COMUNICAZIONI ORALI

CLASS II PI3K MEDIATED REGULATION OF mTOR IN GLUTAMINE SENSING WITHIN PANCREATIC CANCER

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Pancreatic cancer (PC) is on path to become the second leading cause of cancer -related death by 2030. Late diagnosis and resistance to chemotherapy are the main reasons for the poor clinical outcome of PDAC patients. mTOR-dependent metabolic adaptation to nutrient availability, such as glutamine, is a key feature of PC, that can be therapeutically exploited. Targeting key metabolic regulators and their downstream pathways shows efficacy only in subsets of patients but gene modifiers maximising response remain to be identified.

We demonstrated that PI3K-C2g expression was reduced in about 30% of PC cases, associating with aggressive phenotype. In KPC mice, loss of PI3K-C2g increased tumor development, correlating with hyperactivation of mTORC1 pathway, through the newly discovered Asap1/Arf1 axis, and glutamine metabolism rewiring, to support lipid synthesis. PI3K-C2g-KO tumors failed to adapt to metabolic stress induced by glutamine depletion or glutaminase inhibition, resulting in cell death.

In conclusion, we established the combinatorial targeting of mTOR and glutamine metabolism as an innovative therapeutic option in pancreatic cancer. The study of glutamine -mediated regulation of PI3K-C2g activity and the manipulation of different metabolic features, as glutamine addiction, would help to identify the best combinatorial treatment that fits with patients to improve their clinical outcome.

FUNCTIONAL INTERPLAY BETWEEN SPHINGOSINE 1-PHOSPHATE SIGNALLING AND ENDOCANNABINOID SYSTEM IN ENDOMETRIOSIS

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Endometriosis is a chronic inflammatory gynaecological disease associated with infertility and pelvic pain. The pathogenesis of the disease is multifactorial, however the molecular mechanisms involved are complex and far to be fully elucidated.

The signaling of the bioactive sphingolipid sphingosine 1-phosphate (S1P) is deeply dysregulated in endometriosis being the expression of its specific receptors S1P1, S1P3 and S1P5 increased in endometriotic lesions.

The endocannabinoid system (ECS), consisting of the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG), their receptors, CB1 and CB2, and their metabolic enzymes, plays a crucial role in modulating different processes including inflammation. Here, the involvement of S1P and ECS signalling and their possible cross-talk in endometriosis has been investigated.

CB1, CB2 and GPR18, an orphan cannabinoid-like G-protein coupled receptor, have been found to be expressed in endometriotic lesions both at mRNA and protein levels. Furthermore, the effect of 2 - AG and AEA in the modulation of inflammation has been investigated in endometriotic epithelial cells. 2-AG, but not AEA, significantly augmented the expression of proinflammatory cytokines as well as COX2. Interestingly, 2-AG-induced increase of S1P3 expression is crucial for the biological action of the endocannabinoid. Indeed, S1P3 pharmacological blockade or its specific silencing impaired the pro-inflammatory action of 2-AG.

These findings highlight for the first time a functional interplay between S1P signalling and ECS in endometriosis, paving the way for innovative pharmacological approaches for the treatment of the disease.

CD44/NRF2 AXIS DRIVES FERROPTOSIS RESISTANCE IN OVARIAN CANCER STEM CELLS BY ENHANCING CYTOSOLIC AND MITOCHONDRIAL FERRITINS TRANSCRIPTION

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Ovarian cancer stem cells (OCSCs) are iron addicted and, thus, theoretically highly vulnerable to ferroptosis. Colliding with this enthusiasm, experimental data show that OCSCs often resist ferroptosis inducers (FINs), and the biological mechanisms underlying the escape from this cell death are not fully elucidated. Here, 11 ovarian (OVCA) cell lines were grown either as adherent 2D cultures or non-adherent 3D tumor spheroids to enrich the CSCs subpopulation, and then treated with growing concentration of ferric ammonium citrate (FAC) or ferlixit (class IV FINs). We observed that OVCA cells grown in 2D conditions show a variable ferroptosis sensitivity, which was independent from both the genetic make-up and histological grade, but rather nicely correlated with the amount of the labile iron pool (LIP) accumulated within cytosol and mitochondria. When grown in 3D conditions, instead, all OVCA cell lines exhibited a substantial resistance to FAC and ferlixit associated with a remarkable decrease of cytosolic and mitochondrial free iron. Mechanistically, OVCA cell lines grown in 3D conditions overexpressed CD44 stemness marker; this was associated with increased nuclear factor erythroid 2–related factor 2 (NRF2) expression and translocation into the nucleus where it bound to the promoter of genes encoding cytosolic ferritin subunits (*FtH* and *FtL*) and mitochondrial ferritin (*FtMt*) ($p<0.05$), ultimately leading to expanded iron storage capacity and low availability of free iron for the initiation of ferroptosis. In agreement, silencing of *CD44* resulted in NRF2 reduction, lower expression of *FtH*, *FtL* and *FtMt*, higher levels of LIP, mitochondrial reactive oxygen species (ROS) accumulation and lipid peroxidation. Collectively, these findings uncover the CD44-NRF2-ferritin axis as a promising target for the control of ferroptosis resistance of the ovarian-CSC subpopulation.

MODULATION OF MITOCHONDRIAL QUALITY CONTROL THROUGH AUTOPHAGIC PATHWAY IN GENETIC ALZHEIMER'S DISEASE

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Autophagy is a cellular catabolic process by which macromolecules or damaged organelles are degraded via lysosomes, thus being crucial for the cellular homeostasis. Accordingly, autophagic dysfunction is linked to the pathogenesis of neurodegenerative disorders, including Alzheimer's disease (AD), thus its modulation might represent a therapeutic approach. Searching for a compound that stimulates autophagy, led us to identify 37/67 kDa laminin receptor (LR) inhibitor as modulator of Akt-mTOR pathway, the master regulator of autophagy. We found that fibroblasts from genetic AD-affected patients showed impaired autophagic flux and accumulation of damaged organelles. The 37/67 kDa LR inhibitor, NSC47924, by inactivating Akt – mTOR axis, enhanced autophagic flux, restoring the lysosomal turnover of autophagic vacuoles Indeed, NSC47924 influenced the conversion of the cytosolic microtubule-associated LC3-I into the lipidated LC3 II associated to autophagosome. Moreover, qRT-PCR analysis revealed that NSC47924 influenced the expression of autophagy-linked genes. Interestingly, inhibitor treatment modulated the turnover of autophagic receptors, e.g. p62, NDP52 and OPTN, as well as the mitophagy regulators PINK1 and Parkin, consistent with an efficient elimination of dysfunctional mitochondria abnormally accumulated in fAD cells. In fAD fibroblasts, the inhibitor significantly improved mitochondrial morphology, restoring a highly interconnected network. The morphological improvement correlated with a functional recovery of mitochondrial network, as assessed by the measurement of oxygen consumption rate by Seahorse analysis and mitochondrial ROS production. Collectively, our findings suggest that LR inhibitor stimulates an autophagic pathway allowing the efficient removal of damaged organelles, contributing to the recovery of cellular homeostasis.

NEW INSIGHT ON MARINESCO-SJÖGREN SYNDROME: THE ROLE OF ANTIOXIDANT DEFENSES

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Marinesco-Sjogren syndrome (MSS) is a rare autosomal recessive disorder of infancy that causes cerebellar ataxia, cataracts, myopathy, skeletal muscle weakness and sometimes mental retardation. Apart from educational and rehabilitative programs, there is no pharmacological treatment. Most patients suffering from this disease carry a mutation in the Sil1 gene that encodes for a protein that acts as NEF (Nucleotide Exchange Factor) for Bip, essential for the protein folding process.
In case of compromised protein folding, the cell activates the UPR (Unfolded Protein Response) as an attempt to restore cellular proteostasis. The activation of these stress sensors in MSS is well documented, however our proteomic analyses highlighted how antioxidant defenses could also play an important role. Therefore, our attention was focused on the study of both antioxidant pathways and the most common markers of ROS-induced damage in MSS and CTR human fibroblast and in muscle tissue of woozy mice, a model for MSS. Our data highlighted that the activation of these defenses, and in particular of the catalase-superoxide dismutase axis to the detriment of the Gpx-GR system, has a protective effect and represents an adaptive response to counteract damages caused by overproduction of ROS. In fact, by evaluating the common markers of ROS-induced damage (lipid peroxidation, protein damage and DNA damage) we found that these molecules don't undergo permanent modifications/damage. From our data it is clear that, both in patient fibroblasts and in mouse muscle tissue, the same pathways are activated and probably have a protective role, considering the non-lethality of the syndrome. Furthermore, our work highlights how the use of patient fibroblasts can be a good model for studying the disease.

P140CAP COUNTERACTS B-CATENIN PATHWAY IN THE BREAST CANCER STEM CELL COMPARTMENT POTENTIATING THE ANTI-TUMOR IMMUNE RESPONSE

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The p140Cap adaptor protein functions as a tumor suppressor in breast cancer and is associated with a favorable prognosis. Here we show that this protein plays a critical role in coordinating local and systemic events that ultimately inhibit the function of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs), which create an immunosuppressive, tumor-promoting environment both in the primary tumor and in premetastatic niches. Through integrative transcriptomic and preclinical studies, it has been revealed that p140Cap regulates an epistatic axis by inhibiting β-Catenin. This inhibition restricts tumorigenicity and the self-renewal of tumor-initiating cells, thereby limiting the release of the inflammatory cytokine G-CSF. G-CSF is necessary for PMN-MDSCs to perform their tumor-promoting functions both locally and systemically. Mechanistically, p140Cap's inhibition of β-Catenin relies on its ability to localize within and stabilize the β-Catenin destruction complex, enhancing β-Catenin inactivation. Clinical studies in women have shown that low p140Cap expression correlates with a reduced presence of tumor-infiltrating lymphocytes and more aggressive tumor types. This was observed in a large cohort of real-life breast cancer patients, underscoring the potential of p140Cap as a biomarker for therapeutic interventions targeting the β-Catenin/tumor-initiating cells/G-CSF/PMN-MDSC axis to restore an efficient anti-tumor immune response. Additionally, the cancer stem cell are implicated in response to chemotherapy indicating p140Cap as a possible predictive biomarker in breast cancer. Preliminary data indicate that the presence of p140Cap leads to increased doxorubicin uptake and subsequently higher cell death related to chemotherapy.

COLORECTAL CANCER-DERIVED EXTRACELLULAR VESICLES AFFECT THE IMMUNOMODULATORY PROPERTIES OF HEPATOCYTES CONTRIBUTING TO LIVER METASTATIC COLONIZATION

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Colorectal cancer-derived liver metastases represent the leading cause of CRC-related deaths. CRC-derived extracellular vesicles (CRC-EVs) promote in the liver the formation of the pre-metastatic niche (PMN), by affecting the activity of the non-parenchymal cells while few data are available about the involvement of hepatocytes (Heps). Recently, we showed that CRC-EVs induce epithelial to mesenchymal transition in Heps, event preceding the fibrotic injury. Evasion of immune surveillance is another crucial event that define the hepatic PMN and is mainly caused by the activation of immune check-point PD-L1/PD-1. We already demonstrated that CRC-EVs carry PD-L1 and upregulate the expression of PD-L1 in macrophages. Our hypothesis is that CRC-EVs can support liver metastasis inducing an early alteration also of Heps immunomodulatory properties within the PMN, inducing tumor-associated escape from antitumoral immunity. We showed that CRC-EVs induce in Heps the increase of PD-L1 expression also promoting its nuclear translocation according to emerging data that reports that beyond the well-known localization on cell surface and in cytoplasm, PD-L1 is also present in the nucleus (nPDL1) where could activate alternative pathways that support tumor progression. According to that, we observed that the nPDL1 in CRC-EVs treated Heps is associated with the upregulation of GAS6, VISTA and PD-L2, proteins involved in the regulation of immune tolerance mechanisms. To study the correlation between nPDL1 and the increased expression of these factors we used a molecule able to block the nuclear transfer of the SEVs cargo into recipient cells, showing that the immunomodulatory properties of CRC-EVs are blocked in pretreatred Heps. Our results highlighted that CRC-EVs elicit in Heps an alternative activity of PD-L1 contributing to the formation of an immunosuppressive microenvironment.

C-MYC DYSREGULATION CONTRIBUTES TO GLIA-TO-NEURON MISCOMMUNICATION IN AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA

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Amyotrophic Lateral Sclerosis (ALS) is a non-cell-autonomous disease. Severalstudies demonstrated that glia modulates disease progression in a mouse model expressing a mutant form of SOD1. This effect was not validated yet in a transgenic mouse expressing TDP-43^{Q331K}, so we selectively deleted the transgenic TDP-43 in astrocytes and observed an improvement of motor symptoms and a rescue of the cognitive impairment. We performed RNA sequencing of astrocytes purified from this mouse model, observing an increased expression of genes related to proliferation and de-differentiation at the early symptomatic stage. Increased proliferation was also confirmed by EdU staining in primary astrocytic cultures. By interrogating the genomes of 12.577 ALS patients versus 23.475 healthy controls, we revealed the enrichment of SNPs in the responsive elements of transcription factors linked to proliferation with a hub around c-Myc. Phosphorylated c-Myc is increased in primary astrocytes expressing TDP-43^{Q331K} or SOD1^{G93A}, in Hek293 cells overexpressing TDP-43, and in iPSC-derived motoneurons expressing C9orf72, TDP-43^{M337V}, SOD1^{I114T} or derived from sporadic patients. Among its many functions, c-Myc enhances the release of extracellular vesicles (EVs). We observed that WT glia-derived EVs play on receiving neurons a trophic effect lost with glia-derived EVs from TDP-43^{Q331K} cultures or WT cultures overexpressing

c-Myc. Proteomic analysis revealed that ALS EVs and EVs derived from c-Myc-overexpressing cells were deficient in extracellular matrix (ECM) proteins and integrins. Interestingly, those EVs were less prone to enter recipient neurons. Finally, by a cytofluorimetric analysis of ALS patients' cerebrospinal fluid, we measured a decrease in one integrin-interacting protein, suggesting that the role of integrins and ECM proteins should be further investigated in ALS.

DNA METHYLATION ALTERATIONS IN MACROPHAGES UPON *LEISHMANIA* INFECTION IN DIFFERENT CULTURE CONDITIONS

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Leishmaniasis is a neglected tropical disease caused by the protozoan *Leishmania* that presents diverse clinical forms, ranging from cutaneous/mucosal lesions to visceral infections. The flagellated promastigote stage of *Leishmania* enters in macrophages and transform into amastigote. To colonize macrophages, the parasite modulates host cells epigenome, including DNA methylation. Given the lack of effective vaccines and therapy toxicity, leishmaniasis represents a public health problem. The study of methylome alterations caused by *Leishmania* species could lead to a new research area for leishmaniasis therapy. Therefore, we performed a genome-wide methylation analysis in macrophages, in presence/absence of IL6, infected with *L. braziliensis*, *L. amazonensis* and *L. infantum*. Different DNA methylation alterations, focused on CpG islands and promoters, were identified upon infection and among species, suggesting a specie-specific parasite/host interaction. We also detected that the DNA methylation pattern slightly changed in a culture environment with IL6. Moreover, macrophages infected with *L. braziliensis* and *L. infantum* were treated with Glucantime and Amphotericin B, respectively. Distinct DNA methylation profiles were reported between treated and untreated samples. Interestingly, we detected a tendency to the reestablishment of the DNA methylation towards a normal pattern after treatment. The discovery of these methylated based alterations addresses further functional studies and provides a probable new approach for leishmaniasis therapy.

CIRCSRSF5 PROTECTS EWING SARCOMA CELLS FROM YK-4-279 INDUCED CELL DEATH

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Circular RNAs (circRNAs) are a class of covalently closed and mostly non-coding RNA molecules, produced by back-splicing events. Involved at different levels in the regulation of gene expression, circRNAs are new key players in the field of cancer, representing promising tools for tumor diagnosis and therapy. Ewing sarcoma (ES) is an aggressive pediatric tumor affecting bones and soft tissues. The interaction between the oncogenic transcriptional factor EWS-FLI1 and the RNA helicase DHX9 plays a key role in ES onset and progression. Although DHX9 is a known regulator of circRNAs production, the possible involvement of circRNAs in ES disease has not been unraveled yet.

To understand the role of EWS-FLI1 and DHX9 in circRNA functions in ES cells, we performed RNA-deep sequencing after treatment with the anti-tumor small molecule YK-4-279, which inhibits the interaction between DHX9 and EWS-FLI1, highlighting several deregulated circRNAs. We focused on circSRSF5 (circBaseID: hsa_circ_0000549), upregulated after YK-4-279 treatment. Functional analyses showed an oncogenic role for this circRNA, counteracting YK-4-279 effect on ES cell viability. Biochemical analysis allowed identification of the molecular circuit underlying this function. We found that EIF4A3, a key pro-survival protein, positively regulates circSRSF5 biogenesis. Furthermore, circSRSF5, in turn, positively affects EIF4A3 expression stabilizing its transcript.

Current ES therapy is mainly based on the administration of chemotherapeutic agents with deleterious long-term effects on the young patients. The identification of circRNA molecules, like circSRSF5, able to counteract drug-mediated tumor cell death, could represents the first step for the identification of new potential RNA-based therapeutics to counteract drug resistance.

M6A RNA MODIFICATION REPRESSES MIRNA FUNCTION AND FAVOURS MIRNA DELIVERY IN EXTRACELLULAR VESICLES

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Epitranscriptomics (e.g. RNA methylation) is an emerging layer of gene expression regulation so far demonstrated to impact mRNAs stability, degradation and translation; with respect to miRNAs, it has been reported as necessary for their biogenesis, but little is known about the impact of m6A on mature miRNAs function.

Here, by means of omics (miRNA- and mRNA-seq NGS, bioinformatic analyses and native mass-spectrometry), and biochemical (MeRIP, CLIP, RNA pull-down, luciferase assays, EV cargo analysis) assays on 14 selected miRNAs, we describe that m6A plays a central role on the activity and localization of miRNAs.

In brief: i) several mature miRNAs are m6A-modified, ii) high methylation of miRNAs weakens their interaction with AGO2 and their activity on target mRNAs, iii) m6A modification determines hnRNPA2B1-dependent delivery of miRNAs into Extracellular Vesicles (EVs), iv) receiving cells restore the activity of EV-delivered miRNAs through FTO-demethylation activity. Further interest derives from the observation that: a) mature miRNAs are methylated in a cell-specific manner, and b) methylation consensus is shared by a large subset of miRNAs.

Overall, these data provide evidence on an epitranscriptomic regulatory mechanism that distributes the miRNAs function between the intracellular and the cell-to-cell communication allowing producing cells to restrict functional effects to target cells.

The regulatory layer here described implies that in future observation, in addition to miRNA expression levels, it should be taken in account their effective intracellular functionality and/or extracellular delivery as regulated by m6A editing.

DECIPHERING THE ROLE OF THE EPITRANSCRIPTOMIC MODIFICATION M6A IN GLIA DURING BRAIN DEVELOPMENT

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Obtaining a properly developed brain is the result of tightly controlled regulation of gene expression. RNA modifications known as the Epitrancriptome, constitute a dynamic layer of control of gene expression, that allow quick response to developmental changes. N6-methyladenosine (m6A) is an abundant mRNA modification and its localization on the transcripts, levels and biological consequences, depend on the interplay between the components of the m6A machinery: the "writers", that deposit m6A, the "erasers", that remove the m6A and the "readers", that recognize and bind m6a marked transcripts. Previous studies clarified that m6A plays an important role in neurons during development, affecting axons growth in several neuronal circuits, but little is known about m6A role in other CNS populations such as glia. We use *Drosophila melanogaster* as a model to investigate how m6A in glia contributes to neurodevelopment. Our results show that perturbing the m6A machinery in glia leads to developmental lethality, suggesting a crucial role of m6A. Brain analysis at different developmental times highlights several dramatic effects in different classes of glia, such as loss of glia cells, aberrant projection patterns/morphology among the others. Specific m6A perturbation in astrocytes also leads to defects in terms of number of cells and structure of projections. In particular, astrocytes show reduced area coverage of active zones in the CNS and aberrant projection structure. In conclusion, our data indicates that m6A plays a role in glia during brain development and, in particular, affects astrocytes growth and patterning and synaptic association. Currently, we are dissecting the precise molecular mechanisms underlying this function and which are the target of m6A methylation involved in these processes.

ORGANELLE COMMUNICATION CONNECTS GROWTH FACTOR SIGNALLING TO CELL METABOLISM

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The integration of distinct internalization routes is crucial to determine the fate of plasma membrane (PM) receptors and the output of their signalling pathways. Contact sites between cellular organelles adds a further layer of regulation by creating microdomains that governs different signalling and metabolic pathways. These regulatory mechanisms are relevant to the epidermal growth factor receptor (EGFR). Our research has revealed that, while clathrin-mediated endocytosis (CME) is mainly involved in EGFR recycling and sustaining signalling, EGFR internalization through non-clathrin endocytosis (NCE) leads primarily to receptor degradation and signal extinction, representing a crucial safety mechanism to protect cells from overstimulation. NCE involves contact sites between the PM, the endoplasmic reticulum and the mitochondria, that work as platforms for the modulation of localized calcium signalling and mitochondrial energetics. Importantly, this mode of regulation extends beyond EGFR to encompass other growth factor receptors, and is anticipated to be relevant in tumours. In my laboratory, we are currently elucidating how the integration of distinct endocytic pathways and inter-organelle crosstalk regulate growth factor receptor signalling and its interplay with cell metabolic functions in physiology and cancer.

SYNAPTOJANIN-1: A NEW PLAYER IN THE CONTROL OF VESICULAR MEMBRANE TRAFFICKING IN THE EARLY SECRETORY PATHWAY

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Synaptojanin 1 (Synj1) is an inositol-phosphatase belonging to the family of Sac domain-containing proteins, crucial for maintaining homeostasis in the early endocytic pathway. Dysfunction of Synj1 is linked to several neurological disorders, including Parkinson's and Alzheimer's. Although the role of Synj1 in the endocytic pathway is well-established, its involvement in the early secretory pathway had not been explored. Our study shows that in HeLa cells, the interference of Synj1 expression results in a significant reduction of COPII-coated vesicles and ER exit sites (ERESs), along with a severe impairment in the delivery of transmembrane, soluble, and GPI-anchored cargo proteins to the Golgi or plasma membrane. Additionally, the phosphatidylinositol (PI) and PI-4-Phosphate (PI4P) levels, which primarily mark the membranes of ER and *cis*-Golgi, are disrupted in Synj1-depleted cells. To uncover the molecular mechanism underlying these defects we examined Synj1 localization and function during COPII vesicles budding. We found that Synj1 partially localizes to ERES during vesicle assembly and closely associates with Sec23a, a key component of the inner COPII coat. Notably, the interaction between Synj1 and Sec23a begins in the cytosol and modulates Sec23a recycling during coat assembly, as disruption of Synj1 expression results in an increased association of Sec23a with the ER membrane. These findings suggest that Synj1's phosphatase activity, by modulating PI and PI4P levels, is essential for controlling the retention of Sec23a on ER membranes, thereby promoting an efficient export of cargo proteins from the ER. Overall, these results reveal a novel function of Synj1, highlighting it as a new regulator of membrane trafficking in the early secretory pathway. This discovery has relevant implications for therapeutic strategies targeting Synj1-related diseases.

SYNPATOJANIN 1 (SYNJ1) DYSFUNCTION LEADS TO AUTOPHAGY DYSREGULATION: POTENTIAL IMPACT ON PARKINSON'S DISEASE

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The ubiquitous inositol-phosphatase synaptojanin 1 (SYNJ1) is expressed at high levels in neuronal cells, in which it plays a crucial role in synaptic vesicle retrieval. Loss -of-function mutations of *SYNJ1* gene cause early-onset parkinsonism 20 (PARK20), a hereditary form of Parkinson's disease (PD), but the mechanisms through which the functional deficit of SYNJ1 exerts its pathogenic activity have not been fully elucidated. We have recently reported that *SYNJ1* mutations are associated to alterations in early endosomal compartment homeostasis and function, emphasizing the role of endosomal trafficking in the pathogenesis of PD. Here, we investigated whether *SYNJ1* is involved in the regulation of the lysosomal and autophagic pathways. We performed immunofluorescence and Western blot analysis of several lysosomal and autophagic markers in HeLa cells in which *SYNJ1* expression was knocked-down by RNAi, and in fibroblasts isolated from PARK20 patients. *SYNJ1*-silenced HeLa cells present a mild enlargement of the lysosomal compartment, higher levels of the autophagic markers LC3-II and p62/SQSTM1, and a marked increase in the number of autophagosomes with respect to control interfered cells. Interestingly, such alterations are associated to a perturbation of the autophagic flux, to a reduced clearance of autophagic substrates, like the -synuclein mutant A53T, and to endoplasmic reticulum stress. Consistently, the levels of autophagic markers and the number of autophagosomes are increased in PARK20 fibroblasts compared to control ones. Taken together, our data indicate that *SYNJ1* is involved in autophagy regulation, and suggest that the impairment of autophagic flux and the accumulation of autophagy substrates may be important mechanisms by which *SYNJ1* mutations contribute to the pathogenesis of PARK20.

ALPHA-SYNUCLEIN IMPAIRS MACROPHAGE AUTOPHAGY IN ASSOCIATION WITH HYPER-INFLAMMATION, LIPID DYSHOMEOSTASIS, AND DEFECTIVE PHAGOCYTOSIS

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Spreading of excess alpha-synuclein (aS), a hallmark of Parkinson's disease, is shown to promote peripheral inflammation via still unclear intracellular mechanisms. Autophagy plays a functional role in monocytes/macrophages, where it was shown to govern aS proteostasis. However, if aS affects autophagy in these cells specifically remains poorly explored. Here we investigate the subcellular, molecular, and functional effects of aS in human monocytes and macrophages with a focus on autophagy. Human THP-1 monocytic cells (TMs), derived macrophages (TDMs), and primary monocyte-derived macrophages (MDMs) were cultured w/wo recombinant aS (1uM) for 4 and 24h. By Confocal microscopy, Western Blot, qRT-PCR, and Elisa we assessed: i) aS internalization; ii) inflammatory profile; iii) autophagy (LC3II/I, LAMP1/LysoTracker, p62, pS6/S6 ratio); iv) red-oil stained lipid droplets (LDs); v) phagocytic capacity, and the fate of phagocytosed cargo. Extracellular aS was internalized by TMs, MDMs and TDMs, where it induced intracellular accumulation and release of pro-inflammatory mediators. In TDMs, this was accompanied by mild toxicity, increased p62 protein levels, decreased LC3II/I ratio, and decreased LAMP1 at both protein and mRNA levels. This was independent of the mTOR activity index pS6/S6, but was associated with F-actin clumping, and reduction of intracellular LDs, and their co-localization with LC3 and LAMP1. Finally, aS reduced both phagocytosis, and the clearance of phagocytosed cargo, which markedly filled LysoTracker-stained organelles resulting in a co-localization pattern reminiscent of engulfed/stagnant lysosomes. Our results suggest that while monocytic cells well-tolerate excess aS, macrophages undergo an exhaustion status resembling hypophagia, with autophagy-lysosome impairment potentially bridging hyper-inflammation, cell toxicity, and lipid dyshomeostasis.

SPATIAL POSITIONING OF GLIOBLASTOMA CELLS WITHIN THE MASS DICTATES COLLAGEN VI DEPOSITION AND EXTRACELLULAR MATRIX-INDUCED PHENOTYPE

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Glioblastoma (GBM) is the most aggressive adult brain tumour, representing one of the biggest therapeutic challenges in oncology. Despite recent advancements in the molecular characterization of these tumors, the current treatment strategy, including surgery and radio-chemotherapy, has remained the same for nearly three decades. Our research revealed that collagen VI (COL6) is localized in definite areas within the tumour mass, particularly in the inner core, where the most aggressive and immature cells reside. We demonstrated that COL6, produced by glioblastoma stem cells (GSCs), is crucial for maintaining their stem-like properties and even their survival upon the chemotoxic stimuli, possibly through the ATM/ATR axis. Taking advantage of our paired transcriptomic data from the core and periphery of GBM tumours from patients, we analyzed the expression of enzymes involved in glycine, proline, and hydroxyproline metabolism, the major amino acids composing COL6. These enzymes resulted differentially expressed between the core and periphery, indicating that cells residing in the core are characterized by a peculiar metabolic phenotype supporting COL6 synthesis. Interestingly, SHMT2, involved in glycine synthesis from serine, was overexpressed in the core together with the serine transporter SLC1A5, suggesting that glycine synthesis is driven by serine uptake rather than its synthesis. Additionally, enzymes involved in proline synthesis (PYCR1, PYCRL) and prolyl-4-hydroxylases (P4Hs) were both upregulated in the core, indicating an increased capacity of proline synthesis and hydroxylation, eventually impacting on collagen maturation. These findings suggest that GSCs in the GBM core sustain their stem cell -like properties also through a metabolic network favouring COL6 synthesis and deposition that can serve as a relevant target to sensitize GBM cells to treatments.

AUTOPHAGY MECHANISM AND VIRAL CONTAINMENT

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Autophagy is the main catabolic process by which intracellular components or potentially dangerous cytosolic entities can be degraded through delivery to lysosomes (Klionsky DJ 2021). Of note, this mechanism is used to capture pathogens having important implications in both innate and adaptive immunity. We previously demonstrated that authophagy plays an important role in the containment of the human immunodeficiency virus (HIV) in nonprogressor-infected patients, people that remain asymptomatic for more than 10 years in the absence of any antiretroviral therapy (Nardacci R 2014). This study is aimed to tested if autophagy induction can be able to counteract viral replication in HIV-infected people not able to spontaneously control viral replication. It is note that the active form of vitamin D, (1,25 (OH)2D), plays a role in the autophagy induction and in the inhibition of some viruses (Campbell GR 2012). We examined the autophagy modulation in peripheral blood mononuclear cells (PBMCs) of patients. We compared the expression of the autophagy markers AMBRA1, ATG5, BECN1 and LC3 in PBMCs of patients with hypovitaminosis and with normal blood concentration of vitamin D. Results obtained by immunohistochemistry, immunofluorescence and western blot analyses showed a higher level of autophagic markers in PBMCs from both treated and naïve patients with a normal concentration of vitamin D compared to patients who showed hypovitaminosis. Thus, patients with a normal concentration of vitamin D show significantly greater expression of the analysed autophagy factors. Future experiments will be aimed at evaluating whether autophagy induction by vitamin D could be able to counteract HIV yield. Understanding the HIV/autophagy interplay could allow the development of novel therapeutic approaches to eradicate infection and to prevent the pathogenesis.

SEMA6A DRIVES GNRH NEURON-DEPENDENT PUBERTY ONSET BY TUNING MEDIAN EMINENCE VASCULAR PERMEABILITY

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Innervation of the hypothalamic median eminence by Gonadotropin-Releasing Hormone (GnRH) neurons is vital to ensure puberty onset and successful reproduction. However, the molecular and cellular mechanisms underlying median eminence development and pubertal timing are incompletely understood. Here we show that Semaphorin-6A is strongly expressed by median eminence-resident oligodendrocytes positioned adjacent to GnRH neuron projections and fenestrated capillaries, and that Semaphorin-6A is required for GnRH neuron innervation and puberty onset. In vitro and in vivo experiments reveal an unexpected function for Semaphorin-6A, via its receptor Plexin-A2, in the control of median eminence vascular permeability to maintain neuroendocrine homeostasis. To support the significance of these findings in humans, we identify patients with delayed puberty carrying a novel pathogenic variant of *SEMA6A*. In all, our data reveal a role for Semaphorin-6A in regulating GnRH neuron patterning by tuning the median eminence vascular barrier and thereby controlling puberty onset.

CLIC2 MODULATES JAK/STAT SIGNALING CONDITIONING MONOCYTES DIFFERENTIATION IN THE TUMOUR MICROENVIRONMENT

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Chloride intracellular channels (CLICs) are a family of six evolutionarily conserved proteins with heterogeneous functions (ion channels, redox proteins, enzymes, scaffolding proteins) and previously, we reported that the family member CLIC2 was upregulated in gastric cancer (GC). To investigate Clic2 function in GC, we first determined Clic2 distribution in normal and in GC human tissues detecting Clic2 signal in dendritic cells (DCs), endothelial (ECs) and macrophages (MCs), with increased intensity in tumour samples. Since both DCs and MCs are derived from the differentiation of monocytes, we used THP-1 cells, a monocytic cell line, to investigate whether Clic2 could have a role during differentiation or in the function of those cells. We started defining Clic2 intracellular localization in differentiated naïve cells, finding it expressed in the Golgi apparatus and in the plasma membrane. Next, we generated CLIC2-KO THP-1 cells to explore cell differentiation mechanisms and functions. Differentiated naïve KO cells, exhibited a different morphology, suggestive of an activated DC phenotype, as confirmed by increased expression of CD11c, CD80 and CD86 markers. In addition, when we characterized cytokines secretion and Jak/Stat signalling, we observed in KO differentiated naïve cells the increase of chemotactic cytokines CCL7 and CCL8, the reduction of IL 6 secretion, increased phosphorylation of Shp1/Shp2 phosphatases and the absence of Stat3 phosphorylation with the resulting impairment of its signalling. We thereby suggest that Clic2 plays a central role in regulating DCs differentiation and function, by the modulation of inhibitory signals of the Jak/Stat pathway contributing to support GC progression by tumour a tumour-permissive microenvironment.

REGENERATING NASAL PSEUDO-TURBINATES FOR EMPTY NOSE SYNDROME: ADVANCEMENTS IN AIRWAY CARTILAGE AND EPITHELIUM TISSUE ENGINEERING

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Empty nose syndrome (ENS) is a poorly understood iatrogenic condition affecting 20% of patients following turbinate surgeries. It manifests with distressing symptoms such as suffocation, nasal bleeding, crusting, sleep disorders, and severe anxiety and depression, leading to a high suicide rate. Current treatments only alleviate symptoms without offering a cure. Experimental surgical methods focus on restoring nasal cavity volume rather than regenerating the respiratory mucosa, which hosts trigeminal nerve endings, and it is vital for air filtration, warming, and humidification. This study aims to develop a nasal pseudo-turbinate, featuring a human nasal tissue-engineered cartilage graft (N-TEC) covered with a functional airway epithelium. The research delves into the interaction between multiple tissues during *in vitro* regeneration to manufacture a graft able to restore not only the nasal cavity volume and aerodynamics but also the entire respiratory physiology. Our study prioritized identifying optimal culture conditions to maintain the highest regenerative potential of airway epithelial cells and their ability to differentiate into various specialized cell types, validating clinical release parameters for the N-TEC quality. We explored the vital preservation of the airway epithelial stem cell pool after culturing on the N-TEC through single-cell clonal analysis and detailed biological and molecular assessments. The successful regeneration of a 3D pseudo-turbinate holds a revolutionary potential for ENS patients and those with severe respiratory conditions. Indeed, this work not only advances the boundaries of regenerative medicine but also lays the groundwork for innovative *ex vivo* tissue engineering therapies. It provides a model for regenerating complex 3D structures and organs, paving the way for the development of numerous personalized treatments.

THE ROLE OF THE FRAGILE X MESSENGER RIBONUCLEOPROTEIN IN INTRAHEPATIC CHOLANGIOCARCINOMA PROGRESSION

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Intrahepatic cholangiocarcinoma (iCCA) of the bile ducts is one of the most aggressive neoplasms with the worst prognosis worldwide. Some clinicopathological prognostic parameters have been found but the identification of molecular markers and the comprehension of the pathogenetic mechanisms involved in iCCA prognosis are still unclear. We showed that the Fragile X messenger ribonucleorotein (FMRP), a missing or mutated protein in patients with fragile X syndrome (FXS), is overexpressed in iCCA and is localized in invadopodia protrusions of migrating and invading iCCA cancer cells, where it is able to bind several mRNAs encoding key proteins for invadopodia formation and/or function. In particular, we analyzed 65 samples from human iCCA tissues and 13 from the pre-invasive forms of this neoplasm, called Intraductal Papillary Neoplasm of the Bile duct (IPNB). Immunohistochemistry analysis revealed that FMRP is overexpressed in iCCA tissues compared to IPNB, especially on the margin of the tumor nests, close to the neighboring stromal tissue. Accordingly, we found that silencing of FMRP in metastatic iCCA cell lines affected cellular migration and invasion. Moreover, using a Nanostring nCounter approach to investigate key genes involved in invadopodia activity, we found a major dysregulation of 15 genes in iCCA compared to IPNB. Importantly, we observed that FMRP gene expression was positively correlated with major invadopodia target genes. Finally, principal component analysis (PCA) and volcano analysis identified invadopodia mRNA targets discriminating the invasive iCCA to the pre-invasive IPNB. In conclusion, these findings suggest that FMRP may play a role in iCCA progression, promoting invasiveness of iCCA cells by modulating plasma membrane plasticity and invadopodia formation at the leading edges.

THE EFFECTS OF ALDH1A3 INHIBITION ON PLEURAL MESOTHELIOMA MULTICELLULAR SPHEROIDS AND NEUTROPHILS RECRUITMENT

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Pleural mesothelioma (PM) is a rare tumor with a poor prognosis, linked to asbestos exposure. A high number of PM patients progress after the first-line pemetrexed-cisplatin chemotherapy suggesting the urgent need for identifying molecular targets for novel therapies.

It has been recently described that aldehyde dehydrogenase 1A3 (ALDH1A3) is involved in cisplatin resistance and could prognostically stratify PM patients. ALDH1A3 belongs to the NAD dependent subfamily of ALDH1As isoenzymes and is involved in the detoxification of endogenous and exogenous aldehydes.

We detected the expression of ALDH1A3 in different PM derived cell lines by Real-time PCR and Western blot analyses and by the use of a newly developed fluorescent selective probe. We tested NR6, a highly selective ALDH1A3 inhibitor; in different ALDH1A3^{+} PM derived cell lines cultured as 3D spheroids (MCSs). We demonstrated that NR6 caused the accumulation of malondialdheyde and induced DNA damage. NR6 treatment induced apoptosis in *CDKN2A* homozygously deleted PM cells and in derived cisplatin resistant clones. Alternatively, in *CDKN2A* proficient cells, NR6 caused a senescent growth arrest by inducing $p16^{ink4a}$ expression. In these cells, NR6 treatment resulted in a significant increase in IL-6 expression, while a decrease in IL-8 expression and release, preventing both neutrophil recruitment in PM MCSs and generation of neutrophil extracellular traps (NETs).

In conclusion, we demonstrated that in response to ALDH1A3 inhibition, *CDKN2A* loss skews cell fate from senescence to apoptosis. Dissecting the role of ALDH1A3 isoform in PM cells and tumor microenvironment can open new fronts in the treatment of this cancer.

ENDOCRINE DISRUPTORS IN BREASTMILK: EFFECTS ON INFANT NEUROBEHAVIORAL DEVELOPMENT

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Endocrine disruptors (EDs) are environmental chemicals capable of interfering with endogenous hormone balance especially during critical developmental periods. Several studies report evidence linking EDs exposure (in utero or *via* breastfeeding) to a variety of health outcomes. The Life MILCH project (*Mother and Infant dyads: Lowering the impact of endocrine disrupting chemicals in milk for a Healthy Life)* aims at assessing EDs in breastmilk in relation to mothers' life and nutritional habits and their effects on infant development during the 1st year of life. In 3 different hospitals (Parma, Reggio Emilia and Cagliari), 690 pregnant women were enrolled and recalled with their children at 1 (T1), 3 (T2), 6 (T3) and 12 (T4) months of age for assessing infant growth and neurobehavioral development and collecting urine and breastmilk samples. At any timepoint, mothers filled questionnaires on their lifestyle and nutritional habits. Biological samples were analyzed for 13 different EDs. Preliminary results showed that bisphenols, phthalates, and parabens were detected in more than 50% of samples. The detection of EDs in children was more likely when the EDs were detected in maternal samples. Significant associations were observed between mothers' lifestyle and diet and ED levels. Significant negative associations were found between maternal bisphenol levels and infant socio-emotional behavior at T2, and between maternal phthalate and bisphenol levels and neurodevelopmental scores at T3 and T4. These preliminary data indicate breastmilk as a robust biomarker of maternal and infant exposure to environmental EDs and unravel possible early effects of exposure on infant health and neurobehavioral development.

DUPLICATION OF CHROMOSOME 9P INDUCES T-CELL EXHAUSTION AND INCREASES STEM CELL CLONOGENIC POTENTIAL IN JAK2-MUTANT MYELOPROLIFERATIVE NEOPLASMS

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Myeloproliferative neoplasms (MPNs) are a group of blood cancers originating from a single hematopoietic stem cell, characterized by an excessive production of mature blood cells. The most common lesion is a point mutation in *JAK2*, known as JAK2V617F, which leads to constant activation of the JAK-STAT signaling pathway. Moreover, the JAK2V617F variant allele frequency has a significant impact on the disease phenotype. Alongside many described co-occurring mutations, cytogenetic abnormalities are commonly observed in MPNs. As the *JAK2* gene is located on the short arm of this chromosome, we investigated the combined effect of JAK2V617F and Chromosome 9 copy gain (+Chr9) in hematopoietic stem and progenitor cells (HSPCs) and differentiated cells from MPN patients. In-depth analysis revealed that the minimal amplified region was the entire short arm, thus including other gene loci as *CD274*, encoding programmed death-ligand 1 (PD-L1). Single cell genomics analysis and droplet digital PCR on HSPC-derived colonies showed that most +Chr9 patients had two out of three alleles harbouring JAK2V617F, with the genetic mutation being the initial event followed by Chromosome amplification. Functionally, +Chr9 HSPCs displayed high clonogenicity due to PD-L1-mediated overexpression of OCT4 and NANOG stemness factors. Furthermore, PD-L1 expression was increased and exclusively membrane-bound in +Chr9 monocytes compared to diploid cells and was positively correlated with JAK2 mutational burden. As PD-L1 is an immune checkpoint known to curb T cell activation, we analysed the T cell compartment revealing increased levels of exhausted cytotoxic T cells. In conclusion, our comprehensive characterization of the molecular interplay between JAK2V617F and chromosome 9 alterations fills a critical knowledge gap and provides valuable insights into the disease progression of +Chr9 MPNs.

MATTER OF QUANTITY: A NOVEL DISEASE-CAUSING MECHANISM IN HEREDITARY HEMORRHAGIC TELANGIECTASIA?

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Hereditary Hemorrhagic Telangiectasia (HHT) is a rare autosomal dominant vascular dysplasia caused by a pathogenic variant in one among *ENG*, *ACVRL1*, *SMAD4*, *GDF2*, all belonging to the TGFβ/BMPs pathway, leading to haploinsufficiency. Here we present preliminary data about 3 clinically diagnosed HHT families, presenting with an *ACVRL1* intron 4 variant. The first variant (c.526-22 A>G), found in a family and in an apparently *de novo* case, involves a putative branch point, the first case of a branch point mutation in HHT. The other (c.526-80_526-9del) is a 71 bp intronic deletion, immediately upstream exon 5 and potentially affecting the donor splice site. We analysed the *ACVRL1* transcript to demonstrate variants pathogenicity.

We performed a NGS custom HHT-panel (*ENG*, *ACVRL1*, *SMAD4*, *GDF2*) and MLPA analyses on a clinically confirmed case from each family. One index case was also analyzed by WGS. Co-segregation was verified by Sanger Sequencing. RNA was extracted from peripheral blood of two index cases and six healthy controls, retrotranscribed and analysed by qualitative/semi-quantitative and quantitative PCR. Amplicons Sanger sequencing was also carried out.

Semi-quantitative analyses confirmed, in addition to the canonical transcript, the presence of the already known splicing *ACVRL1* exon-5 skipping isoform, in index cases and controls. However, the results suggested an increase in the alternative spliced isoform in the patients. qPCR analysis definitively demonstrated a change in the ratio between the canonical coding and the exon-5 skipping isoforms.

We report for the first time preliminary data about two intronic *ACVRL1* variants involving splicing by affecting the quantity rather than the quality of the transcript. Additional studies are necessary to deepen the molecular mechanisms underlying this alteration and their effects on *ACVRL1* transcription and translation.

COMPARISON OF KNOCK-IN MOUSE AND hIPSC-BASED MODELS OF ARRHYTHMOGENIC CARDIOMYOPATHY CARRYING THE DSG2 p.Q558* PATHOGENIC VARIANT

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Background. Arrhythmogenic cardiomyopathy (ACM) is one of the most common inherited cardiomyopathies, characterized by progressive myocardial fibro-fatty replacement, ventricular arrhythmias and sudden death. Among the known disease genes, those encoding for the desmosomal proteins plakophilin-2 (PKP2), desmoplakin (DSP), and desmoglein-2 (DSG2) are most commonly mutated. The pathogenesis of ACM is not clear and therefore the establishment of reliable disease models is crucial. While human induced pluripotent stem cells (hiPSC) represent a valuable tool to investigate genetic disorders, they are limited, as they don't reproduce the cardiac architecture and function. Therefore, the integration of in vitro and in vivo models for a given condition can result in a better platform to study the disease pathogenesis and therapy. Here, we aim to compare and integrate the phenotype of a novel hiPSC-derived cardiomyocyte (hiPS-CMs) line and knock-in mice carrying the DSG2 p.Q558* pathogenic variant found in an ACM patient. **Results**. All our models showed reduced expression of DSG2 gene and deregulation of components of cardiac mechanical and electrical junctions. RNA-seq suggested alterations in genes involved in electrical transmission and in fibrosis. Moreover, the homozygous mice displayed an abnormally large left atrium and, under forced endurance training, left ventricular hypertrophy. **Conclusion**. We provided a preliminary phenotypic characterization of novel models for ACM harboring the p.Q558* variant in DSG2. Further analyses, such as electrophysiology studies on hiPS-CMs and histological analyses on mouse hearts, will help evaluate the presence of typical ACM features in these models.

ZINC METABOLISM AND ITS ROLE IN IMMUNITY STATUS IN SUBJECTS WITH TRISOMY 21: CHROMOSOMAL DOSAGE EFFECT

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Introduction. Trisomy 21 (T21), which causes Down syndrome (DS), is the most common chromosomal aneuploidy in humankind and includes different clinical comorbidities, among which the alteration of the immune system has a heavy impact on patient's lives. A molecule with an important role in immune response is zinc and it is known that its concentration is significantly lower in children with T21. Different hypotheses were made about this metabolic alteration and one of the reasons might be the overexpression of superoxide dismutase 1 (*SOD1*) gene, as zinc is part of the SOD1 active enzymatic center. **Methods.** The aim of our work is to explore if there is a linear correlation between zinc level and immune cell levels measured in a total of 217 blood samples from subjects with T21. Furthermore, transcriptome map analyses were performed using Transcriptome Mapper (TRAM) software to investigate whether a difference in gene expression is detectable between subjects with T21 and euploid control group in tissues and cells involved in the immune response such as lymphoblastoid cells, thymus and white blood cells. **Results.** Our results have confirmed the literature data stating that the blood zinc level in subjects with T21 is lower compared to the general population; in addition, we report that the T21/control zinc concentration ratio is 2:3, consistent with a chromosomal dosage effect due to the presence of three copies of chromosome 21. The transcriptome map analyses showed an alteration of some gene's expression which might explain low levels of zinc in the blood. **Discussion.** Our data suggest that zinc level is not associated with the levels of immunity cells or proteins analyzed themselves and rather the main role of this ion might be played in alteringimmune cell function.

IDENTIFYING THE ROLE OF HMGA NON-HISTONE CHROMOSOMAL PROTEINS IN TESTICULAR GERM CELL TUMORS TUMORIGENESIS AND CHEMORESISTANCE

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Testicular germ cell tumors (TGCTs) represent the most common malignancy in males aged 15-44 years and are a major cause of death attributable to cancer in this age group. Albeit TGCTs are highly curable with cisplatin-based chemotherapy, 10%-20% of the patients with non-seminomas (non-SEMs) do not respond to treatments. Unfortunately, the main genetic drivers of most TGCTs are still unknown.

Our group first demonstrated that HMGA1 and HMGA2, two well-known non-histone chromosomal proteins that mainly act as oncogenes, are overexpressed in embryonal carcinoma (EC) TGCTs, which are the malignant stem cell component of TGCTs, in seminomas (SEMs) and yolk-sac tumors, while are undetectable in terminally differentiated teratomas. This suggests a role for HMGAs in TGCTs progression. Moreover, we have demonstrated that HMGA1 is regulated by miR-26a and Let-7a in SEMs, playing a key role in HMGA1 expression and SEMs tumorigenicity. Furthermore, we have identified two pseudogenes, HMGA1P6 and HMGA1P7 (HMGA1Ps) for the HMGA1 gene, whose overexpression acts as miRNA decoys to compete with miRNAs that target HMGA1, playing a role in the epigenetic regulation of HMGA1 expression.

To study the impact of HMGAs and HMGA1Ps in non-SEM carcinogenesis and chemoresistance, we analyzed HMGAs expression in embryonal carcinoma (EC) cell lines. We found that both HMGAs and HMGA1Ps are expressed in these cells, suggesting their possible role in EC pathology. Moreover, by analyzing cisplatin-resistant cell lines obtained by chronic exposure to cisplatin, we observed that HMGA1 expression remains constitutively high in cis-r cells, while that of HMGA2 is dampened. A parallel analysis of HMGAPs expression showed that pseudogenes are also expressed in cis-r EC cells. This indicates a possible role of P6 and P7 in supporting HMGA1 expression. Together, these findings suggest the involvement of HMGAs and HMGAPs in EC tumorigenesis and response to chemotherapy.

SAPJE ZEBRAFISH AS A LIVING PLATFORM TO UNVEIL THE PATHOGENESIS OF DUCHENNE MUSCULAR DYSTROPHY

Elena Cannone[1]; Silvia Castagnaro[2]; Martina La Spina[2]; Chiara Tobia[1]; Barbara Gnutti[1]; Valeria Cinquina[1]; Patrizia Sabatelli[3]; Dario Finazzi[1]; Massimo Gennarelli[1]; Annamaria Haleckova[4]; Sébastien Tardy[5]; Laurance Neff[5]; Leonardo Scapozza[5]; Olivier Dorchies[5]; Paolo Bernardi[2]; Chiara Magri[1]; Marco Schiavone[1]

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Duchenne muscular dystrophy (DMD) is a severe disorder characterized by the absence of dystrophin, which leads to the progressive loss of muscle tissue. Several studies showed that mitochondrial dysfunction due to Ca^{2+} overload is the main cause of muscle fiber death triggered by the long-lasting opening of the permeability transition pore (PTP), a mitochondrial high-conductance channel sensitive to high Ca^{2+} levels. It has been demonstrated that PTP inhibition prevents mitochondrial dysfunction and muscle degeneration in both DMD patient cells and *sapje* zebrafish, a severe model of DMD. However, there is still a gap of knowledge about the early events linking the absence of the dystrophin to Ca^{2+}-dependent muscle fiber death. We (i) generated *sapje* fluorescent biosensors to assess cell dynamics at the level of single muscle fibers in a living organism and (ii) performed RNA sequencing to identify differentially expressed genes at different stages of disease progression. RNAseq data from *sapje* zebrafish at 2 days post fertilization (dpf) showed a significant dysregulation of genes involved in Ca^{2+} homeostasis. At 5 dpf we observed dysregulation of genes involved in mitochondrial function, muscle contraction and differentiation. Significant dysregulation of Notch, Shh and Hif1α pathways, together with the impaired mitochondrial patterning and the severe macrophage infiltration, confirmed defects in muscle repair and differentiation. Treatment of *sapje* zebrafish with new PTP inhibitors leads to a full rescue from dystrophic phenotype, showing a complete recovery of normal mitochondrial function, muscle structure, motor behavior, survival rate and signaling pathways activity. Identification of new druggable targets is key to perform new drug screenings and develop novel compounds that can be used in combination with PTP inhibitors to slow down or arrest DMD progression.

POSTER
PRIMA SESSIONE

Giovedì 19 Settembre
17:00 – 18:00

UNRAVELING ATM AND SLFN11 CORRELATION IN REGULATING RESPONSE TO DNA DAMAGE REPAIR INHIBITION IN A MELANOMA MODEL

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The Ataxia-Telangiectasia Mutated (ATM) protein kinase plays a key role in the cellular response to DNA damage. ATM is altered in many cancers; we previously observed that germline ATM variants are enriched in high-risk melanoma patients. However, their functional effect needs careful assessment considering their preventive and predictive implications.
RAD51 is a protein involved in the homologous recombination (HR) pathway and is crucial for repairing double-strand breaks in DNA. The absence of RAD51 foci formation is often used as a marker of HR deficiency (HRD).
HR-Deficient tumours are susceptible to Poly ADP-Ribose Polymerase (PARP) inhibitors, which exploit the DNA repair defects to induce synthetic lethality.
Using multiple melanoma cell line models, either positive or negative for potentially pathogenic variants, we assessed LOH, HRD, downstream DDR pathway activation and response to inhibition of DNA damage repair by PARP-inhibitors.
Despite proven LOH, lack of ATM protein, HRD (absence of RAD51 foci) and downstream pathway dysregulation, our model did not show sensitivity to PARP-inhibition (PARP-i). Therefore, we investigated the potential role of SLFN11, a protein involved in regulating cell proliferation, differentiation, and immune response, whose expression is inversely correlated with PARP -i sensitivity.
Most cell lines lacking SLFN11 expression featured hypermethylation-mediated silencing of the SLFN11 gene promoter. No data on the relationship between ATM and SLFN11 in melanoma are available. Our ATM mutant cell line shows lack of SLFN11 protein and methylation of the *SLFN11* promoter.
Our findings functionally support the pathogenicity of germline ATM variation through disruption of mechanisms maintaining genomic integrity and unravel a potential correlation between ATM and SLFN11 in regulating response to PARP-i in melanoma.

18 kDa TSPO-STIMULATED NEUROSTEROIDOMA ENSURES CHOLESTEROL HOMEOSTASIS IN ACTIVATED HUMAN MICROGLIA PROMOTING THE REPARATIVE PHENOTYPE

Elisa Angeloni[1], Lorenzo Germelli [1], Laura Marchetti[1], Eleonora Da Pozzo[1], Claudia Martini[1], Maria Letizia Trincavelli[1], Barbara Costa[1]

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Excessive cholesterol accumulation in microglia compromises their ability to resolve the neuroinflammatory response, leading to dysfunctional phenotypes associated with neurodegenerative disorders. Cholesterol is the substrate for the *de novo* synthesis of steroids, molecules able to counteract microglial excessive reactivity when exogenously administered. Here, we studied the impact of the cholesterol-binding Translocator protein (TSPO), crucial in the rate-limiting step of *de novo* neurosteroidogenesis, on regulating cholesterol homeostasis and functionality of human microglia activated by immunogenic stimuli. The results showed that adequate TSPO levels are required to maintain steady-state cholesterol concentration, ensuring proper microglial activation. In this context, the biosynthetic pathways of neurosteroidogenesis leading to allopregnanolone/pregnanolone production are involved. Noteworthy, TSPO pharmacological stimulation effectively redirected endogenous cholesterol towards mitochondrial metabolism, inhibiting cholesterol accumulation mechanisms. TSPO-mediated *de novo* neurosteroidogenesis stimulation promoted the shift of biosynthetic pathways towards the production of sex steroid hormones, including oestradiol and testosterone. This steroidogenic profile promoted a reparative phenotype that ensured pro-differentiating effects on human progenitor cells. Overall, the results suggest TSPO-mediated neurosteroidogenesis as a promising pharmacological target to restore the control on cholesterol trafficking and functionality of microglia in neuroinflammatory disorders.

LIFE STYLE INTERVENTION IN MOVIS BREAST CANCER SURVIVORS COHORT MODULATES IGF-1 LEVELS AND REDUCES TRIPLE NEGATIVE CANCER PROLIFERATION

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Background. The effect of lifestyle through the induction of biochemical-metabolic changes can meliorate components of health-related quality of life (HRQoL) in breast cancer (BC) survivors, but its role in IGF-1 system modulation and in controlling cancer cell proliferation remains poorly understood. **Methods.** BC women (n=30, age 52.7 ± 7.6 years), enrolled in the MoviS study (NCT04818359) performed 3-month aerobic training with increase of exercise intensity (40-70% HRR) and followed the Mediterranean diet recommendation. Circulating IGF-1 and IGFBP3 levels were measured before and after the intervention as well as the cardiorespiratory fitness [$VO_{2\ max}$] and HRQoL parameters (i.e., glycemia, insulin, insulin resistance, triglycerides, high- and low- density lipoproteins, total cholesterol, progesterone, testosterone). Serum samples were also collected to evaluate the triple-negative BC cell line MDA-MB-231 growth and spheroid formation in order to correlate the biological effects *in vitro* with physiological parameters. **Results**. Beneficial effects of the lifestyle were observed in HRQoL variables (i.e., VO2max, glycemia, insulin, HOMA-IR index, LDL, total cholesterol, triglycerides, testosterone) after 3-month. Although the mean IGF-1 level did not change (ng/mL: before=164.3±70.9, after=166.8±57.6), IGFBP3 level decreased (µg/mL: before=6.1±1.4, after=4.2±1.5) a negative correlation between pre- and post-training ($r=-0.62$, $p<0.001$) was observed. Moreover, the proliferative inducer IGF-1 was found to be predictor of cell tumorigenic potential among physiological and metabolic data linked to spheroids proliferation. **Conclusion.** This study provides evidence on the positive association between lifestyle and HRQoL in BC survivors. Moreover, our findings emphasize the significance of lifestyle in controlling the progression of cancer.

ADAR-MEDIATED SITE-SPECIFIC RNA EDITING TO TACKLE NEURODEGENERATIVE DISORDERS

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Adenosine Deaminases Acting on RNA (ADAR) enzymes catalyse the conversion of adenosine (A) to inosine (I) within double-stranded RNA molecules. This mechanism, known as A-I RNA editing, is able to modulate gene expression, RNA stability and protein functions.
Recently, new therapeutic approaches based on site-directed RNA editing (SDRE) leverage the recoding ability of ADAR to correct pathogenic G to A nucleotide mutations. SDRE allows for the manipulation of genetic information at the RNA level, without causing damage to the genome. G-to-A point mutations make up nearly 30% of the pathogenic single nucleotide variations in the human genome and can be potentially targeted by ADAR mediated repair.
In order to study the possible therapeutic implications of these enzymes, we have selected two mutations: the LRRK2 p.G2019S (c.6055 G>A), that is one of the most common genetic determinants of Parkinson's Disease (PD) and the TDP43 p.A382T (c.1144G>A), a genetic variant of the TAR DNA-binding protein 43 (TDP-43) gene, which has been associated with the on-set of Amyotrophic Lateral Sclerosis.
Initially, we have set up an in vitro models based on HEK293 cells stably expressing LRRK2 p.G2019S or TDP43 p.A382T. Transient co-transfection of a vector expressing a specific guide RNA, targeting the sequence encoding LRRK2 p.G2019S mutation, together with a vector expressing ADAR1 enzymes, induced a site-direct RNA editing of about 60%, 48h after transfection, with however the presence of off-target effects that deserve further optimization. Considering TDP43 p.A382T, the use of specific gRNAs corrected the G/A point mutation in nearly 30% of the transcripts, indicating that up to one third of mutated mRNA may be corrected at the RNA level.
These preliminary data, to be confirmed in disease models, demonstrate the importance of deepening the role of ADAR enzymes in RNA based therapy.

CADMIUM EXPOSURE TRIGGERS A FERROPTOSIS-LIKE CELL DEATH IN ORAL SQUAMOUS CELL CARCINOMA (OSCC) CELLS DERIVED FROM NEVER-SMOKERS

Anna Martina Battaglia[1], Lavinia Petriaggi[1], Emanuele Giorgio[1], Stefania Bulotta[2], Alessandro Antonelli[2], Sonia Bonacci[2], Marialaura Frisina[2], Laura Berliocchi[2], Antonio Procopio[2], Amerigo Giudice[2], Concetta Maria Faniello[1], Francesco Costanzo[1], Flavia Biamonte[1]

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Cadmium (Cd), a major carcinogenic component of tobacco, is a risk factor for oral squamous cell carcinoma (OSCC). It may exert cytotoxic effects depending on both the extent of exposure and the type of cell exposed to this metal. Here, we explored the effects of Cd in OSCC cell lines derived either from patients classified as non-smokers (CAL27) or smokers (SCC154), cultured in nonadherent 3D conditions. Propidium-iodide flow cytometry assay show that high doses of Cd (>50μM) triggered a remarkable mortality in 3D CAL27 but not in 3D SCC154 cells. By using ICP-MS analysis, we found that 3D CAL27 accumulate 6-fold higher intracellular amount of Cd compared to 3D SCC154, suggesting that the different sensitivity to this metal relies on the diverse ability of OSCC cells of internalizing it. The use of ferroptosis (ferrostatin-1) and autophagy (bafilomycin A1) inhibitors, partially prevent mortality caused by Cd, indicating its cytotoxicity might be driven by ferroptosis- and autophagy-related pathways. In agreement, Cd causes GSH depletion, quantified by immunofluorescence, that leads to lipid peroxidation, measured by BODIPY flow cytometry assay, only in 3D CAL27. This was also associated with increased mitochondrial ROS and membrane hyperpolarization, assessed by MitoSOX and TMRM flow cytometry assays, respectively. As bivalent cation, Cd can interfere with iron metabolism. Notably, Cd causes intracellular iron depletion, assessed by CA-AM assay, by reducing CD71 surface levels in OSCC cells. Iron administration (ferlixit) prevents Cd damage, while chelation of intracellular Fe^{2+} (deferoxamine) enhances Cd cytotoxicity. Of note, the chronic exposure to low doses of Cd (20μM) for 1 month induces tolerance against this metal in 3D CAL27 cells. Overall, we demonstrated that Cd may act as ferroptosis-like inducer in OSCC cells derived from non-smokers.

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF PLANT EXTRACELLULAR VESICLES (P-EVs) FROM POMEGRANATE JUICE: POTENTIAL APPLICATION AGAINST OVARIAN AGING

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Ovarian aging occurs earlier than that in most other organs, leading to a decrease in reproductive potential, and is characterized by oxidative stress. Recently, there has been a growing interest in the role of plant extracellular vesicles (P-EVs) and their contents in counteracting the effects of oxidative stress. P-EVs can transfer their cargo, including proteins, RNAs, metabolites, and lipids, from one cell to another, thereby influencing the function of recipient cells.

In this study, P-EVs were isolated from pomegranate (*Punica Granatum* L.) juice. The experiments were undertaken during the 2023 season on fresh pomegranate fruits, cultivated in Sicily. Using a combination of ultracentrifugation and filtration techniques P-EVs were isolated and concentrated from the pomegranate juice. Zetasizer light scattering and Scanning Electron Microscopy (SEM) were employed to characterize the P-EVs in terms of concentration, size, and morphology. Pomegranate juice contains a homogeneous population of P-EVs with size and morphology similar to mammalian extracellular nanovesicles. Identification and quantification of specific non-coding RNA cargo, especially microRNAs, were conducted by Next-generation RNA sequencing on NovaSeq 6000Dx. The experiments to map their proteomic profile are currently underway. Then, we tested the effects of increasing concentrations (2.5 - 10 µg/ml) of P-EVs on cell viability and metabolism in a senescent model of human granulosa cells (GCs). Preliminary data revealed no effects on cell proliferation, but 5 µg/ml P-EVs increased the NAD^+/NADH ratio in senescent GCs, reaching levels similar to young GCs.

In conclusion, P-EVs mediate cross-kingdom communication and improve the functionality of the recipient cells. This provides new insight into the potential use of P-EVs to counteract oxidative stress and improve reproductive health.

EFFECTS OF MICRO-NANOPLASTICS (NMPs) ON OXIDATIVE AND METABOLIC STATUS OF HUMAN GRANULOSA CELLS

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In recent years, human activities have led to a significant rise in global plastic production. As plastic waste breaks down into micro and nanoparticles (NMPs), they spread in the environment, endangering human health. However, how NMPs impact reproductive functions in mammals, remains unknown. In this context, the aim of the work is investigate molecular mechanisms underlying NMP effects on reproductive cells.
Human granulosa cells (hGC, KGN cell line) were exposed to different NMP sizes (40, 70, 100 and 200 nm) at concentrations ranging from 5 to 1000 μg/ml for 24 h. We demonstrated NMP uptake by hGCs under confocal laser scanning microscopy and observed NMP incorporation starting after about 2 h of culture (Nanolive, Media System Lab). A significant decrease of cell viability, in association with increased apoptosis, was described with all NMP treatments, regardless of size and concentration. Accordingly, increased protein expression of cell cycle regulators p21 and p53 were found upon NMP exposure. All NMP treatments increased reactive oxygen species (ROS) production (Mitosox Red, Invitrogen) and altered gene and protein expression of antioxidant enzymes, catalase (CAT), superoxide dismutase 1 and 2 (SOD1, SOD2). In addition, NMPs induced a reduction of gene expression of sirtuins (Sirt1, Sirt3) in association with an increase of SIRT1 protein level. Finally, ATP production (Cell Titer-Glo ATP assay kit, Promega Ltd) and mitochondrial metabolism (MitoStress Kit, Seahorse XFe 96, Agilent) were altered by all NMPs. Our data suggest that NMPs induce a condition of oxidative stress, which triggers SIRT1 adaptive response leading to activation of antioxidant defenses. This condition alters mitochondrial metabolism and influences cell cycle progression/survival, likely underlying deleterious effects of NMPs on female fertility potential.

UNRAVELING THE INTERPLAY BETWEEN BIOLOGICAL MICROENVIRONMENT AND IMMUNE SYSTEM: THE ROLE OF ECM IN MACROPHAGE RESPONSE

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In recent years, the role of biological microenvironment on the immune system response has garnered significant attention in the clinical field. Several studies suggest that a molecular-level comprehension of biological mechanisms that govern immune pathways could revolutionize therapeutic strategies. Since the extracellular matrix (ECM) modulates the behaviour of immune cells in healthy and diseased tissues, the study of interactions between immune cells and inflammatory stimuli should consider the extracellular microenvironment where these interactions occur. In the present work, our main goal is to study the role played by the microenvironment on the macrophage response to inflammatory inducers embedded in ECM. To this end, we developed ECM-like substrates based on denatured collagen loaded with increasing concentrations of carbon nanotubes (CNTs), as a model of inflammation-inducing fibers. The macrophage-like cells, derived from the human monocytic THP-1 cell line, were grown on the substrates up to 5 days. Preliminary experiments demonstrated that macrophages adhere to all substrates and that CNT treatments do not affect cell viability 24 hours after cell seeding. However, the cell morphology changed at higher CNT concentrations and culture times, showing an increase in filopodia and a decrease in spreading area taking on a round shape. Macrophages can remodel the ECM-like substrates, making CNTs bioavailable to be internalized as indicated by confocal and transmission electron microscopy imaging. Finally, conditioned media from THP-1 cells cultured in the presence of CNTs facilitated endothelial cell migration in the wound healing assay regardless conditioned media from control cells. Overall, functionalization of ECM-like substrates with CNTs can influence the activities of macrophages in a concentration-dependent manner suggesting macrophage activation.

NATURAL COMPOUNDS MODULATES HISTONE CHANGES IN PATHOPHYSIOLOGY OF LUNG CANCER MODEL

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Lung cancer is a leading cause of cancer deaths worldwide; in particular the prognosis for lung cancer patients is poor, with a 5-year survival rate of 18.1%. The management of lung cancer treatment is often ineffective as a result of the development of drug resistance, reactions to treatment, drug-drug interactions or non-specific targeting of the anticancer drugs. In recent years, the use of natural compounds in combination with conventional chemotherapy drugs has gained considerable attention as a potential new approach in the treatment of lung cancer causing very few side effects. At the same time, the combinatorial action of an anticancer drug with a natural compound provides a synergistic action that helps enhance the overall therapeutic action against tumor cells. In this scenario, our research aim to test three different compounds from medicinal plants: *Dolichos Biflorus* and *Punica Granatum* in human adenocarcinoma cell (A549). The present work highlights how natural compounds can modulate apoptosis and autophagy, processes dysregulated during cancer, which facilitate tumour cell survival. Furthermore, we observed by gene expression the modulation of specific lung cancer markers such as AGR2 and simultaneously, of markers involved in the epithelial-to-mesenchymal transition such as AXL and nucleolin. Finally, to characterized chromatin modification by western blot analysis we evaluate the following H3 modification: H3K9/14ac and H3K4me2 (chromatin opening), H3K9me2 and H3K27me3 (chromatin closure). In conclusion, in the present work the combined use of an anti-cancer drug and a natural compound exhibits synergistic effects, enhancing overall therapeutic actions against cancer cells; these results therefore may present the springboard for alternative therapies against chemoresistance induced by conventional therapies.

CELLULAR SENESCENCE AND AGEING: AN EPIGENETIC SWITCH LINKING TRANSCRIPTIONAL CONTROL AND DNA DAMAGE RESPONSE.

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Cellular senescence is responsible for human ageing, but the epigenetic mechanisms that control senescence and their contribution to ageing are not fully understood. Class IIa HDACs (histone deacetylases) are epigenetic regulators that function primarily as platforms that drive the assembly of multi-protein complexes. Among the class IIa HDACs, HDAC4 is downregulated during various forms of senescence via GSK3-beta-dependent phosphorylation and subsequent degradation via the ubiquitin-proteasome system. The degradation of HDAC4 allows the full engagement of typical enhancers and super-enhancers that control the transcriptional landscape of senescence. HDAC4 can also regulate the DNA damage response. Recent evidence suggests that HDAC4 regulates histone 2B lysine 120 acetylation (H2BK120ac) through association with class I HDACs, in particular HDAC2. Alterations in H2BK120ac at the site of DNA damage impair DNA repair via homologous recombination. These results have important implications for understanding the mechanisms of human ageing.
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PRECLINICAL ORGANOIDS MODELS IN INTESTINAL BOWEL DISEASE TREATMENT

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Inflammatory bowel disease (IBDs) are complex chronic inflammatory disorders of the gastro-intestinal (GI) tract characterized by inflammation of small and large intestine. This condition shows up mainly with two major forms, known as Crohn's disease and ulcerative colitis. The conventional treatments include aminosalicylates, corticosteroids, immunomodulators, biologics and/or surgical resection. Natural products have shown the ability to provide a safer choice to reduce inflammatory condition and restore tissues physiology. In order to characterize this pathology, we first assessed the correlation between IBD patients and a novel 3D model, organoids, that recapitulates the IBD disease. Next, we evaluated the effect of natural compounds on PDOs (Patients derived organoids) inflammatory status. In conclusion, this study may provide the development of functional preclinical models and a potential therapeutic strategy for the IBD treatment.

A POSSIBLE ROLE OF BAG3 PROTEIN IN THE PATHWAY FROM STRESS TO FIBROSIS IN SYSTEMIC SCLEROSIS

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Systemic sclerosis (SSc) is an autoimmune disease whose main characteristic is the chronic activation of the fibrotic process involving the skin and internal organs. In this disease, activated immune cells produce a series of cytokines, which can induce the transformation of resident fibroblasts into activated myofibroblasts, characterized by substantial collagen deposition in target tissues. We identified the extracellular BAG3 protein as a biomarker of the progression of the fibrotic process in patients with SSc. Specifically, serum levels of BAG3 are significantly higher in patients with the diffuse form SSc (dcSSc) compared with normal controls and patients with the limited form (lcSSc). In addition, BAG3 serum levels correlate with the presence of interstitial lung disease (ILD). We next identified a correlation of BAG3 serum levels in patients with SSc complicated by ILD to response to Nintedanib therapy. In patients who responded to Nintedanib, we observed a decrease in BAG3 serum levels, whereas, conversely, an increase in serum BAG3 values was observed in non-responder patients. To explain the possible role of BAG3 protein in the pathogenesis of SSc, fibroblasts were isolated from skin biopsies of dcSSc patients and healthy donors. Cytofluorometric analysis evaluated the cells for IFITM2 (BAG3R) protein expression. Notably, fibroblasts from SSc patients showed a statistically higher percentage of positivity for the analyzed protein. In addition, the same cells appeared to release BAG3 protein after stimulation with pro-fibrotic cytokines. Finally, it was verified that in a skin fibroblast cell line, Nintedanib inhibits collagen expression and secretion of BAG3 protein induced by TGFβ1. All our data demonstrate how BAG3 can be a valuable and useful biomarker to monitor the progress of the fibrosing process and the eventual response to the therapy.

GENETIC MODULATION OF CARDIOVASCULAR RESPONSE TO FENOFIBRATE: INSIGHTS FROM AN *IN VITRO* MODEL

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Fenofibrate, a PPAR-α agonist, has shown heterogeneous responses in clinical trials for preventing major cardiovascular events (MACE) in type 2 diabetes patients. A common genetic variant (rs6008845, C/T) in the *PPARA* gene region has recently been identified to modulate fenofibrate's effects on MACE. T/T homozygotes experienced a 51% reduction in MACE with fenofibrate while C allele carriers showed no benefit. To investigate the underlying mechanism, we used Human Umbilical Vein Endothelial Cells (HUVEC) with different rs6008845 genotypes (T/T and C/C) and cultured them in high glucose (HG) to mimic diabetic endothelial inflammation.
Initially, HUVEC underwent a cell viability test to determine the optimal fenofibrate concentration. HUVEC with T/T and C/C genotypes (n=10 each) were then exposed to HG and treated with 50μM fenofibrate. *PPARA* gene expression and activity were assessed using RT-PCR and ELISA, respectively. VCAM1 and NF-κB mRNA (RT-PCR) and protein (flow cytometry) were examined. An *in vitro* assay assessed monocyte adhesion to endothelial cells, critical in atherosclerosis, and Nitric Oxide (NO) bioavailability was evaluated using cGMP ELISA kit.
We found increased *PPARA* gene expression and activity with fenofibrate treatment, especially in T/T homozygotes. T/T HUVEC showed significant reductions in VCAM1 and NF-κB at both mRNA and protein levels ($p<0.001$), while C/C HUVEC showed no significant effects. Additionally, T/T HUVEC showed a significant decrease in monocyte adhesion to the endothelium ($p<0.001$). Fenofibrate treatment also showed a trend of increased NO bioavailability, especially in T/T homozygotes.
These findings suggest a significant benefit of fenofibrate for T/T HUVEC. If confirmed, this supports the clinical evidence for personalized fenofibrate prescriptions for optimal cardiovascular protection.

TELOMERE DYSFUNCTION AND GENETIC PREDISPOSITION IN FAMILIAL PAPILLARY THYROID CARCINOMA: PRELIMINARY STUDY

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Familial papillary thyroid cancer (fPTC) is an uncommon and not well-defined clinical entity. Although an imbalance of the telomere-telomerase complex has also been reported in fPTC, there are conflicting observations in the literature regarding telomere length (TL) between affected and unaffected fPTC and sPTC subjects (Cantara et al., 2012). Moreover, the genetic basis of fPTC remains unclear (Capezzone et al., 2021). In this study we aim to investigate if rare germline variants have a role in the familial form and cause alterations of still unknown cellular and/or molecular mechanisms, including defects in the telomere dysfunctions. A panel of five Sardinia families was identified. Blood samples were obtained from 26 patients: 15 with fPTC, 8 unaffected family members (UFM) and 3 with multinodular goiter (MNG). TL was determined using quantitative fluorescence in situ hybridization (Q-FISH) and Whole Exome Sequencing (WES) to identify new predisposing genes of thyroid cancer and expand our current understanding of the genetic basis of the disease. fPTC and MNG displayed a reduction in telomere fluorescence (Q-FISH value: 86,4±11, 85,51±5, respectively) compared with UFM (110,2±14). fPTC patients have shorter telomeres compared to UFM group ($p<0.001$). Until now, the WES analysis identified c.254C>T (p.Ser85Leu) missense germline variant in PDZK1IP1 gene in two probands from one family. Although preliminary, our results reinforce the hypothesis that TL is significantly correlated with susceptibility to PTC. WES analysis identified PDZK1IP1 variant as a likely novel candidate susceptibility gene for fPTC, but further analysis in other family members is needed to confirm the role of this variant in the pathogenesis of fPTC.

CHD7 DEPLETION VIA CRISPR-CAS9 INDUCES BIOLOGICAL AND TRANSCRIPTOMIC CHANGES IN A MOUSE NEURONAL MODEL OF CHARGE SYNDROME.

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CHARGE syndrome (CS) is a rare genetic disease characterized by developmental defects of many organs including the brain. No pharmacological treatments are available and the patients undergo severe surgical interventions. CS is commonly caused by mutations of *CHD7*, an ATP-dependent chromatin remodeller protein involved in the fine-tuning of gene transcription during early development of different tissues, including the central nervous system. Despite >85% of CS patients carry *CHD7* mutations, the number of symptoms presented by the patients and the grade of severity show high variability. A possible explanation is that mutations in other genes with an epistatic relationship to CHD7 may function as 'modifiers' to alter disease traits expressivity. In this context, the genes encoding for semaphorins (SEMA) and their receptors are good candidates for genetic CHD7 interactors in the CNS, but the underlying biological mechanisms are still largely unknown.

Thus, to better understand how CHD7 regulates SEMA genes during neuronal development and to discover novel genetic pathways, we generated a Chd7-knock-out (KO) CS neuronal model by genome-editing of a murine immortalized cell line (GN11). Two independent KO clones, in which CHD7 protein was significantly reduced or undetectable compared to WT cells, were phenotypically and genetically characterized, by cellular assays and RNA sequencing. Our analyses revealed cellular and genetic signatures related to altered cell migration and proliferation pathways, including genes belonging to Semaphorin signaling and to other families involved in axon guidance/neurogenesis. Overall, these results highlight that CHD7 might act as master regulator of several signalling pathways during neuronal development, that could be used as potential druggable targets for the treatment of neurological defects of CS patients.

IDENTIFICATION OF NOVEL EXTRACELLULAR VESICLE MICRORNA SIGNATURES IN SARCOPENIC HEPATOCELLULAR CARCINOMA

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The critical challenge of sarcopenia in advanced hepatocellular carcinoma (HCC), a condition characterized by muscle wasting and poor prognosis. We aim to develop a novel set of prognostic biomarkers based on extracellular vesicle EV-derived microRNAs (miRNAs) for early detection, improved risk stratification, and potential therapeutic interventions in sarcopenic HCC. The method utilized are isolation and characterization of EVs from patients with HCC and healthy controls: by Global miRNA profiling of EVs to identify specific signatures associated with sarcopenia and HCC progression; Clinical validation of the identified miRNA signatures in a larger patient cohort; Exploration of RNA-based therapeutic strategies to target muscle wasting and metabolic dysregulation. The expected outcomes are development of a sensitive and specific blood -based test for early diagnosis of sarcopenic HCC; improved prognostic accuracy and risk stratification for patients with HCC; Identification of potential therapeutic targets for mitigating muscle wasting and improving patient outcomes. Alignment with Spoke 4, the project directly aligns with Spoke 4's focus on innovative RNA-based therapeutic strategies for metabolic diseases. By targeting the inflammatory and metabolic mechanisms underlying sarcopenia in HCC, the project contributes to the development of personalized medicine approaches and potentially improves patient care and healthcare efficiency. The preliminary data suggests that circulating microRNAs (miRNAs) can be categorized based on their cellular origin and potentially used as tissue -specific biomarkers, diagnostic tools, and therapeutic targets. Unsupervised clustering analysis revealed distinct miRNA signatures associated with epithelial and inflammatory cell types, while miRNA profiling of lung cancer samples identified differences between epithelial and stromal compartments.

CLONAL DYNAMICS AND COPY NUMBER VARIANTS BY SINGLE-CELL ANALYSIS IN LEUKEMIC EVOLUTION OF MYELOPROLIFERATIVE NEOPLASMS

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Myeloproliferative neoplasms (MPNs) are hematopoietic stem cell disorders causing the overproduction of myeloid differentiated cells that can lead to Acute Myeloid Leukemia (AML). MPN driver mutations affect *JAK2*, *CALR* or *MPL* genes; however, additional genetic alterations create complex phenotypes.

Through single-cell (SC) genomics, we sought to define the genomic architecture of CD34+ stem and progenitor cells from 10 MPN patients during disease evolution. Next, SC RNA-seq and ATAC-seq were performed on CD34+ cells from the MPN and AML phase of one patient to uncover chromatin and transcriptional perturbations induced by epigenetic modifiers' mutations.

In most patients, the first mutation affected epigenetic remodelers (*TET2*, *ASXL1*). Driver mutations' allele frequency remained stable during disease progression and did not seem to drive AML transformation, whereas leukemogenic mutations, affecting genes such as *IDH2* and *KRAS*, were already traceable at very low allele frequencies in MPN chronic phase, despite being undetectable by bulk diagnostic NGS analysis. Moreover, leukemic clones displayed increased copy number variations.

SC-multiomic analysis revealed the activation of NF-kB pro-inflammatory signature and the enrichment in GATA family motifs in leukemic clusters. In addition, we identified an AML-specific Megakaryocyte-Erythroid Progenitor cluster harboring chromosome 7 deletion together with the impairment of EZH2 and WT1 activity, as potential triggers of leukemogenesis.

Overall, this work suggests that epigenetic modifiers' mutations are pivotal for MPN onset and progression. Driver mutations do not account for leukemic transformation and genetic alterations driving AML evolution are early identified by SC analysis. Moreover, multiomic analysis enables the identification of pro-leukemic molecular events occurring in different stem and progenitor cell clusters.

TITLE: IDENTIFICATION OF EPIDRUGS LINKED TO TBX1 ACTIVATION

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Background. The discovery of the role of epigenomic mutations in genetic diseases has driven research into the study of epigenetic modifications and epidrugs targeting these changes. DiGeorge syndrome (DGS) is a rare genetic disorder caused primarily by *TBX1* haploinsufficiency. Studies in animal models of DGS suggest that epigenetic changes might contribute to its pathogenesis. Epidrugs capable of modulating *TBX1* expression are of great interest for their potential therapeutic value and for determining the mechanism by which this disease gene is regulated. **Methods**. We use undifferentiated C2C12 murine myoblasts, which expresses low levels of *Tbx1*, to evaluate changes in *Tbx1* expression in response to selected epidrugs. Gene expression is evaluated by RT-PCR and RTqPCR at various time points. Epidrugs were selected on the basis of their predicted function and on the results of a bioinformatic analysis of epigenetic changes at the *Tbx1* locus in tissues with high or low *Tbx1* expression, using the Genome Browser and Encode databases. **Results and conclusions.** We found that SAHA (Vorinostat), a histone deacetylase inhibitor reduced *Tbx1* expression in treated C2C12 myoblasts. We expected that SAHA would promote histone acetylation and, potentially, increase *Tbx1* expression. We are currently testing other epidrugs and combinatorial treatments.

VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR-2 EXPRESSION IS ASSOCIATED WITH ANGIOGENESIS IN A PRECLINIC MODEL OF INFLAMMATORY BOWEL DISEASE

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Inflammatory bowel disease (IBD) is a group of chronic disorders that includes two main forms, Crohn's disease, and ulcerative colitis. Angiogenesis is a key component of both inflammation and pathogenesis of IBD. Vascular Endothelial Growth Factor-A (VEGF-A) and its receptor, Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2), represent the most prominent ligand-receptor complex involved in the formation of new vessels and playing a significant role in angiogenesis in physio-pathological conditions, *in vivo*.
In this study, we have used an animal model of acute colitis induced via rectal administration of trinitrobenzene sulfonic acid solution (TNBS). In TNBS-treated rats were observed clinical signs and symptoms like humans including diarrhea, occult blood, and weight loss. In addition, histological analysis of colon tissue samples showed severe inflammation and ulceration associated with necrosis. The VEGFR-2 expression in vascular endothelium of colitis rats, correlated with angiogenesis, is investigated.
Double label immunofluorescence images showed marked colocalization of RECA-1 (rat endothelial cell marker) and VEGF-R2 in colon sections of colitis rats. Western blot analysis detected significant increased expressions of RECA-1 and VEGF-R2 proteins in treated rats compared to control group. The preliminary data of this study confirm that angiogenesis is a key component of experimental colitis and, that, VEGFR-2 expressed in endothelial cells, is relevant and related to the formation of new blood vessels in an animal model of IBD.

SEPP-1 A KNOWN SELENIUM TRANSPORTER, AS NOVEL BIOMARKER OF ACUTE KIDNEY INJURY: AN *IN VITRO* STUDY

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Acute kidney injury (AKI) is considered as a rapid decline in renal function, manifesting with elevated levels of serum urea and creatinine. The initial indicators of kidney damage are biological and molecular alterations, therefore it is important to identify new biomarkers for early AKI detection and recovery prediction. Selenium deficiency represents a predisposing risk factor for AKI. Based on these assumptions, aim of this study was to investigate the impact of sodium selenite (Na2SeO3) on the expression of selenoprotein-p1 (SEPP-1), a selenium transporter, and on reactive oxygen species (ROS) production using an in vitro model of hypoxia induced in kidney proximal tubular cells (HK-2). HK-2 cells were treated either with Na2SeO3 100nM for 24 hours or left untreated, then the cells were exposed to CoCl2 (500 μM), a chemical hypoxia inducer, for another 24 hours. Our results revealed that the treatment of HK-2 with CoCl2 determined a reduction of cell viability and an increase of cell mortality by MTT and trypan bleu dye exclusion assays, respectively. Moreover, flow cytometer analysis showed an increase of ROS production. Conversely, pre-treatment with Na2SeO3 enhanced cell viability, reducing both cell mortality and ROS production. The morphological cells changes observed in cells treated with CoCl2, was attenuated if cells were pre-treated with Na2SeO3. Moreover, the examination of selenium transporter by Western Blot, after pre-treatment with Na2SeO3, determined an increase of SEPP-1 expression. Our preliminary results shed light on our hypothesis that selenium supplementation could be in the future a therapeutic means of protection for kidney damage and SEPP-1 may be considered as a new promising biomarker.

MODULATION OF THE HISTONE ACETYLATION IN MERKEL CELL CARCINOMA CELLS BY HISTONE DEACETYLASE INHIBITORS

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Histone acetylation regulates the expression of genes involved in multiple pathways, such as cell proliferation, apoptosis, and immunogenicity. Histone acetylation dysregulation leads to cancer onset, while an antineoplastic activity of histone deacetylase inhibitors, such as panobinostat has been proved in carcinoma. The involvement of dysregulated histone acetylation on the onset of Merkel cell carcinoma (MCC), a rare but aggressive and immunogenic viral induced-skin tumor in 80% of cases, is yet unexplored. We investigated the impact of the histone deacetylase inhibitor panobinostat in modulating the acetylation of histone H3 in Merkel cell polyomavirus (MCPyV)-positive/-negative MCC cells, upon tumor cell phenotype and immunogenicity. Panobinostat-treated MCC cells were investigated for (i) cell proliferation, migration, colony forming potential and apoptosis; (ii) acetylation (ac) of H3 lysines 9, 14, 18 and 27 (H3K9/14/18/27); (iii) mRNA expression of antigen presenting machinery genes HLA-A, MICA and MICB. Panobinostat reduced the MCC cell proliferation, migration and colony forming potential. A significant expression of pro-apoptotic genes in panobinostat-treated MCC cells compared to untreated cells was analyzed. No phenotypic/molecular effects were found in panobinostat-treated fibroblast control cells. H3K9ac and H3K27ac were found in all panobinostat-treated MCC cells. H3K14ac and H3K18ac were undetectable in virus-negative MCC13 and MCC26 cells, while a strong expression was found in treated virus-positive WaGa and PeTa cells. Panobinostat treatment induced the expression of HLA-A, MICA and MICB genes in MCC cells. The chromatin remodeling via panobinostat-induced histone acetylation modulation could drive MCC cells towards a reduced cell proliferation and immunogenicity, while favoring apoptosis.

WHEN A CELL IS OUT OF TUNE: TRANSCRIPTIONAL DYSREGULATION, SOMATIC MUTATIONS AND INCREASED CELLULAR DIVISION RATES.

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Abstract. A characteristic of cancer is cellular transformation, a multifaceted process involving both genetic and epigenetic changes. Among these, transcription factor (TFs) dysregulation stands out as a key contributor to the dysregulation of normal cellular processes, ultimately leading to uncontrolled cell proliferation and tumorigenesis. **Aim of the Study.** This in silico analysis aims to elucidate the intricate relationship between TFs dysregulation, aberrant DNA replication, elevated cellular division rates, and the introduction of somatic mutations. **Materials and Methods.** Through the use of an analytic pipeline optimised for promoter regions of tumor-related genes, the functions of TFs in genomic DNA replication integrity are investigated. This four-step in silico method includes analysing genomic sequences using online bioinformatics tools to find conserved motifs that interact with certain target TFs. **Results.** Transcription factor dysregulation was found to lead to conflicts between transcription and replication machineries, causing replication stress and DNA damage. This dysregulation also disrupts cell cycle checkpoints, leading to uncontrolled proliferation and increased cellular division rates. Additionally, impaired DNA repair mechanisms result in the accumulation of somatic mutations, contributing to tumorigenesis. **Conclusions.** Understanding the molecular processes influenced by TF dysregulation is critical for unraveling the complexities of cancer initiation and progression. The insights gained may inform the development of targeted therapeutic strategies aimed at restoring genomic stability and preventing cancer development.

EXPLORING MONOCYTE BIOENERGETIC FOR NOVEL PERSPECTIVE IN METABOLIC DISFUNCTION-ASSOCIATED STEATOHEPATITIS

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Monocytes (Mos) are essential for the transition from metabolic dysfunction associated steatotic liver disease (MASLD) to metabolic dysfunction-associated steatohepatitis (MASH), as they infiltrate the liver in response to Kupffer cells (KCs) depletion and tissue damage. In this study, we examined the possibilities of studying Mos immunometabolism in MASH. In the first step we dissected circulating Mos bioenergetic *ex vivo*, demonstrating that MASH Mos presented a metabolic reprogramming with high glycolysis and mitochondrial respiration (MR) and pro-inflammatory activity, linked to dysfunction and oxidative stress. Moreover, we observed elevated electron transport chain (ETC) complexes I and II activity with significant ROS generation. However, the inhibition of the toll like receptor 4 (TLR4), was able to control this profile. Expression analyses, revealed 7 downregulated and 32 upregulated genes, predominantly associated with ETC complex subunits, especially a notable upregulation was observed in complex II subunits (succinate dehydrogenase [SDH]). Analysing a potential mechanism, we found an increase of mTOR and PGC1a with a reduction of AMPK phosphorylation. In a second step, the modulation of mitochondrial activity with dimethyl malonate (DMM), an SDH inhibitor, normalized glycolysis and MR levels, abrogating cytokine expression, in MASH Mos. Analyzing a public scRNA-seq dataset, we found that in murine models of MASH, liver Mo-derived macrophages exhibit an upregulation of genes included in glycolysis and mitochondrial energy metabolism pathways. Accordingly, the intraperitoneal injection of DMM in MASH mice had an immunomodulatory effect *in vivo,* reducing hepatic inflammation and cellular damage. Overall, these results suggest that Mos study might be considered for promising therapeutic approach in the treatment of MASH.

DISSECTING THE CELLULAR ORIGIN OF A CIRCULATING MIRNA SIGNATURE DIAGNOSTIC FOR ASYMPTOMATIC EARLY-STAGE LUNG CANCER

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MicroRNAs found in extracellular environments, termed cell-free miRNAs (cf-miRNAs), are emerging as significant players in cancer biology, offering insights into innovative biomarkers for cancer detection and therapy monitoring. We investigated a 13-miRNA signature (miR-Test) previously shown to detect asymptomatic lung cancer (LC) in high-risk subjects. Our study revealed a mixed origin of these cf-miRNAs, originating from both tumor cells and the tumor microenvironment (TME), suggesting a synergistic interaction between tumor cells and TME in driving tumor progression. Intriguingly, analysis of serum samples from a large cohort confirmed differential abundances of cf-miRNAs between tumor patients and healthy subjects. Further examination of miR-29a-3p and miR-223-3p expression profiles in lung adenocarcinoma tissues and serum samples revealed distinct distribution patterns between tumor epithelium and tumor-associated stroma, indicating a role in tumor-stromal interaction. Functional studies demonstrated the impact of miR-223-3p on cancer cell migration and invasion, particularly in KRAS mutant LUAD cells, suggesting a potential link to KRAS signaling. Transcriptome analysis upon miRNA modulation revealed downstream regulatory pathways, implicating miR-223-3p in inflammatory processes and tumor metastasis. Moreover, miR-223-3p overexpression in LUAD cells altered immune responses, promoting an immunosuppressive microenvironment and contributing to tumor progression. Analysis of LUAD datasets validated these findings, demonstrating associations between high miR-223-3p expression and immune evasion signatures, neutrophil enrichment and adverse prognosis. Our study underscores the multifaceted roles of cf-miRNAs, particularly miR-223-3p, in LC pathogenesis and immune modulation, offering potential targets for therapeutic intervention and prognostic stratification.

EXTRACELLULAR VESICLE MICRORNAS CONTRIBUTE TO NOTCH SIGNALING PATHWAY IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive T-cell malignancy characterized by genotypically-defined and phenotypically divergent cell populations, governed by adaptive landscapes. Clonal expansions are associated to genetic and epigenetic events, and modulation of external stimuli that affect the hierarchical structure of subclones and support the dynamics of leukemic subsets. Recently, small extracellular vesicles (sEV) such as exosomes were also shown to play a role in leukemia. Here, by coupling miRNome, bulk and single cell transcriptome profiling, we found that T-ALL-secreted sEV contain NOTCH1-dependent microRNAs (EV-miRs), which control oncogenic pathways acting as autocrine stimuli and ultimately promoting the expansion/survival of highly proliferative cell subsets of human T-cell leukemias. Of interest, we found that NOTCH1-dependent EV-miRs mostly comprised members of miR-17-92a cluster and paralogues, which rescued in vitro the proliferation of T-ALL cells blocked by γ-secretase inhibitors (GSI) an regulated a network of genes characterizing patients with relapsed/refractory early T-cell progenitor (ETP) ALLs. All these findings suggest that NOTCH1 dependent EV-miRs may sustain the growth/survival of immunophenotypically defined cell populations, altering the cell heterogeneity and the dynamics of T-cell leukemias in response to conventional therapies.

LOSS OF CIRCADIAN GENE TIMELESS INDUCES EMT AND TUMOR PROGRESSION IN COLORECTAL CANCER VIA ZEB1-DEPENDENT MECHANISM

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The circadian gene Timeless (TIM) provides a molecular bridge between circadian and cell cycle/DNA replication regulatory systems and has been recently involved in human cancer development and progression. However, its functional role in colorectal cancer (CRC), the third leading cause of cancer-related deaths worldwide, has not been fully clarified yet. Here, the analysis of two independent CRC patient cohorts (total 1159 samples) reveals that loss of TIM expression is an unfavorable prognostic factor significantly correlated with advanced tumor stage, metastatic spreading and microsatellite stability status. Genome-wide expression profiling, in vitro and in vivo experiments, revealed that TIM knockdown induces the activation of the epithelial-to-mesenchymal transition (EMT) program. Accordingly, the analysis of a large set of human samples showed that TIM expression inversely correlated with a previously established gene signature of canonical EMT markers (EMT score), and its ectopic silencing promotes migration, invasion, and acquisition of stem-like phenotype in CRC cells. Mechanistically, we found that loss of TIM expression unleashes ZEB1 expression that in turn drives the EMT program and enhances the aggressive behavior of CRC cells. Besides, the deranged TIM-ZEB1 axis sets off the accumulation of DNA damage and delays DNA damage recovery. Furthermore, we show that the aggressive and genetically unstable 'CMS4 colorectal cancer molecular subtype' is characterized by a lower expression of TIM and that patients with the combination of low-TIM/high-ZEB1 expression have a poorer outcome. In conclusion, our results suggest the engagement of an unedited TIM-ZEB1 axis in key pathological processes driving malignant phenotype acquisition in colorectal carcinogenesis. Thus, TIM-ZEB1 expression profiling could provide a robust prognostic biomarker in CRC patients.

SPLICING REGULATION OF *MAPT* GENE IN A HUMAN CEREBRAL ORGANOID MODEL

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The human *MAPT* gene encodes for Tau, a microtubule-stabilizing protein which plays a crucial role in the cytoskeleton organization. Of the 16 *MAPT* exons, six of them are subjected to alternative splicing, generating a plethora of different Tau protein isoforms. Imbalance among these isoforms is associated with tauopathies, a group of neurodegenerative diseases marked by Tau protein misfolding and aggregation. Therefore, understanding how different factors, like RNA-binding proteins (RBPs) can regulate the alternative splicing of these exons could shed new light on the molecular mechanisms underlying Tau pathology. Recently, organoid technology has risen in interest due to its ability to mimic the developmental processes and act as a valuable model for drug design and screening. We developed a brain organoid model suitable to study RBPs and Tau alternative splicing. To produce this cellular model, we differentiated the iPSC line XF-iPS using the StemDiff™ Cerebral Organoid Kit. We collected organoid samples at 20 (20D) and 40 days (40D) for RNA extraction and immunofluorescence. Immunofluorescence analysis revealed cellular changes in the organoids from 20D to 40D, suggesting the development of cerebral phenotype. Gene expression analysis via RTqPCR for the RBPs PTBP1, PTBP2 and RBM20 revealed a significant decrease in PTBP1 expression over time, consistent with differentiative processes taking place *in vivo* and *in vitro*, and a significant increase in RBM20 expression. Additional qPCR analysis revealed an increased *MAPT* expression over time, compatible with the differentiation towards neuronal fate, and the presence of *MAPT* exon 6+ and exon 10+ mRNAs in the 40D organoids. These preliminary results highlight how brain organoids, even at early stages of maturation, could be employed in the study of splicing events potentially crucial to the development of tauopathies.

GENERATION OF NOVEL INDUCED PLURIPOTENT STEM CELL MODELS FOR THE STUDY OF SPINOCEREBELLAR ATAXIA TYPE 17

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Spinocerebellar Ataxia (SCA) type 17 is a rare dominant neurodegenerative disease characterized by severe motor and psychiatric symptoms. SCA17 is caused by an expansion of CAG repeats in the TATA-Binding Protein (*TBP*) gene, which becomes longer than 49 repetition and is translated into an elongated polyQ tract in the coded protein. Mutant TBP tends to misfold forming intranuclear insoluble aggregates both in glial and neuronal cell in the cerebellum (mainly in Purkinje cells). TBP forms containing an intermediate polyQ tract (41-47/49 Qs) show incomplete penetrance. Recently, a concomitant heterozygous mutation in *STUB1* gene has been identified to exacerbate the pathogenicity of intermediate TBP alleles, triggering a SCA17 digenic variant (SCA17-DI). STUB1 encodes for the C terminus of HSC70-Interacting Protein (CHIP), an E3 ubiquitin ligase serving as HSP70 co-chaperone involved in protein degradative pathways, probably modulating TBP levels. To identify molecular mechanisms responsible for SCA17-DI, we developed patient derived iPSC models carrying: i) fully expanded TBP (SCA17), ii) intermediate TBP (healthy), and iii) intermediate TBP/STUB1^{mut} alleles (SCA17-DI). Fibroblasts were successfully reprogrammed obtaining 14, 9, and 2 iPSCs clones respectively. Notably, the reprogramming efficiency of SCA17-DI fibroblasts was lower than expected, possibly due to the role CHIP on Yamanaka factors clearance, already described in literature. Selected clones have been characterized for pluripotency markers expression (NANOG, OCT3/4, SOX2) through RT-qPCR, and IF (OCT4, C-MYC, TRA-1-60), as well as for their ability to originate germinative layers. Karyotype evaluation revealed no alterations in chromosomes numbers and morphology. In conclusion, we generated new SCA17 iPSC models useful to study the role of STUB1 in differentiated Purkinje cells involved in SCA17 degeneration.

MODULATION OF ADIPOSE-DERIVED STEM CELL BEHAVIOR BY EXTERNAL MICROENVIRONMENT

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Adipose-derived stem cells (ADSCs) are crucial in maintaining tissue homeostasis and regeneration. Their proliferation, survival and activation are influenced by specific signals within their microenvironment, also known as niche. The organization of the niche provides anatomical and functional interactions that contribute to the maintenance of stemness and modulate the final fate of these cells. Moreover, ADSCs secrete growth factors and pro-inflammatory cytokines, able to trigger several metabolic complications when tissue physiology is compromised.
Moreover, the microenvironment emerges as a central regulator in influencing cancer cell-stromal interactions in tumor biology. Whitin this context, we exposed ADSCs to plasma samples derived from human patients diagnosed with prostate cancer (PC), or precancerous lesions (PL), or benign prostatic hyperplasia (BPH) for 4, 7 or 10 days, in the attempt to investigate how tumor microenvironment can affect their proliferation and cell -cell interactions. We then analyzed the expression of main stemness-related markers and cell-cycle regulators and measured cytokine production. Cell morphology and collagen production by confocal microscopy were then investigated. Differential expression of microRNAs is a critical epigenetic mechanism involved in the development and progression of human cancer. For this reason, miR-145, miR-148, and miR-185 expression was analyzed by qPCR. Here we show significant changes in the morphology of ADSCs exposed to plasma samples, suggesting that the tumor microenvironment can influence ADSC behavior, promoting their proliferation. These findings could have significant implications for the development of treatments of these diseases, including the use of ADSCs for cell-based tissue engineering, regenerative medicine, and autologous transplantations.

NON-α-SYNUCLEIN NEURODEGENERATIVE DISORDERS POTENTIALLY LINKED TO GBA H294Q MUTATION.

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Background. Pathogenic mutations of ß-glucocerebrosidase enzyme coding gene (GBA1) lead to Gaucher disease (GD) when inherited in the biallelic pattern. However, several heterozygous variants of the glucocerebrosidase gene (GBA1) have been reported to increase the risk of Parkinson's disease (PD) and dementia with Lewy bodies (DLB), irrespective of reduction of the enzyme activity due to genetic variants. Clinically, GBA-PD has been associated with a higher prevalence of non-motor symptoms compared to nonmutation carriers. PD patients with GBA mutations show a younger age at onset with a median onset in the early fifties. To date the role of GBA mutations beyond α-synucleinopathies in the parkinsonism-dementia spectrum is still unclear. **Objective.** We studied a family from Campania (Cilento) that exhibited clinical signs of CBD, PD and dementia. The H294Q rare variant in GBA gene was identified by previous NGS in targeted panel sequencing in the case index and then confirmed with Sanger sequencing, also in the relatives, using ABI Prism 3500xL DNA Sequencer (Applied Biosystems, Foster City, USA). **Material and Methods.** Blood DNA was extracted using standard methods. DNA samples were subjected to mutational screening of the entire gene of GBA by Sanger sequencing upon PCR-based amplification of the gene, and not of the pseudogene, in three overlapping fragments. GBA activity determination was performed by Dried blood spots (DBSs) on standard filter paper. Testing for C9orf72 expansion was conducted using the AmplideX® PCR/CE C9orf72 kit (Asuragen, Austin, TX, USA). **Results.** Molecular genetic analysis identified a heterozygous GBA H294Q mutation in three siblings with a variable neurodegenerative phenotype ranging from PD to CBS to dementia. The family history suggested that more individuals had been affected in the previous generation. The GCcase activity in two of the four siblings was deficient (2.5.-2.7 umol/L/h). Although not considered to be pathogenic, both values were in the lower range of the spectrum (cutoff value 1.5 umol/L/h; Medical Laboratory, Archimed, Vienna, Austria). C9orf72 screening was negative. The detected H294Q variant occurs in exon 7 (NM_000157.4) and it is classified as "likely pathogenic" (PS4, PM1, PP2, PM2) according to the ACGM guidelines and "severe" based on GD. GBA1-PD browser confirmed the severity of the variant. **Conclusion.** Herein, we present a family with GBA1 mutation and different phenotypes ranging both tauopathies and α -synucleinopathies. While GBA mutations have been implicated in increasing the risk of PD and dementia with Lewy bodies, their role in tauopathies like PSP and CBD is less defined. In a systematic review of 58 reported CBS cases, GRN was the most common genetic risk factor, followed by C9ORF72, PRNP, GBA and MRS2/ZHX2. Yet, more recent data suggest that the clinical phenotype of GBA-associated neurodegeneration is more heterogeneous than previously assumed, including phenotypes distinct from α-synucleinopathies. Our results are interesting since they indicate that *GBA* H394Q mutation may cause heterogeneous clinical phenotypes, aside from PD and dementia with Lewy bodies. The mechanisms likely involve lysosomal dysfunction, impaired autophagy, mitochondrial dysfunction, and neuroinflammation. However, the lack of a clear association may suggest that the mechanisms by which GBA mutations contribute to neurodegeneration differ between synucleinopathies (such as PD) and tauopathies. Alternatively, it's possible that the sample sizes in previous studies were not large enough to detect a significant association, or that other genetic or environmental factors play a more significant role in the development of PSP and CBD. Further research is needed to fully elucidate the role of GBA mutations in tauopathies and to understand the complex interplay between genetic, molecular, and environmental factors in the pathogenesis of these disorders. This may be of relevance, once causal therapeutic strategies for GBA-associated neurodegenerative disease are developed.

APOPTOSIS INDUCED IN OSTEOSARCOMA CELLS BY DRUG DELIVERY BIOMATERIALS

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Osteosarcoma (OS) is a bone cancer, which affects pediatric/young adult and elderly patients. In recent years, novel drug-delivery system approaches with bioactive bone substitutes have been proposed with the objectives to induce OS cell death and bone regrowth. Herein, injectable nanostructured strontium-doped calcium phosphate scaffold (SrCPC) was investigated as drug delivery system to combine bone regeneration and anticancer treatment by controlled release of methotrexate (MTX) and doxorubicin (DOX), coded as SrCPC-MTX and SrCPC-DOX, respectively. The osteoinductivity of SrCPC was investigated analysing osteogenic activities in an *in vitro* model of human adipose stem cells (hASCs), such as (i) mineral matrix deposition by Alizarin Red Staining, (ii) osteocalcin protein expression by E.L.I.S.A. (III) osteogenic gene expression by Real-Time PCR Array. Drug-loaded biomaterials were tested in an *in vitro* model of human OS cell lines, named SAOS-2, U2-OS, and genetically engineered SAOS-2-eGFP. The ability of scaffolds to induce OS cell death was assessed investigating cell proliferation, by Caspase-3/7 activities, and Annexin V/IP positivity. To determine if OS cells grown on doped-scaffolds change their migratory ability, wound-healing assay was performed. SrCPC demonstrates a good cytocompatibility and upregulation of osteogenic genes, together with OCN protein expression and mineral matrix deposition. SrCPC-MTX and SrCPC-DOX induced cell-killing effects and apoptosis in OS cell lines up to day 7. The proposed approach, based on the local, sustained release of anticancer drugs from nanostructured biomimetic drug-loaded cements seems to be a promising approach aiming to combine anticancer therapy and bone regrowth.

HIGH-FAT DIET DRIVES GLUTAMATERGIC SYNAPTIC DAMAGE BY SHAPING THE GUT MICROBIOTA AND T CELL DYNAMICS IN MULTIPLE SCLEROSIS

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High-fat diet (HFD) induces a systemic inflammatory condition that can aggravate multiple sclerosis (MS), an unpredictable, demyelinating autoimmune disease of the central nervous system (CNS). HFD effect on inflammatory synaptic dysfunctions is still unexplored despite their role in the silent MS progression.

Here, we studied this aspect in both a large cohort of patients with MS (N=226) and its mouse model, the experimental autoimmune encephalomyelitis (EAE). We observed that overweight or obese patients with MS exhibited higher disability and glutamate levels in the CNS, contributing to silent disease progression. We also showed in the EAE that HFD worsened clinical manifestations, neuroinflammation, and glutamatergic synaptic transmission. Unexpectedly, HFD triggered glutamatergic synaptic alterations in control mice resembling those observed in EAE. Mechanistically, interleukin-1beta (IL-1β) and tumor necrosis factor (TNF) were identified as pivotal mediators in the process. A multi-omics approach revealed that HFD altered blood-brain-barrier permeability and gut microbiota composition, redistributing adaptive immune cells from the periphery into the CNS and leading to glutamatergic synaptic dysfunctions. Importantly, a biphasic dietary supplementation of prebiotics (resistant starch, fibers and oligosaccharides) and probiotics (multistrains of Lactobacillus and Bifidobacteria) was able to reverse both immune and synaptic effects of HFD. Altogether our findings suggest that reducing dietary fat intake can offer protection against immune-inflammatory synaptic damage and MS progression.

RILP, A RAB7A EFFECTOR, INTERACTS WITH TDP-43 AND ITS DOWNREGULATION IN ALS COMPROMISES THE AUTOPHAGY-LYSOSOMAL PATHWAY

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RILP (Rab-Interacting Lysosomal Protein), regulates late endocytic trafficking and lysosomal biogenesis interacting with RAB7A. Both proteins are involved in the regulation of autophagy, an essential and well conserved process responsible for the maintenance of protein and organelle homeostasis. Due to its importance in delivering intracellular components and dysfunctional organelles to lysosomes, autophagy dysregulation has been related to a large number of human diseases such as, for instance, cancer and neurodegenerative diseases.

A pathological phenotype of neurodegenerative disorders consists in the presence of cytoplasmic aggregates which have to be eliminated through autophagy, further supporting the idea that alterations of autophagy lead to neurodegeneration.

TDP-43 participates in RNA metabolism but that also plays a role in the regulation of the autophagy process and is mutated in a number of familial forms of amyotrophic lateral sclerosis (ALS). Considering the recently emerged important functions of TDP-43 in the autophagy-lysosomal pathway (ALP), we decided to investigate the relationship between TDP-43, RAB7A and RILP.

As expected, silencing of TDP-43 or RILP or expression of an ALS-causing TDP-43 mutant protein induced an impairment of the autophagic flux. Importantly, we demonstrated that RAB7A, RILP and TDP-43 are together in a complex and that RILP interacts directly with TDP-43, while the interaction of RAB7A with TDP-43 is mediated by RILP. Moreover, we discovered that silencing TDP-43 or expression of an ALS-causing TDP-43 mutant protein reduce the abundance of RILP affecting the autophagic flux. Notably, overexpression of RILP restores the autophagic flux in cells expressing an ALS-causing TDP-43 mutant protein. Altogether, these data indicate that RILP could be fundamental to rescue dysfunctional autophagy caused by TDP-43 alterations.

INNOVATIVE TECHNOLOGY FOR THE EXTRACTION- AND AMPLIFICATION-FREE DETECTION OF SMALL NUCLEIC ACIDS IN LIQUID BIOPSIES AND SOLID TISSUES

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Over the past two decades, numerous techniques have been developed for analyzing small nucleic acids, such as microRNAs and siRNAs in body fluids and tissues. However, these methods still face technical challenges, particularly when compared to well-established techniques for proteins and metabolites. Recently, we introduced the ODG platform, which is an innovative ELISA-like technology that allows for the direct detection and quantification of microRNAs in liquid biopsies without requiring extraction nor amplification and with single-base specificity. The current study presents the implementation of ODG platform within a semi-automated protocol to create the "SA-ODG" platform, enhancing the efficiency and precision of small nucleic acid testing while reducing hands-on time required by laboratory staff. Moreover, SA-ODG platform capabilities have been widened to directly quantify other small nucleic acids in both liquid biopsies and solid tissues. These developments represent a crucial step forward in advancing the field of extraction and amplification-free nucleic acid detection and quantification.

GUT MICROBIOTA LACTOBACILLI MODULATE HUMAN MICROGLIAL P2Y12 SIGNALLING

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The gut microbiota is a complex ecosystem including a large number of microbial species which influence human health. In particular, *Lactobacilli* are considered as beneficial microorganisms for enhancing immune balance and attenuating inflammation, contrasting adverse stress. Emerging data show how the cross-talk between the gut and the brain is involved in brain development. Metabolites produced by *Lactobacilli* are mediators between the gut and the brain, and are able to reach the central nervous system affecting the microglial cells activities, influencing their phenotype and motility. Microglial cells are the resident macrophages of the brain, playing a crucial role in immune defence. Microglial cell motility is controlled by purinergic stimulation of P2Y12 receptors, playing a crucial role in purinergic microglial activation and influencing neuronal activity . We hypothesized that microglial P2Y12 signalling is modulated by *Lactobacilli* metabolites. This pathway may hamper neuronal hyper excitability in paediatric epilepsies acting directly on the cortical inhibitory circuit

.The aim of this study is analyse P2Y12 in microglia activation and on neuronal activities using human cerebral cortical slices from epileptic pediatric patients. Through immunohistochemistry and immunofluorescence, we analyzed microglial morphology and P2Y12 presence with or without treatment with *Lactobacilli* cell free supernatants (CFSs). Preliminary data indicate that *Lactobacilli* CFSs influence P2Y12 expression and microglia morphology. Furthermore, we studied the excitatory neuronal activities through Multi Electrode Arrays (MEA) recordings and found that the CFSs modulate the hypersynchronous neuronal activity. The microglia-microbiota cross-talk highlights the role of P2Y12 in pathological conditions and the adjuvant action of the intestinal microbiota in the neuronal response.

SHEDDING LIGHT ON MIRNA ROLE IN IDIOPATHIC PULMONARY FIBROSIS FOR PERSONALIZED THERAPY THROUGH BIOINFORMATIC APPROACH

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Idiopathic Pulmonary Fibrosis (IPF) is a chronic lung disease that affects about $3x10^6$ people worldwide and results in progressive scarring of the lungs and accumulation of fibroblasts. We aim to identify the key miRNAs in IPF and unveil their functions for targeted therapy. scRNA-seq analysis was performed using Python language on heathy and IPF lung samples obtained by 4 public scRNAseq datasets (GSE122960, GSE128033, GSE135893, GSE136831). Raw count matrices were merged to perform batch effect correction, normalization, scaling and dimensionality reduction using both PCA and UMAP approaches, identifying DEGs between immune and non immune cells clusters. After, using TargetScan, we identified cell miRNAs and checked databases for their expression during IPF. We found 6 up-regulated miRNAs (miR-4326, miR-129-5p, miR-1299, miR-148a-3p, miR-339-5p, miR-1303) and 4 down-regulated miRNAs (miR-181a-2-3p, miR-143-3p, miR-424-3p, let-7c-5p). In particular, miR-129-5p and miR-143-3p target COL1A1, involved in the deposition of the ECM; miR-4326 targets FN1, which is essential in the differential production of Thy 1+ and Thy1- lung fibroblasts and miR-143-3p targets AGR2 highly expressed in alveolar epithelial AT I cells of patients with IPF. Moreover, these two miRNAs target GSK3β dysregulated during the IPF process and is present in fibroblasts of patients with IPF. Followinfg these analyses, miR-143-3p, miR-4326 and miR-129-5p could represent targeting molecules that are in EVs likely released by fibroblasts that characterize the lung microenvironment typical of IPF. The presence of specific cell miRNAs characterizing the IPF phenotype could represent a target to pharmacologically modulate lung environment during IPF condition. Our in-depth analysis will enable us to verify the targets of each identified miRNA and validate specific activated pathways during IPF.

ROLE OF H3K4ME3 DRIVEN BY HISTONE METHYLTRANSFERASE MLL1 IN EPIGENETIC TRANSMISSION OF ADVERSE PHENOTYPE TO OFFSPRING OF GESTATIONAL DIABETIC WOMEN.

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Gestational diabetes (GD) is characterized by chronic hyperglycemia (HG) during pregnancy, associated to oxidative stress and inflammation, drivers of atherosclerotic cardiovascular disease (ASCVD). Histone changes are key players in HG-induced ASCVD and mediators of the transmission of GD phenotypes to the offspring. This study aims to investigate the link between Mixed Lineage Leukemia 1 (MLL1)-dependent H3K4me3 and oxidative and inflammatory phenotypes. Then, we investigated whether such epigenetic mark is transmitted to the offspring born to GD women.

Peripheral blood mononuclear cells (PBMC) from GD and control women, endothelial cells from human umbilical cord vein (HUVEC) and cord blood mononuclear cells (CBMC) at birth, as well as PBMC from adolescents born to control and GD women were used. NF-kB p65 and VCAM-1 were evaluated by RT-qPCR, immunofluorescence (IF) and flow cytometry, and NOX4 by IF. H3K4me3 on NF-kB p65 and NOX4 promoters was assessed by ChIP and RT-qPCR. NF-kB p65 DNA binding activity by ELISA and HUVEC-monocytes adhesion assay was performed.

We observed a significant increase of NF-kB p65 and VCAM-1 gene expression in GD- as compared to control-HUVEC. We demonstrated that MLL1-driven H3K4me3 on NF-kB p65 promoter is upstream to the inflammatory pathway activation, using MLL1 inhibitor (MM-102), which blunted NF-kB p65 inflammatory pathway activation and NOX4 expression. Interestingly, higher levels of H3K4me3 were observed also in CBMC and PBMC from newborns and adolescents born to GD women.

Our results suggest that the MLL1-induced epigenetic mark promotes the inflammatory and oxidative phenotypes in GD, and it is transmitted to the offspring. This mechanistic study supports potential pharmacological interventions to reprogram chromatin changes, aiming to alleviate early abnormal phenotypes in offspring and mitigate the risk of ASCVD.

INVESTIGATING NON-MYOGENIC MESENCHYMAL CELLS' IMPACT ON MUSCLE DEGENERATION PROCESS IN FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY

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Understanding the complexity of muscle degeneration in facioscapulohumeral muscular dystrophy (FSHD) patients remains a critical challenge. FSHD is a rare, progressive muscle disorder caused by the inappropriate activation of the *DUX4* gene. Our study explores the intricate cellular network within the skeletal muscle stem cell niche in patients, focusing on the role of non-myogenic mesenchymal cells in FSHD pathophysiology to elucidate degeneration mechanisms and identify novel therapeutic targets. In skeletal muscle, different cell types cooperate to maintain tissue homeostasis, and we hypothesize that dysregulation in this network contributes to muscle degeneration in FSHD.

Muscle specimens were obtained via needle biopsies from muscles of FSHD patients and healthy controls following MRI examination. Our findings reveal a significant accumulation of non-myogenic mesenchymal cells in FSHD muscles, positively correlated with intramuscular fibrosis severity. Notably, the expansion of the non-myogenic mesenchymal cell compartment is more pronounced in rapidly deteriorating muscles. Furthermore, non-myogenic mesenchymal cells isolated from FSHD muscles display enhanced proliferation rates and altered adipogenic and fibrogenic differentiation capacities in vitro. Additionally, cells from patients exert different impacts on myoblast proliferation and differentiation using co-culture systems. Examination of DUX4 downstream target expression reveals modulation of DUX4 signaling within patient-derived non-myogenic mesenchymal cells during in vitro differentiation.

Investigating the heterogeneity and contributions of various cell types within the skeletal muscle stem cell niche in FSHD patients offers insights into the diverse cell clusters' roles in muscle degeneration. These findings address critical questions and pave the way for innovative therapeutic strategies in FSHD.

DELIVERY OF ALPHA-KETOGLUTARATE USING GLUTATHIONE-COATED AUNPS IN LUNG CANCER: A NOVEL APPROACH TO EPIGENETIC MODULATION

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Gold nanoparticles (AuNPs) have recently emerged as a promising tool in drug delivery due to their physicochemical properties, such as large surface area, ease of functionalization, and biocompatibility. AuNPs can be loaded with therapeutic agents through covalent or non-covalent interactions, allowing controlled and sustained drug release, thus representing a new cutting-edge drug delivery system. Recently, glutathione (GSH)-coated AuNPs (G-AuNPs) of ≈2 nm size were developed. It was demonstrated that in physiological aqueous environments, these nanoparticles form complexes of ≈100-300 nm and get easily internalized in different types of cells.

To ensure G-AuNPs biocompatibility, the toxicity of these nanoparticles was assessed across various tumor models, including breast cancer MCF7, ovarian cancer OVCAR-3, and lung cancer A549 cell lines. Previous studies have shown that glutamine deprivation leads to de-differentiation of tumor cells due to the lack of alpha-ketoglutarate, impacting gene expression by acting as a cofactor for chromatin-modifying enzymes. Similarly, glucose deprivation depletes alpha-ketoglutarate, leading to inhibition of alpha-ketoglutarate-dependent demethylases, increased histone methylation and suppression of differentiation-related genes. Given these results, we aimed to explore the capability of AuNPs to deliver alpha-ketoglutarate to lung adenocarcinoma A549 cells. Then, G-AuNPs were coated with alpha-ketoglutarate, administered to the A549 cells grown under low glucose conditions. Their effect was assessed at protein level measuring changes in the methylation levels of different de-differentiation markers e gene expression level using RNA-seq.

AuNPs present a versatile platform for targeted drug delivery. Their ability to deliver alpha-ketoglutarate offers new treatment avenues for cancers and diseases with metabolic dysregulation.

miRNA EXPRESSION IN HUMAN MACROPHAGE-LIKE CELLS INFECTED BY LEISHMANIA INFANTUM AND THEIR INTERACTION WITH mRNAs

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MicroRNAs (miRNAs) regulate gene expression and play a crucial role in numerous diseases, including infections. Leishmaniasis is a neglected infectious disease occurring in different forms (i.e., cutaneous, mucocutaneous, and visceral) caused by protozoans belonging to the *Leishmania* genus. The parasite infects mainly the macrophages, establishing a niche permissive for its proliferation. Although *Leishmania* parasites are known to modulate host gene expression, the role of host miRNAs in this process has not been fully characterized.
This work aimed to study miRNA expression profile in human macrophage-like cells infected by L. infantum, the causative agent of visceral and cutaneous leishmaniasis in the Mediterranean region. Moreover, we attempted to identify putative miRNA-mRNA interactions based on the mRNA expression changes previously described[1]. To this end, small RNA-seq was performed in U937 cells infected with *L. infantum* after 24 h and 48 h, and differentially expressed miRNAs were identified and validated through qPCR.
The upregulated and downregulated miRNAs at 24h (n=24, 29) and 48h (n=31, 15) were subjected to functional enrichment analysis. Some enriched ontologies were related to pathways identified in previous work based on mRNA-seq on the same samples, providing the basis for identifying miRNA-mRNA interactions. To this end, miRNAs putative targets were defined using Targetscan 8.0; then, the target lists were compared with dysregulated genes identified in the same experimental conditions, and miRNA-mRNA networks were predicted. Interestingly, the miRNAs commonly dysregulated at both 24h and 48h post-infection targeted several dysregulated transcription factors (TFs), evidencing a relationship among miRNAs and TFs that can contribute to *Leishmania*-induced immune subversion into macrophages.

SARS-COV-2 SPIKE PROTEIN S1 INDUCES MG-H1/RAGE ACTIVATION TO SUSTAIN INFLAMMATION IN HUMAN BRONCHIAL BEAS-2B CELLS

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COVID-19 pathogenesis is associated with a hyperinflammatory response. The mechanisms of SARS-CoV-2-induced inflammation are scantly known. Methylglyoxal (MG) is a potent glycating agent, leading to the formation of the proinflammatory advanced glycation end product (AGE), MG-H1, binding to the receptor RAGE. Here, we investigated the involvement of the MG-H1/RAGE axis as a potential novel mechanism in SARS-CoV-2-induced inflammation by resorting to human bronchial BEAS-2B and alveolar A549 epithelial cells, expressing different levels of the ACE2 receptor (R), exposed to SARS-CoV-2 spike protein 1 (S1). Interestingly, we found in BEAS-2B cells that do not express ACE2-R that S1 exerted a pro-inflammatory action through a novel MG-H1/RAGE-based pathway. MG-H1 levels, RAGE and IL-1β expression levels in nasopharyngeal swabs from SARS-CoV-2-positive and -negative individuals, as well as glyoxalase 1 expression, the major scavenging enzyme of MG, seem to support the results obtained in vitro. Altogether, our findings pave the way for the study of the MG-H1/RAGE inflammatory axis in SARS-CoV-2 infection as a potential therapeutic target to mitigate COVID-19-associated pathogenic inflammation.

G-QUADRUPLEX DYNAMICS IN *RPGR*: UNVEILING NEW BIOMOLECULAR MECHANISMS TO RETINAL DEGENERATION

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This study provides novel insights into the pathogenic implications of G-quadruplex (G4) DNA structures in the *RPGR* gene, known for its involvement in most X-linked retinal degenerations. We hypothesized that the stabilization of G4 structures might disrupt DNA replication and transcription, thereby inducing genetic instability and influencing gene expression crucial t o disease progression. To test this hypothesis, we employed whole genome amplification and next -generation sequencing to investigate the blockade of polymerase activity by G4 structures. Our approach included detailed computational and 3D molecular modeling to visualize the interference in DNA replication and transcription regulation. Focusing specifically on the *RPGR* gene, which is rich in predicted G4-forming motifs, our data confirmed that the stabilization of these structures by the G4 ligand pyridostatin leads to significant polymerase obstruction. This obstruction manifested as reduced amplification of *RPGR* gene regions and shifts in the start/end sites of putative G4 motifs. Importantly, our modeling suggested potential disruptions in critical promote r elements and RNA polymerase binding sites, which could lead to substantial alterations in gene expression. These findings suggest that G4 structures could play a critical role in the pathogenesis of retinal degeneration and potentially other genetic disorders. The study underscores the importance of G4 structures as therapeutic targets and supports the advancement of precision medicine by highlighting novel molecular mechanisms that could be leveraged to treat genetic diseases. Our results contribute to a deeper understanding of the molecular genetics underpinning retinal degenerative diseases and open new avenues for therapeutic intervention.

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

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

IN VIVO APPROACHES TO STUDY THE ROLE OF TNPO3 IN A ZEBRAFISH MODEL OF LGMD D2

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Limb Girdle Muscular Dystrophy D2 (LGMD D2) is a rare neuromuscular disorder with recognizable clinical features such as marked atrophy and muscle weakness. A heterozygous mutation in the termination codon of the *TNPO3* gene has been described as causative of LGMD D2. Several mutations of *TNPO3*, all resulting in a mutated protein that is 15-amino acids longer in its C-terminal domain, have been identified. Thus, the onset and progression of the pathology are highly variable in patients. *TNPO3* encodes for Transportin-3 (TNPO3), an importin that mediates the translocation to the nucleus of SR proteins involved in RNA splicing. However, the pathogenetic mechanism remains unknown. The primary goal of this study has been to establish an in vivo Zebrafish model of LGMD D2 to better understand the disease's pathogenesis, investigate the role of TNPO3 in muscle development, and uncover potential molecular pathways associated with the disorder. We microinjected mRNAs encoding either the wild type or mutated forms of human *TNPO3* into Zebrafish embryos to study their effects on myogenesis during development at different levels. Gene expression analyses showed an altered profile of Myogenic Regulatory Factors (MRFs) and muscle-specific proteins, and phenotypical studies, conducted through immunofluorescence and transmission electron microscopy, displayed in zebrafish embryos microinjected with the human mutated TNPO3 mRNA, an aberrant organization of the muscle fibers. Functional tests seem to confirm that embryos microinjected with the mutant TNPO3 exhibited reduced activity levels during open field tests and displayed diminished responses to changes in light. These findings suggest the effectiveness of our approach in establishing a Zebrafish model of LGMD D2 and highlight the role of TNPO3 in muscle development and differentiation as a potential pathogenetic mechanism underlying LGMD D2.

INVESTIGATING THE ROLE OF LNCRNAs IN CNS PATHOPHYSIOLOGY: *LINC00520* AS A POTENTIAL BIOMARKER FOR PARKINSON'S DISEASE IN HUMAN IN VITRO MODELS

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In recent years, long non-coding RNAs (lncRNAs) have emerged as important players in biological processes, particularly during the development of the Central Nervous System (CNS) and in maintaining proper homeostasis. Even minimal changes in their expression levels have been associated with neurological disorders, including Parkinson's disease (PD). PD is a widespread neurodegenerative disorder, with a global incidence increasing due to factors such as age, sex, environmental influences, and genetic susceptibility.
Results from our previous meta-analysis comparing the transcriptomes of *post-mortem* PD brain samples with those of control patients revealed numerous differentially expressed genes, including a significant number of lncRNAs not previously associated with the disease. Specifically, *LINC00520* and *LINC00641* showed marked up- and down-regulation, respectively, while *LINC00674* remained stable. The study aims to validate these findings in various human in vitro models mimicking neurodegeneration. Neuronal, microglial and astrocyte cell lines (SH-SY5Y, HMC3, and SV-40 immortalized astrocytes) were exposed to pro-oxidative and pro-inflammatory stimuli, such as 6-OHDA, Rotenone, IFNγ and TIC (TNFα, IL-1α, C1q). This resulted in a significant up-regulation of *LINC00520* in all treated cell types. Moreover, RNAi experiments against the lncRNA led to an altered oxidative stress and inflammatory response in neurons and microglia. Additionally, gene expression analyses in human induced pluripotent stem cells (hiPSCs), fibroblasts, and dopaminergic neurons generated from hiPSCs in control conditions revealed that *LINC00520* and *LINC00641* were expressed especially in neuronal cell, while *LINC00674* exhibited consistent levels in fibroblasts. Ongoing investigations in cell lines from PD patients aim to elucidate the role of these lncRNAs in the pathogenetic mechanism.

ENDURANCE EXERCISE TRAINING IMPACTS ON ALTERNATIVE SPLICING CHOICES IN THE SKELETAL MUSCLE OF *SOD1^{G93A}* MICE

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disease characterized by the degeneration of upper and lower motor neurons, leading to muscle wasting and atrophy. Epidemiological and experimental evidence suggests a causal link between ALS and intense physical activity. Although the role of intense physical activity in the aetiology of ALS has been debated for several decades, it did not reach a clear conclusion yet. In this study, we investigated how intense endurance aerobic exercise impacts on alternative splicing programs in the skeletal muscle of *SOD1^{G93A}* mice. In the skeletal muscle, extensive alternative splicing transitions occur postnatally, contributing to the maturation of the contractile apparatus and ensuring proper muscle function. Notably, mis-regulation of alternative splicing pattern contributes to the onset and progression of several neuro-muscular diseases, including ALS.
Methods: In this study, we administered an intense endurance exercise through a motorized treadmill for eight weeks to wild type and *SOD1^{G93A}* mice. We measured muscle strength, weight, and motor skills and compared them with the corresponding sedentary groups to define the disease onset. At the end of the protocol, we analyzed the skeletal muscle-motor neuron axis by histological and molecular techniques.
Results: We identified several exercise-induced splicing changes, dysregulated in ALS muscles, including the isoform 4 of the peroxisome proliferator-activated receptor γ (PPARγ) coactivator 1α (PGC-1α), induced in wild type but not in *SOD1^{G93A}* skeletal muscles.
Conclusions: We found that intense endurance exercise worsens the pathological hallmarks of ALS, such as denervation and neuroinflammation, and accelerates the progression of the disease. These features are paralleled by extensive alternative splicing transitions contributing to muscle wasting.

MODULATING VCP TO MITIGATE PATHOLOGICAL MECHANISMS RELATED TO *C9ORF72* MUTATION IN ALS

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by the degeneration of upper and lower motor neurons. Various pathological mechanisms contribute to motor neuronal death, including the accumulation of toxic misfolded protein aggregates and lysosomal dysfunction. Many ALS cases are linked to a hexanucleotide repeat expansion (G_4C_2) in the *C9ORF72* gene. The expansion produces five dipeptide repeat proteins (DPRs) that aggregate and lead to cell death. DPRs disrupt organelle function and the protein quality control (PQC) system.
We investigated the biochemical behavior of each DPR in immortalized motoneurons, confirming their accumulation and localization, and examined their impact on lysosome stability. All DPRs caused lysosomal membrane damage and activity alterations, exacerbated by impaired autophagy induction. DPRs blocked the nuclear localization of transcription factors (TFEB and TFE3) essential for autophagy and lysosome biogenesis, preventing the removal of damaged lysosomes.
Valosin containing protein (VCP), an ATPase involved in multiple PQC pathways, was analyzed to rescue DPR toxicity.
Overexpressing VCP in these models rescued the accumulation of the most toxic DPRs, enhancing clearance via the ubiquitin-proteasome system (UPS) and/or autophagy. VCP overexpression also reduced lysosomal alterations, likely due to increased DPR clearance. Chemical induction of VCP using SMER28 decreased DPR levels in both immortalized motoneurons and iPSC-derived motor neurons (iPSC-MNs). These findings suggest that VCP modulation could be a potential therapeutic target to reduce DPR-mediated toxicity and improve motoneuron survival in ALS.

EXTRACELLULAR VESICLES FROM OVARIAN CANCER CELLS ENDOW ADIPOCYTES WITH PRO-TUMOR FEATURES

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Ovarian cancer (OC) is still the most lethal gynecologic tumor, due to the rapid and silent development of omental metastasis. Thus, a deeper understanding of the mechanisms regulating OC progression may have crucial impact on the outcomes of this deadly disease.
There is consistent evidence of an association between obesity and increased OC aggressiveness. As omentum is rich in adipocytes, a key pro-tumor role for visceral adipose tissue has been postulated. Indeed, a cross-talk between OC and omental adipose cells has been demonstrated; however, the study of this dialog has been limited to metabolites and adipokines, although recent findings point to a key role of extracellular vesicles (EVs) in the control of tumor evolution.
In the present study, we found that OC EVs could induce the production of multiple adipokines, namely interleukin 6, interleukin 1β, MCP-1 and TNFα, in adipocytes. In particular, these changes were accompanied by ERK1/2, p38 and JNK activation, which was demonstrated to modulate the release of all the above cytokines. Interestingly, conditioned media from EV-treated adipocytes stimulated both macrophage and neutrophil recruitment, also favoring their polarization toward the pro-tumor M2 and N2 state, respectively. More importantly, this media promoted OC cell migration and invasion as well as anoikis resistance; in particular, an increase in tumor spheroid formation ability was observed. Overall, these data indicate that an EV-mediated bidirectional crosstalk exists between OC and adipocytes, endowing the latter with pro-inflammatory properties.

CELLULAR RESPONSES TO STRESS LINKED TO MICROPLASTICS EXPOSURE. AN EDIBLE FISH MODEL FOR AN VIVO EXPOSURE.

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The potential effects of microplastics (MPs) on humans is a big concern and it has been reported that the ingestion of contaminated food is the main route of exposure. In the present study, we used *Perca fluviatilis* as a model to evaluate the possible cellular effects induced by 5 different plastic polymers originated from consumer products and car tire after 4 and 7 month exposure. EtoxyResorufin-O-Deetylase (EROD), Acetylcholinesterase (AChE) and Gluthation Reductase (GR) activity evaluation were used on liver and brain samples to detect nervous damage, oxidative stress and metabolism activity, respectively. The Cytome assay was chosen to check chromatin structure on perch's peripheral blood erythrocytes by the evaluation of micronuclei and other nuclear abnormalities. Results indicated an increase of micronucleated cells in specimens co-exposed to microplastics and car tires (MPs + CT) for 7 months and such a response resulted to be modulated by EROD and GR activity, thus indicating that the involvement of oxidative processes might be at least partially responsible for the chromosomal alterations observed. A cytotoxic effect was found in the same exposure group. Increases in dicentric chromosome frequencies were observed in fish exposed to both MPs and MPs + CT, indicating a clastogenic mechanism of action mainly related with mis-repair of double-strand DNA breaks and/or telomere end fusions. A range of molecular mechanisms are potentially suggested for chromosomal abnormalities formation in response to MPs. Based on our data, we can hypothesize that the interactive effect within the co-exposure may mainly be modulated by oxidative processes. The present experimental model is therefore proven to be a useful tool to understand the cellular mechanisms related with MPs-induced oxidative stress and chromatin structure alterations possibly interfering with human health.

EXPLORING CIRCULAR RNA EXPRESSION IN JAK-INHIBITOR RESISTANT MYELOPROLIFERATIVE NEOPLASMS USING LONG-READ SEQUENCING: TOWARDS NEW RNA-BASED THERAPIES

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Circular RNAs (circRNAs) play key biological roles by modulating microRNA and protein activity, stabilising mRNA, and encoding peptides. Recent studies have identified oncogenic and tumour suppressor circRNAs involved in cancer mechanisms. Specific Oxford Nanopore Technology long-read sequencing protocols (circONT-seq) have been developed to obtain complete circRNA sequences, which are essential to define their functions.
First, we compared the effectiveness of the circONT-seq and Illumina RNA-seq methods on a leukaemia cell line, showing that they provide complementary data. Then, we used circONT-seq to investigate circRNAs in myeloproliferative neoplasms (MPN), with a focus on the acquisition of resistance to the JAK-inhibitor Ruxolitinib (RUXO), a critical unmet clinical need whose underlying mechanisms are still not completely understood. We studied the variation in circular isoform expression in MPN cellular models with JAK2-V617F mutation (PAR), treated with and resistant to RUXO (TRT and RES, respectively). Overall, we detected 25,681 individual circRNAs. Host genes of the most expressed circRNAs were *ARID1A, CDYL, GSE1, RUNX1,* and *ETV6*. Notably, the circONT-seq protocol allowed discriminating circRNA isoforms sharing the same backsplice junctions but having different exon compositions. Most importantly, PAR, TRT, and RES cells showed distinct circRNA expression profiles, which were validated. Several promising circRNAs underwent further study in samples collected during the disease course of patients (baseline, sensitive and then resistant to RUXO).
The results suggest that circRNA expression is modulated in response to JAK inhibition and significantly changed upon developing resistance to RUXO in MPN. Functional studies will further elucidate the circRNA roles in chemoresistance, opening new ways for RNA-based therapies.

TRANSGLUTAMINASE 2 ENHANCES THE SENSITIVITY OF METASTATIC MELANOMA CELLS TO FERROPTOTIC CELL DEATH THROUGH ER STRESS INDUCTION

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Melanoma is one of the most aggressive and difficult to treat forms of cancer, with an incidence that has been continuously increasing in recent years. Currently, BRAF and MEK selective inhibitors alone or in combination with immune checkpoint inhibitors cannot be considered a definitive treatment due to the onset of drug resistance phenomena that limit their use. Therefore, new therapeutic strategies are needed, in particular for those patients carrying BRAF/NRAS mutations, responsible for the development of highly invasive and metastatic phenotypes. In response to certain anticancer agents, such as erastine, cancer cells may undergo a type of iron-dependent cell death, named ferroptosis, induced by the inhibition of cysteine uptake by the XC- system, located on the cell membrane. This causes a reduction of intracellular glutathione, with consequent inhibition of the enzyme glutathione peroxidase 4 (Gpx4) and an increase in the levels of lipid peroxides, which are the main executioners of this form of cell death.

We recently demonstrated that most metastatic melanoma cell lines are resistant to ferroptosis, due to the increased expression of the detoxifying enzymes AKR1C1÷3, belonging to the aldo-keto reductase family, also evidencing a potential correlation with cell differentiation. Therefore, we focused on the study of the relationship between the epithelium-mesenchyme transition (EMT), UPR and resistance to ferroptosis.

Our results indicate that metastatic melanoma cell lines characterized by enhanced EMT-LP (EMT-Like Profile) index are more sensitive to the induction of ferroptosis, compared to those with a lower index. Furthermore, we found that pushing forward the EMT-LP index, by TGFβ, it is possible to re-sensitize these cells to the death process. Finally, we found that the transglutaminase 2 (TG2) might be involved in this signalling through ER stress induction.

THE ROLE OF FERROPTOSIS IN THE DEVELOPMENT OF A PSORIATIC-LIKE PHENOTYPE IN HUMAN KERATINOCYTES

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Psoriasis is a chronic and inflammatory skin disease characterized by destruction of the epidermal barrier, associated with abnormal cell differentiation and death of keratinocytes. Recent studies have shown that psoriatic keratinocytes are characterized by enhanced production of reactive oxygen species (ROS) and lipid peroxidation, which are the main drivers of ferroptosis. The involvement of ferroptosis in the pathogenesis of psoriasis is, though, poorly defined. Here, we exposed the immortalized keratinocytes (HaCaT) to two ferroptosis inducers (FINs), RSL3 (GPX4 inhibitor, 5-10μM) and ferlixit (ferric iron, 50-100μM) for 24h. First, propidium iodide-mediated flow cytometry assay showed that RSL3 induces remarkable and dose-dependent cell death in HaCaT cells, while ferlixit appears almost ineffective. The lipid peroxidation analysis, measured by BODIPY cytofluorimetric assay, confirmed the accumulation of lipid ROS in HaCaT cells treated with RSL3 but not in those treated with ferlixit. Both RSL3 and ferlixit induced an enhancement of mitochondrial ROS production, assessed by MitoSOX Red probe, while the mitochondrial membrane hyperpolarization, quantified by using TMRM flow cytometry assay, was found only in HaCat cells treated with RSL3. Then, we analyzed the expression profile of specific differentiation markers (*KRT1, FLG*) and proinflammatory genes (*IL6*, *IL8*, *CCL20*, *defensins*, *IFNβ, TNFα*) upon administration of RSL3 or ferlixit. Real-time PCR analysis highlighted that both FINs led to the upregulation of *FLG* and the reduction of *KRT1*. Furthermore, RSL3 causes the overexpression also of inflammatory cytokines. Overall, these results suggest that ferroptosis can be associated with the development of psoriatic phenotype, potentially interfering with differentiation- and inflammation-related pathways.

EXPLORING THE INTERPLAY BETWEEN TUMOR GROWTH DYNAMICS, LIPID METABOLISM, AND INFLAMMATION IN NORMAL AND MESOTHELIOMA CELL LINES: THE IMPACT OF FABP5 AND NF-KB

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Background: Malignant pleural mesothelioma (MPM) is characterized by a complex molecular landscape and notorious resistance to therapy. This study explores the role of fatty acid-binding protein 5 (FABP5) in MPM, focusing on its impact on metabolic alterations and inflammation through NF-κB dysregulation.
Methods: We compared human epithelioid mesothelioma cell lines (IST-Mes2 and MPP89) with normal human primary mesothelial cells (HMC35). Growth dynamics, intracellular fatty acid profiles, and key metabolic parameters were assessed using proliferation assays, gas chromatography-mass spectrometry, and various metabolic assays. NF-κB activity and related inflammatory gene expression were analyzed using luciferase reporter assays, ELISA EMSA, qRT-PCR and ChIP assay. FABP5 silencing was performed to evaluate its effects on cell dynamics, metabolism, and NF-κB activity.
Results: Mesothelioma cells exhibited higher proliferation rates, enhanced viability, altered cell cycle distribution, and reduced apoptosis compared to normal mesothelial cells. A significant increase in intracellular myristate, palmitate, and stearate, coupled with decreased extracellular levels, indicated augmented fatty acid uptake in mesothelioma cells. FABP5 overexpression was identified in mesothelioma cells, correlating with increased mitochondrial activity, altered ADP/ATP and NAD+/NADH ratios, elevated ROS production, and lipid droplet accumulation and a concurrent decrease in the mRNA expression levels of key enzymes involved in phospholipid biosynthesis CCTα, CHPT1, and CEPT1. NF-κB activity was significantly heightened in mesothelioma cells, with enhanced p65 and RelB activity, increased p65 nuclear translocation, and elevated IKK phosphorylation. FABP5 silencing reduced proliferation, increased apoptosis, decreased mitochondrial activity, altered key metabolic ratios, and downregulated NF-κB activity and its dependent genes. Given the elevated mRNA expression of phospholipid biosynthesis genes in mesothelioma cell lines, we investigated the putative regulatory role of NF-κB on these genes. ChIP assays revealed an increased recruitment of p65 to the NF-κB enhancers of CCTα, CHPT1, and CEPT1 in mesothelioma cell lines compared to normal mesothelial cells (HMC35) while FABP5 silencing attenuated this recruitment, suggesting a modulation of NF-κB activity by FABP5, whit an impact on phospholipid biosynthesis.
Conclusions: Our findings reveal FABP5 as a crucial driver of metabolic alterations and NF-κB dysregulation in mesothelioma, distinguishing these cells from normal mesothelial counterparts. The observed effects of FABP5 silencing underscore its potential as a therapeutic target, offering new avenues for innovative treatment strategies against mesothelioma.

URIDINE DIPHOSPHATE PROMOTES THE DIFFERENTIATION OF MONOCYTES INTO CD11C$^+$ HLA-DR$^+$ DENDRITIC CELLS

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Uridine diphosphate (UDP) is an extracellular nucleotide signaling molecule implicated in diverse biological processes via specific activation of pyrimidinergic receptor P2Y, G Protein-Coupled, 6 (P2Y6). Recently, we found that uridine diphosphate is strongly enriched in melanoma tumor interstitial fluid, prompting us to investigate the effects exerted by UDP on the immune response. We observed that PBMC treatment with UDP induced a reduction in the proliferation of CD14+ monocytes, accompanied by an increase in the percentage of HLA-DR+ CD11c+ cells. This observation suggested a potential induction of monocyte differentiation into dendritic cells. To confirm this, we analyzed the differentiation of isolated CD14+ monocytes into dendritic cells induced by GM-CSF and IL-4 in the presence or absence of UDP. We found that the addition of UDP significantly increased the number of differentiated immature dendritic cells compared to GM-CSF and IL-4 alone. Moreover, we treated the immature dendritic cells with a cytokine pool (IL-6, IL-1β, Poly I:C and TNF-α) in presence or absence of UDP, to induce their maturation. We observed an increase in the percentage not only of HLA-DR+ CD11c+ cells but also of CD80+ CD86+ mature dendritic cells in presence of UDP, compared to the treatment with the cytokine pool alone. Next, we examined whether UDP could influence the phagocytic activity of PBMCs. Interestingly, UDP treatment stimulated both the phagocytosis of E. coli bacteria cells and the phagocytosis of necrotic cells (efferocytosis). Additionally, we analyzed the kinetics of gene expression induced by UDP treatment in PBMCs, revealing eight distinct clusters of genes activated by UDP at different time points. Bioinformatics analysis of these gene clusters indicated the involvement of different transcriptional factors, with NF-κB, IRF3, and BATF being the primary factors regulating the expression of genes within the clusters. Using ELISA EMSA, we confirmed an increased activity of NF-κB subunits p65, p50, RelB, and p52. Western blot analysis showed elevated phosphorylation levels of ERK, STAT1, and STAT3. In conclusion, our findings highlight UDP/P2YR6 pathway as a critical regulator of immune cell differentiation and function. By enhancing dendritic cell differentiation and phagocytic activity, UDP/P2YR6 pathway may play a vital role in shaping the immune response within the tumor microenvironment. Further investigation into UDP/P2YR6 signaling pathways could provide valuable insights into novel therapeutic strategies for cancer immunotherapy.

SOX2 PROMOTES A CANCER STEM CELL-LIKE PHENOTYPE AND LOCAL SPREADING IN ORAL SQUAMOUS CELL CARCINOMA

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Oral squamous cell carcinoma (OSCC) is a commonly occurring head and neck cancer with poor prognosis. Surgical resection is the best treatment option; however, the existence of a possible small subpopulation of residual cancer cells and cancer stem cells (CSCs) in the OSCC resection margins is a primary cause of local recurrence. Here, we analyzed the gene expression of different CSC markers in three tissue samples (tumor core (T), tumor-free close margin (CM) and adjacent health distant margin (DM)) collected from 24 OSCC patients. qRT-PCR analysis showed that *SOX2*, *BMI1*, *UBE2C*, *OCT4*, *CXCR4*, and *CD44* were significantly upregulated in T *vs* DM while *KLF4*, *FAM3C*, *ALDH1A1*, *CD133*, and *IGF-1R* were significantly downregulated. In both T and CM samples the expression levels of *SOX2*, *CXCR4*, and *CD44* were much higher than those analyzed in DM samples, and clinicopathological analysis revealed a correlation between high *SOX2* levels in T samples and a higher TNM stage. Hence, analyzed the functional role of SOX2 by transiently knocking down its expression in CAL27 and SCC154 cell lines. Notably, we found that *SOX2* down regulation reduced migration in both cell lines, measured by would healing time lapse assay. *SOX2* silencing also decreased the sphere-forming ability of CAL27 and SCC154 cells, as shown by the reduction in number and size of 3D spheroids, measured by Leica Thunder microscope. This was accompanied to the significant downregulation of the CSCs markers. Furthermore, *SOX2* silencing enhanced the sensitivity of CAL27 treated with growing concentration of cisplatin as shown by propidium-iodide/annexinV cytofluorimetric assay. Overall, these results identify SOX2 as useful CSCs marker to predict local spreading and, thus, a suitable therapeutic target to prevent OSCC dissemination.

MITOCHONDRIA AT THE CROSSROADS OF METABOLIC ADAPTATION AND BIOENERGETIC FUNCTION IN CELL STRESS SIGNALLING

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Mitochondrial biology is evolving towards an holistic picture reflecting the multifaceted and multifunctional role of these organelles in a variety of cellular processes. With this perspective, we are studying the involvement of mitochondria in the response to osmostress by using *Saccharomyces cerevisiae* yeast cells as a model. Our attention has been focused on the mitochondrial retrograde signalling, named RTG, which regulates mitochondria-to-nucleus communication in response to mitochondrial dysfunction. We have demonstrated that RTG pathway is activated by treatment with sodium chloride-induced osmotic stress and controls TCA cycle reactions through peroxisomes-mitochondria cross talk. In a late phase of cell response, RTG pathway sustains mitochondrial respiratory capacity in cooperation with *HAP4*, encoding the catalytic subunit of the Hap complex, a master regulator of mitochondrial function. Our data indicate that mitochondrial metabolic, rather than respiratory/bioenergetic function, is essential for osmoadaptation, confirming that metabolic rewiring is part of the cell stress response enabling adaptation and survival. At this regard, we found that the absence of mitochondrial DNA confers an advantage in the kinetics of stress response, by increasing the glycolytic flux and glycerol level. Different regulation of RTG pathway has been found in the absence of *RIM2*, encoding a mitochondrial pyrimidine nucleotide transporter, suggesting a putative role for this protein in cytoprotection. Interestingly, preliminary results identify citrate levels as potential regulators of the RTG-mediated adaptive process. Overall our data point to a dynamic osmostress response in which mitochondria act as signalling hubs for the transduction of metabolic signatures and the maintenance of cell homeostasis.

THE ROLE OF ENDOCYTOSIS AND ORGANELLE COMMUNICATION IN REGULATING CELL RESPONSE TO NANOMATERIALS.

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Endocytosis is a complex process that allows cells to transport particles and molecules across the plasma membrane and has a great influence on the regulation of several cell functions both in physiological and pathological conditions. With the advent of nanomedicine, many nanomaterials (NMs), with size ranging from 1 to 100 nm, have been developed to improve the delivery of diagnostic and therapeutic agents to target tissues overcoming physiological barriers. Due to their small size, NMs interact with cell membranes and utilize endocytosis and trafficking routes to enter the cells and exert their function. In my lab, research interests focus on elucidating the molecular mechanisms of endocytosis, intracellular trafficking and organelle communication in cells exposed to NMs. We demonstrated that the cells use several endocytic pathways to internalize NMs, depending on NM physical-chemical properties and the extracellular microenvironment characteristics, and, hence, NMs accumulate into endo-lysosomal compartment mainly. The endo-lysosomal localization strongly reduces NM extracellular release and, in some cases, increases NM cytotoxicity. We recently reported that, despite their confinement to endo-lysosomes, NMs exert their effects on organelle activities by the formation of organelle membrane contact sites (MCSs). In fact, we demonstrated that platinum nanoparticles (PtNPs) can contrast the unbalance of mitochondrial dynamics and increase the expression of SOD2 mitochondrial enzyme that recovers cells from the oxidative stress induced by an external insult. In cells treated with PtNPs, mitochondria form MCSs with endo-lysosomes containing NPs and rough endoplasmic reticulum. These findings help to shed light on identifying causes of possible NM adverse effects and to exploit cellular mechanisms to improve NM biomedical application.

EFFECT OF IRISIN ON uPA/uPAR SYSTEM IN IN VITRO MODELS OF METASTATIC MELANOMA CELLS

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Melanoma is an aggressive type of tumour that mainly occurs on the skin, with poor prognosis for patients with metastatic disease. Several proteins with proteolytic activity mediate the interaction between melanoma cells and the tumor microenvironment. In particular uPA (urokinase-type plasminogen activator) and its receptor uPAR and gelatinase (MMP-2 and MMP-9) orchestrate melanoma spreading towards the surrounding extracellular matrix (ECM) until the formation of distant metastases. Irisin is a newly discovered 12kDa messenger protein, as part of the fibronectin type III domain containing 5 (FNDC5), involved in energy metabolism and musculo-skeletal homeostasis. Recent studies also showed that irisin reduced the invasion capability of several types of cancer cells, however the effect of irisin on melanoma cells has not been described yet. We treated four metastatic melanoma cell lines with 10nM r-irisin, corresponding to the dose of r-irisin reported to exhibit biological activity *in vitro*. Chemoinvasion-assay showed that r-irisin reduced the metastatic potential of $HBL^{wt/wt}$ and $LND1^{wt/wt}$ cells ($p<0.05$), but didn't affect the invasion of $BRAF^{mut}$ cells ($Hmel1^{V600K/wt}$ and $M3^{V600E/V600E}$). Gelatin zymography analysis showed a reduction of MMP-2 and MMP-9 enzymatic activity in $BRAF^{wt/wt}$ cells compared to untreated cells. Moreover, gene expression analysis (qPCR) of MMP-2 and MMP-9 and of the fibrinolytic system (uPAR, uPA and PAI-1) highlighted a significant reduction of pro-invasive systems ($p<0.01$) in $HBL^{wt/wt}$ and $LND1^{wt/wt}$ cells treated with 10nM irisin compared to untreated cells. In conclusion, our results highlighted that irisin impaired the pro-invasive systems of $BRAF^{wt}$ melanoma cells rather than $BRAF^{mut}$, suggesting a possible role of irisin in reducing $BRAF^{wt}$ melanoma cells invasion potential.

NORMALIZING THE EXPRESSION OF HSA21 GENES IN TRISOMIC CELLS MAY ELUCIDATE THEIR ROLE IN NEURONAL CELL DIFFERENTIATION

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Down syndrome (DS), the leading cause of intellectual disability, is the consequence of alterations that occur during neuronal cell differentiation. DS is due to the presence of 3 copies of Hsa21 genes, which are globally 1.5-fold overexpressed, due to a gene dosage effect. Normalization of individual overexpressed Hsa21 genes could help clarify which of them is responsible for specific phenotypic alterations. So far, we modulated, in trisomic fibroblasts, the expression level of 3 Hsa21 genes, namely *NRIP1*, *RUNX1* and *SYNJ1*, which have been shown to be responsible for mitochondrial, extracellular matrix and endosomal alterations, respectively.

To assess which of the protein-coding Hsa21 genes is responsible for neuronal alterations, we identified candidate Hsa21 genes likely to cause significant dysregulation and ranked them according to parameters such as overexpression in neurons and haploinsufficiency. We then established a model of trisomic iPSCs and found that, at an early stage of neuronal differentiation, they manifest defects in mitochondrial function, number and length of neurites, number of synapsin-positive puncta, and neuron/glia ratio, for which we developed rapid assays. Using this model, we are evaluating the phenotypic consequences of normalizing the expression of single candidate genes on neuronal differentiation of trisomic iPSCs. We expressed an inducible Cas9 in iPSCs and selected clones in which gRNAs related to single genes can be expressed. After activation of Cas9, clones in which only one of the three alleles of the candidate gene is inactivated are selected and differentiated into neurons. The transcripts and phenotype of these neurons are compared with those of non-silenced controls.

These experiments are promising for the identification of Hsa21 genes involved in neuronal cell differentiation and possibly new therapeutic targets for DS.

ORGANOIDS: A REVOLUTIONARY TOOL IN STEM CELL RESEARCH

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2D cell cultures are widely used in research but lack of complexity and structure compared to living tissues limiting their ability to accurately replicate biological processes in vivo. However, recent advances in organoid technology have addressed these limitations offering a revolutionizing tool for in vitro research. 3D cell culture systems show great potential to bridge the gap between cellular and animal models in studies of infectious diseases, genetic disorders, cancer, drug development, therapeutics, and tissue and organ repair and replacement. A new source of mesenchymal stem cells called dental pulp-derived stem cells (DPSCs) has attracted the scientific community. DPSCs play a vital role in maintaining the balance of dentin within the teeth. Moreover, recent research indicates that DPSCs have the ability to transform into various cells types beyond the mesodermal lineage, such as neurons, epithelial and endothelial cells. This study aims to establish a protocol for generating organoids from DPSCs and compare their differentiation ability in 2D and 3D cultures. Initially, DPSCs in 2D were induced to undergo odontogenic differentiation. Alizarin red staining revealed the presence of calcium phosphate deposits, typical of odontoblasts. Gene expression analysis through RT-PCR showed increased expression of differentiation specific genes and decreased stemness genes, trends confirmed by immunofluorescence analysis. Similarly, in 3D cultures, following odontogenic differentiation, immunofluorescence analysis demonstrated increased differentiation markers and decreased stemness markers. Analysis of Ki-67 expression showed a decline in proliferation over time, possibly due to increased cell mortality and spontaneous differentiation. While RT-PCR protocols on organoids are currently in progress, our 3D model shows promise for future translational studies.

EXPLOITING CALVARIAL-DERIVED MESENCHYMAL STROMAL CELLS FOR CRANIOFACIAL DISEASE MODELING: TARGETING ORGANELLES AND GENES TO RELIEVE SKULL CONTRAINT

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Skull bones and sutures house calvarial mesenchymal stromal cells (CMSCs) contributing to perinatal skull morphogenesis and growth. The disruption of homeostasis in the calvarial stem cell niche, due to genetic (e.g. *FGFR2* mutations) and environmental factors, results in accelerated osteogenic differentiation of CMSCs leading to premature suture ossification (craniosynostosis, CS). This study aims to harness CMSCs for modelling and study CS pathophysiology, as a unique platform to test innovative treatment strategies.

We isolated CMSCs from surgical waste of CS patients and tested alternative strategies to modulate osteogenic differentiation and promote CMSC maintenance. Polymeric nanoparticles (NPs) nanoprinted into a hydrogel were used to deliver siRNAs targeting *FGFR2*. We also explored the effect of human amniotic mesenchymal stromal cell-derived conditioned medium (hAMSC-CM) in modulating the proliferation and osteogenic differentiation of CMSCs. Furthermore, we investigated the impact of primary cilium-mediated mechanotransduction in CMSCs to address the role of physical triggers and a way to balance their effects in CS pathophysiology.

NPs showed efficient intracellular uptake; siRNAs yielded a significant inhibition of *FGFR2* and downstream signalling. The nanoink allowed controlled sustained release of NPs supporting cell growth without toxicity and efficient gene knockdown. hAMSC-CM preserved the undifferentiated phenotype, promoted proliferation, and reduced osteogenic differentiation of CMSCs. The expression of primary cilium-associated stretch-induced ion channels was modulated by the mechanical stimulation resulting from matrix deposition during osteogenic differentiation. This comprehensive approach signifies a step forward in developing targeted therapies to modulate aberrant osteogenic signalling and correct mechanobiology due to skull constraint in CS.

2D AND 3D *IN VITRO* MODELS OF CELLULAR SENESCENCE: THE ROLE OF P300

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Cellular senescence and tissue aging have increasingly become attractive in biomedical sciences, mainly for their possible role in the pathogenesis of neurodegenerative disorders. Cellular senescence is a survival program characterized by an irreversible arrest of the cell cycle and a peculiar metabolic activity able to reinforce and spread senescence thus modifying and influencing the surrounding microenvironment. The idea of an active contribution of senescent cells in the pathogenesis of neurodegeneration and in its maintenance opens the possibility to develop new targeted drug strategies that could prevent, stabilize or reverse the progression of neurodegenerative diseases. Recently, it was reported that p300, a lysine acetyl transferase, could represent a primary driver of the replicative senescent phenotype and, interestingly, its depletion was sufficient to downregulate senescence related genes. On these bases, we hypothesized that p300, driving senescence, could have a fundamental role in aging-related diseases. To address this issue, we used a cellular model of *EP300* genetic haploinsufficiency to validate the role of p300 also in stress-induced premature senescence (SIPS). Then, we investigated through 2D and 3D *in vitro* models whether p300 chemical inhibition could rescue DNA damage and the senescent phenotype caused by stressor agents. Specifically, leveraging fibroblast, healthy donor iPSC-derived iNeuron, and brain organoid models we were able to recapitulate cellular senescent hallmarks highlighting both molecular mechanisms underlying senescence and p300 as possible pharmaceutical target. These findings could improve the knowledge on pathological mechanisms and therapies development that are still challenging for neurodegenerative disease.

SINEUPS NON-CODING RNAS TARGETING PRPF31 AS A THERAPEUTIC STRATEGY FOR RETINITIS PIGMENTOSA 11

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Autosomal dominant retinitis pigmentosa type 11 (RP11) is a retinal disease caused by PRPF31 null mutations characterized by an initial degeneration of both rods and retinal pigment epithelium (RPE), followed by the degeneration of cones. PRPF31 protein is ubiquitously expressed and codifies for one of the main components of the spliceosome. Alterations in this protein are only associated with retinal degeneration, although the relationship with the development and maintenance of the retina is still unclear. To develop a novel therapeutic approach, we exploited SINEUPs, natural antisense long non-coding RNAs able to enhance protein translation of specific partially overlapping mRNAs. Five different human miniSINEUPs targeting PRPF31 mRNA were designed, cloned into a pAAV vector and tested in h-TERT-RPE-PRPF31 +/- cells (haploinsufficient for PRPF31). Our results showed that two SINEUP-PRPF31 significantly promote the production of PRPF31protein at post-transcriptional level in h-TERT-RPE-PRPF31 +/- cells, without any increase of PRPF31 mRNA. Furthermore, through confocal microscopy, it was possible to confirm the ability of the two active SINEUP-PRPF31 to rescue the cilia length in heterozygous RPE cells, being ciliogenesis a pathological hallmark of RP11. Ongoing studies are focusing on the characterization of iPSC derived from both RP11 and asymptomatic patients and their differentiation into primary RPE cells.

ALPHA-SYNUCLEIN IMPAIRS MACROPHAGE AUTOPHAGY IN ASSOCIATION WITH HYPER-INFLAMMATION, LIPID DYSHOMEOSTASIS, AND DEFECTIVE PHAGOCYTOSIS.

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Spreading of excess alpha-synuclein (aS), a hallmark of Parkinson's disease, is shown to promote peripheral inflammation via still unclear intracellular mechanisms. Autophagy plays a functional role in monocytes/macrophages, where it was shown to govern aS proteostasis. However, if aS affects autophagy in these cells specifically remains poorly explored. Here we investigate the subcellular, molecular, and functional effects of aS in human monocytes and macrophages with a focus on autophagy. Human THP-1 monocytic cells (TMs), derived macrophages (TDMs), and primary monocyte-derived macrophages (MDMs) were cultured w/wo recombinant aS (1uM) for 4 and 24h. By Confocal microscopy, Western Blot, qRT-PCR, and Elisa we assessed: i) aS internalization; ii) inflammatory profile; iii) autophagy (LC3II/I, LAMP1/LysoTracker, p62, pS6/S6 ratio); iv) red-oil stained lipid droplets (LDs); v) phagocytic capacity, and the fate of phagocytosed cargo. Extracellular aS was internalized by TMs, MDMs and TDMs, where it induced intracellular accumulation and release of pro-inflammatory mediators. In TDMs, this was accompanied by mild toxicity, increased p62 protein levels, decreased LC3II/I ratio, and decreased LAMP1 at both protein and mRNA levels. This was independent of the mTOR activity index pS6/S6, but was associated with F-actin clumping, and reduction of intracellular LDs, and their co-localization with LC3 and LAMP1. Finally, aS reduced both phagocytosis, and the clearance of phagocytosed cargo, which markedly filled LysoTracker-stained organelles resulting in a co-localization pattern reminiscent of engulfed/stagnant lysosomes. Our results suggest that while monocytic cells well-tolerate excess aS, macrophages undergo an exhaustion status resembling hypophagia, with autophagy-lysosome impairment potentially bridging hyper-inflammation, cell toxicity, and lipid dyshomeostasis.

SIGNAL PEPTIDE MUTATION AND LOSS OF EXPRESSION OF APOB AS INDICATOR OF POOR PROGNOSIS IN K19^{+} HEPATOCELLULAR CARCINOMAS

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The expression of the stemness marker cytokeratin-19 (K19) in hepatocellular carcinomas (HCCs) is associated with aggressive tumour behavior and poor outcome.
The objective of our work was to investigate the mutational profile of 43 HCCs, classified into 22 K19^{+} and 21 K19^{-} by Next Generation Sequencing.
We identified a non-frameshift deletion in the signal peptide of *APOB* as the most frequent mutation in K19^{+} tumours. *APOB* encodes for a key player in lipid metabolism also exploited by HBV and HCV. To investigate the impact of the mutation on the conformation of the signal peptide, we conducted molecular docking experiments that suggest an impaired interaction between the mutated peptide and the signal recognition particle. We also evaluated apoB expression and survival rates in the HCCs analyzed by NGS and in 323 additional cases. Loss of apoB expression was observed in most mutated tumours, as well as in the majority of K19^{+} HCCs. Both apoB mutation and deficiency were indicators of poor survival, with the worst-case scenario characterizing apoB^{-} and K19^{+} patients.
In conclusion, our study identified a mutation that potentially affects the mechanism of apoB synthesis providing further support for the clinical significance of apoB loss in HCC and revealing an association with the aggressive K19^{+} tumors.

DYSREGULATION OF EXTRACELLULAR MATRIX AFFECTS INTESTINAL TYPE GASTRIC CANCER PROGRESSION BY INITIATING EPITHELIAL-TO-MESENCHYMAL TRANSITION

Viviana Longo[2], Sabino Russi[1], Simona Laurino[1], Pellegrino Mazzone[3], Giuseppina Di Paola[3], Pietro Zoppoli[4], Elena Amendola[2], Chiara Balzamo[2], Ottavia Bartolo[6], Mario Ciuffi[6], Orazio Ignomirelli[6], Alessandro Sgambato[7,8], Rocco Galasso[8], Mario De Felice[4,5], Giovanni Calice[1], Francesco Albano[1,2,3 ‡], Geppino Falco[2,3‡] .

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Gastric Cancer (GC) is an aggressive and heterogeneous malignancy, with cancer cells that start to invade through the stomach (Stage I) until they metastasize to distant organs (Stage IV) conducing to bad prognosis. Identifying factors involved in this stepwise process is crucial. We compared gene expression profiles of Stage IV versus Stage I patients to isolate molecular players regulating the metastatic process. Our workspace of gene expression profiling data supplemented with clinical information, was composed of 719 GC samples generated by harmonizing seven different public datasets. We performed functional enrichment analysis, identifying a gene signature (APOD, COL1A2, FSTL1, GEM, LUM, SPARC) characterizing the stage IV of iGC which was strongly related to extracellular matrix (ECM) organization and Epithelial-to-Mesenchymal transition (EMT). We generated a novel *in vitro* model to characterize the signalling of the metastatic cells. Our novel molecular signature includes soluble factors capable of defining a metastasis-prone cellular microenvironment promoting iGC cells invasion through the ECM by triggering EMT. Overall, we identified a gene signature that, if upregulated, can influences cell signalling, inducing the activation of the Epithelial-to-mesenchymal transition. Our findings could be translated from bench to bedside during the management of gastric cancer patients, addressing them to a closest follow-up and/or alternative therapeutic strategy to restrain disease progression. Furthermore, our experimental model could be useful for the precise dissection of the molecular signalling which underlies the metastatic spread of cancer cells.

POSTER
SECONDA SESSIONE

Venerdì 20 Settembre
16:00 – 17:00

IDENTIFICATION OF STEM CELL-SPECIFIC MARKERS FOR THE MOLECULAR CHARACTERIZATION OF HUMAN ADULT GASTRIC STEM CELLS (hAGSCs)

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BACKGROUND: Human adult gastric stem cells (hAGSCs), due to their self-renewal capability and multipotency, play a significant role in stomach tissue regeneration and aging. However, hAGSC cellular homeostasis in gastric tissue physiopathology is poorly understood because the knowledge of their identity is still limited.
GOAL: The purpose of this work was to explore the biological dynamics of Neck, Chief, and Isthmus stem cells within a physiological context.
AIMS: To define the hAGSC cytotype-specific biomarkers, we performed bioinformatic data mining on open-access human gastric RNAseq single-cell databases and The Human Protein Atlas portal. Furthermore, to standardize a reliable source of hAGSCs to characterize their molecular features, we generated organoids with bio-marker promoter sequences upstream of the eGFP target gene.
RESULTS: We identified AQP5, MUC6, and A4GNT for the isolation of Neck cells; PGC and 3 novel biomarkers (TMEM97, TMED6, and ELAPOR1) for the isolation of Chief cells; TK1 and EDEM3 as novel candidates for the isolation of Isthmus cells. We standardized an ex vivo organoid line that resembles the in vivo expression of these biomarkers. Finally, we isolated the PGC^+ and $A4GNT^+$ populations, making them accessible for further molecular characterization.
In summary, we defined a methodology for the isolation of gastric epithelial cells and, for the first time, purified enriched populations of PGC and A4GNT positive cells using organoids. These results could help follow the hAGSC during homeostasis of the gastric mucosa.

UNVEILING MOLECULAR PATHWAYS: BIOSENSING ENDOCRINE DISRUPTING CHEMICALS IN THE FEMALE REPRODUCTIVE SYSTEM, SPOTLIGHTING OVARIAN DYNAMICS

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Endocrine-disrupting chemicals (EDCs) interfere with the endocrine system, inevitably affecting the homeostasis of the reproductive system. Among these are isoflavones like Biochanin A (BCA), predominantly found in red clover and known for its anti-inflammatory and estrogen-like effects. This study investigated the complex mechanisms, underlying BCA's effects on transcription, metabolism, and hormone regulation in primary Granulosa Cells (GCs), with a specific focus on its activation of TAS2Rs in the female reproductive system. Our findings suggest that BCA directly influences the TAS2Rs pathway, as evidenced by changes in the expression of TAS2R14 and TAS2R43 following treatment with 10μM BCA. Moreover, we demonstrated that this effect can be blocked by selective antagonists. We also provided evidence that BCA stimulated a 70% increase in STAR mRNA levels ($p<0.05$), a rate-limiting regulator of steroid production. Additionally, CYP11A1, responsible for progesterone production, doubled expression with BCA treatment. Also in this case, antagonist co-treatment significantly reduced STAR and CYP11 expression ($p<0.05$). Interestingly, the exposure of GC to BCA induced a significant reduction in the intracellular Ca2+ content ($p<0.01$), consistent with the reported ability of TAS2Rs to induce PDE activation. We further reported a significant reduction in the size of lipid droplets in GCs treated with BCA ($p<0.001$), while the co-treatment with antagonists reverted this phenomenon. Finally, we demonstrated the BCA allows a rise in mitochondrial network complexity ($p<0.001$), that is blocked by the administration of antagonists. This study suggests bitter taste receptors' involvement in steroidogenesis and underscores the need for further research, alongside government, industry, and social efforts to mitigate EDC and environmental toxicant impacts on human fertility and fecundity.

ANTI-INFLAMMATORY ROLE OF LEMON JUICE-DERIVED EXTRACELLULAR VESICLES IN ENDOTHELIAL CELLS DERIVED FROM THE UMBILICAL CORD OF GESTATIONAL DIABETIC WOMEN.

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Extracellular vesicles (EVs) derived from plant cells (PD-EVs) are small lipoproteins structures that have recently gained increasing attention for their biological properties through the release of plant-derived bioactive compounds. In this context, several *in vitro* and *in vivo* studies have demonstrated the anti-inflammatory and antioxidant role of PD-EVs isolated from *Citrus limon L. juice* (L-EVs). However, the effects of L-EVs on vascular function have never been explored. Therefore, the aim of the study was to investigate their potential anti-inflammatory role in endothelial cells isolated from umbilical cords of women with gestational diabetes (GD-HUVECs) as a useful model for reproducing the pro-inflammatory phenotype of diabetic endothelium *in vitro*. HUVECs derived from healthy women (C-HUVECs) were employed as the control condition. In detail, GD- and C-HUVECs were pre-treated for 24 h with two different concentrations of L-EVs (10 and 25 µg/mL) and then were stimulated for 16 h with the pro-inflammatory stimulus TNF-α (10 ng/mL).
Results from confocal microscopy experiments initially demonstrated the ability of C- and GD-HUVECs to internalize L-EVs after a 4 h incubation. Subsequently, an anti-inflammatory effect was observed following the pre-treatment with 25 µg/mL of L-EVs in TNFα-stimulated GD-HUVECs, as revealed by the reduced expression and membrane exposure of the vascular adhesion molecule-1 (VCAM-1) (flow cytometry and IncuCyte® imaging system). The results were confirmed by the significant decrease in monocyte adhesion to HUVECs (monocytes-HUVECs adhesion test). Overall, these results demonstrated that L-EVs also exert an anti-inflammatory role in endothelial cells, paving the way for further studies investigating their potential use in preventing endothelial dysfunction associated with gestational diabetes.

SARS-CoV-2 SPIKE PROTEIN S1 INDUCES MG-H1/RAGE ACTIVATION TO SUSTAIN INFLAMMATION IN HUMAN BRONCHIAL BEAS-2B CELLS

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COVID-19 pathogenesis is associated with a hyperinflammatory response. The mechanisms of SARS-CoV-2-induced inflammation are scantly known. Methylglyoxal (MG) is a potent glycating agent, leading to the formation of the proinflammatory advanced glycation end product (AGE), MG-H1, binding to the receptor RAGE. Here, we investigated the involvement of the MG-H1/RAGE axis as a potential novel mechanism in SARS-CoV-2-induced inflammation by resorting to human bronchial BEAS-2B and alveolar A549 epithelial cells, expressing different levels of the ACE2 receptor (R), exposed to SARS-CoV-2 spike protein 1 (S1). Interestingly, we found in BEAS-2B cells that do not express ACE2-R that S1 exerted a pro-inflammatory action through a novel MG-H1/RAGE-based pathway. MG-H1 levels, RAGE and IL-1β expression levels in nasopharyngeal swabs from SARS-CoV-2-positive and -negative individuals, as well as glyoxalase 1 expression, the major scavenging enzyme of MG, seem to support the results obtained in vitro. Altogether, our findings pave the way for the study of the MG-H1/RAGE inflammatory axis in SARS-CoV-2 infection as a potential therapeutic target to mitigate COVID-19-associated pathogenic inflammation.

PROGESTERONE RECEPTOR IS CONSTITUTIVELY EXPRESSED IN INDUCED PLURIPOTENT STEM CELLS (IPSCs)

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Induced Pluripotent Stem Cells (iPSCs) are nowadays a common starting point for wide-ranging applications including 3D disease modeling, drug development and regenerative medicine. Physiological processes like homeostasis, cell differentiation and development are tightly regulated by hormones through binding to their receptors of target cells. Considering their pleiotropic effects, it is important to understand their role also during cell differentiation, in particular for those *in vitro* disease models which include steroid hormone cellular response. We explored the expression pattern of estrogen receptor (ERα) and progesterone receptor (PR) in four different iPSCs, obtained from CD34+ progenitor cells and skin fibroblasts with four different methods (episomal vector, Sendai-, Retro- and Lenti-virus). Expression of ERα and PR mRNA were significantly reduced in iPSCs compared to MCF7 breast cancer cells positive control ($p<0.0001$). Surprisingly, immunofluorescence staining detected only the expression of PR protein in all the different iPSCs cell lines, while ERα was not detectable. These results suggested that active translation occurred, therefore to determine the moment in which PR protein expression arose in iPSCs, we extended the analysis to precursor cells. By flow cytometry analysis we observed that the ~65% of the total population of iPSCs cells expressed only PR, with 100% fold increase compared to hematopoietic stem/progenitor cell (HSPCs) and fibroblasts ($p<0.0001$), while ERα was not expressed. Our results collectively highlighted for the first time that the reprogramming of somatic cells into iPSCs leads to the expression of PR receptor. These finding would improve future research in iPSCs cell-based disease modelling, being a starting point to better comprehend the molecular mechanisms involved in development and cellular response to treatments.

IκBα-MEDIATED ENDOTHELIAL ACTIVATION FACILITATES LUNG CANCER METASTASIS.

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The inhibitor IκBα, a regulator of the p65-NF-κB signaling pathway, plays a pivotal role in cancer metastasis. Our previous research has contributed to the identification of IκBα/p65 within mitochondria, indicating its potential role in modulating this cellular compartment. To delve deeper into the role of IκBα, we engineered a construct specifically targeting it to the mitochondria (MTS-IκBα) of cancer cells. Intriguingly, lung cancer cells expressing MTS-IκBα increased their aggressiveness, as measured by their capability to proliferate more than controls, reducing apoptotic response and inducing migration. Additionally, MTS-IκBα led lung cancer cells to activate endothelial cells (ECs), promoting an inflammatory and adhesive state, ultimately priming ECs to facilitate metastasis. This mechanism may be attributed to the metabolic shift observed in lung cancer cells with MTS-IκBα compared to those with wild-type IκBα, related to the induction of mitochondrial dysfunction and an acidic tumor microenvironment. This environment affects ECs conducive to cancer-associated thrombosis, characterized by a hypercoagulable state resulting from the release of procoagulant factors such us vWF, platelet activation, and aggregation, thereby facilitating cancer cell extravasation and metastasis.

POSSIBLE ROLE OF BAG3 IN FIBROGENESIS

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Following acute or chronic injury, fibrosis is involved in the healing processes required to complete the tissue repair. However, when dysregulation of the physiological process occurs, activated fibroblasts produce an excess of extracellular matrix (ECM) components, as collagen and fibronectin, that are accumulated, interfering with tissue architecture features and functions.
BAG3, a member of the BAG co-chaperone family, is a multifunctional protein with a potentially pivotal role in fibrosis outset and sustainment. BAG3 is involved in the regulation of apoptosis, autophagy, and cytoskeleton organization. Furthermore, a secreted form of BAG3 in PDAC is responsible for the generation of a tumor-favoring microenvironment by the activation, through its IFITM2 receptor, of tumor-associated macrophages (TAMs) and fibroblasts (CAFs).
To investigate a possible direct modulation of BAG3 on ECM deposition in fibrosis, in this work, we analyzed BAG3, collagen and fibronectin levels in fibroblast cell lines in response to the pro-fibrogenic cytokine TGF-β1. We observed that BAG3 is released in response to TGF-β1 and collagen in the total and the extracellular fraction associated with BAG3, in a stimulus-dependent manner.
Accordingly, BAG3 silencing caused collagen production impairment, even when fibroblasts were stimulated by TGF-β1. Fibronectin synthesis was instead not affected.
Main results evidence that BAG3 has an essential role in collagen fibers production and extracellular deposition, playing a key role in the fibrogenic process.
The dissection of the molecular mechanisms involved in fibrosis and the contribution of BAG3 to this process may help the emergence of innovative therapeutic approaches.

GENETIC AND MOLECULAR RESPONSE TO QUANTUM DOTS IN *SACCHAROMYCES CEREVISIAE*

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Quantum dots are colloidal semiconductor nanocrystals with peculiar optical properties, widely studied for biomedical applications. Zinc sulphide quantum dots (ZnS QDs) are commonly utilized as shell material in core-shell QD structures, being considered more biocompatible compared to other metal-based QDs. However, limited research has been conducted regarding their effects as ZnS QDs core-only. Here, the yeast *Saccharomyces cerevisiae* was used as a model organism to investigate the genetic and molecular response associated with exposure to this type of QD. Following preliminary analyses on ZnS QD characterization and effects on wild-type growth, a YKO (Yeast Knock-Out) collection, comprising haploid mutants deleted in non-essential genes, was screened in the presence of ZnS QDs. The identification of sensitive mutants deleted in genes encoding for proteins related to mitochondrial functions prompted further investigation into the phenotype of the medium-sensitive mutants *sod1Δ* and *glr1Δ* and the hypersensitive mutant *pos5Δ*. Interestingly, the hypersensitivity exhibited by *pos5Δ,* deleted for a mitochondrial NADH kinase, was specific for ZnS QDs and not for other tested ENMs nor zinc sulphate ($ZnSO_4$). Flow cytometry analyses conducted on the wild-type strain and the *pos5Δ* mutant did not reveal a detectable increase in reactive oxygen species following ZnS QD treatment. RNA-sequencing was performed to analyse the transcriptional changes induced by ZnS QD (or $ZnSO_4$) in the wild-type strain and *pos5Δ*. The analysis revealed that exposure to ZnS QDs selectively induced modulation of genes encoding proteins involved in mitochondrial processes, metal binding, and intracellular trafficking. *Saccharomyces cerevisiae* has proved to be a valuable model organism for studying the intricate dynamics of ENM interaction within biological systems.

ALPHA-1 ANTITRYPSIN IS A PLAYER OF PLEURAL MESOTHELIAL CELL TRANSFORMATION

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Alpha1-antitrypsin (AAT) is a serum acute-phase protein encoded by the SERPINA1 gene. It is mainly produced by hepatocytes, and it is involved in anti-inflammatory processes. Altered expression of AAT in human cells can hamper proliferation and apoptosis, leading to transformation. In this study, the transforming activity of AAT was investigated in human pleural mesothelial cells (MeT-5A). AAT expression was first investigated in Met-5A and pleural mesothelioma (PM) cell lines of different histotypes, such as epithelioid, biphasic and sarcomatoid, by droplet digital PCR, showing mRNA to be lowest in MeT-5A and highest in the PM cell lines biphasic (MSTO-211H) and sarcomatoid (H2052). To assess AAT role "SERPINA1 gain and loss of function" experiments were performed to assay cell viability, proliferation and migration in normal and PM cell lines. Met-5A cells overexpressing SERPINA1 resulted in increased cell viability and proliferation compared to non-transfected cells. Conversely, SERPINA1-silenced PM cell lines MSTO-211H and H2052 showed a decrease in both cell viability and cell proliferation compared to non-transfected cells. Cell treatment with staurosporine, a proapoptotic agent, showed caspase 3/7 signaling activation in SERPINA1-silenced PM cell lines MSTO-211H and H2052 compared to non-transfected and to Met-5A cells overexpressing SERPINA1. Our preliminary data indicate that AAT may play a role in human mesothelial cell transformation, especially in the biphasic and sarcomatoid histotypes, thus representing a potentially novel transforming pathway to be investigated in PM.

INNOVATIVE METHODS FOR NUCLEIC ACIDS DELIVERY TOWARDS CORNEAL EPITHELIA REGENERATION

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Gene therapy approaches offer the opportunity to modulate expression of genes involved in tissue regeneration or diseases. Several corneal dystrophies such as Meesmann, Fuchs and Posterior Polymorphic, are affecting the two corneal epithelia, leading to corneal opacification and loss of vision. These conditions are generally treated through corneal transplantation that, although being the most frequent graft performed worldwide, it is associated to patient discomfort, scarce corneas availability and multiple surgical complications. Advanced therapies are emerging to avoid transplantation and overcome traditional gene delivery limitations such as poor efficiency (tear clearance), cell toxicity and viral associated safety issues. Technologies based on nucleofection, lipid based nanoparticles formulations and advanced materials allow efficient nucleic acids delivery into the cells, specific gene targeting with minimal cell toxicity. We have applied nucleofection and nanoparticles to deliver a CRISPR Cas9 ribonucleoprotein complex and specifically target the mutated gene causing Meesmann's corneal dystrophy on patient's primary corneal epithelial cells. Both these methods demonstrated their efficiency in cell internalization and their low toxicity. We have further demonstrated the first use of biodegradable porous silicon nanoneedles to overcome human corneal endothelial cellular proliferative arrest through RNA interference, while maintaining typical markers and cell viability. This pioneer strategy has been improved by fabricating nanoneedles over a clinical grade flexible membrane that perfectly adapts to the corneal curvature. These approaches represent a significant advance for safe and specific nucleic acids delivery to the corneal epithelia, guaranteeing high quality standards for a minimally invasive procedure to treat corneal diseases.

REGULATORY ACTIVITY OF THE TUMOR SUPPRESSOR GENE RAS ASSOCIATION DOMAIN FAMILY MEMBER 1 ISOFORM A (RASSF1A) ON THE MERKEL CELL CARCINOMA CELL PROLIFERATION AND APOPTOSIS

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Ras association domain family member 1 isoform A (RASSF1A) is a tumor suppressor gene, which plays a key role in multiple pathways such as cell proliferation and apoptosis. Although hypermethylation-induced loss of RASSF1A frequently occurs in cancer, the involvement of this gene in the malignant phenotype of Merkel cell carcinoma (MCC), a rare but highly aggressive skin neoplasm, is unclear. The purpose of this study was to investigate the functional role of RASSF1A in the MCC cell phenotype. RASSF1A transcript expression was evaluated in MCC cells MCC13, MCC26, WaGa and PeTa cells and in various epithelial and fibroblast control cells. RASSF1A mimic and inhibitor transfections were carried out to assess the involvement of RASSF1A in regulating MCC cell proliferation and apoptosis. MCC cell viability was investigated in GFP-transfected RASSF1A knock-out/-in WaGa and PeTa cells. To assess whether RASSF1A expression is under epigenetic regulation through promoter hypermethylation in MCC cells, gene transcript levels were assessed in cells treated with the hypomethylating agent guadecitabine and compared to corresponding untreated cells. RASSF1A tested downregulated in MCC13, MCC26, WaGa and PeTa cells compared to epithelial and fibroblast control cells. Functional experiments indicated that forced RASSF1A expression dramatically inhibited cell proliferation and favored apoptosis in MCC13, MCC26, WaGa and PeTa cells, while strongly reduced cell viability in WaGa-GFP and PeTa-GFP cells. The expression of RASSF1A transcript was restored in all tested MCC cells following treatments with guadecitabine, thus suggesting that this gene might be under epigenetic regulation in MCC. Overall, our data suggest a potential tumor suppressor activity for RASSF1A in MCC cells by regulating cell proliferation and apoptosis.

A NOVEL REGULATORY MECHANISM MODULATES GASTRIC CELL PLASTICITY THROUGH HUNK KINASE

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Adult organs retain architecture and function through a fine-tuned homeostatic mechanism mediated by multipotent stem cells ensuring tissue regeneration following homeostatic cell loss or tissue injuries. Metaplasia is a condition where chronic injury and inflammation cause aberrant cell differentiation altering the tissue's homeostatic state irrevocably and it is associated with an increased risk of cancer development. In the gastric environment, metaplasia is commonly induced by environmental factors such as chronic infection, hormonal stimulation and genetic alteration. Hormonally Upregulated Neu-associated Kinase (HUNK) is a serine-threonine kinase belonging to the AMP-activated protein kinase (AMPK) family. It has been found to regulate cell homeostasis in a context-dependent manner in various human cancers, including colon, ovarian, and breast cancers. Indeed, in breast cancer, HUNK expression is associated with disease progression and metastasis by activating the epithelial-mesenchymal transition (EMT). However, in colon cancer, HUNK expression suppresses the metastatic phenotype by inhibiting EMT. Here, we report that HUNK expression in gastric cells is induced upon inflammatory stimuli, leading to increased cell proliferation through direct binding and phosphorylation of p38 MAPK. Furthermore, we show that HUNK depletion results in decreased cell viability due to the activation of the apoptotic pathway. Moreover, RNAseq analysis demonstrated that HUNK induces a distinct pattern of Mucins, notably expressed in gastric metaplasia. Overall, these findings help to shed light on HUNK molecular mechanisms and suggest its potential as a novel biomarker associated with gastric cancer onset.

STEM CELLS OSTEOGENIC DIFFERENTATION AND CYTOKINE RELEASE INDUCED BY A COMPOSITE BIOMATERIAL

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Bone defects or atrophy may arise as a consequence of injury, inflammation, traumatic processes or as a result of surgical procedures. Regenerative medicine offer novel treatment modalities for such defects, including bone augmentation and repair procedures using osteoinductive scaffolds. The mechanisms by which bone marrow mesenchymal stem cells (BMMSCs) and inflammation interact during physiological and pathological processes to regulate bone turnover are still poorly understood. In the present study, the effects of a composite biomaterial (Geistlich Bio-Oss® Collagen), employed in maxillo-facial surgery, was evaluated using human bone marrow mesenchymal stem cells (BMSCs). The biomaterial is made up of 90% hydroxyapatite (granules) and 10% collagen of porcine origin. A qRT-PCR array was employed to evaluate the expression of genes (n=84) involved in MSCs osteogenic differentiation and osteogenic proteins, osteocalcin (OCN) and osteopontin (OPN) were evaluated by immunofluorescence. Cytokine/chemokine proteins released into BMSCs were analyzed using Bio-Plex Pro Human Cytokine 27-plex Assay. Several genes involved in BMSCs osteogenic signalling (i.e BMP1, COL1A1, TWIST1) and ECM remodelling (ITGA1/A2, MMP2) tested dysregulated in BMSCs grown on biomaterial and OPN and OCN proteins were expressed in BMSCs grown on material at day 7. In BMSCS was observe a significant decrease of pro-inflammatory interleukin (IL)-6 protein while anti-inflammatory/pro-angiogenic factor IL-4 and endothelial growth factor (VEGF) protein were up-regulated. Platelet-derived growth factor-BB (PDGF-BB), a key molecule for angiogenesis and osteogenesis coupling regulatory factor was increased in BMSCS by scaffold. Our data demonstrate that anti-inflammatory innate immune-mediated responses associated to stem cells differentiation are induced by the composite biomaterial investigated herein.

THE INTERPLAY BETWEEN KISSPEPTIN AND ENDOCANNABINOIDS IN THE CONTROL OF MALE REPRODUCTION

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The endocannabinoid system (ECS) and the kisspeptin system (KS) are two signaling systems in the central and peripheral control of reproduction. Since their possible interaction has been poorly investigated in mammals we analysed their possible reciprocal modulation in the control of the male reproduction.
Adolescent male rats were treated *in vivo* with Kisspeptin-10 (Kp10) or anandamide (AEA) ± rimonabant (SR141716A), the cannabinoid receptor 1 (CB1) antagonist. Morpho-functional evaluation of the KS and ECS was carried out in hypothalamus, testis and spermatozoa (SPZ), in parallel to puberty related miRNAs and circulating sex steroids and kisspeptin (Kiss1).
Circulating sex steroids and Kiss1 were not affected by Kp10 or AEA. In the hypothalamus, Kp10 significantly increased *GnRH* mRNA and aromatase Cyp19, Kiss1, and Kiss1 receptor (Kiss1R) proteins. By contrast, AEA treatment affected the hypothalamic KS at the protein levels, with opposite effects on the ligand and receptor, and *miR145-5p*. In the arcuate nucleus the number of Kiss1R+ neurons increased in Kp10- and AEA-treated animals with enlargement of the lateral ventricles in Kp10-treated animals.
In the testis, AEA affected the KS at the protein levels, whereas Kp10 affected the intragonadal levels of CB1 and FAAH, the main modulator of the AEA tone. Changes in puberty related miRNAs and the distribution of Kiss1, Kiss1R, CB1, and CB2 following KP and AEA treatment corroborate the KS-ECS crosstalk. Lastly, Kp10 and AEA got opposite effects on the acquisition of sperm motility in the epididymis. All the AEA dependent effects in brain, testis and SPZ were reversed by SR141716A, indicating CB1 mediated effects.
For the first time in mammals, we report the modulation of the KS in both the hypothalamus and testis by AEA and reveal the KP-dependent modulation of CB1 and FAAH in the testis. KP involvement in the progression of spermatogenesis and sperm motility is also suggested.

CIRCULAR RNAs AS NEW ACTORS IN MOLECULAR MECHANISM UNDERLYING AGE-RELATED DECLINE OF MALE FERTILITY

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A gradual decline of male fertility occurs during physiological ageing process. Spermatogonia and Sertoli cells represent the cell types most susceptible to age-related molecular anomalies underlying an impaired spermatogenesis.
Circular RNAs (circRNAs), covalently closed RNA molecules produced by backsplicing mechanism, are acquiring a key role in the regulation of spermatogenesis and sperm morpho-functional skills. The potential role of circRNAs in age-related male reproductive anomalies remains largely elusive.
Here, we analyzed the reproductive phenotype of the aged male mouse experimental model, pointing our attention on circRNAs as potential markers of sperm quality. Our results confirm several testicular age-related defects including: i) seminiferous epithelium anomalies; ii) spermatogenesis defects; iii) decreased sperm production. In particular, aged spermatozoa (SPZ) show decreased sperm motility and abnormal morphology (sperm head anomalies; sperm tail bents). Interestingly, the acrosome and IZUMO1 protein fertility markers appear highly expressed in sperm with abnormal head hook and, conversely, absent in normal sperm suggesting an enhanced fertilizing ability of poor-quality SPZ. Expression analysis of selective spermatic circRNAs demonstrate a de-regulated expression profile in aged- *vs* young-sperm.
Collectively, our study highlights new findings useful to better understand the role of circRNAs in molecular mechanism behind the age-related effect on male fertility.

UNVEILING THE CRUCIAL ROLE OF B-TRCP PROTEINS IN OSTEOSARCOMA: POTENTIAL THERAPEUTIC INSIGHTS FROM FBXW11 REGULATION

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The ubiquitination process is a pivotal mechanism that profoundly impacts cellular invasion, migration, resistance, and growth, thereby orchestrating tumor development. Central to these cellular processes are the β-TrCP proteins, encoded by FBXW, which function as key regulators within the ubiquitin–proteasome system. Numerous oncoproteins and tumor suppressors are substrates of β-TrCP1/2, and their dysregulation is a common occurrence in human cancers, underscoring their critical role in carcinogenesis.
Thus, we aimed to explore the role of FBXW11 in osteosarcoma by analyzing the levels of Axin, β-catenin, and Phospho-β-Catenin (Ser33/37/Thr41) in osteoblastic cells and osteosarcoma cells using 2D and 3D in vitro models.
Our preliminary results demonstrated that FBXW11 levels are reduced while Axin, Phospho-β-Catenin, and β-catenin levels are increased in tumoral cells compared to controls.
This study highlights the critical role of β-TrCP proteins in osteosarcoma and underscores the potential of ubiquitination studies in identifying novel therapeutic targets.

MICRORNA-27A-3P MODULATES THE CIRCADIAN MOLECULAR CLOCKWORK AND IMPACTS CANCER CELL PHENOTYPE IN HUMAN LUNG ADENOCARCINOMA

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Circadian rhythmicity of cell processes, such as cell cycle, autophagy, mitochondrial function, redox state, metabolism, and DNA damage response, are driven by molecular clockworks hard-wired by transcriptional-translational feedback loops (TTFLs). These loops govern the 24-hour periodicity of tissue, organ, and organ system functions, upholding cycles of approximately a day in mammalian physiology and behavior. TTFLs involve core circadian genes and proteins, with Clock and Bmal1 forming the positive limb and Per1-2 and Cry1-2 constituting the negative limb. Clock heterodimers drive the transcription of Per and Cry genes, whose proteins ultimately inhibit Clock activity. This dynamic, where activators prompt inhibitors that exert their role with a delay, maintains circadian rhythmicity. Disruption of this rhythmicity promotes lung carcinogenesis. MicroRNAs (miRNAs), especially the miR-27 family, modulate circadian gene expression post-transcriptionally. MiR-27a is implicated in cancer processes, like immune evasion and metabolic reprogramming. In animal models, MiR-27a targets Bmal1. Altered miRNA-mRNA interactions and disrupted clock gene expression contribute to carcinogenesis and affect cancer cell behavior and chemotherapy response. Our study aimed to elucidate the interaction between miR-27a and the circadian gene/protein Bmal1 in human lung adenocarcinoma. We used in vitro and in vivo models for transcriptomic, metabolic, and phenotypic characterization, defining the molecular mechanisms involved. Our findings reveal that the miR27-Bmal1 axis critically influences mitochondrial function, cell cycle regulation, metabolism, and the malignant phenotype of lung cancer cells. These results provide new insights into human lung tumorigenesis and highlight targeting the Bmal1-miR27a interaction as a potential therapeutic strategy in lung cancer treatment, offering novel avenues for clinical intervention.

ALDOC DRIVES METABOLIC REWIRING TO SUSTAIN BREAST CANCER CELL SURVIVAL IN 3D NON-ADHERENT CULTURE CONDITIONS: A MULTI-OMICS ANALYSIS

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Metastasis is the major cause of mortality of breast cancer. During metastatic process, cancer cells face a complex biological and metabolic reprogramming to detach from the extracellular matrix and survive in non-adherent conditions of blood circulation. Here, we assessed the comprehensive rewiring that allows MCF7 breast cancer cells to generate 3D spheroids through a multi-omics approach. Transcriptomic analysis identified a panel of 100 differentially expressed genes (DEGs) in 3D *vs* 2D cultures. Gene Set Enrichment Analysis (GSEA) showed "Glycolysis" as the most enriched pathway with 6 out of 10 genes encoding glycolytic enzymes upregulated in 3D *vs* 2D. Among these, ALDOC was also found as differentially expressed protein (DEP) by proteomics analysis. Metabolic profiling showed a significant decrease in glucose paralleled by an increase in lactate amounts, and a reduction of ribose-5-phosphate, inosine monophosphate and glutamine, all precursors for the biosynthesis of the purine guanosine monophosphate which, instead, results significantly enhanced in 3D *vs* 2D. Notably, *ALDOC* transient knockdown significantly reduced the sphere-forming ability of MCF7 as shown by the reduction in number and size of 3D spheroids, measured by Leica Thunder microscope and cell viability quantified through CellTiter-Glo assay. All these phenomena were rescued by the administration of exogenous glutamine. Overall, our results identify ALDOC as a key enzyme in the metabolic rewiring responsible for the sphere-forming ability of MCF7.

A TARGETABLE ANTIOXIDANT DEFENSE MECHANISM TO EZH2 INHIBITORS ENHANCES TUMOR CELL VULNERABILITY TO FERROPTOSIS

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Epigenetic changes are present in all human cancers, and are responsible for switching on or off genes, thus controlling tumor cell transcriptome. These changes involve DNA methylation, histone modifiers and readers, chromatin remodelers, microRNAs, and other components of chromatin. The histone H3 methyl-transferase EZH2 is overexpressed in several cancer types including adrenocortical carcinoma (ACC). EZH2 inhibitors (EZH2i) were tested in several clinical trials, but their effectiveness was limited by the toxic effects of the therapeutic doses. We tested several EZH2i on ACC cells, and observed a significant reduction in cell growth only with doses much higher than those required to prevent H3 methylation. We found that all tested EZH2i doses affected lipid metabolism genes, which supported a rewired metabolism followed by an increase in ROS and glutathione production. Additionally, a remarkable change in lipid composition, with an increased presence of polyunsaturated and monounsaturated fatty, acids was observed. These data prompted us to investigate the effects of EZH2i on ferroptosis. We found that EZH2i dose-dependently increased the SLC7A11/glutathione axis and glutathione peroxidase-4 (GPX4), required to counteract lipid peroxidation and ferroptosis. A GPX4 inhibitor synergized with EZH2i, making low doses, otherwise ineffective on cell viability, able to significantly reduce ACC cell growth. Correcting this antioxidant response to EZH2i by ferroptosis inducers may be a new combination therapy strategy, that will easily find clinical applications.

RAPID DETECTION OF INTERLEUKIN 6 IN CELL CULTURE MEDIA THROUGH SURFACE PLASMA RESONANCE-BASED BIOSENSOR.

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Inflammation, a crucial biological response, necessitates rapid and accurate detection for timely intervention and treatment. Traditional methods like ELISA and RT-qPCR are frequently used to measure levels of inflammation biomarkers in diseases. However, these methods typically require substantial sample volumes, prolonged processing times, and extensive labeling steps for detection, which can hinder swift decision-making. Optical biosensor platforms have revolutionized inflammation biomarker screening, offering rapid and sensitive detection capabilities for early disease detection and progression monitoring. In this study, we present a novel approach for the early diagnosis of inflammation biomarkers directly in cell culture media, using Surface Plasma Resonance (SPR)- based biosensing technology. SPR is an optical phenomenon where light-electron interaction changes the refractive index at a metal interface, enabling precise, label-free detection of biomolecular interactions. We prepared cell culture media, enriched with FBS to mimic a complex sample, containing four different concentrations of interleukin (IL)-6 (100, 500, 700, and 1000 pg/ml). We tested different combinations of antibodies (monoclonal and polyclonal anti-IL-6 antibodies), using them either as receptors (capture antibodies) or as detectors (antibodies recognizing the capture antibody-IL-6 complex). With this method we achieved precise measurements of IL-6 levels within a remarkably short timeframe without any processing of the sample. This innovative technique holds promising implications for expediting the diagnosis and monitoring of inflammation, offering a valuable tool for researchers and clinicians in the field of inflammation-related diseases.

ROLE OF THE SENESCENT STROMA-DERIVED GLUTAMINE IN THE AGGRESSIVENESS OF OVARIAN AND PROSTATE CANCER CELLS

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It is now known that the tumor microenvironment contributes to cancer progression through the acquisition of aggressive traits by cancer cells. Cancer treatments induce cellular senescence (therapy-induced senescence, TIS) in both cancer and non-cancerous cells, contributing to many harmful side effects of therapies.
In this context, we focused our attention on the metabolic component of conditioned medium (CM) secreted by senescent fibroblasts in the induction of tumor cell aggressiveness. Human prostate and ovarian fibroblasts were isolated from explants from patients undergoing surgery. After chemotherapy-induced senescence, CMs were collected from fibroblasts. A metabolomic analysis on CM from fibroblasts highlighted an increase in the level of glutamine in the media of senescent fibroblasts of both the prostate and ovary compared to their controls. We therefore observed that incubation with glutamine-rich CM from senescent fibroblasts increased the ability of prostate and ovarian cancer cells to invade a basement membrane-like membrane. Accordingly, the expression levels of several genes implicated in epithelial-mesenchymal transition (EMT) were modulated in a pro-malignant manner. Furthermore, incubation of tumor cells with senescent fibroblast CMs led to increased intracellular ROS production. The molecular mechanism underlying the increase of ROS and invasion are under investigation. These results strongly highlight the contribution of the metabolic component of the senescent stroma to the acquisition of a more invasive phenotype, with a particular involvement of glutamine metabolism.

INCREASED ER STRESS AND RADICAL OXYGEN GENERATION BY IRIDIUM MEDIATES ENHANCED SUSCEPTIBILITY TO ANTI-PD-1/PD-L1 AGENTS IN NSCLC

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Landscape of cancer treatment has been recently revolutionized by immune checkpoint inhibitors (ICIs). The latter improve the host immune response against tumors. Within this class of inhibitors, programmed cell death ligand-1 (PD-L1) has emerged as a pivotal target for cancer immunotherapy. Nevertheless, the limited success as well as the emergence of resistance mechanisms associated with treatment with PD-L1 inhibitors underscore the need to design novel combinatorial strategies which enhance the therapeutic efficacy of anti-PD-L1 based immunotherapy. In this context, iridium-based complexes have attracted extensive attention for their potent anticancer activities and limited side effects. Here, we aim to investigate whether Ir(III) can enhance anti-PD-L1 based immunotherapy as well as to define the molecular mechanisms underlying their potential synergistic anti-tumor activity.. A series of di- and trisubstituted triazines were synthesized through the conjugation of PD-L1 ligand 1 to a bi-sphenyl-pyridine-Ir(III)-complex 2. Anti-tumor activity of anti-PD-L1 inhibitors and Ir(III) complexes was tested *in vitro* utilizing non-small cell lung cancer (NSCLC) cell lines as a model of tumor cells expressing different levels of PD-L1 as well as peripheral blood mononuclear cells (PBMCs). Ir(III) complexes significantly enhanced recognition and destruction of NSCLC cells co-cultured with PBMCs by increasing ER stress-mediated exposure of calreticulin (CRT) and oxygen species (ROS) generation -mediated mitochondrial membrane alterations. These findings can have clinical relevance since they provide the rationale to combine Ir(III) to anti-PD-L1 based immunotherapy for treatment of NSCLC.

EARLY DYSREGULATION OF ENDOSOMAL PATHWAY DRIVEN BY SYNJ1-OVEREXPRESSION: POTENTIAL IMPACT ON DOWN SYNDROME NEUROPATHOGENESIS

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The endolysosomal system is a critical hub for neuronal viability and functions. Alterations of endosomal compartments were observed in cells from adult individuals with Down syndrome (DS). Recently, we have shown that the structure, dynamics and activity of early endosomes (EEs) are altered in human foetal DS fibroblasts, indicating that these alterations occur early in development. However, whether and how the endosomal dysregulation may contribute to the typical neuronal development defects observed in DS is still unknown. To test this hypothesis, trisomic and isogenic euploid induced pluripotent stem cells (iPSCs) were differentiated into neural progenitor cells (NPCs). Combining quantitative confocal microscopy and Western blot analysis, we show that trisomic iPSCs display an enlargement of EEs and a perturbation in their dynamics, associated to the overexpression of *SYNJ1,* a key regulator of endosomal trafficking located on chromosome 21. These endosomal alterations are exacerbated in trisomic NPCs, which present also an impairment of the recycling trafficking. Remarkably, the overexpression of Synj1 in human neuronal SH-SY5Y cells recapitulates the endosomal alterations observed in DS cells, whereas Synj1 knockdown rescues the dynamics of EEs in trisomic fibroblasts and iPSCs, indicating a causal role for Synj1 in the DS-associated endosomal alterations. Finally, a transcriptomic analysis revealed that *SYNJ1*-overexpression alters the expression levels of hundreds of genes, including important regulators of synaptic activity, neurogenesis, and axon guidance in SH-SY5Y cells.
Altogether, our data indicate that endosomal alterations may represent an early step of DS neuropathogenesis. Moreover, these data also show that Synj1 overexpression has a causal role in endosomal alteration, and point out that proper levels of Synj1 are critical for neuronal physiology.

STING REGULATES INNATE IMMUNITY BY MODULATING MACROPHAGE POLARIZATION

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

CHARACTERIZATION OF THE PLATELET TRANSCRIPTOME IN ACUTE CORONARY SYNDROME PATIENTS: A MULTIOMICS APPROACH

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In acute coronary syndromes (ACSs) platelets play a pivotal role. Recent studies showed the fitting capability of platelet transcriptome in different pathological conditions making this exploitable for prognostic, diagnostic, or therapeutic purposes. This research aimed to identify transcriptomic changes between healthy subjects and patients that, at the time of collection, showed an ongoing heart attack (unstable) or patients undergoing therapeutic treatment after this event (stable). Since noncoding molecules are receiving growing interest from the scientific community for their involvement in multiple pathophysiological states, both coding and non-coding transcripts were analyzed. In detail, by using this approach we aimed to correlate post-transcriptional gene expression modulation in these anucleate cell fragments with deregulation of non-coding RNA species specifically associated with disease state. Non-coding transcriptome profiling identified 3 major classes of molecules in all groups: miRNAs, piRNAs and y-RNAs. Interestingly, miRNAs and piRNAs showed, in numerical terms, discordant trends in the 3 groups. Y-RNAs, unexpectedly, exhibited a fair abundance in our dataset. This could indicate an involvement of y-RNAs in platelet physiology since these molecules have been described to be implicated in inflammatory processes. To link these results with posttranscriptional gene expression modulation, RNA-Seq and Mass Spectrometry of the same samples were performed in parallel. The functional analysis of differentially expressed transcripts highlighted several affected pathways such as those related to platelets activation, cell adhesion or wound healing but also others related to cell damage and inflammation. These results revealed several clinically relevant dysregulated pathways and sets of molecules that could be useful as biomarkers or prognostic factors.

ROLE OF GLYOXALASE 1 AND THE RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS IN THE PATHOGENIC INFLAMMATION OF CYSTIC FIBROSIS

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Cystic fibrosis (CF) is a disease caused by mutations in the *CFTR* gene. Although it affects multiple organs, a decline in respiratory function represents the major cause of morbidity and mortality. We recently demonstrated that glyoxalase 1 (GLO1), the major enzyme metabolizing methylglyoxal, a potent precursor of proinflammatory advanced glycation end-products, contributes to CF pathogenic inflammation in a RAGE-dependent mechanism. In the present study, we investigated if hypoxia, a severe complication in CF and a condition known to inhibit Glo1 activity, could play a role in CF pathogenic inflammation through RAGE control. We found that hypoxia sustains RAGE expression, thus reinforcing the role of this receptor as a nodal point in CF pathogenic inflammation and lung pathology. More importantly, anakinra, the recombinant version of the interleukin-1 receptor antagonist, dampens CF pathogenic inflammation and recovers lung function by regulating RAGE expression in addition to rescue GLO1 functionality. Collectively, our previous and current findings show that at least two mechanisms sustain the expression of proinflammatory RAGE in CF. Notably, both represent targets to mitigate lung damage in CF.

MAU2 ROLE IN CORNELIA DE LANGE SYNDROME

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Cornelia de Lange syndrome (CdLS) is a rare genetic disorder caused by pathogenetic variants in seven genes coding for proteins of the cohesin complex. Around 20-30% of CdLS patients remain undiagnosed at the molecular level. Recently, a pathogenic variant affecting the *MAU2* gene was found in one CdLS patient. MAU2 plays a role in cohesin complex functioning by interacting with NIPBL to load the complex onto DNA.
We decided to study MAU2 involvement in central nervous system development, given that little is known on its function, using two complementary approaches. On one side, we differentiated iPSC towards mini-cerebellum and, on the other side, we implemented the *in vivo* zebrafish model. We successfully generated a 3D model of CdLS cerebellum organoids both with *NIPBL* and *MAU2* variants. Preliminary data reveals a dysregulation in the expression of cerebellar genes compared to the control group, highlighting the potential impact of cohesin abnormalities in this process. We then used morpholino (MO) technology for the *in vivo* zebrafish model. Based on initial data, we observed morphological and behavioral abnormalities in morphant embryos compared to controls. Our work could pave the way to a better understanding of the role of *MAU2* in the context of physiological and pathological cerebellum development and to gain new insights into the molecular basis underlying CdLS.

QUANTUM MOLECULAR RESONANCE SUPPRESSES NLRP3 INFLAMMASOME /NITROSATIVE STRESS, PROMOTING M1 TO M2 MACROPHAGE POLARIZAZION IN VITRO OSTEOARTHRITIS MODEL

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This study investigated the anti-inflammatory effects of Quantum Molecular Resonance (QMR) technology in an in vitro model of osteoarthritis-related inflammation. THP-1-derived macrophages were stimulated with lipopolysaccharide and hyaluronic acid fragments to induce inflammatory cytokine expression and nitrosative stress. QMR treatment inhibited COX-2 and iNOS protein expression and activity, and reduced NF-κB activity. Additionally, QMR treatment significantly decreased peroxynitrite levels, a reactive nitrogen species formed during inflammation, and restored tyrosine nitration to levels similar to those of control cells. The effect of QMR on inflammasome activation and macrophage polarization was also examined. Results indicated that QMR treatment significantly reduced NLRP3 and activated caspase-1 protein expression, as well as downregulated IL-18 and IL-1β protein expression and secretion. Moreover, QMR treatment promoted a shift in macrophage polarization from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype.

THE CFTR CORRECTOR REVERSES THE ER-INDUCED STRESS CONDITION IN HUMAN NEUROBLASTOMA CELLS: POTENTIAL USE IN NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases share a common etiopathogenesis, caused by the accumulation of protein aggregates in the brain that leads to an imbalance in homeostasis, generating an ER stress condition in the cells.
To restore physiological conditions, *unfolded protein response* (UPR) is activated, controlled by IRE1α, PERK and ATF6, which are maintained in an inactive state by binding with molecular chaperone GRP78/BiP.
Misfolded proteins accumulation determines a displacement of this chaperone: PERK, after autophosphorylation, phosphorylates eIF2α, which activates ATF4, that, in turn, induces the transcription of the pro-apoptotic protein CHOP; ATF6 activates UPR target genes; IRE1α determines an upregulation of ER chaperones. Thus, UPR consists in an adaptive response of the cell to an ER stress condition, that, however, continuing over time, leads to permanent damage. The association between reticular stress and programmed death has also highlighted by the discovery of caspase 4, specifically activated by apoptotic stimuli induced by reticular stress.
ER stress is also associated with oxidative stress, caused by high total ROS levels, which have escaped the enzymatic control of Superoxide Dismutases, whose levels decrease as stress increases.
We evaluated the activity of "Corrector" Vx-809, used in Cystic Fibrosis therapy, in SH-SY5Y neuronal cells, in which ER stress condition was induced by Thapsigargin, to test whether the drug could improve protein folding, suggesting its possible therapeutic use in neurodegenerative diseases.
The data obtained highlighted that the corrector is involved in reducing protein expressed under ER stress conditions, reducing ROS levels and decreasing the apoptotic pathway activation. Finally, DARTS assay is used to identify possible interactions between Vx-809 and potential target proteins.

A NEW RNA-BASED THERAPY FOR THE FRAGILE X SYNDROME

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

FERROPTOSIS ACTS AS IMMUNOGENIC CELL DEATH IN ORAL SQUAMOUS CELL CARCINOMA

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Ferroptosis has been classified as an immunogenic cell death (ICD). The biological mechanisms underlying the immunogenicity of ferroptosis in oral squamous cell carcinoma (OSCC) is, though, unexplored. Here, we treated CAL27 and SCC154 OSCC cell lines with two ferroptosis inducers (FINs), RSL3 or Ferlixit, for 2h, 4h, and 8h. Flow cytometry analysis highlighted that SCC154 are more sensitive to both FINs than CAL27, as shown by the higher mortality observed at 10μM RSL3 (8h) (% SCC154 PI^+: 44; % CAL27 PI^+: 18) or 250μM Ferlixit (8h) (% SCC154 PI^+: 36; % CAL27 PI^+: 29). SCC154 treated with RSL3 showed an increase of mitochondrial reactive oxygen species (mitoROS) and mitochondrial membrane depolarization; CAL27 treated with Ferlixit, instead, showed an increase of intracellular labile iron pool and lipid peroxidation. Gene expression analysis and ELISA within culture media, highlighted that both CAL27 and SCC154 cells enhance the production and release of pro-inflammatory cytokines (TNFα, IL1β, IL4, IL6, IL8, IL12, IFNβ) at late stage of ferroptosis (8h) triggered by both FINs. Besides, CAL27 reduced expression and release of the anti-inflammatory cytokine (IL10). In both OSCC cell lines treated with RSL3, IFNγ increased during early ferroptosis (2h). Notably, we also found a progressive emission of ATP, a notorious damage-associated molecular pattern (DAMP), within the culture media by OSCC cells treated with both FINs at each time point. Overall, our results suggest that ferroptosis may act as ICD in OSCC, by releasing pro-inflammatory cytokines and DAMPs.

OXYTOCIN AND CANNABIDIOL MODULATE THE DIFFERENTIATION POTENTIAL OF HUMAN ADIPOSE-DERIVED STEM CELLS

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Some organisms are able to regenerate entire parts of their bodies, while others can suffer permanent damage following even a mild injury.
The term "regeneration" defines a process that aims to restore of the morphological and physiological functions of damaged tissues and organs. The human body continuously uses its own tissue-resident stem cells to repair adult tissues and organs, and to counteract senescence-related processes. Human mesenchymal stem cells show many properties such as self-renewal, multilinear differentiation and isolation from almost all organs, thus representing a particularly useful and promising tool in the field of regenerative medicine.
The aim of the study was to find new cellular strategies that could be useful in cell-based therapy. Molecules related to the well-being in different areas (Leu-enkephalin, Met-enkephalin, β-endorphin, oxytocin and cannabidiol) were tested to evaluate their modulatory capabilities towards the biology of human adipose-derived mesenchymal stem cells (hASCs), aiming to reduce their physiological process of senescence and promote their differentiation capacity.
The results obtained indicated that, while Leu-enkephalin, Met-enkephalin and β-endorphin did not appear to affect the proliferation and cellular senescence of hASCs, oxytocin and cannabidiol were able to modulate their differentiation ability. In particular, oxytocin promoted osteogenic differentiation while cannabidiol promoted adipogenic differentiation, as demonstrated with specific stains, gene and protein analyses. Therefore, these two molecules could be considered in further studies for the therapeutic application of stem cells in regenerative medicine.

INVESTIGATING THE ROLE OF MIR-9-5P IN CELLS FROM A CCD PATIENT

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Mutations within the RUNX2 gene, which codes for the Runt-related transcription factor 2 (RUNX2), recognized as the pivotal regulator of osteogenic differentiation, cause skeletal diseases, such as cleidocranial dysplasia (CCD). MiR-9-5p is involved in several cellular processes and is believed to play a significant role in osteogenesis. With the aim to understand the miR-9-5p modulation in CCD, we used cells derived from a patient carrying the RUNX2 c.505C>T mutation. Circulating progenitor cells were isolated from peripheral blood, while mononuclear cells were reprogrammed into induced pluripotent stem cells (iPSCs). These iPSCs were then differentiated into mesenchymal lineage cells (iMSCs) derived from both a CCD patient and sex- and agematched controls. Gene expression and proteins analyses were performed to understand the role of miR 9-5p in cells of CCD and controls. To further explore the effects of miR-9-5p in osteogenesis, we used miRNA inhibitor (siRNA) to silence miR-9-5p in human bone marrow-derived mesenchymal stem cells (hBM-MSCs).
Thus, miR-9-5p expression was lower in both circulating progenitor cells and iMSCs with the RUNX2 mutation compared to wild type cells from healthy donors. We observed increased levels of TGF-β1 and TGF-βR1 in the RUNX2-mutated cells of CCD patient. Additionally, gene expression levels of RUNX2 were decreased, while TGF-β1 and TGF-βR1 expression levels were higher in miR-9-5p silenced cells compared to controls.
In conclusion, our findings indicate that miR-9-5p increases RUNX2 mRNA and protein levels while reducing TGF-β1 and TGF-βR1 expression in mesenchymal stem cells. The decreased expression of miR-9-5p in RUNX2-mutated cells from a CCD patient suggests its significant role in the regulation of TGF-β1 and TGF-βR1 levels, providing insights into the molecular mechanisms underlying osteogenesis and CCD pathology.

WWP2-INDUCED PERICYTES TO MYOFIBROBLAST TRANSITION CONTRIBUTES TO THE PROGRESSION OF FIBROSIS IN CKD IN DIABETES

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Chronic kidney disease (CKD) in diabetes can be classified as diabetic Nephropathy (DN), non-diabetic renal disease (NDRD) or mixed forms (DN+NDRD). Only in true DN is activated the lysine63 ubiquitination (K63-Ub) pathway involved in the progression of renal fibrosis. WWP2 is an E3 ligase that modulates myofibroblast activation and metabolic reprogramming in CKD. The aim of the study was to evaluate the role of WWP2 in K63-Ub and renal fibrosis in diabetes.
The expression of WWP2, K-63Ub, -sma, PDGFBR, vimentin, NG2 was evaluated by immunohistochemistry, immunofluorescence, qPCR, western blotting or flow cytometry in: i) renal biopsies of patients with DN (n=11), NDRD (n=8) and DN+NDRD (n=3); ii) DBA/2J mice treated with streptozotocin in the presence or absence of a specific K63Ub inhibitor (NSC697923); iii) HK2 tubular cells, EAHY926 endothelial cells and pericytes.
WWP2 was expressed at tubular, glomerular and vascular levels in DN and DN+NDRD compared to NDRD ($p<0.05$). Increased expression of WWP2 in DN was associated with the accumulation of K63Ub proteins in tubular and vascular cells, particularly in pericytes. In diabetic DBA/2J mice, K63Ub inhibition with NSC697923 significantly reduced WWP2 expression in the kidney ($p < 0.05$). Also in vitro, in HK2 cells, NSC697923 significantly reduced hyperglycemia-induced WWP2 and -sma expression ($p < 0.01$). In pericytes, hyperglycemia induced the expression of WWP2 (RR>3), -sma (RR>4), PDGFBR (RR>4), vimentin (RR>2) and reduced NG2 (RR>2) suggesting the transition from pericytes to myofibroblasts, and this effect was blocked by NSC697923 ($p<0.05$).
These findings suggest that WWP2 is involved in the K63-Ub pathway and promotes fibrosis progression in DN patients through pericytes to myofibroblasts transition. WWP2 could therefore represent a novel target in preventing the progression of renal damage in patients with DN.

ROLE OF IRON METABOLISM SENSING IN CANCER CACHEXIA AND TUMOR PROGRESSION

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Skeletal muscle atrophy is a hallmark of cachexia, a wasting condition typical of chronic pathologies, that still represents an unmet medical need. We previously found that alteration in signalling pathway related to iron sensing and homeostasis are pivotal in promoting muscle wasting, cachexia and metastatic progression. To this aim, we investigated pathways intersecting iron and muscle mass homeostasis. Bone morphogenetic protein (BMP)-Smad1/5/8 signaling alterations are emerging drivers of muscle catabolism, hence, characterizing these perturbations is pivotal to develop therapeutic approaches. We identified two promoters of "BMP resistance" in cancer cachexia, specifically the BMP scavenger erythroferrone (ERFE) and the intracellular inhibitor FKBP12. ERFE is upregulated in cachectic cancer patients' muscle biopsies and in murine cachexia models, where its expression is driven by STAT3. Moreover, the knock down of Erfe or Fkbp12 reduces muscle wasting in cachectic mice. To bypass the BMP resistance mediated by ERFE and release the brake on the signaling, we targeted FKBP12 with low-dose FK506. FK506 restores BMP-Smad1/5/8 signaling, rescuing myotube atrophy by inducing protein synthesis. In cachectic tumor-bearing mice, FK506 prevents muscle and body weight loss and protects from neuromuscular junction alteration, suggesting therapeutic potential for targeting the ERFE-FKBP12 axis.

IMPACT OF PIGMENT EPITHELIUM-DERIVED FACTOR ON INTRAHEPATIC CHOLANGIOCARCINOMA CELL SPREADING *IN VITRO* AND *IN VIVO*

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Intrahepatic cholangiocarcinoma (iCCA) is a heterogeneous and lethal hepatobiliary tumor that carries a high burden of morbidity and mortality among primary liver cancers. iCCA aggressiveness is revealed through the high proliferation and invasive abilities of malignant cells that lead a rapid tumor spread into regional lymph nodes due to the predominant sprouting of lymphatic vessels in the tumor microenvironment (TME). A peculiar hallmark of the iCCA is given by the expression and released in the TME of soluble angio-inhibitory mediators, such as thrombospondin 1 (THBS1), 2 (THBS2) and pigment epithelium-derived factor (PEDF). The combined expression of these proteins is able to hind the vascular network and promote the tumor-associated lymphangiogenesis, thus highlighting an indirect role in the iCCA progression. Recently, we demonstrated that both THBS1 and THBS2 have also a direct role by exacerbating the malignant phenotype of the iCCA cells. Nevertheless, a direct role of PEDF in influencing the iCCA cell behavior remains to be investigated. In many cancers, PEDF exhibits an anti-metastatic role despite it is found downregulated in tumor specimens. Since PEDF is found strongly up-regulated in the most of the iCCA patients, we speculated that, despite its indirect role in promoting the tumor-induced lymphangiogenesis, this factor may influence the iCCA cell behavior as antitumorigenic protein. Herein, we collected *in vitro* evidences that PEDF is able to inhibit iCCA cell adhesion, migration, and invasion. These results prompted us to corroborate the anti-metastatic ability of PEDF in promoting iCCA cells diffusion to local lymph nodes *in vivo* xenograft models.

CITRATE AS SIGNALLING MOLECULE FOR METABOLIC RECONFIGURATION UPON OSMOTIC STRESS?

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Metabolic rewiring is part of the cell response to a variety of stress enabling adaptation and survival. In this context, inter-organellar cross talk plays an important role for the regulation of metabolites concentration and trafficking. By using a model of *Saccharomyces cerevisiae* yeast cells, we have been studying the metabolic clues underpinning the peroxisomes-mitochondria cross talk occurring upon osmostress. In particular, we have identified citrate deriving from the glyoxylate and/or TCA cycle as a putative modulator of the adaptive process. Gene expression analysis revealed that *CIT1* and *CIT2*, encoding the mitochondrial and peroxisomal isoforms of citrate synthase, are down and up-regulated respectively by sodium chloride-induced osmotic stress in wild type cells. Metabolomic analysis showed that most of TCA cycle intermediates are reduced by NaCl stress, except citrate, suggesting that a remodeling is required to maintain citrate levels and survive under hyperosmotic conditions. This hypothesis has been supported by citrate synthase activity measurements in cellular extracts. Our data clearly indicate that citrate synthase is activated by osmostress. Additionally, preliminary data reveal a modulation of citrate transport by NaCl stress in mitochondrial extracts. *CIT2* up-regulation is prototypical of RTG pathway activation, which regulates mitochondria-tonucleus communication in response to mitochondrial dysfunction. We have demonstrated that the inhibition of RTG signalling delays osmoadaptation and proposed a model in which this pathway might control peroxisome-mitochondria communication upon treatment with NaCl. These data implement the model by including citrate as a main component of the RTG-mediated metabolic adaptation in osmostressed cells.

COLORECTAL CANCER-DERIVED EXTRACELLULAR VESICLES AFFECT THE IMMUNOMODULATORY PROPERTIES OF HEPATOCYTES CONTRIBUTING TO LIVER METASTATIC COLONIZATION

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Colorectal cancer-derived liver metastases represent the leading cause of CRC-related deaths. CRC-derived extracellular vesicles (CRC-EVs) promote in the liver the formation of the pre-metastatic niche (PMN), by affecting the activity of the non-parenchymal cells while few data are available about the involvement of hepatocytes (Heps). Recently, we showed that CRC-EVs induce epithelial to mesenchymal transition in Heps, event preceding the fibrotic injury. Evasion of immune surveillance is another crucial event that define the hepatic PMN and is mainly caused by the activation of immune check-point PD-L1/PD-1. We already demonstrated that CRC-EVs carry PD-L1 and upregulate the expression of PD-L1 in macrophages. Our hypothesis is that CRC-EVs can support liver metastasis inducing an early alteration also of Heps immunomodulatory properties within the PMN, inducing tumor-associated escape from antitumoral immunity. We showed that CRC-EVs induce in Heps the increase of PD-L1 expression also promoting its nuclear translocation according to emerging data that reports that beyond the well-known localization on cell surface and in cytoplasm, PD-L1 is also present in the nucleus (nPDL1) where could activate alternative pathways that support tumor progression. According to that, we observed that the nPDL1 in CRC-EVs treated Heps is associated with the upregulation of GAS6, VISTA and PD-L2, proteins involved in the regulation of immune tolerance mechanisms. To study the correlation between nPDL1 and the increased expression of these factors we used a molecule able to block the nuclear transfer of the SEVs cargo into recipient cells, showing that the immunomodulatory properties of CRC-EVs are blocked in pretreatred Heps. Our results highlighted that CRC-EVs elicit in Heps an alternative activity of PD-L1 contributing to the formation of an immunosuppressive microenvironment.

POTENTIAL APPLICATIONS OF DPSCs IN REGENERATIVE MEDICINE: DIFFERENTIATION ABILITY AND SUPPORT IN THE ANGIOGENESIS PROCESS

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Dental pulp stem/stromal cells (DPSCs), originating from the neural crest, are physiologically involved in dentin homeostasis, bone remodeling, and differentiation into several tissues including cartilage, bone, adipose, hepatic, and nervous tissues. DPSCs have also been shown to influence the angiogenesis process, through the release of secretory factors or by differentiating into vascular and/or perivascular cells.
Based on this information, this study aimed to study the capacity of DPSCs to differentiate into various cell types, including osteo-odontogenic, hepatic, neurogenic, and perivascular phenotypes. This investigation utilized bi-dimensional (2D) and three-dimensional (3D) culture methods. Experiments conducted involved treating DPSCs with osteo-odontogenic and hepatic differentiation media, hypoxia at 1%, and isolation of specific populations using MACS technology. In addition, a stationary technique was employed to generate organoids. Results showed that DPSCs could differentiate into an osteo-odontogenic phenotype, expressing relevant genes and producing dentin and calcium phosphate matrices in 2D and 3D environments. Hypoxia exposure for 5 and 16 days also revealed a time-dependent differentiation. Moreover, conditioned media derived from DPSCs stimulated by hypoxia had the ability to induce neural differentiation in SH-SY5Y neuroblastoma cells and undifferentiated DPSCs. Using immunomagnetic separation targeting NG2, three subpopulations of DPSCs (Total, NG2-, NG2+) were isolated. NG2+ DPSCs, resembling pericytes, stabilized tubules in vitro for 14 days through interactions with endothelial cells.
This study suggests that DPSCs have the ability to differentiate into different cell types, highlighting their potential as a valuable tool in regenerative medicine and bringing hope for treating various medical conditions.

POLYCHLORINATED BIPHENYLS (PCBs) INDUCE IRON HOMEOSTASIS DISORDER IN BOTH DICTYOSTELIUM AND WHITE BLOOD PATIENTS' CELLS

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Exposure to Persistent Organic Pollutants (POPs) can be dangerous, since their ability to bioaccumulate and interfere with the endocrine, immune, reproductive, and cardiovascular systems. Many of them possess a carcinogenic action, like PolyChlorinated Biphenyls (PCBs). Recent analyses, conducted by Arpa Piemonte, reveal that there are still some areas in Piemonte where the PCBs level is above the standard limits. In this regard, our project aims to study the mechanism of action of PCBs and their relationship with different pathologies, including cancer, through the model organism *Dictyostelium discoideum* (D.d). Our results show that both PCB 138/153 impair the growth and alter the development of D.d. According to literature, PCBs can interfere with iron uptake by regulating the levels of hepcidin, a key component in iron homeostasis. Since iron is fundamental for D.d development, the expression of developmental and iron-dependent genes have been investigated. Our findings reveal a generalized upregulation of developmental genes and a deregulation of iron-related ones, suggesting lower intracellular iron levels. That hypothesis was also confirmed by calcein assay. At the same time, PCBs were also tested on white blood patients' cells. A decreased vitality and an upregulation of *hepcidin* gene were observed, suggesting an iron accumulation inside the cells. Further analyses are necessary to elucidate the role of PCBs in iron homeostasis and cancer, but D.d can be considered as a perfect model to understand the mechanism behind it and possibly develop new inhibitors. A new untargeted metabolomic approach is currently underway to better understand the signaling induced by PCBs. Moreover, Wild *Dictyostelium fruiting bodies,* have been isolated from PCBs contaminated soil, opening the possibility to use this model organism as a cheap tool to monitor soil toxicity.

MORPHOLOGICAL AND BIOCHEMICAL RESPONSE OF RAT HARDERIAN GLAND TO ENVIRONMENTAL AND METABOLIC STRESSES

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Harderian gland (HG) is a peculiar gland located in the medial corner of the orbit of many terrestrial vertebrates and, in rodents, is characterized by the presence of porphyrins, which through the photo-oxidation process, produce reactive oxygen species. For this, HG represents a good model to evaluate the physiological alterations in oxidative stress (OS) conditions. In addition, HG acts as an endocrine organ contributing to the secretion of extra-pineal melatonin (MLT) and extra-gonadal steroid sex hormones.
In this study, we evaluated the effects of two different types of stress on rat HG: environmental, due to *ex vivo* treatment with cadmium (Cd), an endocrine disruptor with pro-oxidant action, and metabolic, via the streptozotocin-induced type 1 diabetes. In addition, we tested, by a co-treatment with Cd, the effects of MLT, a well-known antioxidant molecule with a radical scavenger function.
The HG under stress conditions showed a significant increment of TBARS levels because of increased OS, as well as morphological alterations of the glandular epithelium and increased porphyrin-accumulation.
We also evidenced a significant increase in mast cells and apoptotic cell numbers. Furthermore, OS conditions induced an increment of the LC3B-II/LC3B-I ratio and a decrease in the p62 protein level because of autophagy pathway activation. The stressful treatments had deleterious effects also on steroidogenesis resulting in a significant reduction of 3β-HSD levels.
Interestingly, the co-treatment of Cd+MLT counteracted the stress condition restoring all the above parameters to the control values.
The results obtained confirmed that HG is a valid OS model and suggested that MLT is a good candidate for preventing/counteracting OS damage.

HSA-MICRORNA-210-3P REGULATES HUMAN DERMAL FIBROBLAST CELL PROLIFERATION AND DIFFERENTIATION

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Hsa-miR-210-3p is a key regulator of epithelial cell proliferation and differentiation. We recently found hsa-miR-210-3p expression in dermal cells. In this study, the role of hsa-miR-210-3p was investigated in human dermal fibroblasts. Transfection experiments with hsa-miR-210-3p mimic and inhibitor were carried out in human dermal fibroblast cells (HDFa) to assess the microRNA function on cell proliferation, colony formation and migration abilities. The expression of hsa-miR-210-3p target genes (n=84) was evaluated by qPCR array in miR-overexpressed cells and compared to untransfected cells. Forced hsa-miR-210-3p expression in HDFa cells increased cell proliferation, colony formation and migration abilities, while the opposite effects were determined in miR-inhibited cells. HIF3A and CHN1 resulted as the most significantly downregulated target genes in HDFa cells overexpressing hsa-miR-210-3p, compared to untransfected cells. HIF3A is known to inhibit cell survival and proliferation as a negative response mechanism to hypoxia, while CHN1 positively regulates cell differentiation and inhibits cell migration. Enrichment analyses for biological processes on the entire set of differentially expressed target genes indicated the downregulation of positive regulators of cell differentiation, maturation and axonogenesis in HDFa overexpressing hsa-miR-2103p, compared to untransfected cells. These results indicate that hsa-miR-210-3p is a regulator of cell proliferation and differentiation, independently of the cell type, epithelial or fibroblast. The hsa-miR210-3p activity may be used in RNA-based therapies of different dermatological diseases to restore skin layers.

THE RNA-BINDING PROTEIN HNRNPA2B1 IS A KEY PROTEIN CONTROLLING MIR SORTING INTO FIBRO-ADIPOGENIC PROGENITOR DERIVED EXTRACELLULAR VESICLES, CRUCIAL FOR MUSCLE REGENERATION IN DMD.

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The functional exhaustion of muscle stem cells (MuSCs) contributes to Duchenne Muscular Dystrophy (DMD) progression by compromising the compensatory regeneration of diseased muscles. MuSCs activity is influenced by functional interactions with cell types that compose their niche, including fibro-adipogenic progenitors (FAPs) that regulate the regenerative ability of skeletal muscles in physiological and pathological conditions. We have recently discovered that FAP-derived Extracellular Vesicles (EVs) support functional interactions with MuSCs, and contribute to the beneficial effect of HDAC inhibitors (HDACi) on DMD muscles.
FAP-derived EVs mediate microRNA and proteins transfer to MuSCs, and the exposure of dystrophic FAPs to HDAC inhibitor (HDACi) -TSA- increases the intra-EV levels of a specific message which cooperatively target biological processes of therapeutic interest, including regeneration, fibrosis and inflammation.
The proteomic analysis revealed that the most represented proteins in EV-FAPs HDACi can be classified into i) structural and cytoskeletal proteins and ii) proteins involved in intracellular trafficking and RNA binding, such as hnRNPA2B1. By bioinformatics analysis we recognized and verified the presence of binding sites for hnRNPA2B1 on the sequence of the miRs resulting up-regulated by the HDACi-TSA; we found the motif that specifically controls the binding between non coding RNA and the hnRNPA2B1 and the sorting of myo-miR (i.e.miR-206) into EVs. We focused on clarifying the role of hnRNPA2B1 and the mechanism by which its fine-tuning can lead to ad hoc vesicles loaded with regenerative message.
Our data on dystrophic –mdx- mice, reveal a potential for pharmacological modulation of FAP-derived EV's content as novel strategy to design novel regenerating therapies for the treatment of DMD using engineered EVs (eEVs) and for local therapeutic interventions in muscular diseases.

INHIBITION OF THE BROMODOMAIN-CONTAINING PROTEIN BRPF1 AS POTENTIAL LUMINAL BREAST CANCER THERAPEUTIC APPROACH

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Approximately 70% of Breast Cancer (BC) are hormone-responsive and express Estrogen Receptor alpha (ERα), widely used as molecular marker to predict responsiveness to endocrine therapies (ET). Despite an undoubted clinical benefit of these pharmacological regimes, 1/3 of ERα-positive patients develop acquired or *de novo* ET resistance (ET-R), leading to tumor progression. Furthermore, it was observed that a substantial fraction of these tumors showed an aberrant activity and constitutive ERα expression, maintaining the receptor ability to control gene transcription through the recruitment of multiprotein nuclear complexes.
To identify a new actionable therapeutic target among ERα interactome components, a computational approach was used combining genome-wide 'drop-out' screenings and ERα interactome profiling, identifying a set of essential genes in luminal ERα+ BC. We specifically focused on Bromodomain and PHD Finger Containing 1 (BRPF1), placed among the top fitness genes. BRPF1 belongs to a family of epigenetic readers that act as chromatin remodelers to control gene transcription.
We investigate the functional interplay of BRPF1with ERα by chromatin and transcriptome profiling, gene ablation and specific pharmacological inhibition followed by cellular and functional assays. Results indicate the co-recruitment of both factors onto BC cell chromatin, where cooperate to regulate estrogen target gene transcription. Moreover, BRPF1 inhibition reduced proliferation and survival of antiestrogen -sensitive and -resistant BC cells and in Patient Derived Organoids (PDOs), leads to a profound remodelling of chromatin structure and inhibiting ER-dependent gene transcription.
In summary, these results indicate that BRPF1 is a critical regulator of BC proliferation and survival, offering new actionable therapeutic targets for treatment of these aggressive tumors.

EFFECTS OF miR-23b-3p, miR-126-3p, AND GAS5 DELIVERED BY EVs ON BREAST CANCER XENOGRAFTS DEVELOPED IN ZEBRAFISH.

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Extracellular vesicles (EVs) are lipid bilayer membrane-delimited particles secreted by cells. EVs contain and transport DNA, RNA, proteins, lipids and metabolites to recipient cells for intercellular communication. We previously showed that the multikinase inhibitor sorafenib caused dysregulation of miR-23b-3p, miR-126-3p and the long non-coding RNA (lncRNA) GAS5 in *in vitro* models of breast cancer. These ncRNAs generally act as tumor suppressors in human malignancies. Here, we isolated EVs released from 4 different types of breast cancer cells after sorafenib treatment using 3 different approaches: precipitation, ultracentrifugation and immunoprecipitation. We quantified the levels of miR-23b-3p, miR-126-3p and GAS5 in the EVs by ddPCR technology and found a general increase in the expression levels of these ncRNAs with the same trend in all 3 types of isolation methods used. To evaluate their role as possible nanocarriers of tumor suppressor ncRNAs, we treated breast cancer cells with the EVs enriched in miR-23b-3p, miR-126-3p and GAS5. We found a significant reduction of cell proliferation ability and an increase in the expression levels of the selected ncRNAs. In addition, we injected two breast cancer cell lines into zebrafish embryos and treated the resulting xenografts with the enriched EVs to establish their role as ncRNA carriers *in vivo*. We found a reduction in xenograft tumor mass and inhibition of angiogenesis, as well as the number of micrometastases in the tails. Our results demonstrate: i) an innovative way to enrich EVs with tumor suppressor ncRNAs; ii) the role of EVs as ncRNA carriers; iii) the involvement of miR-23b-3p, miR-126-3p and GAS5 in limiting the aggressive properties of breast cancer *in vitro* and *in vivo*.

MALIC ENZYME 1 IN THE ACTIVATION OF HUMAN MACROPHAGES: TRANSCRIPTIONAL REGULATION AND FUNCTION

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Malic Enzyme 1 (ME1) is a cytosolic $NADP^+$-dependent enzyme that catalyzes the oxidative decarboxylation of malate to pyruvate generating NADPH. NADPH is essential for biosynthetic processes, including lipogenesis, and maintaining the cellular redox balance. Macrophages are highly plastic cells with multiple roles in innate and adaptative immunity in response to external or internal triggers. A complex network of signaling pathways connects stimulation to metabolic and gene expression reprogramming, leading to the formation of distinct macrophage subsets. Macrophage activation involves a broad range of metabolic enzymes and proteins.

Upon infection with lipopolysaccharide (LPS), we found that ME1 is overexpressed. After just 4 hours of LPS stimulation, there was a significant increase in ME1 mRNA, protein levels, and enzymatic activity. An in silico analysis identified a phylogenetically conserved NF-κB binding site in the human ME1 gene promoter, which we confirmed to be active during macrophage activation through ChIP experiments. Furthermore, NF-κB inhibition significantly reduced ME1 protein content.

We demonstrated that NADPH derived from ME1 serves as a substrate for both inducible nitric oxide synthase (iNOS) and NADPH oxidase, responsible for producing nitric oxide (NO·) and reactive oxygen species (ROS), respectively. As a matter of fact, ME1 gene silencing put down levels of NO·, ROS, and prostaglandin E2 (PGE2) inflammatory mediators in LPS-triggered human macrophages.

Newsworthy, ME1 is upregulated in PBMC-derived macrophages from patients with Metabolic Dysfunction-Associated Steatohepatitis (MASH), rheumatoid arthritis, and systemic lupus erythematosus, suggesting a potential role of ME1 in inflammatory diseases.

Overall, modulating ME1 levels offers a promising avenue for immunometabolic regulation, thereby impacting macrophage function.

IMPROVING LUNG CANCER EARLY DETECTION BY DEVELOPING A TUMOR SPECIFIC EXOSOME MIRNA SIGNATURE (EXO-MIR TEST)

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Lung cancer (LC) is the leading cause of cancer-related mortality worldwide with ~2 million deaths per year, with two major subtypes, non-small-cell lung cancer (NSCLC, ~85%) and small-cell lung cancer (SCLC, ~15%). Most cases are diagnosed at an advanced stage (III-IV), resulting in a 5-year survival rate of less than 15%, primarily due to the lack of efficient early-stage LC screening tests. Non-invasive blood-based biomarkers have increasingly become appealing to improve LC early diagnosis. MicroRNAs (miRNAs) are small, conserved, non-coding RNAs crucial in regulating cancer initiation, progression, and therapy resistance. Furthermore, circulating cell-free miRNAs (cfmiRNAs) stability, easy manipulation, detection, and tissue specificity make them useful for developing tumor-specific diagnostic and prognostic signatures. We aimed to identify an innovative Exo-miR Test for early LC diagnosis and uncover relevant cancer molecular mechanisms. We demonstrated that ~30% of the cf-miRNAs included in the miR-test were preferentially expressed in epithelial cells (EPC), while ~30% in hematopoietic cells, with a significant portion encapsulated within exosomes (Exo-miRs), mostly released by EPC of LC and display cell-specific markers. Using a global miRNA expression profile including 30 early-stage NSCLC patients and 15 healthy individuals, we identified a new serum-derived Exo-miR signature that distinguishes LC patients from healthy ones. By comparing the global expression profile of Exo-miRs from 28 cell lines representing different cell populations within the tumor and 10 primary cell cultures isolated from lung tissue and NSCLC, we revealed the origin (epithelial, stromal, or both) of serum Exo-miRs associated with LC. Our data suggest that EP cancer cells' exosomal miRNA-cargo may help increase biomarkers tumor specificity to be used in LC screening trials.

ENDOPLASMIC RETICULUM AMINOPEPTIDASE 1 (ERAP1) AND 2 (ERAP2) SHAPE CELLULAR HOMEOSTASIS IN NEUTROPHILS

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Reticulum aminopeptidases 1 (ERAP1) and 2 (ERAP2) have been recently shown to modulate a plethora of physiological processes relevant for the maintenance of homeostasis in different cellular subsets. These roles become particularly significant once ERAPs are released into the extracellular milieu following stressor-induced secretion. In this frame, the extracellular supplementation of recombinant human (rh) ERAP1 and ERAP2 was used to mimic the effect of secreted ERAPs on neutrophils, a population largely involved in the modulation of many ERAP-associated human diseases.

Neutrophils were isolated from 5 healthy subjects (HCs), stimulated with 300ng/mL of rhERAP1, rhERAP2 or rhERAP1+rhERAP2 (COMBO) for 3h or 24h. Stimulation with rhERAPs triggered neutrophil activation as witnessed by the increased expression of specific markers such as integrin MAC-1 and carcinoembriogenic cell adhesion CD66b (Flow Cytometry). This was recapitulated by both gene expression and secretome analyses (RT-PCR and Luminex). Moreover, neutrophil rhERAP-exposure resulted into an increased migration capacity (trans-well assay), phagocytosis (flow cytometry), autophagy (LC3BII/I and p62 by ddPCR, immunofluorescences and western blot), as well as augmented granule release myeloperoxidase and elastase (ELISA). Surprisingly, rhERAP-exposure also reduced ROS accumulation (DCFDA assay) and did not influence oxygen consumption rate (Oxygraphy) in neutrophils.

Our study provides novel insights into the biological role of ERAPs, and indicates that extracellular ERAPs, contribute to shaping neutrophil homeostasis. This information could contribute both to a better understanding of the biological bases governing immune responses, and to designing ERAP-based therapeutic protocols to control neutrophil-associated human diseases.

EPIGENETIC LANDSCAPE OF BRAIN ARTERIOVENOUS MALFORMATION: ROLE OF VASCULAR SMOOTH MUSCLE CELLS IN ENDOTHELIAL DIFFERENTIATION LOSS

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Arteriovenous malformation of the brain (bAVM) is a vascular phenotype most likely arising due to impaired expression of vascular differentiation markers. This results in the capillary bed lack and, then, in the arteriolar-to-venule direct shunt. Differentially methylated genes were identified by epigenome comparison between bAVM-derived endothelial cells (ECs) and human cerebral microvascular endothelial cells (HCMECs/D3, Millipore). Both coding and noncoding genes were considered. Results were validated by quantitative methylation-specific PCR and quantitative realtime-PCR. Impaired methylation profile occurred in genes regulating endothelial cell proliferation and stemness as *EPHB1*, involved in arteriolar caveolae biogenesis. However, the main findings regard the dysregulation of genes regulating the crosstalk between endothelium and vascular smooth muscle cells (VSMCs) during development, as *DAAM1* and *ROR2*. These are effectors of Wnt5a and Wnt7a suggesting, for the first time, involvement of the Wnt signaling in bAVM pathogenesis. Moreover, aberrant methylation pattern was reported for many lncRNA genes targeting transcription factors expressed during neurovascular development. Finally, in addition to the conventional CpG methylation, the CHG methylation was also considered. Differentially CHG methylated genes were clustered in pathways related to endothelial cell homeostasis, as well as to VSMC-EC adhesion.
Taken together, these data encourage to hypothesize bAVM vascular differentiation loss as the result of a defective communication during neurovascular coupling.

EXERCISE-INDUCED EXTRACELLULAR VESICLES IN BREAST CANCER: UNVEILING BIOMOLECULAR PATTERNS AND ENHANCING THERAPEUTIC RESPONSES

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Increasing evidence suggests that regular physical activity (PA) reduces the risk of breast cancer (BC) and improves functional capacity, treatment efficacy, and disease outcomes in BC patients. These effects are, at least partially, mediated by the secretome of tissues responding to exercise. The secreted molecules can be released carrier-free or enclosed in extracellular vesicles (EVs). Although PA appears to influence EVs and their molecular landscape, their fates, utility, and roles in many diseases, including BC, remain largely unknown.

Here, we studied the impact of a well-structured exercise program on BC biology, specifically exploring the role of EVs released during PA in BC patients undergoing medical treatment. After surgery and before beginning medical treatments, forty newly diagnosed BC patients (ages 5065) were divided into a control group (CG, n=20) undergoing usual care and an exercise group (EG, n=20), which additionally performed a PA program.

Plasma EVs were isolated and characterized before and after the 16-week exercise program. In vitro experiments using human mammary epithelial cells (MCF10A) and BC cell lines (MCF7 and MDAMB-231) were utilized to assess the effects of exercise-induced EVs on cancer features, including proliferation apoptosis, migration/invasion, drug sensitivity, and metabolomic aspects. Finally, EVs' cargo analysis and functional validation of "healthy" molecules (proteins and ncRNAs) related to PA were conducted.

Studying the impact and role of these biomolecules on fundamental cellular processes allows us to better understand the biology of cancer. Furthermore, the identification of EV cargo modulated by PA opens the possibility of using them as innovative and integrative therapies based on exerciseinduced biomolecules in the near future.

EXPLORING THE COMPLEXITY OF REDOX SIGNALLING: IMPLICATIONS FOR EXTRACELLULAR VESICLE BIOGENESIS IN EXERCISE-INDUCED ADAPTIVE RESPONSES

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Regular and moderate physical activity induces a plethora of physiological changes to which skeletal muscle tissue shows a great adaptive capacity. These adaptations depend on the activation of specific cell signalling pathways, such as those involving redox homeostasis. In this scenario, hydrogen peroxide (H2O2) stands out, amongst reactive oxygen species (ROS), as a key signalling molecule capable of stimulating muscle adaptive responses to exercise in a dose-dependent manner. In recent years, the growing body of evidence regarding the prominent role of extracellular vesicles (EVs) in cell-cell communication, has provided a powerful tool to better investigate the molecular mechanisms underlying the complex and not yet fully characterised phenomenon of exercise adaptation. Here, we suggest that EVs biogenesis, transport and release, as well as EV cargo, could be affected by redoxsensitive modulation in muscle cells. To this aim, C2C12 myoblasts and myotubes were exposed to increased physiological doses of H2O2 for 1 hr. Preliminary results show H2O2-induced modulation in the expression of markers of EVs biogenesis (TSG101, ALIX, CD9, CD63, CD81, Rab GTPase and SNARE families' members) at both protein and RNA levels. Based on the assumption that EVs released from muscle cells may serve to facilitate neighbouring cells in their adaptation to oxidative eustress conditions, data on targeted protein analyses of EVs cargo will be presented and discussed.

IN VITRO EXPOSURE TO DIFFERENTIATION TREATMENT REDUCES NSCLC PATHOGENIC TRAITS

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Non-small cell lung cancer (NSCLC) relapse after therapy has been linked to the high aggressiveness, chemoresistance and metastatic potential of tumour cells due, in part, to the presence of cancer stem cells (CSCs), which possess characteristics of self-renewal and differentiation. Pro-differentiation approaches have shown promising results in leukemia and more recently in some solid cancer models, offering a possible route to enhancing the effects of current anti-proliferative therapies. In the present study the NSCLC cell line A549 was exposed to a serum-containing medium supplemented with pro-differentiation factors (DM), and effects on the proliferative, migration and adhesion properties of the cells were assessed in vitro, alongside the analysis of stemness marker expression levels after treatment in 2D or 3D culture conditions. A549 cells exposed to DM exhibited notable morphological changes and significant inhibition of cell proliferation and migration, whereas adhesion properties increased. DM treatment further caused a significant reduction in clonogenic ability (2D) and in spheroid growth (3D), alongside a reduced expression of stemness markers Sox2, Nanog and CD44. These results suggest a decrease in pathogenic features of NSCLC cells after exposure to differentiation medium, indicating that pro-differentiation treatments may represent an option of interest for further preclinical testing.

SPERM AROMATASE AND ITS PRODUCT 17-β ESTRADIOL ACT AS TRANSDUCERS OF PATERNAL EPIGENETIC MESSAGES BY AFFECTING EGG ESTROGEN CONTENT.

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Paternal eating habits, before and at conception, have a strong impact on offspring future metabolism. In murine models, pre-conceptional paternal diet and obesity impact on offspring development and metabolism and increase the risk of diabetes and cardiovascular diseases. By sending specific epigenetic signals through spermatozoa, paternal overnutrition influences developing embryos and promotes hypoandrogenism, growth retardation and diabetes in male pups. The identification of paternal epigenetic signals acting as vectors of intergenerational transmission is of pivotal interest to interrupt the consequence of intergenerational inheritance on offspring health. Until now, DNA methylation at CpG islands, small non-coding RNAs and chromosome architecture have been identified as vehicles of parental epigenetic messages. We here use high resolution mass imaging of murine gametes to show that sperm cyp19a1/aromatase and its hormonal product 17-β estradiol (both overproduced in the gonads of obese males) continue acting during egg fertilization and are able to modulate eggs’ estrogen content. This activity results in a paternal epigenetic message affecting embryo DNA methylation and embryo development ultimately compromising pancreatic β-cells expansion and insulin production.

HDAC1/2 CONTROL MESOTHELIUM/OVARIAN CANCER ADHESION IMPACTING ON TALIN-1-A5B1-INTEGRIN MEDIATED ACTIN CYTOSKELETON AND ECM PROTEIN REMODELING

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Peritoneal metastasis, which accounts for 85% of all epithelial ovarian carcinoma (EOC) metastases, is a multistep process that requires the establishment of adhesive interactions between cancer cells and the peritoneal membrane. Interrelations between EOC and the mesothelial stroma are critical to facilitate the metastatic process. No data is available so far on the impact of histone acetylation/deacetylation, a potentially relevant mechanism governing EOC metastasis, on mesothelial cells (MCs)-mediated adhesion.
In this study, treatment of MCs with non-cytotoxic concentrations of MS-275-a Histone deacetylase (HDAC)1-3 pharmacological inhibitor currently used in combination trials, caused a consistent reduction of EOC-MC static adhesion and peritoneal clearance. Proteomic analysis revealed several pathways altered upon MC treatment with MS-275, including ECM deposition/remodeling, adhesion receptors and actin cytoskeleton regulators. HDAC1-2 inhibition by genetic silencing hampered actin cytoskeleton polymerization by downregulating actin regulators including Talin-1, impairing β1 integrin activation, and leading to abnormal extracellular secretion and distribution of Fibronectin-1, as shown by the use of decellularized matrices. Talin-1 ectopic expression rescued EOC adhesion to MS-275-treated MCs. In an experimental mouse model of metastatic EOC, MS-275 limited tumor invasion, Fibronectin-1 secretion and the sub-mesothelial accumulation of MC-derived carcinoma associated fibroblasts.
Overall, our study unveils a direct impact of HDAC-1/2 in the regulation of MC/EOC adhesion and highlights the regulation of MC plasticity by epigenetic inhibition as a potential target for therapeutic intervention in EOC peritoneal metastasis.

MECHANISM OF PAROXETINE-MEDIATED AUTOPHAGIC INDUCTION IN CELL MODELS OF AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA

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Protein aggregation is a hallmark of neurodegenerative diseases, including amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD). The *C9ORF72*-hexanucleotide expansion G4C2 represents one of the most frequent genetic causes of ALS/FTD diseases. One molecular mechanism underlying *C9ORF72*-pathology is the accumulation of dipeptide repeats (DPRs) encoded by the G4C2 expansion. Additionally, TDP-43 inclusions are present in 98% of ALS and 50% of FTD cases. Persistent aggregates cause physical damage and sequester intracellular components, leading to cell damage. For this reason, enhancement of the proteostasis network represents a therapeutic approach in ALS/FTD.

Here, we dissected the mechanism of autophagic induction of the antidepressant paroxetine in motoneuron-like cells. We show that paroxetine induced the expression of autophagic markers LC3, SQSTM1/p62 and LAMP1, and enhanced the autophagic flux. Further analyses revealed that paroxetine was able to induce lysosomal membrane permeabilization. This event triggered the activation of Transcription Factor EB (TFEB), a master regulator of autophagy, previously shown to induce lysophagy, a selective form of autophagy for the turnover of damaged lysosomes.

Therefore, we tested if paroxetine-mediated autophagic induction favored the clearance of protein aggregates. Indeed, we observed a decrease in high molecular weight insoluble species and aggregates of proteins associated with ALS/FTD. In conclusion, these results indicate that the mechanism underlying paroxetine effects on autophagy involves TFEB-activity in response to lysosomal permeabilization. By activating autophagy, paroxetine displays a beneficial effect in cell models of ALS/FTD.

DIFFERENT PATHWAY DYSREGULATIONS CORRELATE WITH DISTINCT PLEURAL MESOTHELIOMA HISTOTYPES.

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The mechanism leading to the onset of pleural mesothelioma (PM), a fatal cancer linked to asbestos exposure, remains largely elusive due to multiple genetic mutations and pathways dysregulations. There are three main PM subtypes, epithelioid, biphasic, and sarcomatoid, characterized by different survival rate, 19, 12 and 4 months, respectively. Significant dysregulation of epithelial-mesenchymal transition (EMT) and signaling pathways, such as Hippo and Wnt/β-catenin have been reported in PM. This study aimed to elucidate molecular mechanisms underlying different PM histotypes through the analysis of genes involved in key cell transformation pathways, in order to simplify the classification of PM into two only histotypes, epithelioid and non-epithelioid, as recently proposed. To this end, gene expression was investigated in PM cell lines by droplet digital PCR (ddPCR). PM cells were from epithelioid (IST-Mes2, MPP89), biphasic (MSTO-211H), and sarcomatoid (PPM-Mill) histotypes. Our molecular investigation revealed different expression patterns of EMT-related genes, with epithelioid cell lines showing higher E-cadherin, N-cadherin, and fibronectin 1, and lower Vimentin gene expressions compared to non-epithelioid PM. Additionally, in epithelioid cell lines the Hippo pathway tested dysregulated, with a high YAP gene expression. Western blot analyses were in agreement with most of the ddPCR data, with discrepancies in β-catenin and TAZ gene expressions. These findings indicate a complex interplay of different molecular pathways in PM, which are linked to distinct histotypes and cancer cell proliferation. In conclusion, new pathways dysregulations tested herein may account for specific cellular and molecular characteristics of PM contributing to a better characterization of epithelioid and non-epithelioid histotypes.

POLYCOMB REPRESSIVE COMPLEX 2 DYSREGULATION IN NEUROFIBROMATOSIS TYPE 1: BESIDES NF1 FURTHER PLAYERS INVOLVED IN PATHOGENESIS?

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Neurofibromatosis type 1 (NF1) is a tumor-prone genetic disease characterized by neurocutaneous manifestations. The classic form of NF1 (cNF1) is caused by heterozygous loss-of-function variants in the *NF1* gene, while 5-11% of patients show NF1 microdeletion syndrome (NF1 MDS) due to deletions of *NF1* and a variable number of flanking genes. The type-1 NF1 deletion, present in 70% of NF1 MDS patients, includes the *SUZ12* gene, a component of Polycomb Repressive Complex 2 (PRC2), which regulates the transcriptional repression of several genes, often of oncological significance. Interestingly, we previously characterized an atypical microdeletion generating *RNF135-SUZ12* chimeric gene in a patient (PT 171) with an early onset of multiple neurofibromas. To investigate whether the deletion of *SUZ12* may have a role in altered PRC2 activity, possibly related to the NF1 tumor phenotype, we evaluated by qPCR assays the expression levels of PRC2 components and target genes on PBMCs from 11 type 1 NF1 MDS patients, 95 cNF1 patients, and 10 healthy donors.

Expression analysis confirmed the deregulation of PRC2 components in all the NF1 patients, showing *SUZ12* hyper-expression in cNF1 patients and hypo-expression in NF1-deleted patients, and increased expression of *EZH2* and decreased *EED* in the entire NF1 cohort, compared to controls. We also found a statistically significant hypo-expression of 5 and 6 out of 6 analyzed PRC2 target genes in NF1 MDS and cNF1 patients, respectively. The deregulated expression of PRC2 targets suggests a higher transcriptional repression activity mediated by PRC2 that could be linked to the increase of *EZH2*, the catalytic subunit of PRC2, possibly caused by the RAS pathway perturbation.

Functional studies will unravel the role of PRC2 in the NF1 pathogenesis, providing new insights to identify novel druggable target genes.

IN VIVO RARE BRAIN TUMOR MODELING USING *DROSOPHILA MELANOGASTER*

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Brain tumors originating from neural and glial compartments impose a heavy burden on patients and our society. Modeling of rare forms of brain tumors in invertebrate model represents a practical approach to elucidate the genetics of tumors, to uncover potential pathogenic mechanism and to identify future therapeutic options. In the vaccarilab.unimi.it we have recently focused on developing and exploiting models of rare tumors using the fruit fly *Drosophila melanogaster.* In recent years, we have adopted a model of gliomagenesis based of ectopic activation of EGFR and PI3K, two genes controlling pathways coopted by glioblastoma. Using such model, we have defined the role of autophagy and of the vacuolar-ATPase in supporting tissue overproliferation.
More recently, in collaboration with the group of Valentina Massa (Unimi), we have tested whether reduction of cohesin activity could promote tumorigenesis. To this end, we have found that reducing expression *Stromalin (SA),* encoding the ortholog of the core cohesin complex component Stag2, leads to a delay in differentiation of type II neurblasts originated from neural stem cells in the larval brain. Such delay correlates with formation of occasional tumor-like brain masses and reduced lifespan. Consistent with a known role of cohesin protein in DNA repair, we find that reduced *SA* expression results in persistent DNA damage. Thus, we treated animals with Poly ADP ribose polymerase (PARP) inhibitors. We observe that such manipulation or depletion of *Drosophila Parp1* increased genotoxic stress, but rescues the effects of reduced *SA* expression. Considering that we have found that occasional *STAG2* mutations are presents in paediatric Medulloblastoma patients, our unpublished data suggest that PARP1 inhibition could ameliorate tumor-relevant defect associated to cohesin deficiency.
In general, our *in vivo* studies reveal novel tumor-promoting or -soppressive roles of core cellular functions that might represent new targets for future pharmacologic interventions.

TYPE 1 DIABETES ALTERS RAT TESTICULAR ACTIVITY AND SPERM QUALITY PERTURBING THE BLOOD-TESTIS BARRIER AND MICROTUBULE CYTOSKELETON

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Among the causes of reduced fertility, type 1 diabetes (T1D) is frequently correlated with damaged testicular activity. Many studies reported that T1D mainly acts by increasing oxidative stress and impairing the hypothalamus pituitary-testis axis, with consequently altered spermatogenesis and reduced sperm parameters. Herein, using the T1D rat model, several parameters of testicular activity were analyzed. Data showed that T1D induced oxidative stress and, consequently, alterations in both testicular somatic and germ cells, as highlighted by enhanced apoptosis, altered steroidogenesis, and impaired spermatogenesis. In addition, the blood-testis barrier integrity was compromised, as indicated by the reduced levels of structural proteins (N-Cadherin, ZO-1, occludin, connexin 43, and VANGL2) and the phosphorylation status of kinases (Src and FAK). T1D also affected the dynamics of somatic and germ cell microtubule cytoskeleton, as revealed by the altered protein levels of microtubule-associated proteins and reduced sperm motility. Further, the dysregulation of the SIRT1/NRF2/MAPKs signaling pathways was proved, particularly, the reduced nuclear translocation of NRF2, affecting its ability to induce the transcription of antioxidant genes. Finally, the stimulation of testicular inflammation and pyroptosis was also confirmed. Interestingly, immunofluorescent data revealed that NLRP3 was associated with the developing acrosome system, assuming a dot-shaped conformation in round spermatids while, in T1D, its extension and signal intensity increased. The combined data allowed us to confirm that T1D has detrimental effects on rat testicular activity. Moreover, a better comprehension of the molecular mechanisms underlying the association between metabolic disorders and male fertility could help to identify novel targets to prevent and treat fertility disorders related to T1D.

IN VITRO AMELIORATION OF THE SENESCENT PHENOTYPE OF HUMAN GRANULOSA CELLS: ROLE OF NAD^+ BOOSTER STRATEGIES

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Ovarian aging is characterized by the progressive loss of ovarian function with increasing age. Over the past few years, NAD^+ has emerged as a potent regulator in reducing functional decline and agerelated diseases. The relationship between NAD^+ levels and ovarian aging has progressively emerged, although the molecular mechanisms of beneficial effects of NAD^+ supplementation remain to be fully elucidated. Our goal was to established an *in vitro* model of senescent human granulosa cells (hGCs), characterize senescence-associated changes of NAD^+ metabolism, and evaluate the effects of different concentrations of NAD^+ booster molecules.
Senescence was induced by intermittent exposure of hGCs (KGN cell line) to 600 μM H_2O_2 for 144 h, and validated by increased SA-β-Gal activity, high levels of p21, p53 proteins and reduced SIRT1 expression. In comparison to control hGCs, senescent hGCs presented lower NAD^+/NADH ratio (NAD^+/NADH-GloTM Assay, Promega) and higher amount of ROS, quantified by MitoSOX™ Red (Thermo Fisher Scientific). Then, senescent hGCs were cultured in the presence of non-toxic doses of NAD^+ boosting molecules, nicotinamide riboside (NR, 0.3-300 μM), nicotinamide (NAM, 0.3-300 μM) and P7C3 (0.5-30 μM), the activator of NAMPT (nicotinamide phosphoribosyltransferase), the rate limiting enzyme of NAD^+ salvage pathway. Analysis of cell proliferation revealed beneficial effects of NR after 24 and 72 h. NR and NAM were able to revert partially the effect of senescence on NAD^+/NADH ratio, ROS production, and p21, p53 and SIRT1 expression. Taken together, our findings lay the foundation for future research aimed to clarify the efficacy of NAD^+ booster supplements as an anti-aging approach, in order to support their application in clinical settings to improve the quality of the follicular environment in patients with advanced maternal age.

ANTI-INFLAMMATORY EFFECTS OF OLIVE LEAF EXTRACTS ON ENDOTHELIAL CELLS FROM WOMEN WITH GESTATIONAL DIABETES

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Gestational diabetes (GD) affects the health of mothers and offspring, often leading to cardiovascular complications. Management focuses on blood sugar control through diet and lifestyle changes. The identification of bioactive compounds from agricultural waste is now a fundamental topic in the context of environmental protection. Olive leaf extracts (OLE) are a potential source, as their anti inflammatory, hypoglycemic, and antioxidant properties show promise for improving metabolic health and reducing cardiometabolic risks. However, further research is required. We investigated OLE's anti-inflammatory effects on umbilical vein endothelial cells from diabetic women (GD HUVEC), which show a pro-inflammatory phenotype due to chronic hyperglycemia. Initial experiments analyzed the antioxidant phenolic profile of OLE from both cultivars using ultrasound assisted extraction and characterization by HPLC-ESI-TOF-MS. Changlot Real and Picual extracts contained 14.7 and 8.7 mg/g of phenolic compounds, respectively. Various concentrations were tested by assessing cellular vitality (MTT). GD-HUVEC and control cells from healthy women (C-HUVEC) were then pre-treated with OLE (0.1, 10, and 50 μg/mL) for 24 hours before exposure to low levels of the inflammatory cytokine TNF-α (10 ng/mL) for 16 hours. Protein expression of vascular adhesion molecule (VCAM-1, flow cytometry), monocyte-endothelium adhesion and expression of inflammatory markers were evaluated.

These preliminary data show that pre-treatment with Changlot Real and Picual OLE reduces VCAM 1 protein expression ($p<0.05$) and monocyte adhesion ($p<0.001$), suggesting their anti-inflammatory potential. This highlights the importance of bioactive-rich natural extracts in improving food function, reducing inflammation, and enhancing vascular health, particularly for high-risk groups like gestational diabetic women.

DELETION OF THE HR-DSCR IN FIBROBLASTS WITH T21 USING CRISPR-CAS9 SYSTEM: A NEW CELL MODEL

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The highly restricted Down syndrome critical region (HR-DSCR), located on chromosome 21 (21q22.13; 34 kbp long), has been identified as a candidate genomic region for the onset of intellectual disability in DS. The HR-DSCR is partially crossed by two newly identified isoforms of *DSCR4* and *KCNJ6* genes which properties and functions have yet to be elucidated.
We created a new cell model with trisomy 21 (T21) and whole HR-DSCR deletion (T21 E-HRDSCR) using the NHEJ CRISPR-Cas9 system in three T21 fibroblast cell lines. The application of this technique was improved analysing the genome sequence by the NCBI Genome Browser database. We also created a positive deletion control of comparable length of the *DYRK1A* gene (T21 EDYRK1A), which includes the catalytic domain of the protein. To validate the cell model, indel and off-target effects were tested by Sanger sequencing and deletion efficiencies by ddPCR. The relative expression of *DYRK1A* and *KCNJ6-201* was assessed by RT-qPCR.
The ddPCR revealed mean deletion efficiencies of 80.6% for *DYRK1A* and of 58.8% for HR-DSCR. Sanger sequencing showed that deletions were repaired without indels, and no off-target effects occurred. The RT-qPCR showed decreased *DYRK1A* transcript expression in T21 E-DYRK1A cell lines (fold changes= 1.9) consistent with the loss of one copy of the gene in almost all cells. *KCNJ6201,* an isoform expressed in fibroblasts and located directly upstream to HR-DSCR, does not seem to be affected by deletion of the HR-DSCR (fold changes= 1.0).
The creation of this new cell model offers the possibility to investigate the molecular mechanisms in which HR-DSCR and *DYRK1A* might be involved, by studying difference in gene expression and metabolite levels compared to the full T21 condition. The model setting could be applied to other cell types to explore the phenotypic effects of HR-DSCR in DS.

A LIGHT-RESUMING STRATEGY AS A NEW SCREENING METHOD FOR SEC61 INHIBITORS: TOWARDS THE DISCOVERY OF SMALL MOLECULES DOWNMODULATING PD-L1 EXPRESSION

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

EPIGENETIC SILENCING OF TFF1 DURING *Helicobacter pylori* CHRONIC INFECTION

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Over half of the world's population has been estimated to be infected by *Helicobacter pylori*; the peculiarity of this pathogen is that it can persist for decades in the harsh stomach environment, where it causes a strong inflammatory response that, if not solved, elicits damage to the gastric mucosa by helping the progress of malignant transformations in a process where multiple factors are involved, such as the silencing of tumor-suppressor genes expression. In response to the infection, the gastric mucosa releases several protective factors, including Trefoil Factor 1 (TFF1). This peptide participates both in maintaining gastric integrity and in retaining *H. pylori* in the mucus layer, limiting its motility toward the epithelial layer and, therefore, the infection, so it is considered a protective factor of the gastric mucosa.

We showed that TFF1 is downregulated during chronic *Helicobacter* infection, so we are currently investigating the molecular mechanisms driving its silencing. We identified the role of the transcription factor C/EBPβ as a repressor of the TFF1 gene and the essential contribution of the IFNγ, which could turn TFF1 transcription off for up to 72 hours also after a short treatment: this suggests that epigenetic modifications, such as DNA methylation and histone modifications, might play a role in this long-term silencing.

Currently, we are studying these aspects using "mucosoids", a model of polarized primary gastric cells that reproduces key features of normal human gastric epithelium and offers the advantage of recapitulating a prolonged *H. pylori* infection.

THE ROLE OF EXTRACELLULAR VESICLES (EVS) IN LONG COVID: IMPACT ON GENE EXPRESSION AND FIBROSIS

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Long COVID (or post-COVID-19 syndrome) refers to a condition where individuals who have contracted the SARS-CoV-2 virus continue to exhibit symptoms for weeks or months after the acute phase of the infection. Given the importance of extracellular vesicles (EVs) in cellular crosstalk, we aimed to evaluate the impact of EVs from long COVID patients on the modulation of gene expression in various cell types, including lung, skeletal muscle, aortic smooth muscle and mesenchymal cells.
Thus, we investigated the effect of EVs from long COVID patients on gene expression modulation in different cell types. EVs were isolated from the plasma of long COVID patients and healthy donors and characterized using Nanoparticle Tracking Analysis (NTA). Cells were cultured in the presence of EVs from both healthy donors or long COVID patients. As it has been reported that RUNX2 can be involved in fibrotic processes, the focus of our study was on the modulation of RUNX2.
A significant increase in RUNX2 was observed in lung, skeletal muscle, and aortic smooth muscle cells exposed to EVs from long COVID patients. Similar increases in RUNX2 expression were observed in mesenchymal cells, sustained for up to 14 days. Additionally, a significant decrease in miR-34a expression was detected in EVs from long COVID patients compared to healthy controls.
The increased RUNX2 expression across various cell types indicates a potential induction of fibrosis by EVs from long COVID patients. The decreased levels of miR-34a in long COVID EVs suggest a regulatory imbalance that may contribute to fibrosis and other long-term pathological effects.
Our findings highlight the significant impact of EVs from long COVID patients on gene expression related to fibrosis, particularly the sustained increase in RUNX2 and the decrease in miR-34a levels. These results suggest that EVs play a crucial role in the pathophysiology of long COVID, potentially driving fibrosis in various tissues.