian cell models, including liver, pre-adipocyte, and endothelial cell lines. MPs were internalized by cultured cells and ingested by zf larvae, accumulating in the intestine. *In vitro* cytotoxic effects were observed only in cells exposed to very high concentrations of functionalized MPs. In zf larvae, survival, hatching, and heartbeat were monitored until 6 dpf; MPs alone did not lead to macroscopic deleterious effects on developing zf. The adsorption of Bisphenol A (BPA) on MPs was tested using a UHPLC-tandem MS method. Following 24 h incubation, the adsorption yield of BPA (25 µM) on MPs was about 50%. The exposure to MPs preadsorbed with non-toxic concentrations of BPA had no toxic effects. We are currently testing further biological effects, including the induction of oxidative stress, lipid accumulation, and alterations in gene expression. Collectively, our results suggest that polystyrene MPs can be ingested by zf larvae and are internalized by cultured cells, carrying adsorbed contaminants such as BPA, with potential developmental and metabolic effects on cells and organisms.

Reference

1. Capriotti M et al. Chemospere 2021;263:128090.

FROM DIAGNOSTIC WORK-UP TO PATHOGENETIC MECHANISMS: AN INTEGRATE MORPHO-FUNCTIONAL APPROACH

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Postgenomics is rapidly changing the health industry in such a way that it allows for the rapid implementation of predictive and personalized medicine. The secular paradigm «one drug fits all» is wrong. The new sequencing technologies allow us to move towards what is today called P4 medicine: Predictive, Preventive, Personalized and Participatory. Utilizing molecular analyses of the genome it is possible to classify patients into subgroups related to morpho-functional features obtained by different techniques and methods. In this way, for example, different groups of patients can be identified: those who will not respond to a treatment, those for whom the treatment will be toxic, and finally those who will tolerate the treatment. The ability to identify biomarkers for the stratification of patients represents the next great challenge in the race to improve the quality of treatment and realize precision medicine.[1] This will be possible due to newest instrumental technologies and specimen preparation techniques.[2] Liquid biopsy would be defined as obtaining circulating cancer cells, tumor-derived cell free DNA (cfDNA) or other compounds in body fluids such as microvesicles and exosomes, collectively referred to extracellular vesicles, submicron-sized lipid containers released by cells. Genetic disease study must be developed following many steps which consist by identification of the disease-causing gene till the definition of the molecular pathways which are the basis of the pathogenetic mechanism allowing the new therapeutic approach. Electron microscopy can play a key role in the definition of these mechanisms aimed at showing the subcellular alterations due to gene abnormalities.[3,4] One of the most recent applications of this role has been defined during the SARS CoV2 pandemic to understand the specific infectious mechanism and tissues alterations.[5]

References

1. Sharma M et al. Int J Mol Sci 2020;28;21:8018
2. de Boer P et al. Nat Methods 2015;12:503-13
3. Pacilio S et al. Bioengineering (Basel) 2023;10(2):239
4. Costa R et al. Front Neurol 2022;13:840683
5. Fabbri VP et al. Biomolecules 2022;12:629

NUCLEAR INOSITIDE SIGNALLING IN MYELODYSPLASTIC SYNDROMES

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Phosphoinositide-specific Phospholipase C (PI-PLCs) are involved in the phosphatidylinositol (PI) metabolism. The nuclear PI cycle, independent of the cytoplasmic one, is critical in nuclear function

control,[1] and specific inositide molecules are localised within the nuclear speckles, *i.e.* cellular domains regulating gene expression and splicing processes.[2] Therefore, nuclear PIs, and particularly nuclear PI-PLCbeta1, can affect cell cycle, proliferation, differentiation, gene expression and other dynamics. Nuclear inositide signalling pathways regulate proliferation and differentiation of hematopoietic stem cells (HSCs). Therefore, PI-PLCbeta1, PI-PLCgamma1, PI-PLCgamma2 and the PI3K/Akt/mTOR pathway play essential roles in the pathogenesis of Myelodysplastic Syndromes (MDS), a series of hematopoietic malignancies characterised by dysplastic HSCs that can evolve into aggressive forms of Acute Myeloid Leukaemia (AML). Drug-induced modulation of nuclear inositide signalling was associated with cell proliferation and differentiation in MDS.[3] Moreover, MDS losing or lacking response to epigenetic therapy, thus showing an increased blast proliferation, can acquire common mutations on 3 inositide-specific genes (PLCG2, AKT3, PI3KCD).[4] Interestingly, the same refractory patients also showed specific microRNA downregulation.[5] Indeed, high levels of miR-192-5p, that can be regulated by inositides and specifically targets and inhibits BCL2, were associated with higher overall/leukaemia-free survival in responder MDS. All in all, the deep analysis of nuclear inositide pathways in MDS may disclose the molecular mechanisms underlying normal HSC proliferation and differentiation, as well as lead to the identification and development of new targeted therapies.

References

1. Manzoli L et al. Prog Lipid Res 2005;44:185-206.
2. Martelli AM et al. Histol Histopathol 2005;20:1251-60.
3. De Stefano A et al. Adv Biol Regul 2023;87:100955.
4. Follo MY et al. Leukemia 2019;33:2276-90.
5. Mongiorgi S et al. Clin Epigenetics 2023;20;15-27.

CHIMERIC ANIMALS: MICE WITH HUMANIZED LIVERS TO TEST DRUGS OR EVALUATE NOVEL CELL AND GENE THERAPIES

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Recent advances in preclinical models have allowed the creation of animals with genetic alterations allowing human hepatocyte engraftment and repopulation. Over time, the loss of native (murine) hepatocytes is replaced by human cells leading to a complete "humanization" of the liver. Such chimeric mice have served as final proof in preclinical studies, where gene therapies (by CrispR-Cas technology) have been tested to correct congenital disorders, as well as for validation of stem and progenitor cell therapies. Our laboratory has pioneered the use of cell therapy to treat patients with liver diseases. However, for preclinical studies, many small animal models of monogenetic liver disease do not faithfully recreate the phenotype observed in human patients. Using special FAH- and immune-deficient mice (FRGN), we have generated new human-relevant models. Hepatocytes were isolated from normal donors or from patients who received a liver transplant due to inborn errors of metabolism. Isolated human hepatocytes were transplanted into the liver of FRGN mice, replacing 85-95% of the mouse hepatocytes with human hepatocytes, as quantified by plasma human albumin levels. Mice repopulated with normal hepatocytes displayed normal hepatic functions including ammonia levels, while mice repopulated with hepatocytes deficient for one specific liver enzyme displayed increased basal levels of ammonia or other systemic alterations characteristics of donor disease. Such new preclinical models are extremely useful for investigations of the disease process, *in vivo*, and for possible corrective interventions such as gene or cellular therapies. When the strengths and weaknesses of these humanized mouse models are fully understood, they will likely be quite valuable for investigations of human liver-mediated metabolism and excretion of drugs and xenobiotics, patient-specific pharmacological effects, and short- and long-term investigation of the toxicity of drugs or chemicals with significant human exposure. Finally, the effective and functional maturation of stem/progenitor cells isolated or engineered using the most advanced techniques has in this model the final validation.

CAVEOLIN-1 IMPACT ON VESICULAR SECRETOME IN A MODEL OF RHABDOMYOSARCOMA

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Caveolin-1 (CAV-1) is an integral membrane protein required to generate caveolae and cholesterol-enriched lipid rafts of the plasma membrane.[1] It is widely accepted that loss of CAV-1 correlates with early-stage tumor progression, while its overexpression and phosphorylation are associated with metastatic disease.[2] A considerable body of evidence suggests that extracellular CAV-1 may be relevant in cancer cell metastasis.[3] The present work aims to investigate if the increased aggressiveness of RD cells overexpressing CAV-1 correlates with an altered extracellular vesicle (EV) release. The obtained data show that RD-CAV1 cells release more EVs than RD-Ctrl cells. Western Blot and proteomic analyses highlighted that EVs exhibit the exosomal markers Alix, Flot-1, Syntenin-1, and TSG101; however, the tetraspanins CD63, CD81, and CD9 were expressed at very low levels in RD-CAV-1 EVs, which instead were detected in RD-Ctrl EVs, suggesting that CAV-1 overexpression induces an alteration of the EV biogenesis and cargo. Moreover, the treatment of HUVEC with RD-CAV-1 EVs showed an increase in cell proliferation and migration, both in a dose-dependent manner. Altogether, the reported data suggest that, in addition to its well-established structural role, CAV-1 is a key regulatory factor potentially involved in remodelling the tumor microenvironment by stimulating the release of EVs deeply altered in protein composition.

References

1. Cohen AW et al. Physiol Rev 2004;84:1341.
2. Codenotti S et al. Cancer Lett 2019;449:135.
3. Campos A et al. Biomolecules 2019;9:31.

ADIPOSE TISSUE DYSFUNCTION IN LAMINOPATHIES AND AGEING

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Adipose tissue dysfunction is a common feature of many diseases including, among others, obesity, Cushing syndrome, metabolic syndrome, progeroid syndromes and numerous rare inherited diseases collectively known as lipodystrophies.[1] A subgroup of lipodystrophies is referred to as lipodystrophic laminopathies as they are caused by mutations in the nuclear lamins (lamin A/C or lamin B) or related nuclear envelope proteins[2]. Adipose tissue atrophy is also a common feature of very old patients, even in the absence of major pathologies, and it is often associated with sarcopenia and cachexia[2]. Despite different phenotypes such as peripheral adipose tissue loss or accumulation in some districts, presence or absence of diabetes and serum triglyceride abnormalities, adipose tissue dysfunction is characterised in almost all cases by an associated metabolic disorder and inflammatory processes.[2,3] However, the pathomechanism(s) leading to altered adipose tissue homeostasis is poorly understood. We discovered that a main pathway leading to lipodystrophy originates from altered lamin A/prelamin A interplay with associated nuclear membrane proteins[4] and/or nuclear receptors[5,6] leading to aberrant trans-differentiation of white adipocyte precursors towards the brown lineage.[1,4,6] This condition impairs white adipose tissue turnover and metabolic function and activates inflammatory processes affecting different tissues and organs.[3,6]

References

1. Pellegrini et al. Exp Mol Med 2019;51:89.
2. Cenni et al. Ageing Res Rev 2020;62:1568-637.
3. Squarzoni et al. Aging Cell 2021;20: e13285
4. Czapiewski et al. Nature Comm 2022;13:321.
5. Capanni et al. Human Mol Genet 2005;62:2669-78.
6. Schena et al. Cells 2023, in preparation.

ULTRASTRUCTURAL INSIGHTS IN ASSISTED REPRODUCTION: THE OOCYTE PERSPECTIVE

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The positive outcome of Assisted Reproductive Technologies (ART), finalised to preserve/restore fertility in the human female, strictly depends on oocyte quality. Thus, oocyte structural preservation during ART, characterised by both completion of maturative changes and absence of degenerative alterations, is essential to the acquisition and maintenance of oocyte competence to fertilisation and guarantees – together with other factors – proper early embryo development. Our studies were aimed to define, evaluate, and compare structural and ultrastructural markers of quality in human oocytes subjected to different ART protocols such as cryopreservation and *in vitro* maturation, also emphasising the importance of patient's age when she faces ART (the fertility "age factor"). Our main results revealed that: a) different oocyte microdomains can be affected by the application of ART protocols, including the number and the position of cortical granules, the number and the dimensions of agglomerates between mitochondria and membranes of endoplasmic reticulum (mitochondria-smooth endoplasmic reticulum aggregates and mitochondria-vesicle complexes), and the incidence of vacuolization;[1-5] b) all these alterations could be worsened by aging.[6] In conclusion, further studies, including morphological ultrastructural analyses, are needed to improve ART outcomes by optimising and standardising the best protocol of oocyte acquirement, selection, and storage, to contrast infertility and increase perspectives of pregnancies in a greater number of couples.

References

1. Nottola SA et al. Reprod BioMed Online 2009;19 Suppl 3:17.
2. Coticchio G et al. J Assist Reprod Genet 2010;27:131.
3. Coticchio G et al. Mol Hum Reprod 2016;22:110.
4. Nottola SA et al. J Assist Reprod Genet 2016;33:1559.
5. De Santis L et al. Cryobiology 2020;95:143.
6. Bianchi S et al. J Assist Reprod Genet 2015;32:1343.

INVOLVEMENT OF AUTOPHAGIC-RELATED PROTEINS (ATGs) IN HUMAN BRAIN TUMOURS OF ASTROCYTIC LINEAGE

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Autophagy, a catabolic cellular process, that maintains cellular homeostasis through the degradation, elimination and recycling of damaged substrates such as organelles, macromolecules and misfolded proteins.[1,2] Activation of autophagy starts with the formation of the autophagosome, and the substrate degradation is controlled by several autophagy-related proteins (ATGs).[1,2] Autophagy exhibits a dual role in promoting or suppressing tumour initiation and growth in different types of cancer, including gliomas.[2] We reported the role of autophagic-related proteins in human brain gliomas, mainly of astrocytic lineage and also new developments in autophagy target-treatments. Taking into consideration the 2021 WHO classification of brain tumours, several studies have shown how autophagy acts as a tumour suppressor in gliomas and how its decreased activity is associated with high grade gliomas (HGGs) compared to low-grade gliomas (LGGs) that show a more sustained autophagic activity. Lower Beclin-1 and LC3-II expression has been reported in glioblastomas (GBMs) compared to LGGs;[3,4] conversely, high expression of Beclin-1 and LC3 is correlated with a better survival in GBM patients.[3] Moreover, the suppression of ULK1, ATG7 and ATG13 favours the reduction in tumour growth, while overexpression of LC3 and p62 are correlated with poor prognosis in high-grade glioma, as

well as an overexpression of ULK1/2 and TFEB[4]. Additionally, high levels of p62 and Dram1 have been reported to induce cell migration in GBMs and to be associated with poor prognosis in these tumours. Overexpression of LC3 and Beclin-1 are also associated with shorter survival in LGGs and HGGs.[3-4] High Atg4c levels have also been observed in gliomas, while the decrease in this protein is associated with apoptosis, autophagy inhibition, and a greater sensitivity to classical treatment by Temozolomide (TMZ). Some studies have shown that autophagy inhibition increases the cytotoxicity of chemo- and radiotherapy; by contrast, other reports suggested that the activation of autophagy can induce apoptosis and, consequently, the therapeutic efficacy of several treatments. New technologies should be applied in the contest of inhibition of autophagy in combination with TMZ, helping to select patients with responsible gliomas.

References

1. Yun CW, Lee SH Int J Mol Sci 2018;19:1-18.
2. Li X et al. Mol Cancer 2020;19:12.
3. Yun CW et al. Int J Mol Sci 2020;22:179.
4. Ieni A et al. Oncol Lett 2022;24:1-9.

THE CRUCIAL ROLE OF MAST CELLS IN CHRONIC INFLAMMATION AND TUMOR GROWTH

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Although mast cells (MCs) have been identified over 170 years ago, their physiological role in the body has remained a mystery. Since ancient times, mast cells have probably been part of protective mechanisms. Their original function, indeed, is to be found in parasite and bacterial defense of the host, and as a general inducer of inflammation. This early type of cell has differentiated toward a more complex cellular entity involved in different regulatory processes, such as immunomodulation, tissue repair and remodeling after injury, and angiogenesis. Mast cells are long-lived and survive in body tissues involved in innate and adaptive immunity. It is likely that a complex interplay between MCs and surrounding cells, mediators, and extracellular matrix proteins ensures the prolonged survival of tissue MCs. The selective placement of MCs near the vasculature may ensure that the release of MC-derived pro-inflammatory products has instantaneous effects on the endothelium. Mast cells have multiple roles extending beyond their classical role in IgE-mediated allergic reactions. Although MCs secrete many pro-inflammatory agents, they also release many anti-inflammatory agents. Mast cells can change from protective immune cells to potent pro-inflammatory cells which influence the progression of many pathological conditions, including autoimmune diseases and tumors. The role of MCs in tumor biology is controversial due to the variety of processes they are involved in enacting both pro- and anti-tumorigenic effects depending on the context. The accumulation of MCs in tumors was described by Ehrlich in his doctoral thesis. Since this early account, ample evidence has been provided highlighting the participation of MCs in the inflammatory reaction that occurs in many clinical and experimental tumor settings. The involvement of MCs in tumor development is debated. Although some evidence suggests that MCs can promote tumorigenesis and tumor progression, there are some clinical sets as well as experimental tumor models in which MCs seem to have functions that favor the host. One of the major issues linking MCs to cancer is the ability of these cells to release potent proangiogenic factors.

STEM CELLS FOR MUSCLE REPAIR AND 3D PATHOPHYSIOLOGICAL MODELS

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Cell-based regenerative therapies become a promising treatment for patients affected by muscle disorders, but also underline the need for reproducible results in preclinical and clinical studies for safety and efficacy.[1] Due to the poor engraftment, survival and differentiation of injected adult stem cells, researchers are now challenging pluripotent stem cell derivatives and exploring cell-free therapies. The latter comprise llama antibody-derived nanobodies and extracellular vesicles,[2] which could be used to deliver targeted drugs, including small noncoding RNAs[3] to muscle cells. However, the pathogenesis of many muscle disorders is still unclear since the available cellular and animal models are not fully recapitulating the human disease phenotype. Therefore, human engineered muscle models are instrumental for investigating muscle pathophysiology and testing novel therapeutic approaches. We generated cardiac organoids from Duchenne muscular dystrophy (DMD) patients using induced pluripotent stem cells and CRISPR/Cas9 gene editing system, showing that DMD-related cardiomyopathy and disease progression occur in the organoids upon long-term culture.[4] Despite displaying relevant functionalities, cardiac organoids show heterogeneity in the cyto architectures and cardiac cell sub-types and lack of the endocardium layer, where the pathological trabeculation remodelling occurs. It is therefore desirable to develop advanced 3D human cardiac models that adequately exhibit the disease phenotypes with high reproducibility at an anatomical and pathophysiologically relevant spatial resolution for testing novel therapeutics.[5]

References

1. Cossu G et al. Front Genet 2023;13:1056114.
2. Yedigaryan L, Sampaolesi M. Front Physiol 2023;14:1130063.
3. Yedigaryan L et al. Front Immunol 2022;13:977617.
4. Marini V et al. Front Cell Dev Biol. 2022;10:878311.
5. Sampaolesi M et al. Expert Opin Ther Targets 2023;27(2):91-5.

NOVEL PLAYERS THAT CONTROL MUSCLE MASS IN DISEASE

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The ability to activate compensatory mechanisms in response to environmental stress is an important factor for survival and maintenance of cellular functions. The systems that are often activated both in short and prolonged stress conditions are autophagy lysosome and ubiquitin proteasome systems. Autophagy is required to clear the cell from dysfunctional organelles and altered proteins and is reported to be involved in muscle wasting during cancer growth and age-related sarcopenia. The regulation of protein breakdown as well as protein synthesis is under the control of transcription factors belonging to different signaling pathways.[1] Different histochemistry and immunofluorescence approaches have been developed to monitor autophagy, myofiber innervation and signaling pathways that control muscle mass in catabolic conditions.[2] Here the last findings about novel genes as well as new cross talk between signaling pathways that control protein breakdown in cancer cachexia will be presented.[3]

References

1. Sartori R et al. Nat Commun. 2021;12(1):330.
2. Sartori R et al. Sci Transl Med 2021;13(605):eaay9592
3. Leduc-Gaudet JP et al. Nat Commun 2023;14(1):1199.

MESENCHYMAL STROMAL CELLS FROM THE AMNIOTIC MEMBRANE OF HUMAN TERM PLACENTA: POTENT TOOLS FOR REGENERATIVE MEDICINE

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For over 2 decades our research has provided greater clarity about the therapeutic properties of stromal cells from human term placenta. Term placenta is an appealing cell source because it is discarded after birth and considered biological waste; its procurement does not require an invasive procedure, nor does it pose ethical issues. Our studies have been aimed at understanding how mesenchymal stromal cells from the amniotic membrane (hAMSCs) contribute to tissue regeneration. We have shown that hAMSCs contribute to the resolution of inflammation that limits pro-inflammatory pathways and boosts mediators/cells responsible for tissue repair. Our *in vitro* studies demonstrate that hAMSC and their conditioned medium (CM) reduce T and B cell proliferation, enhance T regulatory and reduce Th1 and Th17 populations, inhibit the differentiation of monocyte-derived dendritic cells, induce macrophage differentiation toward M2 macrophages, and block of formation of antibody-secreting B cells. We have also shown that hAMSCs promote functional recovery when applied in preclinical models of inflammatory diseases such as lung and liver fibrosis, myocardial ischemia, autoimmune diseases, and traumatic brain injury. In addition, we show that macrophages generated in the presence of CM enhance wound healing in diabetic mice, and CM improves motor deficits, ameliorate brain pathology, and decrease microglia activation in mice with Huntington's disease. Collectively, these results contribute to the development of novel therapeutic strategies based on the ability of hAMSC to contribute to tissue regeneration by resolving inflammation. As we move forward, an improved understanding of the mechanisms of action will provide a solid basis so that hAMSC can be safely and efficiently used in patients.

ORAL COMMUNICATIONS

SUPER RESOLUTION TRACK DENSITY IMAGING: BRIDGING THE GAP BETWEEN *IN VIVO* BRAIN IMAGING AND HISTOLOGY

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The development of novel techniques for the *in vivo*, non-invasive visualisation and identification of brain structure has represented a major challenge for human neuroimaging research in the last decades. The present work aimed at describing a novel protocol for histologically guided delineation of the human thalamic and subthalamic area based on short-tracks track-density imaging (stTDI), which is an advanced imaging technique exploiting high angular resolution diffusion tractography to obtain super-resolved white matter maps.[1] In the present work, we devised an optimised tractography protocol for reproducible reconstruction of thalamic nuclei and tracts of the subthalamic area in a large data sample from the Human Connectome Project repository. First, we leveraged the super-resolution properties and high anatomical detail provided by short tracks track-density imaging (stTDI) to identify the white matter bundles of these regions on a group-level template.[2] Structure identification and manual segmentation on the stTDI template was also aided by visualisation of histological sections of human specimens. To reconstruct white matter bundles, we employed this anatomical information to drive tractography at the subject-level, optimising tracking parameters to maximise between-subject and within-subject similarities as well as anatomical accuracy. Finally, we gathered subject level nuclei as well as tractograms reconstructed with optimised tractography into a large-scale population atlas. We suggest that this atlas could be useful in both clinical anatomy and functional neurosurgery settings, to improve our understanding of the complex morphology of these important brain regions.

References

1. Calamante F et al. Neuroimage 2011;56:1259-66.
2. Basile GA et al. Cerebral Cortex 2021;31:5613-36.

NUCLEAR BEHAVIOUR IN HUMAN CHONDROCYTE DURING CHONDROPTOSIS: COMPARISON BETWEEN *IN VITRO* AND *IN VIVO* CONDITIONS

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Chondrocyte apoptosis is known to contribute to articular cartilage damage in osteoarthritis and is correlated to several cartilage disorders[1]. Micromass cultures[2] represent a convenient means for studying chondrocyte biology, and, in particular, mechanisms underlying their death. In the present study we focused our attention on the different kinds of the death of these cells, which show necrotic features and, occasionally, apoptotic ones, but usually undergo a new form of death called chondroptosis. Chondroptosis[3] has some features in common with classical apoptosis, such as cell shrinkage and vacuolization, chromatin condensation, and, not always, a controversial involvement of caspases. The most crucial peculiarity of chondroptosis relates to the ultimate elimination of cellular remnants. It may serve indeed, in the absence of inflammation, to delete cells in conditions in which phagocytosis would be difficult. This cell death mechanism is probably due to the unusual chondrocyte biology both *in vivo* and in micromass culture. In this study we describe and highlight the morpho-functional alterations appearing in chondrocyte nuclei during chondroptosis and we compare the alterations present in *in vitro* cells and, *in vivo*, in human articular cartilage and menisci.

References

1. Millucci L et al. Calcif Tissue Int 2017;101:50-64.
2. Battistelli M et al. Micros Res Tech 2005;67:86-95.
3. Salucci S et al. Cell and tissue Research 2022;389:159-70.
4. Battistelli M et al. Apoptosis 2014;19:1471-83.

NEW INSIGHTS ON AORTIC VALVE INTERSTITIAL CELL CALCIFICATION AS REVEALED BY von KOSSA SILVER REACTIONS ADAPTED TO ELECTRON MICROSCOPY

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The silver reaction introduced in 1901 by von Kossa (vKr) persists as a method of choice for the visualisation of calcium-binding sites in light microscopy, being based on phosphate- or carbonate-bound calcium ion replacing by silver ions and subsequent reduction to metallic silver.[1] Later, procedural adjustments were made to adapt vKr to electron microscopy.[2] In previous ultrastructural studies on aortic valve interstitial cell (AVIC) calcification,[3,4] a combined procedure consisting in vKr on semithin sections after pre-embedding phthalocyanine reaction on samples and before semithin re-embedding and conventional contrast of derived thin sections revealed major hydroxyapatite nucleators to consist in acidic phospholipid containing layers (PPLs) edging the degenerating AVICs. Here, analogous combined procedure was employed to assess whether ribosomal RNA (rRNA) and nuclear chromatin contribute to AVIC mineralization in *in vitro* pro-calcific cell cultures and *in vivo* aortic valve leaflets undergone experimental or pathological calcification. At early stages, mineralizing AVICs showed superimposition of metallic silver particles on both free and membrane-bound ribosomes. Subsequent melting of ribosomes with PPLs was observed, with the resulting pro-calcific substratum being additionally susceptible to decoration by anti-rRNA immunogold particles. Silver particle deposition onto nuclear chromatin was also found, in absence of apoptotic or oncotic cell death signs. In conclusion, the use of vKr so adapted to electron microscopy enabled the identification of rRNA and nuclear chromatin as additional sites of calcium salt nucleation during AVIC mineralization, providing more information on this type of pro-calcific cell death.

References

1. von Kossa J Beitr Path Anat 1901;29:163-202.
2. Danscher G Stain Technol 1983;58:365-72.
3. Ortolani F et al. Histol Histopathol 2003;18:1131-40.
4. Bonetti A et al. J Thorac Dis 2019;11:2126-43.

EFFECT OF VENETOCLAX AND AZACYTIDINE ON PLCs-DEPENDENT PATHWAYS IN HEMATOPOIETIC-TO-LEUKEMIC STEM CELLS

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Adding Venetoclax (VEN) to Azacytidine (AZA) is effective in Myelodysplastic Syndromes (MDS)[1]. Phospholipases C, mainly PLCβ1, are increased in AZA responders[2], where they can regulate apoptotic pathways. Preliminary data obtained in hematopoietic stem cells (HSCs), obtained from MDS patients treated with AZA and AZA+VEN, showed a decreased expression of BCL-2 and increased BAX in responders, as compared with HSCs at baseline. Stemming from these data, here we further investigated the molecular effect of AZA and AZA+VEN in hematopoietic cell lines. THP-1 and MV4-11 leukemic cells, used as resistant and sensitive *in vitro* models, were treated with AZA/AZA+VEN for 24h. AZA+VEN strongly and rapidly increased *in vitro* cell death in both cell lines. This was partially confirmed by apoptotic markers expression: anti-apoptotic BCL-2 decreased in MV4-11 cells, pro-apoptotic genes (BAX, BIM, PUMA) increased in THP-1 and decreased in MV4-11 cells, and pro-apoptotic BAK1 gene increased in both cell lines. Moreover, AZA+VEN significantly decreased the G_2-M phase in both cell lines, while it early induced PLCβ1, related with G_0/G_1 regulation and myeloid differentiation, only in THP-1 cells, while it decreased in MV4-11 cells. Despite that, both cell lines showed an increase in differentiation markers (CD11, CD14). Finally, preliminary immunocytochemistry data revealed an increase of p-Akt in THP-1 and MV4-11 cells after AZA and AZA+VEN: interestingly, in MV4-11 the localization of p-Akt was homogeneous, while in THP-1 cells it appeared to be more heterogeneous. Ongoing analyses are therefore deepening the role of p-Akt in hematopoietic cell lines. All in all, our results may pave the way to new molecular mechanisms involving PLCs-related pathways and apoptosis regulation in AZA and AZA+VEN treatment in the regulation of leukemic stem cells.

References

1. Bazinet A et al. Treat Options Oncol. 2022;23(5):668-87.
2. Cocco L et al. J Lipid Res. 2015;56(10):1853-60.

LONG-TERM EFFECTS OF MELATONIN ON ESTROGEN SIGNALING IN CHOLANGIOCYTES DURING PSC

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The neurohormone melatonin is synthesized by aralkylamine N-acetyltransferase (AANAT) and acts as a modulator in FSH and estrogen signaling during different diseases.[1] Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by biliary damage and inflammatory infiltrate.[2] We have shown that: (i) cholangiocytes expressed ERα and β in male bile duct ligation (BDL) rats and (ii) melatonin administration ameliorates liver phenotype in male cholestatic model.[3] We aimed to evaluate the long-term effects of melatonin on estrogen signaling in cholangiocytes, in biliary inflammation and senescence in female $Mdr^{2-/-}$ mice (PSC-model). We evaluated: (i) melatonin levels in serum and cholangiocyte supernatant, (ii) liver damage by H&E in liver sections and ALKP levels in serum, (iii) biliary senescence by β-galactosidase and p21 IHC, and (iv) inflammation by IHC for F480, CD3 and CD20. Next, we evaluated the expressions of ERα/β in human PSC and murine samples. We observed that $Mdr^{2-/-}$ mice have elevated levels of melatonin in serum but reduced in supernatant. While the estrogen levels are increased in serum and cholangiocyte supernatant of $Mdr^{2-/-}$ mice compared to FVB/NJ mice. In addition, $Mdr^{2-/-}$ mice treated with melatonin showed less liver damage, biliary senescence and inflammation compared to $Mdr^{2-/-}$. Both $Mdr^{2-/-}$ mice and PSC have elevated expression of ERα/β compared to control group suggesting that: (i) cholangiocytes express estrogen receptors during damage and (ii) there is a possible correlation between melatonin and estrogen signaling during cholestatic liver diseases. Chronic administration of melatonin improves liver damage in the cholestatic murine model, and its administration may reduce the estrogen action in female PSC modulating the cholestatic liver disorders.

References

1. Saptadip SJ Environ Pathol Toxicol Oncol 2022;41(4):55-84.
2. Franchitto A et al. Ann Transl Med 2013;1(3):27.
3. Ceci L et al. Cell Mol Gastroenterol Hepatol 2022;14(4):877-904.

VISUALIZING THE SUBCELLULAR ACTION OF ANTI-HER2 THERAPEUTICS AT NANOSCALE IN BREAST CANCER CELLS

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ERBB2/HER2+ breast cancer affects 20-30% of patients and is characterized by amplification of the ERBB2/HER2/Neu gene or overexpression of the ERBB2 receptor. Neratinib (NE) is an irreversible pan-ERBB tyrosine kinase inhibitor approved for treating ERBB2+ breast cancer. NE inhibits ERBB2 downstream signaling kinases like pERK and pATK to exert anti-cancer effects. NE also affects autophagy and mitochondrial homeostasis, processes that cancer cells exploit to survive. High-resolution microscopy and molecular approaches revealed that NE inhibited kinases involved in cancer cell survival after 2 h, while kinases involved in DNA damage response were inhibited after 72 h. NE transiently enhanced autophagy and affected mitochondrial dynamics and energy metabolism. NE increased the expression levels and nuclear localization of the TFEB and TFE3 transcription factors, promoting autophagic flux. Additionally, NE increased the release of extracellular vesicles (EVs) with reduced ERBB2 positivity. In conclusion, NE activates TFEB and TFE3, suppressing cancer cell survival by promoting autophagy, disrupting mitochondrial function and response to DNA damage, and reducing ERBB2 dissemination through EVs.

References
1. Santamaria S et al. J Histochem Cytochem 2021;69(7):461-73.
2. Marconi S et al. Membranes 2021;11(3):199.
3. D'Alesio C et al. Biol Open 2019;8(4):bio038323.
4. D'Alesio C et al. J Exp Clin Cancer Res 2017;36(1):154.
5. Cortese K et al. Mol Biol Cell 2013;24(2):129-44.

LAMIN A-DEPENDENT MECHANO-RESPONSE IS ALTERED IN MYOTUBES FROM YOUNG AND OLD SUBJECTS

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Myotubes must sustain mechanical stress that is correlated to a relative risky rupture at the junctional sarcolemma level. Through mechanosignaling, the tension forces are sensed by membrane receptors and transduced to the nucleus. In particular, external signals (mechanical stimuli) are converted into biochemical signals and transmitted through the cytoskeleton to the nuclear envelope *via* LINC (Linker of Nucleoskeleton and Cytoskeleton) complex, which includes nesprins, SUN1/2, A-types lamins and emerin, that in turn interacts with different signalling pathways, leading to changes in cell architecture, gene expression, and cellular functions [1]. Our study is aimed at identifying the main molecular interactions between the LINC complex and signalling pathways that regulate mechanosignaling crosstalk under mechanical strain conditions (uniaxial mechanical strain), comparing young and old myogenic cells obtained from human derived samples. We showed that, upon induction of mechanical strain, differentiating myoblasts modulate expression of A-type lamins, downregulate LINC complex proteins at the nuclear envelope and rearrange the muscle-specific intermediate filament desmin network. In this context, lamin A/C binding to histone deacetylase 2 (HDAC2) is modulated. Consequently, p21, one of the main HDAC2 target genes that is upregulated during myoblast differentiation, undergoes different modulation in cells from old individuals. These preliminary results pave the way to a more comprehensive understanding of mechanosignaling pathways under mechanical strain in human skeletal muscle cells.

Reference
1. Osmanagic-Myers S et al. Genes Dev 2015;29:147.

THE SARCOGLYCAN SUB-COMPLEX DURING TRANS-DIFFERENTIATION FROM WHITE TO BROWN ADIPOCYTES

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The Sarcoglycan sub-complex (SGC) is a transmembrane protein system involved in cell-extracellular matrix interactions in muscle and not muscle tissues such as the adipose tissue.[1] Previous data have shown that the entire SGC is expressed both in white and brown adipocytes. In particular, sarcoglycans seem to be more expressed in brown adipocytes if compared to the white ones. Although that, the role played in adipose tissue is still unknown. The aim of the present study was to verify the possible sarcoglycans involvement during trans-differentiation processes from white to brown adipocytes. Culture of 3T3L1 cells were induced to transdifferentiate using agonists of β3-receptors and the cells were processed by immunofluorescence, RT-PCR and Western blot techniques. Our results have shown that all sarcoglycans are expressed in white adipocytes and their expression increases after trans-differentiation from white to brown. These data confirm that sarcoglycans are not muscle specific and suggest their involvement in the transdifferentiation process in adipose tissue.

Reference
1. Groh S et al. J Biol Chem 2009;284(29):19178-82.

INVESTIGATION OF CELLULAR AND MOLECULAR MECHANISMS IN PERICYTES OF PATIENTS AFFECTED BY COL6-RELATED MYOPATHIES

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Collagen VI (COL6) is a ubiquitous extracellular matrix (ECM) protein that exerts a broad range of mechanical function in organising and anchoring the fibrillar collagen network in many tissues, including skeletal muscle.[1] Mutations in genes encoding the three major α-chains of COL6 result in a group of inherited disorders known as COL6-related myopathies. COL6 deficiency alters ECM structure and biomechanical properties, resulting in impaired muscle regeneration and consequently progressive muscle weakness and wasting. Recent evidence has shown that pericytes, mesenchymal stem cells closely associated with endothelial cells of vascular vessels, contribute to the regenerative microenvironment by releasing trophic factors.[2] Pericytes are dominantly quiescent cells but, following muscle injury, they exhibit increased reactivity and differentiation into muscular cells by enhancing tissue healing. Therefore, to gain insight into the involvement of pericytes in the pathogenesis of COL6-related myopathies, we explored cellular and molecular mechanisms coordinating pericyte state of affected patients compared to healthy patients. Firstly, we evaluated the expression and distribution of COL α6 chain and its cell surface receptor Neural/glial antigen 2 in pericyte cell cultures confirming the α6 chain defect in affected patients. Interestingly, our findings show a disruption in cell cycle progression correlated to a quiescent state in affected pericytes. Further analysis uncovered significant changes in key cellular mechanisms, including proliferation, inflammation, and survival by analysing signalling cascades, as well the expression of proliferative markers. Thus, our findings recognize pericytes as crucial players in maintaining tissue homeostasis in COL6-related myopathies, highlighting their importance in contributing to muscle repair in response to injury.

References

1. Di Martino A, et al. Int J Mol Sci 2023;24:5095
2. Moyle LA, et al. Adv Exp Med Biol 2019;1147:319-44.

IMMUNOHISTOCHEMICAL PROFILE OF PRIMARY SMALL B-CELL LYMPHOMAS OF THE CENTRAL NERVOUS SYSTEM

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Primary small B cell-lymphomas (PSBCLs) of the central nervous system (CNS) are rare entities,[1] mainly represented by lymphomas with plasmacytic differentiation, such as marginal zone lymphoma and lymphoplasmacytic lymphoma.[2] These lymphomas are all made up of small to medium-sized lymphoid cells, unlike primary CNS diffuse large B-cell lymphomas (PCNS DLBCLs), which consist of large lymphoid elements. CNS PSBCLs are usually characterised by a good prognosis and should be differentiated from mantle cell lymphoma, which shares some morphological similarities with them, but shows a much more aggressive clinical behaviour and is never a primary CNS disease. In case of suspicion of CNS PSBCL, a standard panel of immunohistochemical stains (IHC) should at least include CD20, PAX5, CD3, CD5, CD23, CD10, Bcl-6, Bcl-2, CD43, Bcl-1, SOX11, EBV in situ hybridization (ISH), and Ki-67, with addition of kappa and lambda immunostains in the presence of plasma cell differentiation[3]. Even if marginal zone lymphoma and lymphoplasmacytic lymphoma are the most frequent CNS PSBCLs, their diagnosis requires the exclusion of other CNS PSBCLs. For this purpose, follicular lymphoma can be ruled out by negative expression of CD10 and Bcl-6, small lymphocytic lymphoma by negative expression of CD5 and CD23, and mantle cell lymphoma by negative expression of CD5, Bcl-1/cyclin D1, and SOX11. By using IHC and/or in ISH studies for kappa and lambda light chains, it is possible to determine the clonal nature of the B-cells in marginal zone lymphoma and lymphoplasmacytic lymphoma. Nevertheless, it can be difficult to distinguish between marginal zone lymphoma and lymphoplasmacytic lymphoma, and additional clinical and laboratory findings (such as the presence or absence of lymphadenopathy, hepatosplenomegaly, bone marrow involvement, and M-protein) must be integrated in order to rule out CNS involvement from a systemic lymphoma. Finally, the detection of MYD88 L265P mutation favours lymphoplasmacytic lymphoma over marginal zone lymphoma because it is more prevalent in the former.[4]

References

1. Jahnke K. et al. Ann Neurol 2006;59:755-62.
2. Nomani L et al. Am J Clin. Pathol 2020;154, 124-132.
3. Lauw MIS et al. Diagnostics 2020;10(12):1076.
4. Swerdlow SH et al. Virchows Archiv 2016;468, 259-75.

TROP-2 INDUCES APOPTOSIS *VIA* TRANSCRIPTIONAL ACTIVATION OF TRAIL, FAS/FASL, CD40/TNFRSF5

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The transmembrane Ca^{2+} signal transducer Trop-2 is a driver of tumor growth and metastasis[1,2] and a therapeutic target for anticancer therapy.[3] Trop-2 is upregulated in the majority of human carcinomas, where it is generally associated with worse prognosis. However, distinct tumor subtypes (*e.g.* of breast, vulva and lung cancers) show better outcomes for increased Trop-2 expression. In normal tissues Trop-2 is expressed at high levels in multistratified epithelia by non-proliferating cells, with a prototypic gradient from the supra-basal to the corneum layer, which parallels induction of programmed cell death in skin keratinocytes. This led us to

hypothesise that Trop-2 could act as a trigger of apoptosis. Dynamic cell morphometry, phosphatidyl-serine membrane exposure, propidium iodide permeability, nuclear pyknosis/karyorrhexis and DNA fragmentation showed that Trop-2 expression induces a dramatic increase of apoptotic cell death in NS-0/Trop-2 myeloma cell tranfectants. Transcriptomic analysis indicated early overexpression of TRAIL, FAS/FASL and CD40/TNFRSF5 transmembrane death receptors and corresponding upregulation of downstream death-inducing signaling complex components and of executioner Caspases 8 and 3, suggesting Trop-2 as a trigger of extrinsic apoptosis pathways. Consistent with apoptosis induction, Trop-2 expression levels are inversely correlated to tumor growth *in vivo*. Parallel induction of anti-apoptotic transcription factors, Rb-binding protein, survivin, Myd-118, and stimulation of NS-0 cell growth *in vitro* was also observed. These findings indicate a previously unappreciated balance between cell growth stimulation and triggering of cell death by Trop-2 at the crossroads of proliferation and terminal differentiation.

References

1. Trerotola M et al. Oncogene 2013;32:222-33.
2. Guerra E et al. Neoplasia 2021;23:898-911.
3. Guerra E et al. Mol Cancer Ther 2023;22-0352.

INNOVATIVE CHIMERIC ARCHAEAL-HUMAN FERRITIN DELIVERY SYSTEM IN MYELOID LEUKEMIA CELL LINES

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In the last decades, nanovehicles have emerged as an innovative molecular technology for drugs and small RNAs delivery. In our works, we developed a delivery system based on a chimeric archaeal-human ferritin. In particular, we melted the hollow cage-like structures, able to incorporate cargos, and the unique 24-meric assembly of *Archaeglobus fulgidus* ferritin together with the human H ferritin binding to CD71 receptor, which is highly expressed in tumor cells. We were able to encapsulate, deliver and release bioactive full-length cytochrome C in Acute Promyelocytic Leukemia (APL) NB4 cell line, in order to induce apoptosis.[1] Since Myeloid Leukemia cells are typically hard to transfect with the conventional liposome-based transfection methods, we decided to use the ferritin cage, introducing a positively charged polyamide dendrimer (Poly(amidoamine) or PAMAM) that acts as an anionic sponge for negatively charged molecules, in particular nucleic acids. We demonstrated that the HumFt-PAMAM nanoparticle delivers a nucleic acid, a pre-miRNA (miRNA-145-5p) into APL cells and can release it into the cytoplasm, where it is processed to mature miRNA.[2] Moreover, we also observed that this delivered miRNA can induce functional effects, like granulocyte differentiation. Our goal is to insert small interference RNA (siRNA) into HumFt-PAMAM nanoparticles to silence target genes in acute leukemia cells, by overcoming the issues of transfection methods and allowing a cell-specific targeting.

References

1. Macone A et al. Sci Rep 2019;13;9(1):11749.
2. Palombarini F et al. J Nanobiotechnology 2021;19:172.

SARCOGLYCANS AND OBESITY: NEW POSSIBLE MARKERS?

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Obesity is an unhealthy condition that represents one of the main risk factors for cardiovascular diseases and diabetes. The induction of the browning process has been identified as a promising anti-obesity therapy. By that, the discovery of new and specific markers for transdifferentiation could be useful for anti-obesity treatment. Sarcoglycans (SGs) are transmembrane proteins[1] that seem to be expressed in adipose tissue. In particular, previous data have shown an increased expression of these proteins in brown adipose tissue. On this basis, the aim of the present study was to investigate the expression of Sarcoglycans in adipose tissue of obese Male Zucker Crl:ZUC-Leprfa rats before and after treatment with anti-obesity natural drugs products; in detail, rats were treated with lycopene and hydroxycitric acid obtained from Garcinia Cambogia fruit to browning. The samples of visceral adipose tissue were processed for immunohistochemistry using anti sarcoglycans antibodies. Results have shown that all SGs are expressed in visceral adipose tissue of control rats at plasmalemma level and that their expression increases after treatment with drugs that induce the transdifferentiation. These data confirm that sarcoglycans are expressed in adipose tissue and could be involved in the transdifferentiation process. Moreover, their increased expression after anti-obesity treatment makes them possible new markers in obesity issues.

Reference

1. Anastasi G et al. Ital J Anat Embryol 2010;115(1-2):19-24.

ENVIRONMENTAL EXPOSURE TO FLUORO-EDENITE FIBERS AND ITS CORRELATION WITH MALIGNANT MESOTHELIOMA: AN IMMUNOHISTOCHEMICAL INVESTIGATION

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Malignant mesothelioma is a deadly cancer linked to asbestos fibers exposure and to other types of naturally occurring asbestos fibers, such as fluoro-edenite, a silicate mineral found in the Monte Calvario quarry in Sicily, Italy.[1,2] Malignant mesothelioma is often diagnosed at an advanced stage due to the lack of diagnostic and

prognostic biomarkers, resulting in poor outcomes for patients.[3] Several studies have shown that stathmin, a cytosolic protein that regulates cell growth and migration, is overexpressed in human malignancies, its expression has not been correlated with the survival and clinical-pathological variables of malignant mesothelioma patients.[4-5] We investigated the stathmin immunoexpression in malignant mesothelioma patients induced by fluoro-edenite fibers environmental exposure. The study aim was to determine if stathmin could serve as a prognostic biomarker and to evaluate its potential to identify patients' prognosis. We analyzed ten MPM tissue samples from patients with available clinical and follow-up data, using histological and immunohistochemical techniques. Our findings revealed that malignant mesothelioma patients with stathmin overexpression had a trend towards shorter overall survival and that exists a significant correlation between stathmin expression and malignant mesothelioma. These results suggest that stathmin immunohistochemical expression may represent a promising prognostic biomarker for malignant mesothelioma and could help clinicians to choose a therapeutic approach. Overall, our study highlights stathmin potential as a prognostic biomarker in malignant mesothelioma and provides insights into the role of environmental factors of this deadly cancer development.

References
1. Brims F et al. Cancers 2021;13:16.
2. Filetti V et al. Biomed Rep 2020;13:6:60.
3. Loreto C et al. Eur J Histochem 2020;64:265-77.
4. Filetti V et al. J Pers Med 2021;11:1205-18.
5. Broggi G et al. Int J Env Res Pub Health 2021;18:6249-62.

CAN PRELAMIN A ACCUMULATION OPEN UP NEW PERSPECTIVES FOR GLIOBLASTOMA?

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Glioblastoma is the most lethal brain tumour in adults. Despite the progress made in understanding the molecular pathogenesis of glioblastoma, the survival rate of patients remains unsatisfactory. This project aims to identify a new potential pharmacological strategy, exploiting the peculiar feature of glioblastoma cells to express lamin A, one of the main components of the nuclear lamina, differently from the healthy nervous tissue.[1,2] It is known that, when the maturation of prelamin A, the precursor of lamin A, is compromised, reactive oxygen species (ROS)-activate DNA damage response cannot properly occur leading to premature cellular senescence, as observed in laminopathies.[3] In this context, we decided to take advantage of the acquired deficiency in recovering DNA damage, resulting from the accumulation of prelamin A, and to evaluate the consequences of this event on both cell lines and patient-derived glioblastoma stem cells, which are responsible for tumour resistance and recurrence. According to this aim, a combination of two drugs was suggested. Prelamin A accumulation was obtained using the farnesyl-transferase inhibitor SCH66336, while ROS-injury was achieved using Menadione. This study shows how the combined treatment leads to morphological alterations of the cells with evident nuclear reorganisation, to a reduction of their ability to migrate, invade, to grow in colonies and to an impairment in cell survival and aggressiveness. A better understanding of the effects of the proposed treatment on the structure, nuclear lamina interactions with the cytoskeleton and on the related signalling pathways, could pave the way for new therapeutic approaches for this tumour that is currently incurable.

References
1. Gatti G et al. Biomedicines 2021;9:1343.
2. Jung H J. et al. Mol Neurobiol 2013;47:290-301.
3. Cenni V et al. Ageing Res Rev 2020;62:101073.

RETINOIC ACID AND PROTEOTOXIC STRESS INDUCE AML CELL DEATH OVERCOMING BONE MARROW STROMAL CELL PROTECTION

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Acute myeloid leukemia (AML) patients bearing ITD mutation in the tyrosine kinase receptor FLT3 (FLT3-ITD) have a poor prognosis and a high risk of relapse after remission. We set up a therapeutic strategy combining low doses of differentiating agent retinoic acid (R), ER stress inducer Bortezomib (B) and oxidative stress inducer arsenic trioxide (A), that exerted cytotoxic activity on FLT3-ITD$^+$ AML cell lines and primary blasts. Upon RBA treatment, morphological and molecular analysis of AML blasts (Giemsa staining, confocal microscopy, TEM, RT-qPCR, and flow cytometry) show that proteotoxic stress leads leukemic cells to death. Since it is established that the bone marrow niche favors AML therapy resistance,[1] we analyzed the impact of bone marrow stromal cells (BMSCs) on AML treatment responsiveness. A co-culture system of AML cells and BMSCs confirmed that the niche provides protection from RBA by attenuating oxidative stress. However, the use of pharmacological doses of ascorbic acid[2] as an adjuvant pro-oxidant agent tamper with this protective effect. Intriguingly, BMSC-AML crosstalk involves BMSCs microfilament dynamics and the Hippo pathway. Upon combined treatment, rearrangements in BMSC actin cytoskeleton and actin cap depend on the presence of AML cells. Of note, this combination showed anti-leukemic effects in a murine model of human AML and no toxicity. In conclusion, our study identifies proteotoxic stress as a target in FLT3-ITD$^+$ AML, providing new insights into BM niche-mediated AML resistance mechanisms.

References
1. Forte D et al. Cell Metab 2020;32:829-43.e9.
2. Ngo B et al. Nat Rev Cancer 2019;19:271-82.

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF LANGERHANS CELLS IN THE SKIN OF THREE AMPHIBIAN SPECIES

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The amphibian taxon includes three orders which present different morphological characteristics: Anura (frogs and toads), Caudata (salamanders) and Apoda (caecilians). The object of this study was to characterize Langerhans cells in the skin of *Lithobates catesbeianus, Typhlonectes natans,* and *Amphiuma means*, three species each belonging to one of three orders of amphibians. Amphibians skin has a crucial role, it acts as an immune organ constituting a physical, chemical, immunological, and microbiological barrier to pathogen insult and it also conducts essential physiological processes. In addition, amphibians have developed specialized features to protect the vulnerable skin barrier, including a glandular network beneath the skin surface that is able to produce antimicrobial and toxic substances, thus contributing to the defense against pathogens and predators.[1] The aim of this study was to characterize Langerhans cells in amphibians' skin with the following antibodies: Langerin/CD207 (c-type lectin), MHC-II (Major Histocompatibility Complex), and TLR2, expressed by different types of DCs. Our results showed Langerhans cells langerin CD/207 positive[1] in the epidermis of three amphibian species; moreover, some cells present in the connective tissue expressed TLR2 and MHC-II, demonstrating their belonging to Antigen presenting cells (APCs).[2] We conclude that the distribution of Langerhans cells is very similar in the three amphibian species examined, despite their different aquatic and terrestrial habitats. A greater knowledge of amphibian immune system could be useful to better understand the phylogeny of vertebrates and to safeguard amphibians from population declines. The similarities between the skin of frogs and that of humans, in terms of anatomical features and physiological processes, could also offer insights into many areas of skin research that may be useful both for biologists and investigative dermatologists.

References

1. Lauriano ER et al. Acta Histochemica 2020;122:151471.
2. Lauriano ER et al. Int J Mol Sc. 2023;24:2316.

EXPRESSION OF TLR2 AND *α*-SMA IN INFLAMMATORY BOWEL DISEASE

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Inflammatory bowel disease (IBD) represents multifactorial, chronic, inflammatory conditions in the gastrointestinal tract. The main IBD are Crohn's disease (CD) and ulcerative colitis (UC).[1,2] CD causes perforation**,** which can occur discontinuously in the entire Gastrointestinal tract (GIT). UC causes superficial inflammation, bleeding and mucosa atrophy in the distal rectum and the colon. Innate immunity is considered the first line of defense against microbial invasion; among Toll-like receptors, TLR2 is the most important for defense against mycobacterial infection. TLR2 has been reported to have many functions in infectious diseases, and also in other pathologies, such as chronic and acute inflammatory diseases. Alfa-Smooth Muscle Actin (α-SMA) is an important biomarker in IBD. All myofibroblasts express α-SMA, which has been found to be upregulated in CD and UC. Paraformaldehyde-fixed intestinal tissues, from patients with CD and patients with UC, were analyzed by immunostaining for TRL2 and α-SMA. Our results showed in the samples obtained from IBD patients with inflamed mucosa TLR2-positive epithelial cells concentrated on the mucosal surface and scattered immune cells in the connective tissue; furthermore, numerous α-SMA-positive cells (subepithelial myofibroblasts) were detected in lamina propria and around glands, while some myofibroblasts co-localizing with α-SMA and TLR2, could be inflammatory macrophages. In control samples a low positivity to α-SMA and to TLR2 was observed respectively in subepithelial myofibroblasts and in scattered immune cells of the lamina propria.[3] These data showed the recall of α-SMA positive myofibroblasts during the inflammatory state; furthermore, it has been observed that TLR2 expression changes in the intestinal epithelium in IBD, demonstrating that changes in the innate system response may contribute to the pathogenesis of these diseases.

References

1. Pergolizzi S et al. Acta Histochemica 2021;123:151715.
2. Pergolizzi S et al. Biomedicines 2022;10:765.
3. Marino A et al. APMIS 2015;123:163-8.

EFFECTS OF HYPERGLYCAEMIC CONDITIONS ON AKT SIGNAL TRANSDUCTION IN BREAST CANCER CELLS

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Breast cancer (BC) represents one of the most diagnosed malignancies and the first cause of cancer-related death in women. Insulin resistance and hyperglycemia, both hallmarks of type 2 diabetes mellitus (T2DM), have been involved in the aetiology of BC[1] and it has been recently demonstrated the involvement of NOTCH and EGFR superfamilies in drug resistance and breast cancer recurrence in patients with diabetes[2]. So far, T2DM represents a risk factor for BC. Hyperglycemia and insulin resistance are signatures of T2DM, but their role in BC must be better elucidated. With this in aim, we explored the relevance of hyperglycaemia in the triple-negative BC cell line MDA-MB-231 exposed to three different glucose concentrations [5.5 mM (physiological), 13 mM (mild hyperglycemia) and 25 mM (supraphysiological hyperglycaemia)] for 24, 48 and 72 h and then treated with 100 nM insulin for 15 min to assess whether those conditions may reflect insulin resistance. Insulin signalling was investigated by evaluating with Western Blotting the phosphorylation of AKT and its substrate AS-160 (160 kD) and of the downstream AKT target mTOR. Furthermore, by means of immunocytochemistry, the intracellular

distribution of p-AKT was assessed. While AKT, AS-160 and mTOR phosphorylation increased in response to insulin when cells were maintained in 5.5 mM glucose, this response was blunted by hyperglycaemia in a dose- and time-dependent fashion. The same held true when assessing the phosphorylation of AKT and its membrane translocation induced by insulin with immunocytochemistry, with both parameters being impaired independently on whether cells were exposed to mild or supraphysiological hyperglycaemia already after 24 h of treatment. This signal transduction pathway impairment puts the basis to better understand the relevance of hyperglycaemia and insulin resistance in the malignant transformation and tumour progression of breast cancer.

References

1. Cao M et al. Nature Cell Biology 2022;24:954-67.

2. Lee C et al. Nature Commun 2023;14:439.

THE ROLE OF IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF SELLAR LESIONS MIMICKING PITUITARY NEUROENDOCRINE TUMOURS

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The sellar region is the site of a large variety of developmental, inflammatory, vascular and neoplastic lesions. The most common entities occurring in this area are pituitary neuroendocrine tumours (PitNETs), accounting for 85% of the sellar masses. Clinical features of PitNETs include mass effect symptoms (headaches, visual field disturbances or central diabetes insipidus) as well as endocrine hormones hyperproduction (acromegaly or galactorrhoea). PitNETs consist of a neoplastic proliferation of monomorphic cells from anterior pituitary gland, arranged in different histological patterns. They are identified by the presence of immunohistochemical expression of neuroendocrine markers, and then classified in subtypes due to the expression of different hormones (PRL, GH, ACTH, TSH, FSH, or LH) and transcription factors (Pit1, TPIT, SF1, GATA3, and ERα).[1-2] Other sellar lesions can range from not neoplastic to high-grade malignant conditions with different cell origin, including Rathke's cleft cyst (28-33%), lymphocytic hypophysitis (LH) (5%), meningioma (3-8%), and metastasis (1-3%).[3-5] Although clinical and radiological features may suggest a preoperative diagnosis, in some cases they may mimic PitNET features. These lesions do not show positivity for neuroendocrine markers but may exhibit different histological and immunohistochemical profiles. In detail, LH is characterised by diffuse infiltration of lymphocytes that show immunoreactivity for markers such as CD20 and CD3.[3] Also, meningioma is a neoplastic proliferation of meningothelial cells positive for epithelial markers (EMA) and progesterone marker.[1,4] Finally, metastasis presents a wide range of features attributable to their primary origin.[5] Therefore, in a case of a sellar lesion, beside to clinico-pathological and neuro-imaging assessments, the immunophenotypic profile is mandatory to reach a definitive strongly supported diagnosis.

References

1. Louis DN et al. Neuro Oncol 2021;23:1231-51.

2. Trouillas J et al. Cancers (Basel) 2020;12(2):514.

3. Langlois F et al. J Clin Endocrinol Metab, 2022;107(1):10-28.

4. Sathananthan M et al. Pituitary 2013;16(2):182-8.

5. Kameda-Smith MM et al. J Clin Neurosci. 2021;93:231-40.

AUTOSOMAL DOMINANT LEUKODYSTROPHY (ADLD): ARE WE FACING AN ASTROCYTOPATHY?

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Autosomal Dominant Leukodystrophy (ADLD) is a rare and fatal late-onset neurodegenerative disorder that affects the central nervous system myelination. The disease is caused by Lamin B1 (*LMNB1*) gene alteration with the mechanisms remaining unknown. This study investigated the changes in different cell populations in ADLD, focusing on the role of oligodendrocytes, astrocytes, and Leukemia Inhibitory Factor (LIF) in myelination. Morpho-functional aspects of primary patient derived cells and engineered cellular models overexpressing LMNB1 were analyzed. It was proven that astrocytes overexpressing LMNB1 displayed nuclear alterations not present in oligodendrocytes.[1] Astrocytic LMNB1 accumulation reduced the levels of LIF and LIF-R, down regulating the Jak/Stat3 and PI3K/Akt pathways.[1] Exogenous LIF administration indicated a partial reversal of the toxic effects of LMNB1 accumulation in astrocytes, but not in oligodendrocytes.[1] Additionally, LMNB1 overexpression inactivated GSK3β without upregulating β-catenin targets, resulting in a reduction of astrocyte survival.[1,2] LMNB1 accumulation also affected proliferation and cell cycle progression with PPARγ and p27 increase and Cyclin D1 decrease, reducing cell viability and causing apoptosis.[2] Astrocytes overexpressing LMNB1 displayed increased immunoreactivity for GFAP and vimentin together with NF-kB translocation and c-Fos increase, suggesting astrocytes reactivity and substantial cellular activation.[2] Furthermore, ADLD patient cells showed activation of proinflammatory mechanisms and increase in reactive oxygen species.[2] Concluding, it was indicated for the first time that LMNB1 accumulation causes cell sufferance, due to astrocyte reactivity. Astrocyte-specific pathological phenotypes were also suggested by post-mortem ADLD brain tissues. Thus, astrocytes play a crucial role in ADLD, hinting the pathology as an astrocytopathy.

References

1. Ratti S et al. Cell Mol Life Sci 2021;78:2781-95.

2. Ratti S et al. Cells 2021;10:2566.

NUCLEAR PHOSPHOLIPASE C DELTA 4 IS A CRUCIAL PLAYER IN RHABDOMYOSARCOMA CELL CYCLE PROGRESSION

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Human rhabdomyosarcoma (RMS) is the most common paediatric soft tissue sarcoma and due to its aggressiveness, the current chemotherapy is often unsuccessful.[1] RMS arises from mesenchymal precursors with the potential to differentiate into skeletal muscle cells but failing the myogenesis program because of the presence of chromosomal aberrations which lead to uncontrolled cell growth.[2] A crucial player in RMS aberrant proliferation could be the nuclear protein known as PLC delta 4, the role of which in driving proliferative processes in mesenchymal stromal stem cells has already been described.[3] Our molecular and morpho-functional data reveal that PLC delta 4 is expressed in embryonal RMS cells where it localises in the nucleus. PLC delta 4 overexpression seems to indirectly interact with Cyclin in delaying cell cycle progression of RMS cells. Therefore, the modulation of PLC delta 4 expression and of its downstream targets could represent a crucial signalling pathway to block embryonal RMS cell proliferation through apoptotic cell death induction.

References

1. Piazzi M et al. Molecules 2022;27:2742.
2. Salucci S et al. Nutrients 2023;5:740.
3. Kunrath-Lima M et al. Cell Signal 2018;49:59-67.

DEVELOPMENT OF A NEW CURCUMIN-SILVER NANOPARTICLES SYNTHESIS METHOD AS A PROMISING FORMULATION TO TEST ON HUMAN PTERYGIUM-DERIVED KERATINOCYTES

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The aim of the present study has been to synthesize curcumin-caged silver nanoparticles (Cur-AgNPs) to employ in the treatment of human pterygium. Pterygium is a progressive disease of the human eye arising from sub-conjunctival tissue and extending onto the cornea. Due to its invasive growth, pterygium can reach the pupil compromising visual function. At present, there is no treatment of choice. There is the need for alternative therapeutic strategies. Previous studies have demonstrated that both *Curcuma Longa* and its active principle curcumin are able to induce, respectively, apoptosis of human pterygium keratinocytes and fibroblasts in a dose- and time-dependent manner.[1,2] However, both molecules are not very soluble in water, neither at neutral nor at acidic pH and are only slightly more soluble in alkaline conditions. Here, we propose an innovative nano-formulation to solubilize and make more bioavailable the active principle of *Curcuma Longa*. The synthesis of this new compound (Cur-AgNPs) was achieved through a modified Bettini's method adapted to improve the quality of the product for human use.[3,4] Hence, the pH of the reaction was changed to 9, the temperature of the reaction was increased from 90°C to 100°C and, after the synthesis, the Cur-AgNPs were dispersed in Borax buffer using a dialysis step to improve the biocompatibility of the formulation. This new compound will be able to deliver both components, curcumin and silver, at the same time to the affected tissue, representing an alternative and a more sophisticated strategy for the treatment of human pterygium. Further *in vitro* and *in vivo* assays will be required to validate this formulation.

References

1. Zhang M et al. J Huazhong Univ Sci Technol 2007;27:339-42.
2. Sancilio S et al. BioMed Res Int 2017;201:2956597.
3. Bettini S et al. Nanoscale 2014;6:10113-7.
4. Stati G et al. Molecules 2022;27(1):282.

POSTERS

P1.1 HISTOPATHOLOGICAL BASIS OF EXPERIMENTAL CHRONIC BANKART MODEL

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In our study, we aimed to create a rat model of chronic bankart defect that causes post traumatic recurrent glenohumeral instability. 48 Wistar albino rats were divided into 3 groups. In the first phase of the study (week 0), all rats were operated and a penrose drain was placed between the capsulolabral tissue and the anteroinferior margin of the glenoid. In the second stage penrose drains were removed 2 weeks after the first surgery for the first group, 4 weeks for the second group, and 6 weeks for the third group. The glenohumeral region from the shoulders of 5 animals from each group were sectioned to take tissue specimens for histological examination after the penrose drain was removed. Paraffin sections were performed, and immunohistochemistry has been applied to the sections to reveal vascularization. After the recovery period, all animals were sacrificed. Histopathological scoring of sections was performed based on disordered collagenous fibers, leukocyte infiltration and fatty degeneration. In all experimental groups, when compared to the control group, a disordered collagenous fiber organization and increased neutrophilic infiltration were observed in Masson's trichrome-stained sections taken from the glenohumeral region of the shoulders. An increase in the histopathological score ($p<0.001$) was determined in the bankart experimental groups compared to the control group. In the tissue sections stained with Oil Red O, fat cells were increased in all experimental groups. The intensity of CD31 immunocytochemistry was similar for all experimental groups. The breaking tensile strength of group 2 and group 3 was found to be higher compared to group 1 and the control group. In conclusion, we demonstrated that chronic bankart lesions can be performed in an experimental rat model in 4 weeks as a sufficient time period to prevent the healing of the defect created to form a chronic bankart lesion.

References

1. Abe H et al. Tohoku J Exp Med 2012;228:103-8.
2. Arnoczky SP et al. Am J Sports Med 1983;11:131-41.
3. Baker CL et al. Am J Sports Med 1990;18:25-8.

P1.2 LOCALIZATION OF GLUTATHIONE S-TRANSFERASE P NUCLEAR AND INTERACTING PROPERTIES OF NRF2/KEAP1 AS BIOMARKERS OF DRUG RESISTANT HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) is highly drug resistant. The oncogene and phase 2 detoxification enzyme Glutathione S-transferase P (GSTP) is proposed to play a key role in both liver carcinogenesis and GSH-dependent drug resistance (DR) mechanisms of HCC. In this study, we evaluate the roles of GSTP and its physical and functional interaction with Nrf2, a *GSTP* transcription factor and a key player of cancer-specific mechanisms of stress response and drug resistance in HCC. The hypothesis was investigated in the mouse model of N-nitrosodiethylamine (DEN) induced HCC and *in vitro* in human HCC cell lines. GSTP increased its expression and enzymatic activity during tumor development, also changing its subcellular localization during the preneoplastic phase of liver tissue transformation, from cytosolic to prevalently nuclear. These changes were associated with increased levels of protein glutathionylation (PSSG), suggesting increased GSTP activity in this redox-dependent protein-protein regulation pathway. These changes of GSTP expression, subcellular localization and activity were recapitulated in the HCC cell lines HepG2 and particularly in Huh-7 cells, but not in the non-cancerous cell line HepaRG. HCC cells also showed increased cellular levels and reduced efflux of free GSH, which supports increased activity of GSTP in the DR mechanism of these HCC cells. DEN-HCC animals also showed liver induction of Nrf2 compared to control animals, as well as of the Nrf2 inhibitory protein Keap1 and protease β–TrCP. *c*-Jun transcription factor, that cooperates with Nrf2 to control *GSTP* expression, was also induced. Immunoprecipitation experiments demonstrated physical interactions of GSTP with both Nrf2 and Keap1 in the liver of DEN-HCC animals. Oligomeric forms of GSTP were involved in these interactions, the levels of which appear to vary in a redox-dependent manner. In conclusion, GSTP expression, nuclear distribution, and Nrf2-interacting properties mark tumor development and may provide a cancer-specific mechanism of DR in HCC.

P1.3 ARISTOLOCHIA A POTENTIAL COAUDIUVANT IN GASTRIC CANCER THERAPY

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Infectious diseases caused by the multidrug resistant bacteria are a major cause of morbidity and mortality worldwide.[1] *Helicobacter pylori* is one of the most common human infectious agents worldwide. The prevalence of *H. pylori* varies with geographic regions, age, socioeconomic status, education level, living environment, and occupation. The mean worldwide incidence of *H. pylori* infection was 58%.[2] Since its discovery in 1982, *H. pylori* has been closely linked to a diverse spectrum of gastrointestinal diseases.[3] Although most individuals infected with H. pylori remain asymptomatic for life, essentially all develop chronic inflammation. Among infected individuals, approximately 10% develop peptic ulcer disease, 1-3% progress to gastric cancer (GC), and 0.1% develop mucosa-associated lymphoid tissue lymphoma (MALT).[4] *Aristolochia olivieri* is a plant belonging to the family of Aristolochiaceae widely used in Kurdish Folk Medicine for the treatment of gastrointestinal ailments. Here an *A. olivieri* leaves methanolic extract (AOLM) was tested on gastric cancer cells and *H. pylori* through morphofunctional analyses. Furthermore, this extract was chemically characterised. Our data suggest that AOLM may provide substances endowed with antibacterial effects against *H. pilory*. In addition, morphological analysis showed that AOLM induces apoptosis in different gastric cancer cells. HPLC-MS analyses revealed the presence of several polyphenols, in particular phenolic acids and flavonoids. These preliminary results suggest this phytocomplex may be further investigated in order to obtain phytocomplexes to be used in the field of gastric cancer prevention.

References

1. Linz B. et al. Nature 2007;445:915-8.
2. Carter FP et al. West Indian Med J 2011; 60:662-5.
3. Marshal BJ, Warren JR Lancet 1984;1:1311-5.
4. Noto JM and Peek RM Methods Mol Bio 2012;921:7-10.

P1.4 THE EVALUATION OF TEMPOROMANDIBULAR DISORDERS IN RHEUMATOID ARTHRITIS: IMMUNOHISTOCHEMICAL STUDY

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Temporomandibular disorders (TMDs) are pathological conditions due to alterations in the musculoskeletal system and cause temporomandibular joint and masticatory muscle dysfunctions. Most patients with Rheumatoid Arthritis (RA) have symptoms and positive HRCT findings of the temporomandibular system. Almost half of our patients with RA also have Myofascial Pain Dysfunction (MPD). MPD is a group of the stress and anxiety-related disorders associated with parafunctional habits such as bruxism. But the temporomandibular joint involvement pattern is symmetrical in patients with RA, the patients with MPD tend to have asymmetric involvement. Interestingly, TMJ dysfunction mainly impairs the functionality of the masseter and temporalis muscles. Furthermore, in masticatory muscles, an association between pain and muscle hardness was observed due to excessive muscle contraction. Of note, Kang *et al*. have already focused their attention on myofascial pain in TMD patients suffering from migraines, showing that pain is mostly localized in the masseter and temporalis muscle. The aim of this work was to provide a transcriptomic profile of masticatory muscles obtained in patients with RA compared to patients with MPD of the temporomandibular system and control patients. We used: Next Generation Sequencing (NGS) technology to evaluate transcriptomes in masseter and temporalis muscle samples and confocal immunofluorescence microscopy. In conclusion, our findings suggest that it is important to determine the cause of temporomandibular pain because of different therapeutic approaches. The studies are required to develop effective therapeutic strategies for these patients.

References

1. Sindona C et al. Medicina 2021;57:354
2. Cutroneo G et al. Eur J Histochem 2016;60:2605
3. Vermiglio G et al. J Funct Morphol Kinesiol 20205;5:80
4. Runci Anastasi M et al. J Funct Morphol Kinesiol 2020;5(4):90
5. De Ponte FS et al. Chin J Traumatol 2017;20(1):14

P1.5 HISTOCHEMICAL STUDY ON ETIOPATHOGENESIS OF CONDYLAR HYPERPLASIA

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Unilateral condylar hyperplasia is a rare disease that causes facial asymmetry as a result of excessive vertical or horizontal growth, or both, of the mandibular condyle. Investigation should address the patient's concerns and establish whether the disease is active with the use of single positron emission tomography (PET). Proportional reduction of the condyle arrests active disease and restores mandibular height, and any residual asymmetry can be corrected according to conventional orthognathic principles. The pathological condition is not described in any other joint and it is generally observed in young patients between the ages of 11 and 30 years. Some patients present as first symptoms pain and joint noise as a result of functional pathology; the secondary symptom is the difficulty in opening the mouth. Many authors have studied this pathology, because it is a frequent clinical condition and of great interest for maxillofacial surgeons for therapeutic solution, but the etiology remains unknown. The aim of the present work was to evaluate, with different methods, the morphological characteristics of the disease, to try to identify the cause that triggers the increase of this pathology. For this study we used 5 patients

with condylar hyperplasia (4 female and 1 male) who were treated with proportional condylectomy and a control patient. The samples were observed under the optical microscope, the SEM and the confocal laser microscope. Our results support the hypothesis that indicates an increase of cartilage tissue activity, with metabolic disorders of the matrix.

References

1. Vasquez B et al. Int J Oral Maxillofac Su 2015.
2. Jingshuang Yu et al. Orphanet Journal of Rare Diseases 2019;14:293.
3. Maffia F et al. J Craniofacial Surg 2019; 47(12):1898.
4. Runci Anastasi M et al. J Funct Morphol Kinesiol 2020;5(4):90.
5. Runci Anastasi M et al. Helyon 2020;6(8):e04651.
6. Cascone P et al. J Craniofacial Surg 2021;32(5):1836.
7. Runci Anastasi M et al. J Funct Morphol Kinesiol 2021;6(1):22.

P1.6 ULTRASTRUCTURAL EXAMINATION OF *POST-MORTEM* COVID-19 LUNG SPECIMENS REVEALS THAT ALVEOLAR DAMAGE IN FATAL CASES IS NOT DUE TO VIRAL REPLICATION

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We used a transbronchial cryobiopsy approach to collect post-mortem specimens from COVID-19 patients and immediately fixed them for electron microscopy. We analyzed samples from six patients and identified ultrastructural associations for various phases of diffuse alveolar damage, including detachment of alveolar epithelium and accumulation of extracellular material. Coronavirus particles were detected exclusively in and around a very limited number of cells in only one of the six samples. Alveolar damage did not correlate with viral presence or structural impairment due to continued replication during later stages of the disease. This finding suggests that lung damage in these patients may involve alternative mechanisms, such as an inadequate immune or stress response. Our data demonstrate the importance of timely tissue collection and preservation for detailed ultrastructural analysis in COVID-19 patients.

References

1. Cortese K et al. Virchows Arch 2022;480(5):967-77.
2. Barisione E et al. Virchows Arch 2021;478:471-85.
3. Rendeiro AF et al. Nature 2021;593:546-69.
4. Dittmayer C et al. Lancet 2020;396:e64-5.
5. Bullock HA et al. Emerg Infect Dis 2021;27:1023-31.

P1.7 EXPRESSION OF TLR2 AND LANGERIN/CD207 IN THE HAIR CELLS OF *STYELA PLICATA* CORONAL ORGAN

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Ascidians are sessile, filter-feeding, protochordate animals also known as tunicates due to the presence of a tunic covering the entire organism. Adult ascidians present two touch-sensitive siphons: an atrial siphon and an oral siphon. In the velum of the oral siphon and in the tentacles, there is a sensory structure, the coronal organ that consists of ciliated sensory cells (hair cells) functioning as mechanoreceptors that detect incoming water. Sensory hair cells show evident similarities with vertebrate hair cells, leading to the hypothesis that such cells are evolutionarily conserved. The presence of these cells has been demonstrated in the mouse auditory sensory epithelium of the cochlea. Hair cells are sensitive to pathogenic insults and therefore implicated in the immune response of many ear disorders.
The purpose of this study was to characterize for the first time the hair cells in the coronal organ of *Styela plicata,* using TLR2 and Langerin/CD207 antibodies. TLR2 is an evolutionarily conserved recognition receptor (PRR) expressed by numerous vertebrate immune cells and at the level of the tunica and endostyle of *S. plicata.*[1,2] Langerin/CD207 is the receptor for C-type lectins expressed by several types of dendritic cells.[3]
The coronal organ samples were processed according to immunohistochemical techniques and observed by light and confocal microscopy. The results show the presence of numerous TLR2 and langerin/CD207 positive hair cells, involved in immune defense against pathogens. Therefore, this animal model allows us to study the phylogeny of molecular mechanisms underlying innate immune responses.

References

1. Alesci A et al. Microsc Res Tech 2022;1-8.
2. Lauriano ER. et al. Tissue and Cell 2021;71:101584.
3. Lauriano ER et al. Fish Shell Immunol 2018;74:380-5.

P1.8 ULTRASTRUCTURAL STUDY OF EXTRACELLULAR VESICLES IN PANCREATIC DUCTAL ADENOCARCINOMA

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Pancreatic ductal adenocarcinoma (PDAC) is a disease with a poor prognosis due to its early tendency to metastasize and chemoresis-

tance. Extracellular vesicles (EVs) play an important role in the metastatic process, cell-cell communication, immune regulation, and drug resistance and have been considered as promising tools to be used as pancreatic cancer biomarkers.[1,2] Ultrastructural investigations can help in characterizing the nature, the origin, and the trafficking of EVs inside and outside the cell. Here we report the ultrastructural findings obtained from 4 subjects (mean age 60.5+1.7, all males) affected with PDAC in the IA, IIA, or B stage of the disease who underwent surgery at the Pederzoli Hospital, Peschiera del Garda (VR), Italy. All the samples were assayed for BRCA2, Ca19.9, CEA and CD10 with immunohistochemistry in light microscopy. At the ultrastructural level the presence of different types of cytoplasmic vesicles of different size and content was found. Interestingly, round cytoplasmic vesicles of bigger size were particularly abundant in two patients expressing $BRCA2^{mut}$. Furthermore, the detection of remnants of mitochondrial cristae inside several vesicles suggested ascribing them to different stages of mitochondrial swelling and disruption. These observations let us hypothesize the occurrence of an apoptotic mechanism of cell death in $BRCA2^{mut}$ PDAC samples which could increase the inflammatory components of the tumor microenvironment (TME) contributing to a worse prognosis.

References
1. Chang CH et al. Cell Death Dis 2021;11:973.
2. Ariston Gabriel AN et al. Mol Cancer 2020;19:132.

P1.9 A LIGHT MICROSCOPY STUDY ON SEROTONIN IMMUNOREACTIVE NEURONAL CELL BODIES AND PROCESSES IN THE HUMAN CEREBELLUM

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The cerebellum has not been considered a serotonergic region. Although, studies evidenced a cerebellar role in serotonin (5-HT) motor, emotional and cognitive functions and in 5-HT brain disorders. Data of an intrinsic neuronal cerebellar serotonergic system are lacking. Currently, in the cerebellum only serotonergic afferents originated by the reticular brainstem serotonergic groups, and a widely presence of 5-HT receptor subtypes has been observed. Therefore, the aim of this study is to evaluate in the human cerebellum the existence of serotonergic neurons. The study was carried out on postmortem fragments of the human cerebellar cortex and dentate nucleus, fixed in a picric acid-aldehyde solution, embedded in paraffin, cut into 5µm sections and subjected to light microscopic immunohistochemistry with rabbit polyclonal antibody for 5-HT. In the cerebellar cortex the 5-HT immunoreactivity (ir) in traditional neurons (*i.e.*, basket neurons, Purkinje neurons) and in non-traditional large neurons (*i.e.*, candelabrum neurons, perivascular neurons) have been observed. In the dentate nucleus the 5-HT ir in perivascular neurons, associative and projective neuron types have been detected. Accordingly, a role of 5-HT in the intrinsic and extrinsic cerebellar circuits, and in regulatory functions of the cerebellar blood flow and of the blood brain barrier is suggested. In addition, these results suggest a cerebellar role in 5-HT related disorders (*i.e.*, ataxias, mood disorders), and may be an innovative target for the development of pharmacologic and non-pharmacologic therapies for neurologic and psychiatric disorders.

P1.10 *IN SITU* ANALYSIS OF HUMAN AMNIOTIC MEMBRANE FROM WOMEN WITH GESTATIONAL DIABETES MELLITUS

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Gestational diabetes mellitus (GDM) is a recurrent glucose intolerance that arises during pregnancy. Experimental evidence suggests that related hyperinsulinemia and hyperglycemia can be responsible for nutritional stress which might promote the reprogramming of fetal precursor cells.[1] Hyperglycemia seems to disrupt the invasive profile of cytotrophoblast cells[2] and to enhance angiogenic abilities of amniotic mesenchymal stem cells (AMSCs).[3] Since amniotic membrane stem cells could represent a good indicator of how the maternal environment impacts the fetus[2], a deeper morpho-functional characterization of the human amniotic membrane (hAM) in women with GDM is required. Thus, we performed a detailed *in situ* analysis of hAM by means of light and transmission electron microscopy (TEM) and investigated the expression of markers of pluripotency and proliferation/differentiation ability with immunohistochemistry. We first collected term placentas from five women with GDM (±30) and five control healthy women (±30) undergoing cesarean section at the SS. Annunziata Hospital of Chieti and isolated the 4 different regions of hAM as previously detailed.[4] In comparison with hAM from control healthy women, all the hAM regions from GDM women appeared thicker due to an evident cell hyperplasia and hypertrophy. At the ultrastructural level the presence of wider intercellular spaces and cytoplasmic inclusions was detected in all the regions analyzed. Furthermore, changes in the cellular markers expressed by the different regions of hAM were detected with immunohistochemistry, suggesting that the GDM environment can modify the phenotype of stem cell populations belonging to hAM.[3]

References
1. Algaba-Chueca F et al. Stem Cells Transl Med 2020;9(3):351-63.
2. Cawyer CR et al. Am J Obstet Gynecol 2014;211(5):541.e1-8.
3. Klid S et al. Stem Cell Res Ther 2021;12(1):608.
4. Centurione L et al. Cell Transplant 2018;27(1):12-22.

P2.1 POSSIBLE ROLE OF FERROPTOSIS AND AUTOPHAGY IN THE DEVELOPMENT OF CHOLESTATIC LIVER DISEASES

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Liver fibrosis represents an important stage for the development of liver diseases such as cirrhosis and liver cancer.[1] Its progression is mainly mediated by activated hepatic stellate cells (aHSCs), the major source of the collagen producing. Hepatic fibrosis also involves other liver cells in crosstalk with HSCs by paracrine and autocrine signaling.[2] We demonstrated that neurohormones, such as secretin, stimulate the senescence of cholangiocytes and regulate it in HSCs.[3] Hence, we aimed to investigate the role of two cell death mechanisms: autophagy and ferroptosis during cholestasis. We performed two set of experiments: (i) C57Bl/6 mice underwent BDL surgery several times; and (ii) C57Bl/6 mice were treated with carbon tetrachloride (CCl_4). We measured: (i) the amount of collagen by Masson's trichrome, (ii) the distribution of iron deposits by Perls staining, (iii) IHC and IF for specific markers of autophagy (p62 and LC3β) and for ferroptosis (CD71 and Hepcidin) in liver cells. Both CCl_4 and BDL mice have increased collagen deposition compared to their respective controls. There was an increase of iron deposition in CCl_4 mice, but not in the BDL mice. At last, CCl_4 mice have increased immunoreactivity of p62 and LC3β as well as CD71 and Hepcidin. BDL mice have elevated immunoreactivity expression of autophagic but not ferroptotic markers compared to control groups. In conclusion, autophagy could be considered a protective mechanism activated in the liver in response to cholestatic injury. Whereas ferroptosis could be related to the acute damage of liver fibrogenesis. Targeting ferroptosis and autophagy in liver fibrosis may represent a novel target to better study the mechanisms during cholestatic liver injury.

References
1. Hernandez-Gea V et al. Annu Rev Pathol 2011;6:425-56.
2. Casini A et al. Life 2022;12(12):21-8.
3. Wu N et al. Am J Pathol 2018;188(10):2264-80.

P2.2 EVOLUTIVE ADAPTATIONS IN SKIN OF GREEN WHIP SNAKE *HIEROPHIS VIRIDIFLAVUS* (LACÉPÈDE, 1789)

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The skin of snakes (Squamates: Serpentes) has more contact with the substrate than other reptiles. Often the entire circumference of the body interacts with the ground during locomotion. We analyzed the structure of Green whip snake skin by Mallory trichrome and Masson trichrome histological staining.[1] The richly keratinized skin shows a peculiar morphology, probably associated with the specific environmental and functional demands of these reptiles. The skin appears normally divided into several cell layers. From outside to inside, epidermis, dermis, and hypodermis are distinguished. The epidermis is composed of densely organized keratinocytes. In the basal layer of the epidermis and in the dermis, immediately below the epidermis, there are numerous melanocytes with intense proliferative activity. An abundant layer of adipose tissue forms the underlying hypodermis. Because the skin is constantly exposed to contact with the external environment, it is rich in mechanosensors, which are also useful for the locomotion of these animals. Previous studies have described cutaneous tactile corpuscles densely distributed in the cephalic scales of some species of small land snakes.[2,3] Histologically, the tactile corpuscles appeared as an organized group of dermal cells and nerve fibers that pushed into the epidermis, forming a capsular structure. In our study for the first time we showed Meissner-like tactile corpuscles by using monoclonal and polyclonal antibodies, particularly serotonin and tubulin, both involved in mechanosensory transduction, in the ventral skin of *Hierophis viridiflavus* (Lacépède, 1789). Our data show significant evolutionary structural adaptations in Green whip snake skin, highlighting the connection of these animals with their environment.

References
1. Alesci A et al. Acta Histochemica 2023;125:152031.
2. Jackson MK J Herpetology 1977;11:7-15.
3. Crowe-Riddell JM et al. Royal Society open science 2019;6:182022.

P2.3 PROTECTIVE EFFECT OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE-ADNP AXIS IN THE CORNEA

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The cornea is a transparent tissue covering the eyeball's anterior portion. It is mechanically strong, ensures protection and provides about 70% eye refractive power. Due to its direct connection with the external environment, the cornea is highly exposed to different types of insults, such as ultraviolet B (UV-B) radiations. For this reason, corneal injury is one of the main causes of blindness in the world. In the last few years, several studies have shown the beneficial effects of pituitary adenylate cyclase-activating peptide (PACAP) in different eye diseases. PACAP plays its effects through the activation of G protein-coupled receptors. Moreover, some PACAP effects are mediated by the stimulation of activity-dependent protein (ADNP). However, the role of the PACAP-ADNP axis on the cornea has not been investigated, yet. Therefore, first, we evaluated the expression of PACAP and ADNP on the human cornea. Our results have shown, for the first time, that ADNP, PACAP, and related receptors are largely expressed in corneal epithelium and endothelium.[1-3] So, we analyzed the role of ADNP on corneal epithelial cells exposed to UV-B radiations. Our results showed that the treatment with ADNP mimicking peptide, NAP, decreases ROS production and inflammatory cytokine IL-1β expression, by counteracting UV-B-rays-induced apoptotic cell death.[3,4] Overall, these data suggested that PACAP or NAP might represent a valid strategy for the treatment of some corneal diseases.

References
1. Maugeri G et al. J Cell Biochem 2019;20:10097-105.
2. Maugeri G et al. Appl Sci 2022;12:3435.
3. Maugeri G et al. Antioxidants 2022;11:128.
4. Maugeri G et al. Int J Mol Sci 2023;24:6895.

P2.4 IMMUNOHISTOCHEMISTRY OF THE NASAL CAVITY ASSOCIATED LYMPHOID TISSUE (NALT) IN THE DOLPHIN (*STENELLA COERULEOALBA*)

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The striped dolphin (*Stenella coeruleoalba*) is a small pelagic dolphin presenting a single external nasal opening (blowhole), which is in the dorsal and rostral part of the skull. The nasal cavity can be divided into three parts: vestibular, respiratory, and olfactory regions. The surface epithelium lining the *regio vestibularis* is the first tissue in the nose to be directly injured by antigens present in the environment. Cetaceans have large amounts of mucosa-associated lymphoid tissue (MALT) scattered in their body. The lymphoid tissue present in the nasal mucosa can be named: nose- (or nasal cavity) or nasopharynx-associated lymphoid tissue (NALT). Moreover, NALT has already been observed and described in laboratory rodents and humans, whereas it has never been studied in the dolphin. This study aims to characterize, for the first time, immune cells in the *mucosae regio vestibularis* of *S. coeruleoalba* nasal cavity by confocal microscopy immunofluorescence techniques using specific antibodies: toll-like receptor 2 (TLR2), CD4, Langerin /CD207 and inducible nitric oxide synthase (iNOS).[1,2] The results of the present study showed aggregates and scattered immune cells immunoreactive to the antibodies tested, present in the epithelial tissue lining the nasal cavity vestibulum and in the underlying connective tissue. This study helps us to better understand the immune system of cetaceans, with particular interest for the development of intranasal vaccines, considering the increasing marine pollution.

References
1. Lauriano ER et al. Acta Histochemica 2020;122:2.
2. Lauriano ER et al. Tissue and Cell 2014;46:113-21.

P2.5 THE EXERCISE EFFECT ON TELOCYTES MORPHOLOGY

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Skeletal muscle atrophy, resulting from states of hypokinesis or immobilization, leads to morphological, metabolic, and functional changes within the muscle tissue, a large variety of which are supported by the stromal cells populating the interstitium. Telocytes represent a recently discovered population of stromal cells, which has been increasingly identified in several human organs and appears to participate in sustaining crosstalk, promoting regenerative mechanisms and supporting differentiation of local stem cell niche. The aim of this morphologic study was to investigate the presence of telocytes in the tibialis anterior muscle of healthy rats undergoing an endurance training protocol for either 4 weeks or 16 weeks compared to sedentary rats. Histomorphometric analysis of muscle fibers diameter revealed muscle atrophy in sedentary rats. Telocytes were identified by double-positive immunofluorescence staining for CD34/CD117 and CD34/vimentin. The results showed that telocytes were significantly reduced in sedentary rats at 16 weeks, while rats subjected to regular exercise maintained a stable telocytes population after 16 weeks. Understanding of the relationship between telocytes and exercise offers new chances in the field of regenerative medicine, suggesting possible triggers for telocytes in sarcopenia and other musculoskeletal disorders, promoting adapted physical activity and rehabilitation programmes in clinical practice.

References
1. Ravalli S et al. Biomedicine 2021;9:807.
2. Manetti M et al Sci. Rep. 2019;9:14515.

P2.6 THE ROLE OF EXERCISE AND "MECHANOKINES" TO PREVENT KNEE OSTEOARTHRITIS

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The purpose of this study was to investigate the influence of moderate physical activity (MPA) on the expression of osteoarthritis (OA)-related (IL-1, IL-6, TNF-α, MMP-13) and anti-inflammatory and chondroprotective (IL-4, IL-10, lubricin) biomarkers in the synovium of an OA-induced rat model. The MPA-based approach may support joint tribology and synovial lubrication, leading to improved joint function and pain relief. In addition, in pathologic conditions, synoviocytes type A secrete cathepsins, MMPs, and pro-inflammatory cytokines/chemokines into the extracellular matrix, triggering tissue damage. A total of 32 rats were divided into four groups: Control rats (Group 1); rats performing MPA (Group 2); anterior cruciate ligament transection (ACLT)-rats with OA (Group 3); and, ACLT-rats performing MPA (Group 4). Early OA was induced through the anterior cruciate ligament transection (ACLT) technique. Analyses were performed using Hematoxylin & Eosin staining, histomorphometry and immunohistochemistry. In Group 3, OA biomarkers were significantly increased, whereas IL-4, IL-10, and lubricin were significantly lower than in the other groups. The results from MPA experimental group (Group 4) highlighted the decreased expression of OA-related biomarkers (IL-1, TNF-α, MMP-13) and the increased expression of chondroprotective ones (IL-4, IL-10, and lubricin). We hypothesise that MPA might partake in rescuing type B synoviocyte dysfunction at the early stages of OA, delaying the progression of the disease and finally postponing the need for joint replacement.

References
1. Ravalli S et al. 2019;10(7):262-267.
2. Di Rosa M et al. 2019;4(1):11.
3. Castrogiovanni P et al. Int J Mol Sci. 2019;20(3):511.

P2.7 SYNOVIAL FLUID DERIVED EXTRACELLULAR VESICLES: A POSSIBLE ROLE IN THE PROGRESSION OF INFLAMMATION

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Exosomes are membrane-bound extracellular vesicles (EVs) surrounded by a phospholipid bilayer secreted by all humans' cell types. EVs can differ in size, origin and biomolecular composition and are divided into three categories: exosomes, microvesicles and apoptotic bodies[1]. Inflammatory joint disease is a complex disorder that unfortunately still lacks appropriate clinical therapy options. The role of EVs in inflammatory diseases has become the focus of many studies since they are involved in cell-to-cell communication and can be used as potential biomarkers for diagnosing and monitoring the progression of inflammation. EVs have been detected in a variety of biological fluids such as plasma, urine, and synovial fluid (SF).[3] Understanding the role of SF-derived EVs in joint physiology is the first step in improving the treatment of joint diseases. The isolation of EVs from synovial fluid is less well characterised than other biological fluids. In this study, we initially collected SFs from the joints of patients with femoroacetabular impingement (FAI) classified as having "high or low-level" of inflammation based on cartilage damage score, and from patients with osteoarthritis (OA). We performed the isolation of EVs from human SFs using two of the most applied EVs isolation methods: ultracentrifugation (UC) and size-exclusion chromatography (SEC). Using the Nanoparticle tracking analysis (NTA), our preliminary data showed that UC is a better technique of separation of EVs from SF samples. Following the separation, we confirmed the presence of EVs-CD63 positive in our three groups. NTA and dot blot analysis showed a higher number of EVs CD63 positive in the high inflammation and OA SFs compared to low inflammation condition. Our preliminary data suggest that EVs are involved in the progression of the inflammation. Further characterization using specific markers are being done to better investigate the cellular origin of the SF-derived EVs.

References
1. Battistelli M, Falcieri E. Biology 2020;9:21.
2. Luchetti F Plos One 2012;10:1371.
3. Battistelli M Diagnostics 2021;11:1391.

P2.8 NEUROPROTECTIVE AND ANTI-INFLAMMATORY EFFECTS OF EPA AND DHA ON OLFACTORY ENSHEATHING CELLS EXPOSED TO LIPOPOLYSACCHARIDE. A PRELIMINARY *IN VITRO* STUDY

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Neuroinflammation is a common symptom in the onset of different neurodegenerative diseases and growing interest is directed towards the development of active drugs for the reduction or elimination of its negative effects. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), belonging to the class of ω-3 polyunsaturated fatty acids, have been largely investigated for their anti-inflammatory activity and their potential as neuroprotective agents has been evaluated on some neural cells[1]. Most of the observed biological activities of these fatty acids are maintained, and in some case enhanced, in the corresponding amide derivatives or oxygenated metabolites[2]. Our study aims to elucidate the protective effect of both EPA and DHA, as well as the corresponding *N*-ethanolamides EPA-EA and DHA-EA, on Olfactory Ensheathing Cells (OECs) exposed to lipopolysaccharide (LPS)-induced neuroinflammation. OECs are glial cells located in the olfactory system, which is the first to show a deficit in neurodegenerative diseases. To verify the anti-inflammatory effect of these compounds on OEC cultures and on cell morphological features, the expression of some cytoskeletal proteins, such as Vimentin and Glial Fibrillary Acid Protein (GFAP), was evaluated by immunocytochemical procedures. In addition, MTT test was carried out to establish the non-toxic concentrations and the optimal time of exposure. Our results show a decrease of GFAP and Vimentin expression in OECs treated with EPA or DHA acids or EPA-EA or DHA-EA and stressed with LPS when compared with OECs exposed to LPS alone. While a protective role on cell morphology is predominantly observed for EPA and DHA, the amides EPA-EA and DHA-EA mainly show anti-inflammatory effects, superior to those of free acids. These results highlight that all the tested compounds have anti-inflammatory activity on LPS-exposed OECs and may provide an innovative tool to contrast neuroinflammation, which plays a key role in several neurodegenerative diseases.

References
1. Dyall SC Front Aging Neurosci 2015;7:52.
2. De Bus I et al. Prostaglandins and Other Lipid Mediators 2019;144:106351.

P2.9 PROTECTIVE ROLE OF TART CHERRY AGAINST WHITENING OF BROWN ADIPOSE TISSUE OF OBESE RATS

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Obesity has a great impact on adipose tissue biology, based on its function as the master regulator in energy balance. The excess of energy affects the adipose tissue with an overstoring of lipids droplets. Brown adipose tissue (BAT) undergoes remodeling, and its activity declines in obese subjects, mainly as a result of the conversion of brown adipocytes to white-like unilocular cells (whitening process). In addition, obesity is associated with endoplasmic reticulum stress in adipose tissue, and free fatty acids induce reactive oxygen species formation. Studies have identified inflammation and immune cell infiltration as contributors to BAT dysfunction. Reduction of oxidative stress and inflammatory processes have been reviewed in animal models of obesity treated with bioactive natural compounds. Thus, we investigated in interscapular BAT (iBAT) the effects of *Prunus cerasus* L. in obese rats fed with a high-fat diet (HFD) called DIO, an HFD supplemented with seed powder (DS), and with seed powder plus juice (DJS) of tart cherries. Rodents were monitored for 17 weeks of HFD and compared to CHOW rats fed with a standard diet. Morphological staining revealed in DIO rats an enlargement of white adipose tissue in iBAT. Tart cherry supplementation reduced obesity-induced whitening of iBAT both in DS and in DJS, compared to DIO rats. A modulation of uncoupling protein 1 (UCP1) expression, specifically in brown adipocytes, was detected in obese phenotype and after tart cherries intake. Predictably, based on the brown-to-white conversion in obesity, the gene expression results showed a downregulation of UCP1 in DIO compared to CHOW rats. Moreover, an upregulation of the thermogenic genes was found in the supplemented rats compared to DIO. Metabolic adaptations, endoplasmic reticulum stress, protein carbonylation, and inflammatory process in the BAT were reported in obese rats, modulated by tart cherries supplementation. In addition to our previous results, these data suggest the protective effect of anthocyanins-enriched fruit consumption in obesity.

P2.10 MORPHOLOGICAL STUDY OF COLONIC MUCOSA IN MICE WITH DEXTRAN SULFATE SODIUM-INDUCED COLITIS: THE IMPACT OF PROBIOTIC SUPPLEMENTATION

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Inflammatory bowel diseases (IBD) are chronic gastrointestinal disorders that can impair the patient's quality of life.[1] Dextran sulfate sodium (DSS)-induced colitis is one of the most common mice models of chemically induced IBD.[2] The treatments for IBD showed insufficient therapeutic efficacy. Many studies identified dietary supplementation with probiotics as a promising intervention by alleviating clinical symptoms. The potential properties of *Weissella paramesenteroides* A1 (*Wp*) and *Pediococcus acidilactici* 46A (*Pa*) were evaluated on a murine model of DSS-induced colitis. 8-week-old mice were used. Colitis was induced by administering 3% (w/v) DSS in drinking water for 7 days. Probiotics were supplemented orally (1×10^8 CFU daily) for 10 days before DSS administration. Weight loss, stool consistency and intestinal bleeding were monitored to evaluate the clinical progression of colitis. Microscopically, histological damage, inflammatory cells infiltration and pro-inflammatory cytokines expression were assessed on proximal and distal colon sections. *Pa* supplementation was able to reduce the macroscopic severity score while not affecting weight loss. The histological damage was recorded for impairment of crypts architecture, goblet cells depletion and inflammatory infiltrate. The colitis severity was reduced in the *Pa* pretreated mice compared to the DSS group. The presence of $CD3^+$ cells was lower in the *Pa* pretreated animals compared to DSS. The same pattern was observed in the sections incubated with TNF-α antibodies. Particularly in the *Pa* treated mice was appreciated a reduction of inflammatory cells in the area in which the colonic wall cytoarchitecture was maintained. These results showed the potential use of specific strains of bacteria to treat intestinal disorders. *Pa* is active against intestinal inflammation in DSS-induced colitis although further studies are necessary to better characterize its possible implication as a therapeutic agent against IBD.

References

1. Mentella MC et al. Nutrients 2020;29;12:944.
2. Chassaing B et al. Curr Protoc Immunol 2014;104:15.25.1-14.