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Abstracts

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Speakers' Abstracts

In chronological order of presentation
(presenting authors are shown underlined)

Hippo pathway regulation by Bcl-2 in melanoma cells

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Melanoma represents one of the most aggressive forms of skin cancer with an increasing occurrence worldwide, often characterized by resistance to standard therapies, finally causing patient death. For these reasons, there is an urgent need to find novel druggable targets to improve melanoma treatment strategies. We have previously demonstrated that the anti-apoptotic members of the Bcl-2 family proteins, such as Bcl-2, Bcl-xL and Bcl-2L10 can enhance disease aggressiveness and progression through their non-canonical functions, promoting the development of a tumor microenvironment that favors metastasis formation, angiogenesis, tumor stage advancement and resistance to therapies. Here, our aim was to delineate a transcriptional signature and novel cellular pathways uniquely modulated by Bcl-2 respect to Bcl-xL in human melanoma cells. To this end, we performed RNAseq analysis after siRNA-mediated transient knockdown of Bcl-2 or Bcl-xL. Subsequently, we conducted a gene ontology analysis to unveil a distinct Bcl-2 transcriptional fingerprint, finding involvement in the modulation of different genes belonging to the Hippo pathway. Further validation was carried out through qRT-PCR assays to confirm the expression of genes influenced by Bcl-2 and associated with the Hippo pathway. Additionally, Western blotting analysis was employed to study the protein expression of upstream regulators of YAP in relation with varying levels of Bcl-2 protein. The impact of YAP silencing in Bcl-2 overexpressing melanoma cells was functionally assessed through *in vitro* migration assays, demonstrating that YAP can be one of the mediators of Bcl-2 functions. In conclusion, our findings suggest that the interplay between Bcl-2 and the Hippo pathway could be exploited for design novel therapeutic strategy.

The *in vivo* characterization of EPSIN 3 in breast cancer tumorigenesis and metastasis

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Endocytosis is a crucial mechanism regulating cellular signaling and its dysregulation is linked to cancer. We showed that the endocytic protein Epsin 3 (EPN3) is overexpressed in 50% of breast cancers (BC) and amplified in 10%. EPN3 overexpression correlates with increased tumor invasiveness and distant metastasis. Interestingly, the well-characterized oncogene *HER2* is co-amplified with *EPN3* in half of the BC cases, suggesting the possible cooperation of these genes. By overexpressing EPN3 in non-transformed mammary epithelial cells, we showed that it induces a partial epithelial-to-mesenchymal transition (pEMT); a state linked to the expansion of the stem cell pool and to invasion. Mechanistically, EPN3 overexpression induces pEMT by enhancing ECAD endocytosis and turnover, and destabilizing cell-to-cell junctions. To better understand the role of EPN3 in mammary gland tumorigenesis, we generated a conditional EPN3 knock-in (KI) mouse model which revealed that although EPN3 overexpression is insufficient *per se* to induce mammary tumors, it alters the mammary morphogenesis by increasing ductal branching. This aberrant morphogenesis was associated with the upregulation of proliferation and an expansion of the stem/basal cell subpopulation. To investigate whether EPN3 and HER2 cooperate in tumor progression, we generated an EPN3-KI:MMTV-NeuN mouse overexpressing EPN3 and the HER2 rat homologue (NeuN) in the mammary gland. These mice displayed an accelerated tumor onset and pulmonary metastatic dissemination compared with MMTV-NeuN mice. We are now investigating the role of EPN3 overexpression in branching morphogenesis and invasive phenotypes by performing single cell RNA-seq and immunohistochemistry analysis of primary mammary epithelial cells and mammary organoids derived from EPN3-KI *vs*.WT mice. Additionally, we are using these cells to perform *ex vivo* assays to assess the role of ECAD endocytosis in determining EPN3-dependent tumorigenic and invasive phenotypes.

Unveiling the RUNX2 functions in thyroid cancer: from gene expression regulation to metabolic reprogramming

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Cancer progression depends on specific transcriptional programs, established by multilayered regulatory networks directed by transcription factors (TFs). RUNX2 is a TF involved in thyroid cancer (TC) metastatization, but the molecular mechanism describing how it supports cancer progression is still unknown. In this work, we used a multi-omics approach to explore the RUNX2 regulatory landscape in TC and its downstream effect on cancer-supporting biological processes. ChIP-seq analyses of RUNX2, RNA-PolII, and chromatin histone marks distribution were carried out to map and functionally characterize the RUNX2-associated genomic elements. RNA-seq profiling was performed on RUNX2-silenced cells and integrated with the ChIP-seq data to infer the RUNX2 transcriptional program. By combining our profiles with transcriptomic and clinical data retrieved from the TCGA-THCA project, we computationally reconstruct the RUNX2-regulatory network in TC. GO enrichment and correlation analyses underlined metabolism regulation as a RUNX2-dependent biological function significantly correlated to TC metastasis. Untargeted metabolomics and functional assays showed that the RUNX2-silencing lead to a profound reshaping of cancer cell metabolism. On one side, RUNX2 inhibits the oxidative capacity of cancer cells, by influencing mitochondria morphology and functionality. On the other, RUNX2 affects lipid metabolism by controlling the expression of several genes playing key roles in lipid biosynthetic pathways, including SREBF1 and SREBF2. We characterized the functional interplay between RUNX2 and SREBF1, showing that the two TFs cooperate in regulating the expression of a common subset of target genes. Overall, our data describe for the first time the RUNX2 transcriptional landscape in TC and identify a previously underscored function of RUNX2 in orchestrating the metabolic rewiring of these neoplasms.

RCoR2 sustains non-canonical oncogenic transcription as a member of the ADR neuroblastoma core regulatory circuitry

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Childhood high-risk neuroblastomas (NB) with MYCN gene amplification are difficult to treat effectively. This has focused attention on a set of critical dependency genes in MYCN-amplified NB that form tumor-specific core regulatory circuitry (CRC), auto-regulatory feed-forward networks that positively drive tumorigenic transcriptional programs and are essential for cell state and survival in this tumor.
Here, we identify RCoR2 as a new member of the adrenergic NB CRC. RCoR2 is mostly known as a component of CoREST repressive complexes (RCoR1/2/3:LSD1:HDAC1/2), yet paradoxically our genome-wide ChIP-seq analysis demonstrated that RCoR2 binds coordinately with other CRC members to regions of open chromatin marked by H3K27ac and Pol2. Additionally, RCOR2 is a super-enhancer regulated gene, it is a prognostic marker for NB and its expression is elevated in this malignancy compared to other tumor types, highly correlating with MYCN.
Moreover, as opposed to the other RCoR factors, RCoR2 seems to act as a potential and non-canonical transcriptional coactivator, since its downregulation causes a general decrease of transcription and a reduction of histone markers typically associated with active transcription. Finally, our HiChIP data suggest how RCoR2 could possibly act as an essential looping component to promote enhancer-promoter interactions and maintenance of the 3D chromatin architecture. Based on these findings we suggest an unprecedented model whereby RCoR2 could maintain a transcriptional counterbalance between histone deacetylases activity and core regulatory TFs, thus defining RCOR2 as a critical vulnerability in MYCN-amplified neuroblastoma.

CITK catalytic activity inhibition leads to DNA damage, cytokinesis failure and cell death in brain tumors

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Medulloblastoma (MB) and gliomas are the most frequent high-grade brain tumors (HGBT) in children and adulthood, respectively. The general treatment for these tumors consists in surgery, followed by radiotherapy and chemotherapy. These therapies are only partially effective, and many patients still die, making these diseases an unmet medical challenge. Citron kinase (CITK), product of the primary microcephaly gene MCPH17, is required in neural progenitor cells for cytokinesis, mitotic spindle positioning and chromosomal stability. In vivo studies in xenograft models and in SHH MB arising in transgenic mice have shown that CITK deletion inhibits tumor progression. On this basis, we are working on the development of CITK inhibitors as a possible strategy for HGBT treatment.
Stemming from published binding data between kinase inhibitors and the kinome, we discovered Lestaurtinib as CITK inhibitor. We therefore tested the biological effects of this inhibitor on different MB and GBM patient derived cell lines and in vivo injecting the drug in MBs arising in SmoA1 transgenic mice. In parallel, we developed a screening aimed at obtaining CITK specific inhibitors.
Similar to CITK knockdown, treatment of MB cells with 100 nM Lestaurtinib reduces phospho-INCENP levels at the midbody and leads to cytokinesis failure. Moreover, Lestaurtinib impairs cell proliferation, leads to accumulation of DNA double strand breaks and cell death in MB and GBM cells, recapitulating CITK knockdown effects . Finally Lestaurtinib treatment reduces tumor growth and increases mice survival. Moreover, our screening campaign produced several interesting hits that we are functionally and in vitro validating.
Reduced cell proliferation and increased mice survival indicate that Lestaurtinib and more specific CITK inhibitors are promising candidates for HGBT treatment, deserving deeper investigation.

It’s a match! Finding effective drug combinations for genetically defined cancer subtypes

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The identification of cancer-driver mutations and the consequent development of drugs that selectively target the effectors of the oncogenic signal, known as precision medicine, has reshaped cancer treatment strategies and clinical outcomes. Among cancer-driving oncogenes, *KRAS* is one of the most frequently altered, with mutations found in 11% of all human tumors, in particular pancreatic, colorectal (CRC), and non–small cell lung cancers (NSCLC). Therefore, many targeted therapies have focused on directly inhibiting mutant KRAS as well as kinases in the downstream signaling pathways, like the RAF-MEK-ERK (MAP Kinase) pathway and the PI3K-AKT pathway. Unfortunately, targeted therapies that act in the MAPK or PI3K signaling routes, are often ineffective as single-agents, due to adaptive engagement of feedback mechanisms that reactivate the same mitogenic pathway, or a parallel one. Identifying and disabling such feedback mechanisms by concomitant inhibition of key effectors can be highly successful in improving clinical responses. For example, inhibition of mutant BRAF in colon cancer leads to feedback activation of EGFR, precluding a response to BRAF inhibitor monotherapy, but the combination of BRAF and EGFR inhibitors is highly effective, and now an approved therapeutic strategy for these tumors. By applying both rational combinations and CRISPR-based genetic screenings, we have uncovered multiple effective “matches” to improve the efficacy of MAPK inhibitors or of the new generation direct RAS inhibitors, in KRAS-mutant tumors. Those include both “usual suspects” like the SHP2 phosphatase or the mammalian target of rapamycin (mTOR), as well as novel targets like MAP2K4. In parallel, we are carrying out efforts to translate those combinations in the clinical setting.

Melusin modulation of AKT-GSK3β signalling axis mitigates cancer cachexia-induced skeletal muscle atrophy

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Background: Cachexia-induced skeletal muscle atrophy is the most severe comorbidity associated to advanced cancer and still represents an unmet clinical need. Chaperone proteins exert pivotal functions in cellular proteostasis and recent data suggest their involvement in different type of muscle atrophy. Herein, we evaluated whether the chaperone protein Melusin exerts an anti-atrophic role in cancer cachexia-induced muscle atrophy.
Methods: Cachexia-induced muscle atrophy was induced by subcutaneously injecting 10^6 of colon 26 (C26) cancer cells in BALB/c mice. Atrophy occurrence was evaluated by measuring both skeletal muscles weight and myofibers cross-sectional area (CSA). Melusin overexpression was achieved by intramuscular injection of AAV-9 vectors carrying human Melusin construct. RT-qPCR, Western blot, immunofluorescence, co-immunoprecipitations, proximity ligation assay and gel filtration chromatography were utilized to investigate the molecular role of Melusin in cancer cachexia-induced muscle atrophy.
Results: Melusin expression is significantly reduced during cancer cachexia-induced muscle atrophy. Remarkably, AAV9-mediated Melusin overexpression in C26 tumor-bearing mice partially protects from cachexia-induced muscle weight reduction, CSA reduction and atrogenes rise. Melusin overexpression blocks GSK3β activation that occurs during muscle atrophy. Specifically, Melusin binds AKT, favouring its inhibitory phosohrylation on GSK3β. Melusin-mediated GSK3β inhibition leads to β-catenin accumulation and rise of its pro-trophic gene targets, c-Myc and Cyclin D1.
Conclusions: Melusin maintenance counteracts cancer cachexia-induced skeletal muscle atrophy by modulating, via AKT interaction, GSK3β-βcatenin signalling axis.

The mitotic protein CENP-F is functionally repurposed during neurodifferentiation

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Centromere protein F (CENP-F) is a large multidomain protein that interacts with kinetochores, microtubules and motor proteins during mitosis. CENP-F mutations are also growingly identified in neurodevelopmental disorders (NDDs) associated with primary microcephaly (MCPH), suggesting that CENP-F may be functionally repurposed in cells that exit mitosis and undergo neurodifferentiation. The mechanism through which mutant CENP-F may contribute to these NDDs however remains elusive.
We have now characterised three neurogenesis-relevant aspects in which CENP-F is implicated.
First, we focused on a C-terminal region of CENP-F, within which viable MCPH mutations fall. That region contains a nuclear localisation signal (NLS) flanking a KEN box driving ubiquitin-dependent proteasomal degradation at mitotic exit. Our results show that “saturating” the NLS by overexpressing the nuclear transport factor importin beta impairs both CENP-F localisation at the microtubule/kinetochore interface during mitosis, and its degradation at mitotic exit. Both of these defects can impair cell division in neuronal precursors.
Second, we examined CENP-F in cell cycle-arrested cells that form the primary cilium, a crucial structure with architectural roles during the organisation of the cortical layers. We find that CENP-F is required for the formation of a functional axoneme and for proper positioning of the cilium.
Third, by following up CENP-F during neurodifferentiation, we have identified a fraction that localises at MT-based structures required for neurite formation and branching.
These findings suggest that CENP-F acts at multiple steps during neurodifferentiation, potentially affecting the proper generation of the neuronal precursor pool, the process of ciliogenesis, and the formation of neuronal networks. These results begin to shed some light on processes through which CENP-F mutations can yield neurodevelopmental disorders.

The cytoskeleton regulator inverted formin INF2 regulates the SHH pathway and is involved in medulloblastoma tumorigenesis

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Medulloblastoma (MB) is a lethal pediatric malignancy of cerebellum. Identifying effective therapies for MB is extremely difficult, mainly due to its high molecular heterogeneity. The Sonic Hedgehog subgroup (SHH-MB) is the most abundant and genetically understood. SHH-MB is characterized by mutations in the key components of SHH signaling, a developmental pathway emerged as an attractive therapeutic target for MB treatment. However, the molecular mechanisms governing SHH-MB remain unclear. Recently, defects in cytoskeleton remodeling are emerging as an important hallmark of cancer.
Here, we identified INF2, a formin involved in the regulation of actin cytoskeleton dynamics, as a negative regulator of SHH signaling, with a putative opposite effect to the one previously described for mDia formins. We found that the overexpression of INF2 counteracts the positive effects of mDia on GLI1 (the final effector of SHH signaling) by reducing its activity. Accordingly, the genetic silencing of INF2 increases mRNA and protein levels of GLI1 as well as the proliferation of granule neuronal progenitors (GNPs), the cells of origin of MB. Moreover, a correlation between increased INF2 protein expression and time dependent switching off of the SHH pathway was observed during normal cerebellum development in mice, showing an opposite trend to mDia. Interestingly, INF2 protein levels were strongly reduced in murine and human SHH-MB samples, contrary to what observed for mDia. Notably, the overexpression of INF2 in SHH-MB primary cells significantly inhibits the cell proliferation as consequence of the reduction of GLI1 expression levels, and increases the stiffness, thus suggesting that the absence of INF2 affects tumor cell motility and invasiveness. Overall, these findings unveil INF2 as new player of the SHH pathway paving the way to study cytoskeletal remodelling proteins as a novel area of investigation in SHH-MB.

Role of TBC1D7 in lipid metabolism in breast cancer cells

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TBC-containing proteins are key players in membrane trafficking and cell metabolism. To evaluate the involvement of TBCs in breast cancer aggressiveness and metabolic rewiring we investigated the prognostic values of alterations of TBCs genes using the METABRIC database. We found that high mRNA levels of four TBCs correlate with worse survival in multivariate analyses. Among them, we focused on TBC1D7 because this gene is mostly expressed in glucose-avid Triple-negative tumours and its functional depletion reduces the cell energy metabolism. TBC1D7 binds to TSC1 and stabilize the TSC1/TSC2 complex, which negatively regulates mTORC1. However, additional TSC-independent functions have also been proposed prompting us to include, in our study, a mutant form of TBC1D7 that does not bind to TSC1. Metabolic tracing analyses reveals that cells overexpressing TBC1D7 and its mutant have elevated glucose metabolism which is used to generate fatty acids, while glutamine is mostly employed to sustain the Tricarboxylic Acid cycle. Accordingly, the lipid storage compartment is increased in these cells. Moreover, overexpression of TBC1D7 and its mutant stimulates the growth of tumour cell spheroids, a pro-neoplastic feature that mirrors the increased levels of TBC1D7 found in the most aggressive breast tumours. Treatment of the TBC1D7 overexpressing cells with an inhibitor of the lipid enzyme SCD1, which impairs the formation of lipid droplets, blocks spheroids overgrowth. In addition, TBC1D7 delays autophagy, an activity might contribute to the accumulation of intracellular lipids. Altogether these data suggest that TBC1D7 promotes *de novo* lipogenesis and lipid consumption, independently from the mTORC1 pathway, contributing to breast cancer cell growth.

3D Gastruloids: stem cell embryo models for in vitro teratogenicity testing

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Gastruloids are polarized 3D cellular aggregates generated *in vitro* from pluripotent stem cells, which display the axial organization of post-implantation embryos. 3D gastruloids are obtained by seeding a defined number of embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) in ultra-low attachment plates to generate spherical cell aggregates, which undergo symmetry breaking, self-organize into the three germ layers and express the mesendodermal markers in a polarized manner . Gastruloids are emerging as robust model systems to study early embryonic development and to perform genetic and pharmacological/teratogenic screenings. We have developed a robust gastruloid formation assay (GFA) and evaluated the effect of glucocorticoids, including budesonide, dexamethasone, fluticasone and hydrocortisone on gastruloids development. We found that at a high micromolar regimen budesonide inhibits gastruloid elongation/polarization and prevents exit from naïve pluripotency by stabilizing cell-cell adhesions. This correlates with increased accumulation of E-cadherin (E-cad) at the cell membrane. Moreover, in the search for more active molecules, we used structural analogues of budesonide and identified a new compound, which favors the maintenance of pluripotency state and prevents 3D gastruloid elongation with higher efficacy compared to budesonide. Our findings indicate a possible negative impact of budesonide on embryo development and support the idea that gastruloids are a promising platform for drug screening and to identify potential teratogenic drugs.

Rejuvenating and repairing aged bones

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Half of all people will break a bone during their lifetime. Fracture repair can take years in some cases, limiting mobility and placing a significant burden on patients and health care systems alike. Therefore, developing strategies to improve bone health and repair, particularly for elderly individuals, is critical.
The Habib lab has recently engineered the bone stem cell niche to study human bone formation and pioneered novel bandages that can promote bone repair. In this presentation, Prof Shukry James Habib will discuss the advancement of these technologies and their application in aging research.

Selected recent publications:
Habib SJ and Acebrón SP (2022) Wnt signalling in cell division: from mechanisms to tissue engineering.*Trends Cell Biol*. 2022 Jun 15:S0962-8924(22)00137-4
Okuchi Y, Reeves J, Ng SS, Doro DH, Junyent S, Liu KJ, El Haj AJ, and Habib SJ (2021) Wnt-modified materials mediate asymmetric stem cell division to direct human osteogenic tissue formation for bone repair. *Nature Materials* Jan;20(1):108-118
Junyent S, Reeves J, Szczerkowski JLA, Garcin CL, Trieu TJ, Wilson M, Lundie-Brown J, and Habib SJ (2021) Wnt- and Glutamate-receptors orchestrate stem cell dynamics and asymmetric cell division. *eLife* 2021;10:e59791
Junyent S, Garcin CL, Szczerkowski JLA, Trieu TJ, Reeves J, Habib SJ (2020) Specialized Cytonemes Induce Self-Organization of Stem Cells. *Proc Natl Acad Sci U S A* 31;117(13):7236-7244

The RNA-binding protein PCBP2 is a regulator of microRNAs partition between cell and extracellular vesicles

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While it is accepted that Extracellular Vesicles (EVs)-mediated transfer of microRNAs contributes to intercellular communication, the knowledge about molecular mechanisms controlling the selective and dynamic partition of miRNAs between intracellular and EV compartments is still largely limited. As yet, the interactions between specific RNA-binding proteins (RBPs) and short RNA sequences, have been proved causal for the loading of multiple miRNAs in EVs. With respect to intracellular miRNAs, the interacting protein/s remained unknown although in silico and mutagenesis analysis demonstrated the presence/function of specific sequence determinants (the cellular CL-motifs and the CELL-motifs).
Here, the RBP Poly-C-binding protein 2 (PCBP2, also known as hnRNPE2 or αCP2), was identified as a direct interactor of the CELL-motif AUUA/G: RNA immunoprecipitation (RIP) after UV cross-linking, coupled to RNA pull down, demonstrated that this protein directly binds to miRNAs embedding this sequence and mutagenesis of the motif proved the specificity of its binding. Functionally, PCBP2 knock-down allows the EV-loading of specific intracellular microRNAs. Furthermore, a second requirement for PCBP2 specific binding was identified in SYNCRIP, a previously characterized miRNA EV-loader. SYNCRIP and PCBP2 may contemporarily bind to miRNAs endowed of both hEXO and CELL motifs, as demonstrated by RIP and EMSA assays. Mechanistically, SYNCRIP knock-down appears to limit PCBP2 recruitment.
Overall, this body of evidence i) extends PCBP2 known pleiotropic functions to the role of intracellular determinant of miRNAs retention acting as a dominant inhibitor of SYNCRIP function and ii) highlights that multiple proteins/miRNA interactions govern miRNA compartmentalization.

A subset of oncomiRs released in melanoma EVs govern resistance to immunotherapy through the inhibition of CXCL9 cytokine

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Immunotherapy with Immune Checkpoint Inhibitors (ICIs) has become the most impactful therapy for patients with metastatic melanoma. However, drug resistance occurs in a significant proportion of patients mainly due to the development of an immunosuppressive tumor microenvironment (TME). During last years, miRNAs have emerged as orchestrators of resistance to therapy in melanoma. A miRNA-seq profiling analysis of 48 serum samples collected from melanoma patients before starting ICI therapy, conducted in our laboratory has led to the identification of a set of 19 miRNAs strongly upregulated in non-responder vs responder patients. This initial finding has inspired my PhD project. First of all, we have selected 4 miRNAs from the initial set of 19, as most promising candidates for further studies according to the highest level of deregulation and the association with patients’ overall survival based on TCGA data. Moreover, we found by digital PCR that these miRNAs are released by melanoma cells in extracellular vesicles (EVs). Of note, bioinformatics analyses unveiled that they regulate the expression of cytokines like CXCL9, which are pivotal for the recruitment of T-cell to the tumor. Interestingly, we found that high levels of CXCL9 are associated with a better response for melanoma patients treated with ICIs. Moreover, we have observed by luciferase assays the capability of one of these miRNAs to directly bind the 3’UTR of CXCL9 gene. The targeting has been confirmed also by measuring CXCL9 relative mRNA and protein levels by qRT-PCR and ELISA, respectively. These experiments have been performed by miRNA transient transfection on Tumor-associated macrophages (TAMs) because they are the main suppliers of CXCL9 within the TME. Finally, I also found that cell media derived from miRNA-transfected TAMs are less able to recruit T-cell using transwell migration assays. These promising results will be validated by exploiting co- cultures and advanced Organ on Chip technologies in order to deepen the crosstalk among melanoma cells-TAMs and T cells.

FRG2A is part of a novel family of lncRNAs affecting nucleolus-associated chromatin interactions and function in FSHD cells

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Facioscapulohumeral muscular dystrophy is a hereditary myopathy, associated with the reduction of copy number of the D4Z4 macrosatellite on chromosome 4q35 leading to the inappropriate expression of nearby genes. Among the 4q35 genes, *FRG2A* is overexpressed in FSHD muscles and stabilized upon genotoxic damage, with an extent inversely correlated with the number of D4Z4 repeats.
We found that *FRG2A* is a heterochromatic long non-coding RNA, which associates with centromeric α-satellites and the Intergenic Spacer (IGS), a regulatory region of the rDNA arrays. We found that *FRG2A* localizes at the Dense Fibrillar Compartment (DFC) of the nucleoli and that FSHD-derived primary myoblasts showed alteration of Nucleolus-Associated Domains (NADs), one of the greatest heterochromatin hubs of the nucleus, where centromeres and rDNAs reside. Indeed, in FSHD myoblasts centromeres relocalize to strictly interact with nucleoli. We also detected increased H3K9me3 and H3K27me3 histone marks at the IGS and determined that this epigenetic effect impairs rRNA synthesis resulting in reduced protein synthesis. Both the centromere localization and rRNA transcription are restored upon *FRG2A* silencing. Interestingly, the T2T genome assembly revealed other 13 *FRG2* paralogs interspersed within the genome, with identity varying from 80% to 99.5%. Also, these paralogs are heterochromatin-associated non-coding RNAs, expressed in a subject and tissue-specific manner.
Based upon these results we propose that *FRG2A* belongs to a novel family of lncRNAs involved in the organization of nuclear chromatin architecture, causing the compaction of NADs regions by anchoring centromere satellites to the nucleoli, and as epigenomic modulators impacting ribosome function and protein synthesis in muscle cells. These observations provide evidence of the long-range effects of *FRG2A* increased expression opening new perspectives on the molecular mechanism involved in FSHD pathogenesis.

SCALT: automatic identification of cell types from single-cell RNA sequencing data

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Single cell RNA sequencing (scRNAseq) marks a key methodological breakthrough for the study of the organization and function of cells and cell types. The key concept behind scRNAseq is relatively simple: gene expression profiles are collected at single cell resolution by micro fluidic devices or equivalent methods; subsequently gene expression patterns are used as a proxy to define similar cell types and infer their identity.
Dimensionality reduction and unsupervised clustering currently represent the *de facto* standard methods for the analysis of scRNAseq data. These techniques however suffer from inherent limitations including: 1) need for manual/expert curated annotation of cell types; 2) general lack of reproducibility (since manual annotation); 3) limited resolution in the identification and annotation of scarcely represented cell types. As a result, current analytical workflows require extensive analyses and the application of different sets of parameters for an accurate delineation of cell type clusters and their annotation.
Here, we present SCALT, an innovative method which introduces a paradigm-shift for the analysis of scRNAseq data. In our approach, cells are annotated to a specific type at individual level, by using a simple but elegant method based on maximum likelihood, without the need for clustering, dimensionality reduction or manual annotation. SCALT leverages a collection of 471 lists of cell-type specific genes, constructed by extensive re-analysis of comprehensive and expert curated catalogues (HPA and DISCO).
Applied to the reference benchmark dataset by Abdelaal et. Al 2019, SCALT performed comparably or better than other the methods therein tested. Further, it recognized the correct cell type for 98.7% and 98.8% of the over 553411 and 4339209 distinct cells included in HPA and DISCO, respectively.
In conclusion, SCALT represents an innovative and highly useful method for the analysis of scRNAseq assays.

Gold nanoparticles and endothelial progenitor cells: a win-win alliance for targeting tumors

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Plasmonic photothermal therapy utilizes biologically inert Near Infrared (NIR) gold nanoparticles (AuNPs) that convert light into heat capable of eliminating cancerous tissue. In this work, we propose tumor tropic cellular vectors, called Endothelial Colony Forming Cells (ECFCs), enriched with gold chitosan-coated nanorods (AuNRs). ECFCs display a great capability to intake AuNRs without losing viability and exerting an *in vitro* and *in vivo* antitumor activity per se. Conventional optical and Transmission electron microscopes (TEM), Photoacoustic imaging (PA) were used to evaluate AUNRs intracellular uptake in Melanoma cells (M6) and ECFCs. Melanoma spheroids were employed to investigate the behavior of AuNRs-ECFC in 3D-culture. The tumor tropic properties of AuNRs-ECFC were confirmed in vivo, using a human melanoma xenograft rat model. The PA signal provided from ECFC loaded with AuNRs exhibited a stronger enhancement compared to AuNRs-M6. As expected, ECFCs loaded with AuNRs, thanks to their ability to enter the spheroid, exert their antitumor activity by reducing the volume of the sphere, compared to control spheroids plated with unloaded ECFCs. Besides, the PA signal provided from AuNR–ECFCs inside spheroids exhibited a strong enhancement compared to M6-AuNRs ones. Histological analyses of explanted tumor mass demonstrate that gold is still retained after 1 week from injection and organs did not show any morphological alterations. We demonstrated in vitro that AuNRs-loaded ECFCs are able to generate higher photoacoustic signals than AuNRs loaded in M6 cells. 3D cultures confirm the cytostatic effect of AuNRs-ECFC on tumor. In vivo, we show, via immunohistochemical analysis, a great tumor-homing efficiency of AuNRs–ECFCs after a bolus intravenous administration and their permanence inside the tumor masses 1 week after administration. These important AuNRs properties will be exploited to perform NIR-infrared photothermal ablation.

Extracellular matrix guides human neuromuscular organoid morphogenesis

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Morphogen and growth factor contribution during organogenesis has been deeply studied in animal models and is routinely applied *in vitro* for the generation of human organoids. In fact, by regulating key pathways involved in neuromuscular (NM) system morphogenesis, it is possible to derive human neuromuscular organoids (NMOs) from induced pluripotent stem cells (hiPSCs), self-assembled spheroids that contain functional neuronal and muscular compartments. During development another key extrinsic factor, the extracellular matrix (ECM), is guiding NM system morphogenesis, maturation and function. However, little is known about the role of ECM during human NM system differentiation and morphogenesis and there is a lack of knowledge on how this extrinsic factor can affect human NMO derivation.
Here, we found that skeletal muscle (SkM) ECM, provided as decellularized SkM (dSkM), can guide the morphogenesis of differentiating hiPSCs toward tissue engineered and functional NMOs (t-NMOs) that do not self-assemble into spheroids. By providing different ECM-enriched culture environments, we also show that hiPSCs can give rise to human NM system *in vitro* models that display differential morphogenesis, function and gene expression profiles. Moreover, we found that the ECM provided as dSkM is sufficient to induce hiPSC commitment toward neuromesodermal progenitors, and that this cell commitment requires direct dSkM-hiPSC contact. Finally, to investigate the ability of t-NMOs to reproduce dysfunctional muscle function we used hiPSCs derived from patients affected by Duchenne Muscular Dystrophy (DMD) to produce DMD t-NMOs. Upon neuronal stimulation, DMD t-NMOs were able to mimic the reduced SkM contraction and altered calcium dynamics typical of the disease. Altogether, our study shows that the ECM can impact *in vitro* the development of human NM system models and opens new perspectives for future applications of the ECM as an extrinsic factor to engineer patient-specific NMOs.

Aptamer to ErbB-3/HER3 as innovative therapeutic agent in HNSCC

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Cetuximab (CTX) treatment induces an acquired resistance in the majority of head and neck squamous cell carcinoma (HNSCC) patients, leading to local and distant treatment failures. The emergence of resistance is associated with compensatory up-regulation of HER3, making it a new therapeutic target. In this regard, our approach involves targeting the HER3 receptor axis with an innovative drug, known as aptamer. Aptamers are DNA-based molecule synthesized *in vitro* through SELEX technology.
Briefly, a large library of random DNA sequences was incubated with purified IgB-3, a fusion protein combining the extracellular domain of HER3 and the Fc domain of a human IgG1. Any sequences that bounded to the target were retained, amplified by PCR to generate a larger pool of molecules, and lastly subjected to cloning and sequencing. Using the HNSCC cell line FADU, we then confirmed the binding specificity of the selected aptamer (A33) to HER3, comparing it to a control primer.
The evaluation of higher HER3 protein level already after 24hrs of CTX treatment support its potential involvement in the bypass pathway resistance mechanism.
Based on these results, we proceeded to assess the biological function of A33.
Through Western Blot analysis, A33 showed a promising inhibition of HER3 phosphorylation both alone and in combination with CTX, with a consistent downregulation of the downstream signaling pathway, pAKT. Using Livecytetechnology, we observed that incubation with A33 resulted in a decreased cell track speed. Furthermore, when combined with CTX, A33 increased cell doubling time and decreased cell confluence.
Their addictive effect was also confirmed in both monolayer cultures, with the reduced ability of single cells to grow as colonies, and in 3D growing conditions, where there was a decrease in spheroid volume.
Collectively, our promising results shed new light on the potential use of DNA-aptamers as therapeutic agents in a combinatorial setting for cancer therapy.

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. Indeed, the expression of its specific receptors $S1P_1$, $S1P_3$ and $S1P_5$ is increased in endometriotic lesions.
The endocannabinoid system (ECS), consisting of the cannabinoid receptors, CB1 and CB2, the endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and their metabolic enzymes, plays a crucial role in modulating different processes including inflammation and pain. 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 receptor that belongs to the class A family of G-protein coupled receptors, have been found to be expressed in ovarian and deep infiltrating endometriotic lesions (n=15) both at mRNA and protein levels. Furthermore, the effect of 2-AG in the modulation of inflammation has been investigated in endometriotic epithelial 12Z cells. The endocannabinoid significantly augmented the expression of proinflammatory cytokines as well as COX2. Interestingly, the 2-AG-induced increase of $S1P_3$ expression is crucial for the biological action of the endocannabinoid. Indeed, $S1P_3$ pharmacological inhibition by the antagonist VPC23019 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.

Amyloid-β peptide alters VDAC1 electrophysiology and reduces mitochondrial respiration in an *in vitro* model of Alzheimer's disease

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Voltage-Dependent Anion-selective Channel 1 (VDAC1) is the most abundant pore-forming protein of the outer mitochondrial membranes (OMM). VDAC1 allows the passive diffusion of ions such as Cl-, K+, Na+, and small metabolites, including ATP/ADP and NAD/NADH+, thus participating in the proper maintenance of the organelles' bioenergetic functions. Being a channel, VDAC1 has peculiar electrophysiological properties: when reconstituted in artificial planar lipid bilayer (PLB), VDAC1 forms pores with a conductance of about 3.5 nS in 1 M KCl, which decreases with the application of both positive and negative voltages. From several studies, VDAC1 stands out as a preferential mitochondrial binding site for misfolded proteins in neurodegenerative contexts. In Alzheimer's disease (AD), intracellular oligomers deriving from amyloid-β (Aβ) peptide aggregation form toxic aggregates by directly binding VDAC1 on OMM, promoting thus mitochondrial dysfunction and an increase of the oxidative stress. To deepen this aspect, here we analyzed the effect of Aβ1-42 oligomers on VDAC1 channel activity at the PLB. We found that, at the transmembrane potential around ± 10 mV, Aβ increases the conductance of VDAC1 of about 30%, as well as the channel propensity to switch towards closed states already at low voltages, contrary to what observed in the presence of VDAC1 alone. To analyze the effect of VDAC1-Aβ1-42 interaction for the overall mitochondrial functionality, we investigated the respiratory profile of permeabilized SH-SY5Y cells exposed to Aβ by high-resolution respirometry. We found that Aβ reduces the basal oxygen consumption and the maximal respiratory capacity, affecting the oxygen flow linked to the ADP phosphorylation. Although further experiments with VDAC1-antagonizing peptides are ongoing, this work could shed a light on the molecular mechanisms behind the VDAC1-Aβ interaction and, at the same time, offer the basis for the development of pharmacological tools in AD treatment.

Dysregulation of lipid droplets dynamics by OCRL-dependent PI4,5P2 accumulation contributes to mitochondrial stress and increased fibrosis in in vitro models of Lowe syndrome

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Lipid metabolism is essential for energy homeostasis, membranes composition and intracellular signalling. Neutral lipids are stored in the core of Lipid Droplets (LD). In physiological conditions, triglycerides stored in LD are metabolized into fatty acids (FA) by cytosolic lipases and transferred to peroxisomes and mitochondria for beta-oxidation and energy production. Lipids stored in LD are also protected from peroxidation, thus preventing lipotoxicity and cell damage. Here we show aberrant accumulation of PI4,5P2 in LD membranes in cells lacking the PI4,5P2 5-phosphatase OCRL, which is mutated in Lowe syndrome, a rare genetic disease mainly affecting kidney function. This influences LD dynamics, reduces their consumption and results in altered trafficking of FA to mitochondria causing impaired mitochondrial function. We also demonstrate that the PI4,5P2 pool on LD is produced *in situ* by two pathways: i) phosphorylation of PI5P by the PI5P4Kα and ii) local increase of PI4P by the activity of the lipid transport protein ORP4. Excessive PI4,5P2 on LD prevents them from efficiently contacting peroxisomes and mitochondria, leading to impaired FA uptake, mitochondrial stress, and increased ROS production. These phenotypes are hallmarks of chronic kidney disease (CKD) that Lowe patients experience early in their lives. To understand the association of these pathways with the progression of kidney disease towards CKD in Lowe syndrome we developed kidney organoids from Lowe patients-derived iPSCs, we mimicked aging and observed LD accumulation and increased fibrosis, clear signs of CKD. In summary, we described a novel pool of PI4,5P2 on LD regulated by OCRL, that negatively impacts on LD dynamics, FA metabolism and mitochondrial functions. We finally envisage that we can rescue these dysfunctional phenotypes and ameliorate kidney function in Lowe patients by restoring physiological levels of PI4,5P2 on LD.

Shaping membrane-related PKA microdomains

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Cells perceive various extracellular signals that are transduced in function using a few intracellular signalling cascades. Among them, the cAMP/PKA signalling axis is well known to regulate a plethora of cell functions. However, the mechanisms underlying the ability of this relatively linear pathway to couple specific cues to distinct roles are not understood. The leading hypothesis points to the rearrangement of both, second messenger and effectors in subcellular locations to constitute functional units finely regulated called microdomains. A significant body of evidence connects the equilibrium between cAMP production and hydrolysis (via ACs/PDEs) as a crucial component of cAMP microdomains, however, recent work points to a significant role for phosphatases (the terminators of PKA signals). How these two modalities are integrated in generating microdomains remains elusive. We used cAMP and PKA-dependent phosphorylation FRET sensors targeted to specific subcellular locations. We decoupled the cAMP levels from the regulation of PKA and found that cAMP is crucial for the activation of microdomains, however, the dephosphorylating actions of phosphatases determine the termination of PKA-dependent signals and dictate the duration of the microdomain's effects. To test the spatial distribution of PKA microdomains we developed and validated a series of targeted FRET sensors, that were distanced from their original location for 10 or 30 nm using rigid aminoacidic nanorulers. Using *HeLa* and *U-2 OS* we found that cAMP levels did not affect the spatial extent of the microdomain while on the contrary, phosphatases were crucial and their activity acted as a physical boundary for PKA-phosphorylated targets. Overall, our findings contribute to design a picture where two microdomain-forming modalities are necessary. The cAMP levels are indispensable for the initial activation of local PKA, while the temporal and spatial length of the microdomain is mostly regulated by phosphatases.

Phosphoinositide 3-Kinase C2α controls cardiac contractility through regulation of β_2-adrenergic receptor recycling

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Phosphoinositide 3-kinase C2α (PI3KC2α) is a ubiquitously expressed class II PI3K isoform, which has been previously shown to be involved in the control of senescence and vesicular trafficking. However, its role in the myocardium has not been investigated. The primary aim of this study is to elucidate the mechanism by which mechanism PI3KC2α controls cardiac pathophysiology.
Our findings indicate that cardiomyocyte specific PI3KC2α knockout (PI3KC2α KO) mice display reduced cardiac contractility. Similarly, PI3KC2α morphants zebrafish exhibit lower heart rate and fractional shortening compared to controls, both at baseline and after isoproterenol (ISO) stimulation. A similar unresponsiveness to ISO was found in vivo in PI3KC2α KO mice where chronic treatment with the β-adrenergic receptor (β-AR) agonist failed to induce the classical β-AR-mediated remodeling, characterized by an increase of the left ventricular mass and of cardiomyocyte area. These findings suggest that PI3KC2α plays a critical role in the regulation of β-AR signaling. In agreement, cAMP levels failed to increase in response to ISO treatment in PI3KC2α morphants. Furthermore, silencing of PI3KC2α in HEK293-GFP-β2-AR cells resulted in an increased GFP-β2-AR internalization compared to control cells, both at baseline and after ISO stimulation. Interestingly, preliminary findings indicate that overexpression of constitutively active Rab11Q70L induced a redistribution of GFP-β2-AR at the plasma membrane in PI3KC2α-silenced cells.
Overall, we identify a key role for PI3KC2α in the control of cardiac contractility through the regulation of β_2-AR trafficking to the plasma membrane through a Rab11-dependent mechanism.

Sestrin2 drives ER-Phagy in response to protein misfolding

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Protein biogenesis within the endoplasmic reticulum (ER) is a vital process essential for the development and functioning of organisms. However, the inherent propensity for errors during protein folding necessitates the efficient removal of faulty products to avoid cellular toxicity. While the ER-associated protein degradation (ERAD) pathway governs the disposal of misfolded clients in the proteasome, ER-phagy directs ERAD-resistant misfolded polypeptides to lysosomes for degradation. The signaling mechanisms underlying the initiation of ER-phagy in response to misfolded protein accumulation remains unknown. In this study, we show that protein misfolding within the ER elicits a response mediated by the transcriptional regulation of a handful of genes, including SESTRIN2, a leucine-binding protein that controls mTORC1 signaling. SESTRIN2 induction is primarily driven by Xbp1, and its activation inhibits mTORC1 kinase function in a nutrient-rich state. Consequently, the transcription factors TFEB/TFE3 are no longer phosphorylated and translocate into the nucleus orchestrating the upregulation of the ER-phagy receptor FAM134B, alongside lysosomal and autophagy genes. This coordinated response effectively facilitates ER-phagy mediated lysosomal degradation of misfolded cargoes. Moreover, we demonstrate that targeted pharmacological manipulation of FAM134B enhances the clearance of misfolded proteins in cellular models of ER storage diseases. Our study sheds light on the interplay between nutrient signaling and ER quality control, opening new potential therapeutic avenues for managing disorders associated with aberrant protein folding in the ER.

TMEM65 controls mitochondrial activity through respiratory complex I assembly

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Transmembrane protein 65 (TMEM65) is a protein localized in the inner mitochondrial membrane. A homozygous loss-of-function mutation in the *TMEM65* gene was identified in a patient with a clinical presentation resembling a mitochondrial disorder [1]. In addition, knock-down of TMEM65 expression in human fibroblasts was shown to severely affect mitochondrial content and respiration [1], but the exact mechanism remains unknown. Interestingly, by performing a quantitative proteomic screening, we observed an accumulation of TMEM65 protein in osteosarcoma cells that lack fully assembled respiratory complex I (CI) and accumulate CI assembly intermediates.
Thus, in order to get a deeper understanding of the function of TMEM65 we obtained a KO human cell line and analysed the effects on mitochondrial function of the absence of the protein. Ablation of TMEM65 resulted in a mild reduction of mitochondrial OXPHOS capacity, associated with a reduction of fully assembled functional CI and abnormal accumulation of CI subassemblies. Moreover, TMEM65 KO cells showed an impairment in the kinetics of CI biogenesis, suggesting the presence of an assembly defect.
Taken together, the data suggest that TMEM65 plays a role in mitochondrial respiratory chain function by assisting CI assembly. Gaining deeper knowledge on the molecular function of this protein will help understanding the mitochondrial disorders caused by mutations in the *TMEM65* gene.

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Integrative analysis of protein-protein interaction networks reveals aryl hydrocarbon receptor (AHR) crosstalk in von Hippel-Lindau (VHL)-associated clear cell renal cell carcinoma (ccRCC): implications for targeted therapies

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The intricate regulatory mechanisms governing the crosstalk between the Aryl hydrocarbon receptor (AHR) and hypoxia-inducible factor (HIF), both members of the basic helix–loop–helix (bHLH) PER-ARNT-SIM (PAS) transcription factor family, is central to understanding cancer pathogenesis, particularly in clear cell renal cell carcinoma (ccRCC). In ccRCC, loss of function mutations in the von Hippel Lindau tumour suppressor (pVHL) leads to persistent HIF activation, driving tumorigenesis. In this study, we explore the interplay of AHR and HIF pathways, shedding light on potential therapeutic routes for ccRCC. Employing protein-protein interaction network analysis and gene expression profiling, we delineate the impact of pVHL loss on AHR activity and its associated pathways. Our findings reveal distinct expression patterns of AHR interactors following exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and in ccRCC. Through Kaplan-Meier survival analysis, we identify AHR interactors significantly associated with poor survival rates in ccRCC patients. Notably, the upregulation of androgen receptor (AR) and retinoblastoma-associated protein (RB1) by TCDD exposure, followed by their downregulation in ccRCC, suggests potential therapeutic targets. Strategic activation of AHR via selective AHR modulators (SAhRM) emerges as a promising approach for treating VHL-mutated ccRCC, thereby reducing reliance on surgical interventions. Our study provides comprehensive insights into the complex interplay between AHR and VHL pathways in ccRCC pathogenesis, offering novel avenues for targeted therapeutic interventions.

Poster Abstracts

(presenting authors are shown underlined)

P.1

Suppressing PDAC invasion and enhancing AKT inhibitors efficacy via RNA-based targeting of p130Cas scaffold protein

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Pancreatic cancer is the third most lethal cancer in the world, with a 5-year survival rate of around 10%, mostly due to lack of early diagnosis and poor therapeutic options. In this context, the p130Cas adaptor protein has garnered attention for its pivotal role in enhancing PI3K activity, critical for tumor initiation. However, targeting p130Cas, a scaffold protein devoid of catalytic activity, presents significant challenges. Here, we explore the potential of RNA therapy as an effective approach to target p130Cas protein expression. Utilizing siRNA-mediated knockdown, we observe a notable reduction in the migration of pancreatic cancer cells in vitro, coupled with a substantial impairment in the invasion of matrix-embedded tumoroids into the surrounding environment. Notably, pharmacological inhibition of AKT significantly curtails PANC1 cell growth following p130Cas knockdown, while exerting a milder effect on wild-type cells. At the molecular level, p130Cas-silenced cells exhibit diminished basal activation of the mTOR and MAPK pathways, leading to a pronounced reduction in phosphorylation of the S6 ribosomal protein. To facilitate efficient delivery of RNA molecules into tumor cells, we encapsulate them within PLGA nanoparticles coated with hyaluronic acid (HA). Given that hyaluronans are prevalent components of PDAC desmoplasia and their receptor CD44 is often overexpressed in tumor cells, HA-PLGA nanoparticles hold promise for targeted delivery. Preliminary results affirm the internalization and perinuclear localization of HA-PLGA nanoparticles, alongside a reduction in target protein expression. These findings confirmed the importance of p130Cas-PI3K pathway in PDAC tumor cell migration and invasion, while affirming nano- RNA therapeutics as an effective approach for combating pancreatic cancer.

Investigation of the role of MiT/TFE family members in breast cancer metastatic dormancy

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Delayed relapses at distant sites are a common clinical observation for certain types of cancers, i.e. breast and prostate cancer, after removal of primary tumor. Thus, understanding the molecular divers and pathways contributing to the long-term survival and outgrowth of disseminated tumor cells, defined as dormancy, is of utmost importance. Our research group has developed and validated an in vitro model of breast cancer persistence in the lungs. This system combines the quiescence contest-dependency of lung microenvironment (Alveolar type 1 cells, alveolar type 2 cells, and lung fibroblasts) with breast cancer model cellular systems. This organotypic system has revealed the importance of TFEB, member of MIT/TFE proteins and master regulators of autophagy-lysosomal pathway, for the survival of disseminated cells. We have investigated the genetic requirement for these transcription factors via CRISPR and RNAi mediated loss of function experiments. Preliminary data have suggested a genetic role for these factors in lysosomal accumulation in our coculture systems. This approaches however, highlighted counter selection of knock-down cells; hence, more efficient strategies are under development to study the contribution of TFEB and TFE3, other potent member of MIT/TFE proteins, with better resolution.
In perspective, the development of inducible gain/loss-of-function approaches, i.e. models expressing either constitutively active forms or down regulation of MIT/TFE proteins would bypass the aforementioned limitation and allow us to test the role of these transcription factors in in-vivo assay for experimental metastasis.

P.3

Blocking the Hedgehog-dependent tumor growth by a new selective Endoplasmic Reticulum Aminopeptidase 1 inhibitor

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The Sonic Hedgehog (SHH) signalling pathway plays a crucial role during organogenesis and stem cell maintenance. Its aberrant activation is responsible of a wide range of human cancers including medulloblastoma (MB), the most common malignant paediatric brain tumor, which shows a high drug-resistance to the current therapies. Therefore, is extremely overriding to unveil the molecular mechanisms that regulate the SHH pathway, in order to develop novel therapeutic strategies for the treatment of SHH-dependent cancers. We identified Endoplasmic Reticulum Aminopeptidase 1 (ERAP1), a key player of the immune response, as a new positive regulator of the SHH pathway. We found that genetic or pharmacological inhibition of ERAP1 suppresses SHH-dependent tumor growth both *in vitro* and *in vivo*, placing ERAP1 as a promising therapeutic target for SHH-driven tumors. However, the lack of availability for highly specific chemical inhibitors for ERAP1 has limited progression in this area. To address this issue, we performed a docking-based virtual screening of a library of natural compounds against the crystallographic structure of ERAP1 catalytic domain and we identify the alkaloid compound N1 as a potential inhibitor for ERAP1. We found that N1 directly binds ERAP1 and disrupts its function on SHH pathway leading to a reduction of the signaling. N1 impairs the association between ERAP1 and USP47, promoting βTrCP protein stability and degradation of GLI1, the powerful activator of the SHH pathway. Furthermore, SHH-responsive cells genetically depleted for ERAP1 are insensitive to N1 treatment both in physiological and pathological condition, demonstrating the specificity of N1 for ERAP1 in counteracting SHH signaling. Notably, N1 blocks SHH-MB growth both *in vitro* and *in vivo* in heterotopic and orthotopic allograft mouse models. Overall, our finding strongly indicates N1 as a good candidate for further preclinical studies in the treatment of SHH-driven cancers.

Dissecting the crucial role of heme synthesis in supporting Non-Small Cell Lung Cancer (NSCLC) and Pancreatic Ductal Adenocarcinoma (PDAC) progression

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The Feline Leukemia Virus Subgroup C Receptor 1 isoform a (FLVCR1a) serves as a membrane transporter originally associated with exporting heme. Recently, its involvement in choline import has also been acknowledged. While there's an ongoing discussion about its specific role as a transporter, it's been established that FLVCR1a functions within a coordinated axis alongside the heme synthetic enzyme ALAS1. Notably, FLVCR1a has been observed to be overexpressed in multiple types of cancer. Additionally, the dependence of KRAS-mutated Non-Small Cell Lung Cancer (NSCLC) and Pancreatic Ductal Adenocarcinoma (PDAC) on heme biosynthesis has been confirmed.
In this study, we employed FLVCR1a silencing to disrupt heme synthesis in NSCLC and PDAC. This approach led to reduced ALAS1 activity and significant metabolic rewiring, restricting the glycolytic profile of KRAS-mutated tumor cells. The altered metabolic profile has been associated with diminished proliferation, dissemination, and compromised tumor initiation. Our findings highlight the critical role of the ALAS1-FLVCR1a axis in determining cellular metabolic status and its essential role in regulating cell proliferation.
These data point out the potential of targeting the ALAS1-FLVCR1a axis for therapeutic intervention. To achieve this, we downregulated ALAS1 activity using the safe and FDA/EMA-approved 5-Aminolevulinic acid (ALA). Notably, our study reveals that ALA treatment results in heme accumulation and ALAS1 downregulation, mirroring both the metabolic changes and the reduction in tumor cell proliferation.
In summary, our findings suggest that the FLVCR1a-ALAS1 axis plays a crucial role in regulating the progression of NSCLC and PDAC. We propose targeting this axis through the repurposing of ALA as a potential anticancer therapy.

Targeting mitochondrial calcium by RNA-based therapy in inflammatory bowel diseases

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Inflammatory Bowel Diseases are a group of inflammatory chronic diseases affecting the gastrointestinal tract, including ulcerative colitis, Crohn's disease, and less common IBD-unclassified.
Due to the heterogeneous etiology and the multifactorial mechanism of the disease, the therapies that are now used are not completely efficient. Therefore, a comprehensive understanding of the pathways responsible of the chronic inflammation in IBD is essential for the development of targeted medications. Nowadays, the implication of mitochondria in the modulation of the inflammatory process is emerging, highlighting their potential role as therapeutic targets for chronic inflammatory diseases. Mitochondria are intracellular organelles able to modulate cellular calcium homeostasis thanks to their Ca^{2+} buffering activity. Ca^{2+} enters the mitochondria through the Mitochondrial Calcium Uniporter (MCU), a highly selective channel located in the mitochondrial inner membrane. Within the mitochondria, Ca^{2+} activates different physiological processes. However, an impairment in mitochondrial Ca^{2+} homeostasis can lead to dysfunctional mitochondria that can alter the production of ROS and the release of components such as mitochondrial DNA and mitochondrial formylated peptides into the cytoplasm. These components can act as DAMPs and induce the activation of different molecular pathways involved in the inflammatory response such as NLRP3 inflammasome and cGAS/STING. The aim of our research is to study the role of MCU in IBD. To this aim we analyzed inflammasome activation in differentiated HT29 cell line upon different treatments (LPS, TNFα and macrophage-derived conditioned medium). Preliminary data indicate that LPS activates NLRP3 inflammasome. In the future we will test MCU silencing by siRNA or pharmacological inhibition could counteract NLRP3 inflammasome activation. Finally, we aim to develop an RNA-delivering system for siRNA delivery in intestine through the production of LNPs.

P.6

Interplay of heme and gluco-lipid metabolism: implications for liver pathophysiology

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The liver is a pivotal regulator of systemic glucose and lipid levels, serving as a central hub for metabolism. Dysfunctions in hepatic nutrient sensing and processing contribute to the development of metabolic syndrome and progression to non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD), two liver pathologies exhibiting sexual dimorphism. The metabolism of iron and of its bioavailable form, heme, are intricately linked to nutrient utilization, responding to nutritional cues such as glucagon during fasting and insulin post-prandially, and influencing the expression of glycolytic and oxidative enzymes. Our previous works have shown that FLVCR1a, a plasma membrane transporter first recognized as a heme exporter but recently identified as a choline importer, regulates ALAS1-mediated heme synthesis. Our recent findings indicate that deletion of *Flvcr1a* in hepatocytes (LivKO mice) results in decreased heme biosynthesis and in heme-dependent reduction of glucose uptake and glycolysis, along with elevated fatty acid uptake and oxidation, oxidative phosphorylation and cholesterol production. Based on these results, we hypothesized that the metabolic dysregulation elicited by impaired hepatic heme synthesis in these mice predisposes them to metabolic syndrome. To test this in a gender-specific manner, male and female LivKO mice were subjected to high-fat high-fructose (HFF) diet. Preliminary data indicate exacerbated insulin resistance and compromised gluco-lipid metabolism in LivKO males and females under HFF diet compared to matched controls. These results suggest that rewiring of hepatic heme metabolism contributes to systemic gluco-lipid dysregulation, hinting at heme biosynthesis as a potential target for the prevention/treatment of metabolic syndrome and NAFLD.

P.7

High-content imaging approaches to identify new druggable targets to tackle Batten disease

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Neuronal Ceroid Lipofuscinoses, also known as Batten disease (BD), is a group of lysosomal storage disorders (LSDs) characterized by rapid progressive visual loss, early mental deterioration, and seizures. Interest exists in developing small molecule-based therapeutics to target relevant pathways and counteract the difficulties in replacing the mutated genes with other therapeutic strategies in difficult-to-reach tissues such as the central nervous system. We recently discovered a novel accumulation of the glycosphingolipid Gb3 as a pathological marker for multiple BDs (Soldati et al., 2021), although the molecular players causing this accumulation are unknown. More importantly, the identification of these genes might represent a novel strategy to develop small molecule therapies. Here, we use our cell-based high content imaging (HCI) screening assay to detect the accumulation of Gb3 in ARPE19-CLN3 KO cells to screen the Human Druggable Genome siRNA Library composed of 11000 siRNAs. We identified 115 genes whose depletion reduces Gb3. We validated 34 siRNAs hits by their ability to reduce the accumulation of the subunit c of the mitochondrial ATP synthase (SCMAS). We focus our attention on the estrogen-related receptor alpha (ERRα), recently identified as a molecular player in autophagy. Interestingly, also the pharmacological inhibition of ERRα reduces Gb3 accumulation in ARPE19-CLN3 KO cells. Together, we developed a high-content screening platform to discover novel druggable targets reducing phenotypic hallmarks of BD cells and found that targeting ERRα might represent a novel therapeutic strategy to treat BD.

P.8

The role of RNA and RNA-binding proteins in EGFR non-clathrin endocytosis and its relevance in physiological contexts

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The epidermal growth factor receptor (EGFR) plays a pivotal role in several physiological processes; however, its aberrant regulation can lead to cancer progression. Endocytosis is a critical regulator of EGFR; indeed, the levels of activity and the fate of the receptor depend on the internalization route. We recently discovered a novel endocytic route of EGFR, non-clathrin endocytosis (NCE), that is activated only at high concentrations of ligand, EGF and targets receptor to lysosomal degradation. In contrast, clathrin-mediated endocytosis (CME) of EGFR, which recycles receptor, is activated at low EGF concentrations. Some aspects of the regulation of EGFR-NCE remain to be elucidated: *i*) proteomics analysis of EGFR-NCE vesicles identified RNA-binding proteins (RBPs) as critical regulators of this mechanism; however, their role in EGFR-NCE and whether RNA species are also involved are unknown; *ii*) EGFR-NCE is known to be active in some cell lines and not in others, but its relevance in different physiological cellular contexts is unknown. Here we show that degradation of cytosolic RNAs by RNaseA inhibited EGFR-NCE but not -CME. Based on these data, we are: *i*) dissecting the role of selected RBPs in EGFR-NCE by performing, immunofluorescence-based and *in vivo* internalization assays; *ii*) identifying specific RNAs associated to EGFR-NCE vesicles regulated by EGF by using different approaches (*e.g.,* CLIP or APEX-seq). To investigate the relevance of EGFR-NCE in physiological contexts, we generated isogenic cell models by differentiation of human induced pluripotent stem cells (hiPSCs) into fibroblasts, cardiomyocytes, hepatocytes and keratinocytes in which we are preliminary setting *in vivo* internalization assays. The analysis of EGFR-NCE activation coupled with single-cell RNAseq analysis in these lineages will allow us to unveil if specific endocytic proteins are expressed in different contexts thereby controlling the EGFR endocytic route and signaling output.

PARP1 significantly influences the self-renewal and maintenance of cutaneous squamous cell carcinoma

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Cutaneous squamous cell carcinoma (cSCC) ranks as the second most prevalent skin cancer in humans, arising from excessive UV exposure and conventionally managed through surgical excision or radiotherapy. Given the established treatments, uncovering novel pathways pivotal to cSCC development is imperative for exploring less invasive therapeutic approaches. Our investigation revealed a pronounced upregulation of poly(ADP-ribose) polymerase PARP1, an intranuclear enzyme crucial for DNA repair, in cSCC compared to healthy skin. Nonetheless, the precise role of PARP1 in this malignancy remains elusive.
Employing RNA interference, we targeted PARP1 to elucidate its function in cSCC, observing G1-phase cell cycle arrest concomitant with diminished DNA synthesis upon PARP1 depletion in cSCC cell lines. Additionally, exploiting CRISPR-Cas9 technology, we engineered various cSCC cell lines with PARP1 knockout. Specifically, targeting the first exon of the PARP1 gene, we efficiently introduced loss-of-function mutations (InDels) via nucleofection-mediated delivering a ribonucleocomplex comprising Cas9 protein and a single guide RNA. Notably, PARP1 deletion markedly attenuated clonogenic capacity and oncosphere formation in cSCC cells. In summary, our study underscores the pivotal role of PARP1 in regulating cSCC proliferation, self-renewal, and maintenance. To further validate these findings, we propose investigating the effects of PARP1 depletion using skin organoids derived from cancer cells.

P.10

The MRN complex regulates primary ciliogenesis to sustain neuronal progenitor proliferation

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Hypomorphic mutations in MRN (Mre11/Rad50/Nbs1) complex, the main player in DNA Damage Response (DDR), cause DDR defective syndromes, which are characterized by immunodeficiency, cerebellar hypoplasia, microcephaly and predisposition to cancer. Interestingly, microcephalic phenotype is a common outcome of mutations in proteins operating at the centrosome or at the primary cilium (PC) .PC is a solitary, non – motile organelle essential for the regulation of many mitogenic pathways involved in neurodevelopment and cancer, such as the Sonic Hedgehog (SHH) one .We recently showed that the Central Nervous System (CNS)-restricted Knock Out of Nbs1 (Nbs1-KO) completely abolishes SHH-driven medulloblastoma (MB) in mice, and this is correlated to SHH pathway downregulation.
Since MRN proteins localize at the centrosome, and Mre11 mutations are involved in ciliopathies, we hypothesize that MRN complex, in addition to its role in DDR, may regulate the SHH pathway through a new uncanonical function on primary ciliogenesis, which subsequently may impact on the development of the cerebellar cortical architecture and on Granular Cells Progenitors (GCPs) tumorigenic transformation.
We show that depletion of Mre11 and Nbs1, but not of Rad50, as well as Mre11 pharmacological inhibition via Mirin, induces striking elongation and severe dysmorphism of the PC. Importantly, PC phenotypes induced by Nbs1-KO resemble those observed in NBS patient derived fibroblast. Moreover, GCP-restricted KO of Nbs1 reduces proliferation, impairs SHH-dependent cerebellar development and abrogates MB insurgence. Additionally, Nbs1 depletion inhibits SHH pathway, in *in vitro*, *ex vivo* and *in vivo* models and alters the PC-dependent GliA/R ratio which SHH pathway activation depends on
Our data reveal that the MRN complex exerts a previously undisclosed role on the structure and function of PC through which it regulates SHH pathway and consequently impacts on cerebellar development and tumorigenesis.

Intestinal organoids as a model to study autophagy flux in celiac patients

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Celiac disease (CeD) is a small bowel disorder characterized by mucosal inflammation, villous atrophy and crypt hyperplasia, stimulated by gliadin peptides that are not digested. Alteration of intracellular vesicular trafficking in CeD has been demonstrated in the intestinal epithelial cells with a delay from the early to the late vesicles. Moreover, in CeD biopsies alteration of proliferation, inflammation and autophagy has been demonstrated.
The aim of the present study is to investigate the interplay between autophagy and inflammation in intestinal biopsies and organoids derived from CeD patients.
Intestinal biopsies from controls (CTRs) and gluten-containing diet-celiac disease (GCD-CeD) were used to test pE4BP, LC3 I/II, p62 and pNF-kB by Western Blot analysis (WB). Intestinal organoids were isolated from biopsies of CTR, GCD-CeD and gluten-free diet celiac disease (GFD-CeD), pE4BP, LC3 I/II, p62 and pNF-kB were also evaluated. Bafilomycin and rapamycin were used to interfere with autophagy flux. P62 levels was evaluated in biopsies and intestinal organoids by immunofluorescence. GCD-CeD supernatant was evaluated by Bioplex.
The autophagic flux is reduced (p62 and pE4BP levels are higher in GCD-CeD than in CTRs) and inflammation is increased (evaluated by pNF-kB) in both intestinal biopsies and organoids from CeD patients compared to CTRs. In CeD, both GCD and GFD, organoids Rapamycin induces the autophagic flux (p62) and reduces inflammation (pNF-kB) while in CTRs organoids Bafilomycin reduces the autophagy flux (p62) and increases inflammation (pNF-kB). 21 out of 27 cytokines were found significantly increased in GCD-CeD supernatant. CTRs organoids treated with GCD-CeD supernatant presented reduction of autophagic flux and inflammation respect to the untreated.
A crosstalk between autophagy and inflammation is present in both CeD biopsies and intestinal organoids.

P.12

Role of Epsin3-mediated E-Cadherin endocytosis in partial EMT, breast cancer cell invasion and metastasis

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Aberrant endocytosis is implicated in the development and progression of cancer. Our lab identified the endocytic protein Epsin3 (EPN3) as an oncogene and prognostic marker in breast cancer (BC). EPN3 overexpression in normal mammary epithelial cells (MCF10A) caused morphological changes, increased mesenchymal markers (N-Cadherin and Vimentin), and partially decreased the epithelial marker E-Cadherin (ECAD), indicating a partial epithelial to mesenchymal transition (pEMT). Interestingly, ECAD downregulation occurs via increased endocytosis rather than transcriptional repression. ECAD endocytosis induces β-catenin translocation into the nucleus, where it activates the pEMT transcriptional program and established an autocrine TGFβ positive feedback loop, sustaining pEMT.
In genetically modified mouse model, EPN3 overexpression cooperates with ErbB2 oncogene in accelerating mammary tumor onset and metastasis in the lung. Importantly, EPN3 restricted expression in adult tissues, along with its overexpression in breast cancer (BC), positions it as a promising therapeutic target. Since increased ECAD endocytosis is the initial event driving EPN3 invasive phenotypes, our project aims to investigate the potential of inhibiting ECAD endocytosis to counteract EPN3-driven BC, invasiveness and metastasis. Thus, we dissected the ECAD endocytic mechanism in MCF10A cells. Our findings demonstrate that ECAD endocytosis occurs through a mechanism mediated by Dynamin, EndophilinA2 and Galectin-Glycolipid interaction. Our current focus lies on the investigation of ECAD endocytosis inhibition as an approach to reverse partial EMT (pEMT) and invasiveness in *in vitro* and *ex vivo* models, exploiting breast primary cells from genetically modified mouse models.

P.13

Identification of an exosite contributing to high-specificity interaction between alpha-1-antitrypsin and neutrophil elastase

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Neutrophil elastase (NE) is a protease released by activated neutrophils during inflammatory responses and mediates anti-microbial function and proteolytic activity on elastin and other extracellular matrix components. Alpha-1-antitrypsin (AAT), an abundant plasma serpin, acts as an irreversible inhibitor of NE, thus preventing excessive damage by NE on elastin-rich tissues. This protective activity of AAT is highlighted by the development of early onset emphysema in patients with the alpha-1-antitrypsin deficiency.
The structure of the Michaelis complex formed by AAT and NE was studied by computational techniques to simulate the docking process. Analysis of the interaction interface reveals key contacts between the protease catalytic pocket and the central part of the reactive center loop (RCL) of AAT. Moreover, a region located upstream strand 4C of AAT, comprising three acidic residues (D202, E199, E204), was found to strongly interact with Arg 147 of NE. These observations suggest that the interaction between AAT and NE, while primarily mediated by the RCL, may also involve an exosite represented by these acidic residues.
Recombinant AAT variants in which all three acidic residues were mutated to either alanine or serine, as well as the single D202R mutant, showed no significant alteration in their stoichiometry of elastase inhibition when compared to the wild type, meaning that these substitutions do not alter the ability to inhibit NE. However, analysis on the association rate of AAT variants to NE revealed decreased inhibition rate constants (k_{inh}), confirming that, as predicted by molecular dynamics studies, the acidic residues form an exosite contributing to high specificity interaction to NE.

UHRF1 acts as positive prognostic factor in gastric and colorectal cancer repressing embryonic morphogenesis genes

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UHRF1 is an epigenetic regulator involved in the maintenance of DNA methylation, DNA repair and other cellular processes. Methylation of gene promoters is involved in transcriptional repression; in cancer, hypermethylation of oncosuppressor gene promoters is correlated with worse prognosis. UHRF1 is widely described as an oncogene. Surprisingly, in colorectal (COADREAD) and stomach (STAD) cancer, UHRF1 overexpression (UH) is associated with a better prognosis (BP). Particularly, in UH-BP, UHRF1 expression is anti-corelated with the expression of genes enriched in the embryonic morphogenesis (EM) process; hence, suggesting a repressive role of DNA methylation in these set of genes. Moreover, patients with BP in STAD manifest UH and low expression of the embryonic morphogenesis (GL) genes signature. Concomitantly, The methylation level of cytosines with a significant methylation difference in all the conditions, is higher in UH-GL patients. The higher level of cytosines methylation in the regulatory regions of the EM genes of UH-GL condition, provides an improved prognosis. Our hypothesis is that UHRF1, in STAD and COADREAD, inhibits the expression of EM genes as an epigenetic heritage deriving from embryonic chromatin configuration, that guides UHRF1 access to regulatory sites. According to our preliminary interpretations, the favourable or unfavourable prognosis in UH tumours, is established by the gene set that UHRF1 is programmed to regulate during the embryonic morphogenesis. If chromatin is permissive to UHRF1 binding onto oncogenes regulatory sites, then better prognosis is expected; contrarywise, if UHRF1 access is allowed in differentiation genes regulatory regions, it results in a negative outcome.

P.15

Investigating on the biological mechanisms underlying the macrophages-ECM crosstalk

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Understanding how to modulate immune pathways is attracting great interest to develop new therapeutic strategies. In recent years, nanomedicine has demonstrated promising results in modulating the immune response. To boost the application of nanomedicine in immunotherapy, it is fundamental to understand the mechanisms of interaction between immune cells and nanomaterials (NMs). Since the extracellular matrix (ECM) modulates the behaviour of immune cells in healthy and diseased tissues, the study of interactions between immune cells and NMs should consider the extracellular microenvironment where these interactions occur. In this work, we aim at studying the role of the ECM in influencing macrophage response to NMs. To address this issue, we prepared ECM-like substrates based on denatured collagen, the most abundant protein present in the ECM, and then loaded with increasing concentrations of carbon nanotubes (CNTs), known to affect macrophage activity when dispersed in cell culture media. Macrophage-like cells, derived from the human monocytic THP-1 cell line, were grown on the substrates up to 5 days. Preliminary data demonstrated the ability of macrophages to adhere to all the substrates and no effect on cell viability was observed after 24 h from seeding, regardless the presence of CNTs. However, at higher CNT concentrations and culture times, cell morphological changes were observed with an increase in filopodia, a decrease in spreading area by assuming a round shape. Furthermore, scanning electron microscopy analysis indicated that macrophages remodelled the ECM-like substrates, making bioavailable the CNTs that are internalized by THP-1 cells. Finally, conditioned media by THP-1 cells grown on CNT-loaded substrates promote cell migration of endothelial cells in wound healing assay compared to conditioned media of control cells. Taken altogether these findings indicate that the presence of NMs embedded in the matrices can modulate the macrophage response.

Impaired cAMP/CREB1 signaling drives mitochondrial dysfunction in skeletal muscle during cancer cachexia

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Cancer-associated cachexia affects most of cancer patients and consists in a severe loss of skeletal muscle mass and functionality. At early stages of cachexia onset, several key genes involved in mitochondrial biogenesis, dynamics, and function are down-regulated in the skeletal muscle, thus leading to mitochondrial dysfunction and contributing to muscle wasting. Nevertheless, the mechanisms underlying the impairment of muscle oxidative capacity are still poorly understood. In the muscle fibers, the activation of the cAMP/CREB1 axis represents a major regulator of skeletal muscle metabolism, indeed, CREB1 coordinates a complex transcriptional network of genes encoding for mitochondrial function and mitochondrial proteins. Here, we propose a model in which dysfunctional cAMP signaling in the skeletal muscle leads to a defective CREB1 transcriptional activity on its target genes, thus contributing to mitochondrial dysfunction and muscle wasting. Moreover, we demonstrate through *in vitro* and *in vivo* studies that boosting cAMP signaling by targeting cAMP-hydrolyzing enzymes phosphodiestarases 4 (PDE4) restores the oxidative metabolism transcriptional program, thus rescuing mitochondrial content and muscle oxidative capacity. Furthermore, we identify PDE4D isoform as the major responsible for tumor-induced impairment of cAMP signaling *in vitro*. Notably, the possible key role of PDE4D in mediating cAMP dysfunction also *in vivo* is supported by the finding that Pde4D is induced in human biopsies of cancer patients, in murine cachectic muscles, and that the relative abundances of its transcript variants undergo a dramatic reassortment in skeletal muscles of C26 tumor-bearing mice.

In conclusion, our study suggests that targeting the defective cAMP/CREB1 signaling, through the pharmacological inhibition of PDE4, could represent a strategy to counteract cancer cachexia-induced metabolic dysfunction and muscle wasting.

P.17

BMP7 promotes cardiomyocyte regeneration

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Zebrafish exhibit astonishing lifelong regenerative ability of the injured heart, driven by the proliferation of pre-existing cardiomyocytes. While neonatal mammals share this potential, it sharply declines in early postnatal life. Consequently, the loss of cardiomyocytes after injuries in adult mammals results in scarring and impaired cardiac function.
In this study, we hypothesised that the postnatal decline in expression levels of specific growth factors may contribute to the loss of cardiomyocyte regenerative potential in mammals. Our findings in the mouse model support this hypothesis, demonstrating a decline of known pro-regenerative factors soon after birth, paralleling the decline in cardiomyocyte regenerative capacity. Furthermore, our study identified novel potential pro-regenerative candidates, among which BMP7, a bone morphogenetic protein, exhibited the most pronounced effect in stimulating neonatal cardiomyocyte cycling and division. Knockdown or knockout of BMP7 reduced the proliferation of cardiomyocytes isolated from neonatal mice *in vitro*, and after cardiac injury in adult zebrafish *in vivo*, underscoring the crucial role of BMP7 in sustaining cardiomyocyte regeneration across different regenerative models. Moreover, BMP7 overexpression or delivery enhanced *in vitro* cell cycle activity of cardiomyocytes isolated from postnatal day 7 mice and promoted cardiomyocyte regeneration in adult zebrafish after cardiac cryoinjury and in adult mice after myocardial infarction.
Mechanistically, we elucidated the involvement of type I and type II BMP receptors in mediating the pro-proliferative effect of BMP7, along with both canonical and non-canonical downstream signalling players.
Overall, our study highlights the potential of BMP7 delivery as a promising strategy to stimulate cardiomyocyte regeneration following injuries.

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Involvement of Lysophosphatidic acid receptor 4 (LPAR4)- dependent signaling in direct cardiac reprogramming

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Cardiovascular diseases are the leading cause of morbidity worldwide. Following an ischemic event, such as myocardial infarction (MI), necrotic cardiomyocytes, which induce tissue damage and inflammation, are replaced by cardiac fibroblasts (CFs). Lacking any regenerative ability, thisleads to the loss of cardiac function. Recently, cardiac regenerative approaches based on induction of Direct Cardiac Reprogramming (DCR) of CFs into induced cardiomyocytes (iCMs) have emerged, although to date none of the available protocols meet the yield and quality necessary to translate DCR into clinical practice. In order to identify new biochemical pathways possibly involved in boosting DCR efficiency, we analyzed the expression profile of CFs pre-treated for 24h with PTC-209, a chemical inhibitor of Polycomb Repressive Complex 1 (PRC1), which increased the efficiency of DCR up to 30%. Among the genes up-regulated following PTC-209 treatment, we focused on Lysophosphatidic acid receptor 4 (LPAR4), a membrane GPCR recently identified as a specific marker for cardiac progenitor cells. To investigate whether LPAR4 activation could play a role in increasing the efficiency of the DCR, we are evaluating the efficiency of DCR by both stimulating LPAR 4 with the selective agonist octadecyl phosphate (ODP) and by knocking down (KD) LPAR4 expression with specific shRNAs. Our preliminary data suggest a possible role of LPAR4 in the regulation of DCR process.

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Immunodetection of extracellular vesicles in experimental mouse melanoma

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Beside intrinsic features of tumor cells, malignancy also relies on various non-malignant cell types, infiltrating and surrounding the lesion, that constitute the tumor microenvironment. Extensive cell interactions occur via extracellular vesicles (EVs), differing in size based on their origin (endosomes, plasmalemm, or cell debris by death processes and autophagy) and thought to play relevant roles into inflammation and establishment of immune tolerance. Previous studies in *PML*-knockout mice demonstrated that PML loss led to uncontrolled inflammatory response arising from NLRP3 activation, favoured by liaison with P2X7, resulting in cytokines blast (IL-1β and IL-18) boosting melanoma growth and recruitment of immunosuppressive cells.
We are currently immunodetecting EVs in the melanoma mass and inside blood vessels, aiming to assess their (co)expression of inflammatory factors and size, as initial step to further explore their roles in immune tolerance, cancer progression and dissemination.

P.20

Molecular and structural investigation of the p53 regulatory pathway mediated by the Numb isoforms

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Numb is a tumor suppressor that functions by inhibiting the oncogene Notch and stabilizing the tumor suppressor p53. This latter function is mediated by the binding of specific Numb isoforms (1 or 2) to p53 and to the ubiquitin-ligase, Mdm2, responsible for p53 ubiquitination and degradation. The restoration of the Numb:p53 axis in breast cancer cells defective in Numb expression/function, rescues p53 activity and represents a potential therapeutic strategy for these Numb-defective tumors (20-30% of all breast cancers). To better understand the differential functions of Numb isoforms, we identified isoform-specific interactors, such as the endocytic protein SNX9 which is a specific interactor of Numb -1 and -2. We found that SNX9 also binds to p53 and is required for an efficient Numb -p53 interaction. To investigate the role of SNX9 in the Numb/p53 circuitry, both at the molecular and functional level, we characterized the surfaces necessary and sufficient for the interactions in all three proteins. Interestingly, we found two different regions in the Numb phosphotyrosine binding domain required for the interaction with p53 and SNX9: one is necessary for the direct binding with SNX9 while the other is required for the proper localization of Numb-1 and -2 isoforms at the plasma membrane, where we have indications that the Numb-SNX9-p53 complex is localized.
Finally, by silencing SNX9 expression in human non-tumorigenic breast cells, MCF10A, we established that the functional role of the Numb-SNX9-p53 complex seems to be related to the control of p53 signaling in the microenvironment by extracellular vesicles.
Our results point to the existence of a novel complex involving Numb-SNX9-p53 in which the presence of SNX9 seems to be necessary to maintain p53 at the plasma membrane. The balancing of the interaction between Numb 1/2 and SNX9 and Mdm2 might finely regulate the levels and the activity of p53 both intracellularly and in the surrounding microenvironment.

P.21

Evaluation of effectiveness therapeutics of small molecules in human glioblastoma cells

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Glioblastoma (GBM) is the most frequent malignant tumor among the tumors of the central nervous system . The therapy involves maximal surgical resection, followed by radiotherapy and adjuvant chemotherapy with Temozolomide. Despite this, the probability that the tumor will recur is very frequent. The objective of the study was to test three small molecules alternatives to Temozolomide for the treatment of GBM: Ipatasertib, Regorafenib and Selinexor. The therapeutic efficacy of these drugs was evaluated both in immortalized GBM cell lines (A172 (TP53 wildtype) and LN18 (TP53 p.Cys238Ser mutated) and in GBM cells isolated from patients treated at the Tor Vergata Polyclinic. Ipatasertib, a selective inhibitor of the PI3K/AKT pathway, inhibits the PI3K/AKT pathway and reduces cell survival both in immortalized glioblastoma lines and in one of three primary cultures of GBM isolated from patients. Regorafenib, a multikinase inhibitor directed against EGFR, is able to inhibit the MAPK pathway by abrogating the levels of both pTYR and pERK1,2 and has also been shown to be effective in inducing cytotoxicity in both cell lines and GBM cultures isolated from the three patients. Selinexor, a nuclear export inhibitor, was effective in inducing cytotoxicity in both cell lines causing a notable increase in the expression levels of TP53 and MDM2, which are therefore retained in the nuclear compartment due to the inhibition of CRM1 and perform an enhanced oncogenic action. These results show how Ipatasertib, Regorafenib and Selinexor are effective in inducing cytotoxicity with a higher efficiency than TMZ, regardless of TP53 status and MGMT methylation. This is a relevant fact as the prognosis for patients with GBM is poor due to continuous relapses. Therefore, the use of these drugs as adjuvant therapy or as an alternative to current radio-chemotherapy could represent a real benefit in terms of survival.

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Role of RDS 3337 heparanase inhibitor on crosstalk between apoptosis and autophagy in human glioblastoma cells

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BACKGROUND-AIM: Heparanase (HPSE) is an enzyme known as an endo-ß-glucuronidase, which plays a significant role in cleaving heparan sulfate side chains. This enzymatic activity results in the disassembly of the extracellular matrix, ultimately promoting cell invasion and the dissemination of metastasis. In our research, we focused on examining the impact of a novel HPSE inhibitor, RDS 3337, on the regulation of the autophagic process and its interplay between apoptosis and autophagy in U87 glioblastoma cells. We aimed to assess its potential as a therapeutic agent for modulating these cellular processes in glioblastomaMETHODS: The investigation focused on analyzing the autophagy and apoptosis processes in human glioblastoma cells. Both untreated cells and cells treated with the benzazolyl derivative RDS 3337 were analyzed by immunoblotting and flow cytometry analysis, aiming to gain a comprehensive understanding of how the benzazolyl derivative RDS 3337 influences autophagy and apoptosis processes in human glioblastoma cells.
RESULTS: We observed, an increase of LC3II expression together with a significant increase of p62/SQSTM1 levels in U87 cells incubated with RDS 3337.. These findings suggest an accumulation of autophagosomes and an inhibition of autophagic-lysosomal flux, indicating an impairment of the autophagic clearance. Conversely, the suppression of autophagic flux could activate apoptosis mechanisms, as revealed by the activation of caspase-3, the increased level of cleaved PARP1 and DNA fragmentation.
CONCLUSIONS: The findings of our study underscore HPSE’ssignificant role in promoting autophagy,compelling evidence that the novel HPSE inhibitor, effectively blocks autophagic flux in U87 human glioblastoma cells. This observation implies that the HPSE inhibitor has the potential to influence thebalance between apoptosis and autophagy in these cells, suggesting a potential role for this new class of compounds in the control of tumor growth progression.

P.23

Chronic stress on neuronal cells causes endogenous TDP43 cleavage and aggregation

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease affecting upper and lower motor neurons. It is characterized by the presence of proteinaceous inclusions containing the TAR-DNA binding protein 43 (TDP43), which form following protein misfolding, subcellular mislocalization, and subsequent prion-like aggregation. Physiologically TDP43 resides mainly in the nucleus, but it was shown to be mobilized in the cytosol upon stress induction. To date, cell-based studies aimed to dissect TDP43 behaviour were conducted following acute insults. Although this kind of stress elicits a strong biological response in terms of TDP43 localization and aggregation, it is far from representing the slow and persistent alterations occurring in the years-long disease process, which instead would be better mirrored by long-term chronic stress conditions. Since little is known about TDP43 biology upon milder and prolonged insults, a condition closer to pathology compared to acute stress, here we applied different chronic stress protocols and described TDP43 aggregation in SH-SY5Y human neuroblastoma cell line by combining solubility assays, thioflavin-based microscopy and flow cytometry. This approach allowed us to detect, for the first time to our knowledge in vitro, the formation of 25 kDa C-terminal fragment of TDP43, a pathogenic hallmark of ALS. Our results indicate that chronic stress, compared to the more common acute stress paradigm, better recapitulates the cell biology of TDP43 proteinopathies. Moreover, we optimized a protocol for the flow cytofluorimetric detection of bona fide prions in living cells, suggesting that TDP43 may form amyloids as a stress response.

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Stem cell-based disease model of hepatic organoids to study the cellular phenotype of ATP7B mutant genotype

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H1069Q substitution is the most frequent mutation of the copper transporter ATP7B causing Wilson disease in Caucasian population. Normally ATP7B localizes to the Golgi compartment in hepatocytes, but in the case of copper overload it moves to the endo-lysosomal compartment to excrete copper through the bile duct. A lack or dysfunction of this enzyme results in a progressive accumulation of copper in many organs, especially in the liver, but also in the nervous system, corneas, kidneys, and heart. Since this pathogenic mechanism has never been tested in patients' hepatocytes, we generated a model of H1069Q mutant hepatocytes by reprogramming homozygous patient's fibroblasts and heterozygous familiar controls into induced pluripotent stem cells (iPSCs) and by differentiating them into hepatocyte-like cells (HLCs). Using this model, we demonstrated that in patient's HLCs only one third of ATP7B-H1069Q localized in the Golgi complex and can move to the endo-lysosomal compartment upon copper stimulation, resulting in toxic copper accumulation. However, the liver is composed also of other populations beyond hepatocytes, like cholangiocytes. Thus, the study of the molecular mechanisms underlying Wilson disease can be improved using a model more representative of liver organization and cellular composition. To this aim, we have generated hepatic organoids starting from ATP7B-H1069Q and control iPSCs. This 3D model recapitulates the main step of embryonic liver development giving rise to complex structure formed by different cell types such as bile ducts and canaliculi. We have found that ATP7B-H1069Q iPSCs were able to generate hepatic organoids, but they showed some structural differences compared to control ones. Moreover, upon copper overload, ATP7B-H1069Q hepatic organoids showed a different behavior compared to the control ones. Interestingly, we observed that ATP7B is expressed also in cholangiocytes thus indicating a still unknown role of ATP7B in this population.

Identifying epigenetic changes linked to TBX1 activation

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Background: The discovery of the contribution of epigenomic mutations to genetic diseases has driven research into epigenetic modifications and epidrugs that target such 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 particular interest because of their potential therapeutic value and to increase out understanding of the mechanism by which this disease gene is regulated.
Methods: We are using an undifferentiated C2C12 murine myoblast cell line, 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 different time points. Selection of epidrugs was based in part upon their prediction function and in part upon a bioinformatic analysis of epigenetic modifications at the *Tbx1* locus in tissues expressing high or low levels of *Tbx1*, using the Genome Browser and Encode databases.
Results and conclusions: To date we have tested SAHA (Vorinostat), a histone deacetylase inhibitor. Surprisingly, results obtained in multiple experiments showed a modest reduction in *Tbx1* expression after treatment. We expected that SAHA would promote histone acetylation and thereby (potentially) increase *Tbx1* expression. We are now testing alternative epidrugs and combinatorial treatments.

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Optimizing liquid biopsy approaches for ovarian cancer patients

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Liquid biopsy (LB) has emerged as a promising and minimally invasive strategy for detecting and monitoring cancer. Its efficacy could be particularly ground-breaking in High Grade Serous Ovarian Cancer (HGSOC), where 70% of patients are diagnosed at the advanced stages, resulting in high mortality. However, there are still some challenges that need to be overcome, including the lack of standardization, and the assessment of analytical techniques. We here lay the groundwork for a methodological procedure by exploring (1) circulating tumor cell (CTC) and (2) circulating tumor DNA (ctDNA) detection in simulating standard and patient-derived blood samples. First, we validated and established a CTC-specific gene expression panel, achieving 44% detection rate in 9 LBs from high-stage OC patients. Additionally, we compared the efficiency of two different CTC enrichment methods in the same study, demonstrating comparable performances for Parsortix® and negative CD45 selection. In the same patient cohort, we set up a ctDNA assay based on untargeted Next-Generation Sequencing (NGS) of *TP53*. We identified *TP53* mutations in 6 out of 9 OC patients' plasma (66%), with a limit of detection set at 0.2% of variant allele frequency (VAF). For the first time in the context of OC, we compared the efficiency of CTC versus ctDNA detection, revealing a complementary approach for a comprehensive HGSOC-derived material identification in blood. Lastly, we are currently investigating a new label-free technology for CTC identification, exploiting Artificial Intelligence (AI) to precisely distinguish HGSOC cells from monocyte models with a high level of accuracy (97.44%). Overall, these results demonstrate the feasibility and the potential of an LB strategy based on CTC and ctDNA analysis, holding a promising advancement in HGSOC patient care.

MicroRNA "miR-30d" suppresses innate immune signalling in tumor cells by attenuating the cGAS/STING/IFN-I pathway

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Interaction of cancer cells with the immune tumor microenvironment is critical for tumor growth, metastatic dissemination and response to therapy. Recently, we discovered that oncogene-induced miR-30d alters the secretory pathway in cancer cells, thereby impacting the tumor-stroma crosstalk and fostering tumor growth and metastatic progression. We further observed that miR-30d overexpression attenuates the cGAS-STING pathway, a cytoplasmic DNA-sensing cascade leading to IFN-I mediated activation of anti-tumor innate immune surveillance, while miR-30d inhibition was able to induce this response in BC cells and mouse models. Our experimental evidence is consistent with a model in which inhibition of miR-30d may both trigger upstream induction of the cGAS/STING pathway in cancer cells, by causing release of dsDNA in the cytosol, and further sustain its execution by normalizing the structure of the secretory pathway. We are currently dissecting the mechanistic underpinnings of these effects of miR-30d inhibitor. In addition, we observed that miR-30d inhibition synergized with chemotherapeutic treatments in activation of IFN signaling in BC cells. Experiments with ex-vivo and in-vivo preclinical models are currently in progress to investigate whether inhibition of miR-30d could reactivate immune surveillance in immune-cold tumors and sensitize to chemotherapeutic and immune-modulatory treatments.

Keywords: Cancer cells; tumor microenvironment; cGAS/STING/IFN-I pathway; miR-30d

Molecular characterization of calcium homeostasis upon dormancy induction in the tumoral context

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Quiescence is the reversible state of proliferative arrest where cells are not actively dividing yet retain the ability to re-enter the cell cycle upon appropriate stimuli. Despite being physiological, also neoplastic lesions have dormant tumour cells (DTCs), which participate in metastatization, chemoresistance and relapse. Intracellular calcium homeostasis is fundamental for several cellular functions, and it's proposed to regulate quiescence although the molecular mechanism and its meaning for anti-tumoral therapies remains debated. Here we aim to elucidate the mechanism linking Ca^{2+} homeostasis to regulation of quiescence and its potential as therapeutic approach. We induced dormancy in tumour cells deriving from distinct organs using two different protocols. Aequorin measurements highlighted the significant decrease of Ca^{2+} transfer from ER to mitochondria upon dormancy induction. More specifically, the reduced Ca^{2+} released from the ER was shown to be due to decreased levels of expression of IP3R3 protein. Furthermore, single cell analysis using fluorescent probes revealed that Ca^{2+} suppression is a central event prior to quiescence entry. Altogether, our data suggest that Ca^{2+} homeostasis might represent a strategy for quiescence manipulation, potentially leading to tumour eradication.

Targeting pancreatic cancer stem cells in pancreatic ductal adenocarcinoma for enhanced therapeutic efficacy

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Pancreatic ductal adenocarcinoma (PDAC), a highly lethal diseases with an average 5-year survival rate below 10%[1], is also driven by cancer stem cells (CSCs) endowed with enhanced resistance to chemotherapy[2]. Piperlongumine (PL), an alkaloid derived from *Piper longum Linn*., exhibits promising anti- cancer effects. Despite previous *in vivo* and *in vitro* evidence supporting PL's efficacy[3], its specific impact on CSCs and clinical potential remain unclear. This study explores the effect of PL's on CSCs in PDAC. PDAC cells treated with PL show a significant reduction in spheroid formation capacity, indicating the inhibition of CSC function. Notably, PL does not induce apoptosis, and spheroids regrow post-treatment cessation. Cell cycle analysis reveals S phase arrest in PL-treated spheres. In immunocompromised mice model with subcutaneously implanted PDAC cells, combined treatment with PL and Gemcitabine, the gold standard for PDAC therapy, results in significantly lower tumor growth compared to control and single-agent-treated mice. In conclusion, our findings suggest that PL exerts a reversible cytostatic effect on spheroids, showcasing its potential for combination therapy with gemcitabine to enhance the efficacy of PDAC treatment.

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MiR-148b induces mitochondrial dysfuncton and affects tumor progression in melanoma

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microRNAs affect cancer progression by coordinating a variety of cell processes, including metabolism. The antimetastatic miR-148b is often downregulated in cancer, including melanoma, where it inhibits malignancy by impairing tumor cell dissemination. Since a complex interplay occurs between tumor progression and metabolic rewiring, we investigated the impact of miR-148b on cell metabolism. We characterized the cell metabolic profile upon miR-148b modulation and investigated its intervention on glycolysis and mitochondrial functions *in vitro* and *in vivo*. We evidenced decreased glucose uptake, glycolysis, and lactate production in mi-148b-overexpressing cells, while the opposite was found in miR-148b depleted cells. Decreased mitochondrial oxidative phosphorylation was also found upon miR-148b overexpression, as revealed by decreased electron transport chain (ETC) activity and mitochondrial ATP production. In parallel alterations in mitochondrial morphology and function were detected, including increased mitochondrial permeability transition pore opening and ROS production. We focused on the mitochondrial defect and found that miR-148b overexpression led to the downregulation of mitofusin 1 and OPA1, essential for mitochondrial fusion. Some preliminary data suggest that the miR-148b-dependent impairment of mitochondrial function may depend on the downregulation of peroxisome proliferator activated receptor-gamma coactivato-1alpha (PGC1α), a master regulator of mitochondrial biogenesis and predicted target of miR-148b. In parallel and in line with our previous data, a proteomic analysis revealed the modulation of several ETC complexes subunits and of different players involved in the cell antioxidant defense system upon miR-148b overexpression. Altogether, these data suggest that miR-148b affects mitochondrial function, which can contribute to metastasis inhibition.

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Assessing a molecular signature for induction chemotherapy response in laryngeal and hypopharyngeal cancers

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Laryngeal (LA) and Hypopharyngeal (HYPO) cancers are two types of head and neck carcinomas diagnosed in an advance status (stage III or IV) due to lack of early symptoms and biomarkers. First line therapies for advance LA and HYPO cancers include surgical and non-surgical approaches. Since total laryngectomy is a very invasive approach, which negatively affects patient quality of life, preservation strategies seem to be the best choices for maintaining organ functionality, being less invasive methods. Induction chemotherapy (IC) is a good functional preservation approach, but it cannot be offered to all patients due to restricted criteria (TNM staging). Moreover, not all patients benefit because of the high toxicities of chemotherapeutic drugs, such as cisplatin, docetaxel and 5-fluorouracil. The identification of a molecular signature of IC in responding or non-responding patients could allow to predict IC's response in patients with advanced disease, resulting in a great advantage for therapy decisions. Through the PRESERVE study (https://www.frrb.it/it/progetti-finanziati-preserve), we conducted a retrospective study on 200 FFPE samples obtained from patients with advanced LA and HYPO cancers treated alone with IC from different European hospitals. We performed laser capture microdissection on samples sections, followed by RNA-extraction and sequencing. Data analyses generated a molecular signature, through machine algorithms, which identified the most important genes in the prediction of IC's response. We trained the algorithm on 80% of samples and validated its prediction on the remaining 20%, with a great accuracy (more than 80% for all the categories). We will validate these data *in vitro*, in a panel of HNSCC cell lines, then in other cohorts of patients and in a PRESERVE prospective clinical trial. This project may lead to a great improvement in treatments of LA and HYPO cancers, predicting patients' response to IC, through personalized medicine approaches.

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Matched germline and somatic whole exome sequencing: analysis of male breast cancer patients with multiple primary malignancies

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Male breast cancer (MBC) is a rare disease, associated with genetic factors. MBC patients are at elevated risk of developing second primary tumors. Whole Exome Sequencing (WES) of multiple primary tumors from the same MBC patient may provide a comprehensive view of shared driver alterations and explain the genetic susceptibility to several types of cancer.
The aim of this study was to apply WES for a matched and integrated germline and somatic analysis of high-risk MBC cases with multiple primary malignancies to appropriately identify and prioritize candidate pathogenic variants (PVs).
We performed 35 WES from a series of 13 MBC cases with multiple primary tumors, for which both germline and tumor DNA were analyzed, on an Illumina NovaSeq 6000 platform. To evaluate WES quality, we measured the uniformity of coverage (%), which resulted an average of 96.4% and 88.7% for germline and somatic WES, respectively.
We identified an average number of about 37.000 germline and about 70.000 somatic PASS variants per sample.
To identify candidate germline PVs, all PASS variants were filtered to include: exonic non-synonymous or splice site variants with 30%≤variant allele frequency (VAF)≥70%, a total read depth≥20 and a Global Allele Frequency<1% in the gnomAD database. To identify the driver somatic PVs, we used similar filters, with exceptions of 5%≤VAF≥90%, total read depth≥40, variants classified as somatic in the Cancer Gene Census database.
For germline variants, 8 candidate PVs were identified, including PVs in *ATM*, *BRCA2*, *ERCC3* and *ZFHX3* genes. For somatic variants, an average of 38 and 41 candidate cancer-specific PVs were identified for MBCs and multiple tumors, respectively. Among these, an average of 8 PVs were shared between MBC and second tumor of the same patient.
Further ongoing analyses from this study are expected to contribute to give a comprehensive view of multi-cancer carcinogenesis that could allow for a better understanding of the aetiology of cancer.

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Role of ABCG2 transporter in chemoresistance mechanisms in ovarian cancer

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High grade serous ovarian cancer (HGSOC) has a recurrence rate of 75% of patients within two years due to the developing of chemoresistance to taxanes and platinum compounds. ATP-binding cassette G2 (ABCG2) protein carrier mediates drug transport across the plasma membrane and is known to be an HGSOC stemness marker highly expressed in chemoresistant cancer cells. To investigate the role of ABCG2 in metabolism and chemoresistance, we generated cisplatin-resistant OV90 and OC314 cell models, in which we observed higher expression levels of *ABCG2*, hinting that they acquire a stem-like phenotype. This was associated with the activating acetylation of mutant p53, in response to platinum, which has been reported to regulate *ABCG2* expression. Although ABCG2 does not transport platinum, it is a known carrier of estrone sulphate (E1S), i.e. the inactive form of estrone, whose occurrence as a circulating precursor prompted speculation of a role for ABCG2 overexpression in feeding a loop of estrone release in the HGSOC microenvironment. E1S formation in the ovary depends on the activity of two enzymes that mediate the addition or removal of the sulphate group, i.e. sulphotransferase (SULT1E1) and steroid sulphatase (STS) respectively. The detection of *STS* and, surprisingly, *SULT1E1* transcripts in HGSOC cell lines revealed the possibility of a cell-autonomous E1S synthesis, especially in OV90 upon the acquisition of resistance to platinum, where the *SULT1E1/STS* expression ratio reached 1. SULT1E1 is regulated by Peroxisome proliferator-activated receptor gamma coactivator 1-alpha and in HGSOC cell models *PPARGC1A* reflects the same expression levels of *SULT1E1*, suggesting the possible role of the coactivator in estrogens metabolism. Overall, these findings lead to the hypothesis that platinum resistance may lead to ABCG2 overexpression via mutant p53, triggering a feed-forward loop of estrone abundance which may have paracrine or autocrine effects fostering tumor relapse.

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miR-214 induces cell metabolism rewiring which leads to tumor progression

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microRNAs (miRNAs) are involved in the regulation of multiple events in cancer from proliferation, to cell metabolism. Reprogrammed energy metabolism, as the Warburg effect, is one of the most common tumor metabolic phenotypes. We previously demonstrated that miR-214 is pro-metastatic and overexpressed in both melanoma and breast cancer. Specifically, miR-214 silences Transcription Factors, such as TFAP2C, suppresses the anti-metastatic miR-148b and upregulates adhesion molecules (ALCAM and ITGA5). Evidences indicates an intense crosstalk between tumor progression and metabolic rewiring, hence we aim at understanding the role in this of miR-214. We evaluated the metabolic profiles of miR-214 overexpressing melanoma and breast cancer cells in vitro and in xenotranplants in mice. We worked on the identification of the main metabolic players coordinated by miR-214 and on the impact of the metabolic rewiring on metastatic features. We showed that miR-214 promotes a Warburg effect. In fact, an increase in glucose uptake, glycolysis, lactate production, glycolytic enzyme activity was observed in miR-214-overexpressing cells. Consistently, a decrease in Tricarboxylic acid, electron transport chain and fatty acid oxidation enzymes activity was also evidenced. In line, a diffused mitochondrial damage was found. We are currently investigating the molecular mechanisms underlying the observed metabolic and mitochondrial altered phenotype. Mitofusin 2, a mitochondrial GTPase, and a proven miR-214 direct target, key for maintaining mitochondrial network and bioenergetics, is potentially responsible for the mitochondria damage. When we assessed the impact of miR-214-dependent metabolism change on metastatic traits, we evidenced altered cell adhesion and motility suggesting a direct interplay between metabolic rewiring and tumor dissemination. Our data support the key role played by miR-214 in promoting malignancy by driving metabolism reprogramming in melanoma and breast cancer.

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HSPB3 loss impairs motor neuron differentiation and the maturation of neuromuscular junctions

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The small heat shock protein HSPB3 is expressed in motor neurons (MNs) and skeletal muscle cells (SkMCs), although its physiological functions in these cell types are still uncharacterized. Previously, we reported that HSPB3 participates in SkMC differentiation, and we found that it is upregulated during MN differentiation. *HSPB3* gene variants have been associated with Charcot-Marie-Tooth disease type 2, Amyotrophic Lateral Sclerosis (ALS), and congenital myopathy with signs of neuropathy. Peripheral neuropathies and ALS are characterized by repeated cycles of muscle denervation and reinnervation, occurring at the very early stages of the disease. Defects in axonal regeneration and in the maintenance of neuromuscular junctions (NMJs) have been suggested to play an important role in the progression of these diseases, which mainly manifest late during life. Of note NMJs are highly plastic and NMJs build-up during adulthood, as well as muscle regeneration and reinnervation rely on stem cell differentiation. Based on these data, we hypothesized that defects in the differentiation processes and NMJ development may contribute to the development of HSPB3-associated diseases. Here we investigated whether HSPB3 participates to the differentiation of SkMCs and MNs and to the correct build-up of NMJs. We found that HSPB3 loss in human induced pluripotent stem cells (hiPSC)-derived MNs downregulates the expression of genes involved in neuronal differentiation, NMJ development and maintenance. We further demonstrate that loss of HSPB3 impairs the development of a proper dendritic arborization in hiPSC-MNs and it compromises the maturation of NMJs in hiPSC-derived MNs-SkMCs co-cultures. Altogether, these data suggest that HSPB3 is required for the development of NMJs by promoting the postnatal differentiation of MNs and SkMCs.

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Dissecting cellular mechanisms underlying Δ^9-tetrahydrocannabinol effects on morphine-mediated signaling to identify more effective and safer combinations of analgesics

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Chronic pain is a debilitating condition exerting an enormous socio-economic burden. Existing medications provide only modest relief. Opioids in particular have considerable side effects, including sedation and the liability of addiction and abuse. Cannabinoids are increasingly being studied for their opioid-sparing potential, i.e. enabling a reduced opioid dose without loss of analgesic efficacy. In the framework of implementing a Quantitative Systems Pharmacology platform to identify novel combinations of existing drugs for chronic pain treatment, here we aim to investigate Δ^9-tetrahydrocannabinol (THC) co-administration effects on morphine-mediated signaling in different neuronal cell models.
Brain region-specific rat and mouse primary cells, HEK-293 cells, and differentiated SH-SY5Y cells were employed. Cells were tested under basal conditions and after exposure to morphine, THC, or the combination. To investigate opioid receptor activation, cAMP ELISA assay was performed. Confocal microscopy analysis was conducted to study the potential heteromerization of μ opioid receptor (MOR) and cannabinoid receptor 1 (CB1). Site-directed mutagenesis was performed to mutate MOR/CB1 interfacial residues predicted through Computational Alanine Scanning as relevant contributors to dimer formation.
Our results showed that morphine's ability to inhibit adenylyl cyclase was improved by THC co-administration at low concentrations in human neuron-like cells and in most, but not all, rodent primary neurons. After MOR desensitization, THC co-administration partially rescued morphine-mediated signaling. The p.V82A and p.F86A mutations in MOR respectively reduced and increased morphine potency in inhibiting adenylyl cyclase when morphine and THC were co-administered, confirming that these residues are involved in MOR-CB1 interaction. To conclude, our findings show a complex MOR/CB1 interaction and support the opioid-cannabinoid combination as a promising treatment for chronic pain.

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Exploiting glucocorticoid receptor antagonization to enhance NRG1-induced mitogenic activity: a promising strategy for cardiac regeneration

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The regenerative ability of the adult mammalian heart is very limited, leading to cardiomyocyte loss after injuries and often resulting in heart failure. However, during fetal and neonatal stages mammals possess a robust ability for heart regeneration driven by the proliferation of endogenous cardiomyocytes. This regenerative potential declines during early postnatal development. Our recent study demonstrated that glucocorticoids, a group of steroid hormones, contribute to this decline by activating the Glucocorticoid Receptor (GR), prompting heart cells to exit the cell cycle (*Pianca [...] Da Pra [...] & D'Uva, Nature Cardiovascular Research 2022*).
Using primary cultures of neonatal cardiomyocytes treated with the GR agonist corticosterone and selective GR modulators, we unveiled that the anti-proliferative effect of GR primarily operates through its direct transcriptional activity. RNA sequencing data of glucocorticoid-treated cardiomyocytes suggested the negative regulation of the MAPK cascade among the most significantly regulated pathways. Consistently, glucocorticoid treatment led to an increased expression of DUSP1 and ERRFI1, inhibitors of MAPK signaling. Neuregulin 1 (NRG1) and its co-receptor ERBB2 are known to stimulate cardiomyocyte proliferation by activating MAPK. However, glucocorticoid treatment suppressed NRG1-induced ERK activation, nuclear translocation, and the transcription of Immediate Early Genes (IEGs). In line, glucocorticoid administration inhibited cardiomyocyte proliferation induced by NRG1 or overexpression of a constitutively active ERBB2. Importantly, Glucocorticoid Receptor antagonization or ablation rescued NRG1 mitogenic capacity in post-mitotic cardiomyocytes.
In summary, we propose that glucocorticoids suppress NRG1-induced proliferation by intercepting MAPK signalling. Therefore, we suggest Glucocorticoid Receptor antagonization as a strategy to enhance the efficacy of cardiac regenerative therapies based on NRG1 administration.

Endothelial FLVCR1a controls Notch signalling and lipid metabolism to accomplish angiogenesis

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The Feline Leukemia Virus Subgroup C Receptor 1a (FLVCR1a) is a transmembrane protein critically required by endothelial cells (EC) to accomplish angiogenesis. Indeed, endothelial-specific *Flvcr1a* deletion in mice is embryonically lethal and strongly compromises both developmental and tumor angiogenesis.
FLVCR1a has been identified as a controller of intracellular heme balance, and, more recently, it has emerged as a choline importer. In this view, FLVCR1a stands out as a crucial determinant of EC metabolism. Nethertheless, the mechanisms underlying vascular defects due to FLVCR1a-loss have been poorly understood.
Here, we show that endothelial FLVCR1a controls lipid metabolism and angiogenic signalling. FLVCR1a modulation in ECs affected Notch activation and stability. Consistently, inhibition of γ-secretase by DAPT rescued defective angiogenesis due to endothelial FLVCR1a loss. Furthermore, FLVCR1a targeting influenced the availability of tricarboxylic acid (TCA) cycle intermediates by impinging on pathway interconnected with the TCA cycle (i.e. heme synthesis, fatty acid oxidation, ketogenesis). In this view, FLVCR1a inversely correlated with acetyl-CoA shuttling into the cholesterol biosynthetic pathway. Accordingly, dysregulated cholesterol synthesis led to cholesterol accumulation in models of FLVCR1a-deficiency. Remarkably, cyclodextrins-mediated cholesterol efflux rescued angiogenic defects due to FLVCR1a loss. Additionally, in line with its involvement in lipid metabolism, FLVCR1a deficiency diminished phosphatidylcholine levels in EC. Notably, choline supplementation in FLVCR1a-null EC was able to restore defective angiogenesis.
In conclusion, functional FLVCR1a is required by EC to accomplish angiogenesis by controlling Notch and lipid metabolism. Based on these findings, we hypothesize that FLVCR1a controls Notch-driven angiogenesis by shaping lipid membrane composition.

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Cerium oxide nanoparticles counteract UV-induced cell damage and mutagenesis, thus promising preventing UV-promoted skin cancer development and progression

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UV radiation is a major cause of skin diseases, including cancer, due to direct cell damage and secondary induction of oxidative stress. Commercial sun lotions shield UV, whereas antioxidant protection is still unsolved. Modern sunscreens are based on inorganic nano-formulations such as titanium oxide nanoparticles (TNPs). TNPs absorb UV, dissipating the extra energy in the form of reactive oxygen species (photocatalysis). If this allows stable activity, it also produces extra-ROS. If ROS are not a concern for intact skin, they become dangerous in cases of lesions to the stratum corneum. The cosmetic industry is thus seeking UV-stable antioxidant agents that may re-cycle once oxidized by UV. Cerium oxide nanoparticles (CNPs) possess both efficient UV-shielding, and stable antioxidant abilities; in fact, these biocompatible nanozymes act as superoxide-dismutase and catalase mimetics through a self-regenerating, energy-free redox cycle driven by $Ce^{3+}/^{4+}$ valence switch, thus preventing UV-induced oxidative stress. We tested the anti-cancer effect of CNPs vs. TNPs on UV-irradiated non-tumor human keratinocytes and melanoma cancer cells, in a system where the direct NP-cell contact mimic the real UV exposure of compromised skin. TNPs strongly increase UV toxicity, whereas CNPs reduce DNA damage and almost eliminate UV-induced mutagenesis on both cell types. Notably, CNPs efficiently counteract the noxious effects of UVA irradiated-TNPs, scavenging photocatalytic ROS. CNPs prevent damage also if administered immediately after irradiation, i.e. when the shielding effect is not necessary, showing that protection is not due their UV-absorbing activity, but rather, to their antioxidant effect. CNPs are thus ideal bi-functional sunscreens. In particular, these results suggest that CNPs, coupling their UV-shielding and stable anti-oxidant abilities, may protect against UV-induced damage preventing mutagenesis, possibly acting as to prevent cancer genesis and progression.

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Unravelling the role of the TGN export machinery for basolateral proteins in Amyloid Precursor Protein (APP) transport and processing

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The secretory pathway is involved in the synthesis, processing, and transport into specific compartments of around 30% of human proteins. The **Trans-Golgi Network (TGN)** is one of the major sorting stations within the secretory pathway, targeting newly synthesized proteins and lipids to different subcellular destinations such as the apical and basolateral plasma membrane domains. In the TGN **basolateral proteins** transport is regulated by a control system, which includes the orphan receptor **GPRC5A**, G-protein αi3, PLCβ3, specific isoforms of Protein Kinase C, and Protein Kinase D. The mis-sorting of basolateral proteins and/or dysregulation in the TGN-sorting machinery have been associated with different human diseases, including neurodegeneration. Our aim is to test the role of the basolateral sorting system on the **Amyloid precursor protein (APP)** transport and processing, a basolateral protein normally processed by **α-**, **β-**, and **γ-secretases**. In neurons, the β-secretase forms the β-amyloid peptide, one of the etiologic factors of Alzheimer's disease. We are characterising APP intracellular and extracellular levels and fragments in HeLa cells upon GPRC5A depletion (GPRC5A-KD) and treatment with secretases inhibitors. Here we show that APP N-terminal fragment secretion increases upon GPRC5A-KD in an α-secretase dependent way. Instead, the intracellular levels of APP C-terminal fragment decrease in GPRC5A-KD. We speculate that these alterations could be due to an increased α-secretase activity, deriving from defects in APP or α-secretase transport/localisation. Indeed, the APP C-terminal fragment changes its intracellular localisation in GPRC5A-KD cells. Moreover, we are characterizing APP and secretases expression upon GPRC5A-KD. Here, our goal is to identify a possible mechanism that couples the TGN regulatory system with APP transport and processing to reduce the production of the amyloidogenic peptide and favour the production of the non-amyloidogenic peptide.

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Deciphering the impact of a diagnostic/prognostic three-miRNA signature in the response to treatments in human gliomas

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Gliomas are diffusely growing brain tumours and challenging cancers for diagnosis and treatment. Therapeutic options for gliomas include surgery followed by radiotherapy and chemotherapy with the DNA alkylating agent temozolomide (TMZ). However, the prognosis of patients remains poor especially due to the mechanisms of resistance. O6-methylguanine-DNA methyltransferase (MGMT) expression is a top correlating feature for TMZ resistance and one of the main reasons for glioma treatment failure. MiRNAs are important players in glioma biology modulating many cancer-related processes, including response to treatment. We previous identified a diagnostic/prognostic three-miRNA signature (miR-1-3p, miR-26a-1-3p and miR-487b-3p) that displays an oncosuppressive role on several glioma biological functions.
To study the miRNA signature involvement in TMZ treatment response we overexpressed the miR-1/-26a-1/-487b signature in MGMT unmethylated neurospheres (NS; BT314 and BT453). We found that ectopic miRNA signature expression in TMZ-treated NS has a negative impact on proliferation with a concomitant increase of cell mortality that is mainly mediated by necroptosis, a type of inflammatory programmed cell death. We also identified, by in silico analyses, two pro-oncogenic transcription factors (TFs) as novel targets of the miRNA signature. Interestingly, both TFs act as master regulators of an important pathway that promotes acquisition of drug resistance and it is involved in glioma progression. Our on-going experiments also suggest that the impacted pathway is ‘druggable’ in glioma and its downstream effectors could be modulated by the three-miRNA signature.
Altogether, our data suggest that miR-1/-26a-1/-487b signature having an impact on treatment response, can pave the way for miRNA-based complementary therapies useful for patients’ management.

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Identify molecular pathway regulating cell proliferation through glycosphingolipids biosynthesis

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Glycosphingolipids (GSL) are a subtype of glycolipids localized on the plasma membrane. They have a role in the regulation of signal transduction and through this control several functions of cells including cell adhesion, cell motility, and growth. Contact Inhibition of Proliferation (CIP), a mechanism that ensures proper tissue homeostasis, is known to regulate GSL biosynthesis, and the GSLs in turn exert feedback control on CIP. The molecular details of this feedback circuit are not known. We have (1) identified GRASP55, a Golgi matrix protein, to be a key molecular player in this feedback circuit. The absence of GRASP55 determines cell density-dependent alteration in GSL biosynthesis and also CIP. We are now dissecting the molecular details of how GRASP55 contributes to this feedback circuit.

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Development of cell imaging-tools to identify prognostic and predictive biomarkers in Hereditary Spastic Paraplegia (HSP)

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Hereditary spastic paraplegia (HSP) are motor neuron diseases characterized by axonal degeneration involving the lateral corticospinal tracts. HSPs are caused by several types of mutations and the most common are haploinsufficient mutations in the SPG4 gene, which encodes spastin, a microtubule severing ATPase, that controls cytokinesis, endosomal traffic, lipid droplet (LDs) homeostasis and axonal transport. Truncating and missense mutations in the SPG4 gene have been identified in HSP patients; most missense mutations are located in the ATPase domain and affect the MT severing ability. As spastin-elevating therapies are emerging, there is a need to identify biomarkers that can be used to monitor the effects of spastin recovery treatments.
We have developed an automated, simple, rapid, and non-invasive cell imaging-based method to quantify the organization of the MT cytoskeleton, which can distinguish HSP-SPG4 from healthy donor lymphoblastoid and peripheral blood mononuclear cells. It is also able to detect changes in spastin protein levels.
We are now extending the imaging-based method to a large cohort of SPG4 patient cells to evaluate its sensitivity and specificity in relation with molecular and clinical patient features and to detect the effects of different spastin-elevating drugs. Additionally, we are focusing on other subcellular components affected by spastin mutations, such as LDs, exploring their behaviour in HSP-SPG4 patient-derived cells.

Searching for NF-Y-dependent signalling pathways in the progression from colorectal primary tumor to liver metastases

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Colorectal cancer (CRC) represents a significant clinical challenge, with over 50% of patients developing liver metastases in the course of the disease. Identifying molecular signatures associated with the epithelial-mesenchymal transition (EMT) leading to metastatic CRC could detect new specific therapeutic targets. The role of the transcription factor NF-Y in tumour proliferation and progression has already been demonstrated. NF-Y is composed of three subunits: NF-YA, -YB and -YC; the NF-YA subunit has two splicing isoforms, NF-YA long (NF-YA^{+EX3}) and NF-YA short (NF-$YA^{\Delta EX3}$). We have demonstrated that the long isoform is associated with a mesenchymal phenotype while the short with an epithelial one. Preliminary RNA-seq analysis has revealed the presence of 182 genes deregulated between primary CRC and synchronous liver metastases, containing NF-Y binding sites in their promoter. Notably, the upregulated genes in metastases belong to the apolipoproteins (APOs) family, prompting further investigation into their functional role in CRC metastasis. We have demonstrated, in silico and in vitro, that APOs are upregulated in liver metastases compared to the primary tumour. We will conduct ChIP experiments to confirm NF-Y binding to the promoters of APO genes. Additionally, functional studies will be performed using 3D cell cultures, involving overexpression and/or silencing of APO genes. The identification of NF-Y-mediated regulation of APO genes in CRC metastasis offers promising possibilities for prognostic and therapeutic strategies. Understanding the functional significance of these genes in EMT will not only advance our knowledge of CRC progression, but also open the way for the development of targeted therapies to inhibit metastatic spread and improve patient outcomes.

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Calcium and mitochondrial dysfunctions in sensory neurons: insights into the cellular mechanisms of neuropathic pain in Fabry disease

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Neuropathic pain is a hallmark symptom in Fabry disease (FD), a hereditary X-linked lysosomal storage disorder caused by a reduced activity of α-galactosidase A (α-GalA). The α-GalA deficiency results in the progressive accumulation of globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3) in the body fluids and lysosomes of various cell types, including sensory ganglia. The FD neuropathy affects the small thinly myelinated Aδ fibers and unmyelinated C fibers leading to the loss of intra-epidermal neuronal terminations, along with altered thermal and mechanical perception. Lipid accumulation, such as Gb3 and lyso-Gb3, leads to an altered function of ionic currents. It has been recently shown that administration of Gb3 to human umbilical vein endothelial cells leads to the downregulation of the calcium-activated K^+ channel $K_{Ca}3.1$ whereas lyso-Gb3 evokes cytosolic calcium transients and an enhancement of voltage-activated calcium currents in murine dorsal root ganglia (DRG). Therefore, we examined the mechanism underlying Ca^{2+} regulation in primary afferent neurons from the α- GalA gene KO mouse model. The obtained results suggest that other transport proteins participate in Ca^{2+} homeostasis in FD and their dysfunction may be directly involved in nociception. In this context, plasma-membrane Ca^{2+} ATPases (PMCAs) exhibit reduced activity in FD, leading to an increased resting $[Ca^{2+}]_I$ in sensory neurons. The reduced activity was associated with a decrease in cytosolic pH which weakened the PMCA-dependent calcium extrusion. We also evaluated the contribution of mitochondria to the calcium signaling and we observed an impairment of the mitochondrial buffer capacity as well as dysfunctional mitochondria and enhanced autophagy/mitophagy. These findings provide a basis for future insights into the alterations of calcium signaling underlying the onset of neuropathic symptoms in FD.

Transcriptional drivers at the root of the cutaneous squamous cell carcinoma

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Cutaneous squamous cell carcinoma (cSCC) is the second most common human cancer. It is caused by chronic long-term sun exposure which leads to an accumulation of somatic mutations in multiple tumor suppressor genes and increased expression of tumor promoter proteins. Given the high frequency of cSCC in the human population, identification of uncharacterized pathways underlying the pathogenesis of cSCC is of crucial interest. In this study, we investigated the functions and the crosstalk between several transcription regulators and cancer related proteins (p63, p73, YAP1 and PARP1) whose interplay is not fully characterized in the context of the cSCC.
We performed transcriptomic analysis in cSCC cells depleted for our proteins of interest and we observed a consistent overlap between the differentially expressed genes (DEGs). The most significant biological process affected in the absence of these transcription factors is the cell cycle progression, and further analysis of DEGs showed an enrichment in genes involved in DNA replication and revealed an involvement of the E2F family of transcription factors. Co-Immunoprecipitation (Co-IP) and Proximity ligation assays (PLAs) performed in cSCC cell line demonstrated a physical interaction between our proteins of interest only in the presence of the DNA suggesting the presence of a novel chromatin associated and transcription regulatory complex involved in the regulation of cell cycle related gene expression.

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New strategies for control Programmed Death Ligand-1 endocytosis to improve cancer checkpoint inhibitor therapy

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Immune checkpoint inhibitors (ICI), such antibodies against PD-L1 and PD-1, have shown effectiveness against a large number of cancer types. The therapeutic efficacy of PD-1/PD-L1 inhibitors is high in patients with high PD-L1 expression. Recent data showed that targeting extracellular Plasminogen Activator Inhibitor (PAI-1) by its inhibitor tiplaxtinin (TPX) synergizes with anti-PD-L1 checkpoint blockade in a model of murine melanoma, improving the efficacy of melanoma treatment. PAI-1 induced the internalization of surface-expressed PD-L1, resulting in the reduction of surface PD-L1. Binding of PAI-1 to uPA/uPAR complex results in the recruitment of low-density lipoprotein receptor protein 1 (LRP1), which also mediates PD-L1 internalization. Moreover, PD-L1 on exosomes surface can inhibit antitumor immune responses. We propose to inhibit PDL-1 endocytosis by uPAR/LRP1 complex blockade to maintain high-cell-surface levels of PD-L1 and to reduce the expression of exosomal PD-L1. A375M6 (metastatic melanoma) and A549 (non-small cell lung cancer) cells were treated with tiplaxtinin to evaluate the modulation of TPX on exosomal PD-L1. uPAR/LRP1 inhibitors were synthetized starting from the binding site of uPAR. 2D and 3D cultures of A375M6 and A549 were treated with TPX and uPAR/LRP1 inhibitors. Our results evidenced that in 2D and 3D cultures PAI-1 inhibition by TPX and uPAR/LRP1inhibitors are able to block the PD-L1 internalization and, consequently, to increase PD-L1 membrane levels. Moreover, we demonstrated that exosomes from TPX-treated A375M6 and A549 show a decrease of exosomal PD-L1 levels, compared to untreated cancer cells. Our results open the way for new combined therapeutic strategies of uPAR/LRP1 inhibitors with anti-PD-1/PD-L1.

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The effects of plastic nanoparticles on human and mouse primary and immortalized cell lines

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Plastic is a synthetic product consisting of organic polymers and chemical additives. It is the mostly produced and employed material for the production of objects used in everyday life. Large plastic waste exposed to UV radiation and atmospheric phenomena degrade giving rise to little particles called microplastics (MPs) and nanoplastics (NPs). Through the food chain MPs and NPs are ingested by living organisms and can accumulate within organs and tissues. The aim of this project is to study the effects of plastic nanoparticles on *in vitro* human and mouse primary and immortalized cells and, in case of cell damage, to understand the mechanisms by which tested NPs induce such damage. First results show that cells are able to internalize polystyrene nanoparticles, but this appears to have no adverse effects on the proliferative capacity of cells. Interestingly, the analysis of adhesion molecules expression levels shows that the exposure to polystyrene (PS) or polyethylene-terephthalate (PET) nanoparticles enhance the adhesion capacity of *in vitro* Sertoli cells, human glioblastoma cell lines and human immortalized keratinocytes.

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Modelling celiac disease: intestinal organoids for precision medicine, dream or reality?

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Celiac disease (CeD) is an autoimmune enteropathy caused by an abnormal immune response to gliadin peptides in genetically predisposed subjects. The therapy today is a gluten free diet to keep for life. New therapies include pro/post-biotics as nutritional supplements that can benefit the host. As no animal models are available for CeD, our first aim was to produce intestinal organoids derived from biopsies of CeD subjects as an attempt to model CeD at cellular level. Such model has been used to study the inflammation pathways and the role of postbiotics in preventing this one. Intestinal organoids were derived from CeD biopsies of patients at GCD (Gluten Containing Diet), with villus atrophy, and controls (CTR). Postbiotics, metabolites released by bacteria fermentation, from L. Rhamnosus GG; L. Plantarum; L. paracasei were obtained. Western Blot analysis was used to evaluate pNFkb and pERK levels as markers of inflammation in presence or not of gliadin peptides. GCD-CeD organoids at basal were more inflamed then CTRs, expressing higher levels of pNFkB and pERK. Gliadin peptides induced inflammation both in GCD-CeD and CTR, but in GCD-CeD organoids only low concentrations of gliadin peptides were enough to increase pNFkB and pERK levels, indicating that CeD organoids were more sensitive than CTRs to gliadin. Pretreatment with all postbiotics tested could prevent the inflammation in CeD-GCD organoids, both gliadin-induced and basal. In conclusion our data show that intestinal organoids are a good model to study CeD inflammation, and that postbiotics have been found effective in the prevention of CeD organoids inflammation both basal and induced by gliadin peptides. Moreover, Intestinal organoids is a good model for pre-clinical studies to predict the response to anti-inflammatory agents in CeD patients.

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Unraveling tuberous sclerosis pathogenesis with CRISPR-modified kidney organoids

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Tuberous Sclerosis (TS) is a rare genetic disorder resulting from mutations in the TSC1 or TSC2 genes, forming the Tuberous Sclerosis Complex, which also includes TBCD17. Both TSC1 and TSC2 are tumor suppressor genes regulating cell growth and proliferation via mTOR signaling. The absence of TSC1/TSC2 leads to tumor and cyst formation, primarily in the brain and kidneys. Renal manifestations predominantly involve angiomyolipomas and renal cell carcinoma, yet details on cellular pathogenesis remain elusive. In recent years, kidney organoids derived from human pluripotent stem cells (hPSCs) have emerged as a promising platform for studying kidney development and diseases. Here, we employed a CRISPR/Cas9 based OPTimized inducible gene KnockOut method for conditional loss of function in hPSCs. This approach relies on a tetracycline-inducible system for the conditional expression of sgRNA driving Cas9 activity. To ensure homogeneous and stable expression, transgenes were inserted into the AAVS1 genomic safe harbor locus. Following nucleofection, correct transgenes integration in the AAVS1 loci was assessed, and the selected clones were then treated with different concentrations of tetracycline to induce sgRNA expression and TSC2 protein downregulation. These clones were differentiated into kidney organoids and treated with tetracycline at different stages of the differentiation process to identify early pathways and developmental processes affected by TSC2 loss. We observed that TSC2 absence triggers cystogenesis in kidney organoids, with cysts likely arising from distal tubules. Notably, organoids also exhibited loss of cell polarity in the proximal tubules, although the organoids were correctly differentiated. The TSC2 inducible-KO kidney organoids will be instrumental for identifying early and late dysregulated pathways leading to cystogenesis and tumorigenesis, opening the possibility to discover new druggable targets/pathways.

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Walking down the road of neurodevelopment to obtain both 2D and 3D cortical cultures from hiPSCs using one single protocol

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Current advances in disease modelling have raised the urgency to develop culture systems that can align as much as possible to the physiology of the tissue/cells. A lot of effort is spent on organoids and 3D cell culture pathophysiological modelling since cell monolayers are far from resembling the native organization of tissues and organs. However, some assays are suitable for 2D cultured cells only and this does not exonerate us completely from using both these systems to gain solid results. Thus, we explored the possibility to derive from a single differentiation process both 2D and 3D cortical neuron cultures. Our optimized differentiation protocol is feeder and transgene-free, it mimics key embryogenesis stages and has been adapted from existing solid protocols. Experimentally, human iPSCs are detached to obtain embryoid bodies (EB). Upon early cortical induction, EB are plated for the formation of neural epithelium (NE) composed of typical neurorosettes. Neurorosettes colonies are then picked for the assembly of neural spheroids (NS), which can be either plated to obtain the terminal 2D differentiated cortical neurons or kept for the desired time in low attachment plates for 3D cortical neurons culture. Both 2D and 3D cultures result enriched in cortical neurons positive for Reelin, CTIP2, Tuj-1 and MAP2. The presence of some GFAP-positive cells from DIV 70 suggested that a percentage of heterogeneity is maintained along the differentiation process giving rise to a model that is enriched but not solely made of cortical neurons.
This protocol offers several advantages: i) a NE formation step that aids in selecting neural fate committed cells; ii) does not require a bioreactor for the 3D colture; iii) a single differentiation experiment can yield two models to fulfill different experimental needs. It can be easily adapted to different neural fates by adjusting the induction step and is suited both for neurodegeneration and neurodevelopment.

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Elucidating MLK4 role in pancreatic cancer progression and metabolism

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Pancreatic cancer (PC) represents a highly malignant neoplasm characterized by a dismal prognosis. The preponderance of PC cases is characterized by the presence of the oncogenic KRAS mutation, which activates intracellular signaling cascades, fostering cellular proliferation and regulating metabolic alterations to meet the increased energy and biosynthetic demands of PC. In order to develop alternative therapeutic approaches based on the specific metabolic features of PC, it is crucial to identify critical molecular mechanisms underpinning the pathogenesis of this malignancy. Mixed lineage kinase 4 (MLK4) is a serine/threonine kinase downstream KRAS, encompassing both oncogenic and tumor-suppressing functionalities. Although MLK4 is downregulated in approximately 20% of PC patients, its precise role in the development and progression of PC remains enigmatic. Our findings demonstrate that MLK4 loss promotes PC metastatic spread both in vitro and in vivo. Moreover, MLK4 has a critical role in regulation of glucose metabolism, primarily directed toward energy production. Specifically, MLK4 loss induces a metabolic rewiring toward aerobic glycolysis, accompanied by heightened glucose consumption and increased lactate production, both in vitro and in vivo. These metabolic changes are accompanied by an increase in oxidative stress levels and mitochondria fragmentation. In conclusion, our study sheds light on the multifaceted role of MLK4 in pancreatic cancer, highlighting its significance not only in metastatic potential but also in the metabolic adaptation that fuels tumor progression. These findings underscore the importance of further exploration into MLK4 signaling to identify new potential therapeutic avenues for pancreatic cancer.

Effects of NLRP3/PML axis on obesity-driven inflammation

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Adipose tissue (AT) has recently been indicated as the largest endocrine organ of the human body. Due to its plasticity, it can release a variety of cytokines depending on its trophic state. Obesity, identified as an excessive accumulation of AT, has been associated with the promotion of different pathologies including tumors, due to the instauration of a pro-inflammatory environment in the whole organism. The most studied effector of inflammation is the NLRP3 inflammasome that is responsible for the release of the pro-inflammatory cytokines IL-1β and IL-18. Our research group has recently proven that the Promyelocytic Leukemia Protein (PML) is able to reduce NLRP3 activation leading to a decrease of the inflammatory state in tumor microenvironment. Consequently, we hypnotize that NLRP3/PML axis can play a key role in obesity-driven inflammation.
In this study we demonstrated that visceral AT derived from PML^{KO} mice shows increased IL-1β release *in vitro* once treated with NLRP3 activating stimuli compared to WT one. Similarly, visceral AT obtained from obese mice, which were fed on high-fat diet (HFD), exhibited increased IL-1β release *in vitro* if compared to the control group (CTRL), fed on regular chow diet. The possible correlation between PML expression, and the increased pro-inflammatory state of AT, has been confirmed by a population analysis, conducted on 150 subjects, which revealed that PML expression in visceral AT of obese women is significantly decreased compared to normo-weight subjects. The data obtained so far, led to the hypothesis that decreased PML expression might contribute to the development of obesity-driven inflammation, indicating PML/NLRP3 axis as a promising target for pharmacological interventions in inflammatory-associated disease like most types of cancer.

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Activation of ALK signaling pathway induces DNA damage response in colorectal cancer

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In recent years, there have been several attempts to classify colorectal cancer (CRC) into well-defined molecular subgroups that reflect the inherent heterogeneity between patients. Consensus molecular subtypes (CMS) classification successfully achieves this purpose and provides an important tool for personalized medicine. We previously identified an inverse association between high levels of anaplastic lymphoma kinase (ALK) expression and recurrence-free survival, only in the CMS1 subtype, confirmed by a wide array of *in vitro*and *in vivo* assays.
To support the hypothesis of ALK having a strong implication in CRC patient tumor initiation, we overexpressed ALK receptor in a normal human colon epithelial cell line (NCM460). ALK overexpression was confirmed both in 2D and in 3D spheroid models, cultured in 3D.
The role of ALK pathway activation in colonocytes was then investigated by examining its effects on proliferation, survival, migration, and invasion through various 2D and 3D *in vitro* assays. Immunohistochemistry analysis revealed an increase in the proliferation marker KI67, and a decrease in the E-cadherin (E-CAD) expression in ALK-overexpressing spheroids compared to the control ones suggesting a correlation with tumor metastasis, progression, and invasion. Moreover, we identified a strong correlation between high level of expression of ALK and the downregulation of MSH2, a DNA Mismatch Repair (MMR) protein associated to microsatellite instability, which is typically found in CMS1 patients.
Finally, the mass density, size, and weight of *in vitro* NCM 460 spheroids were analyzed with an emerging microfluidic technology provided by CellDynamics isrl company.
Overall, these results suggest that ALK overexpression itself is sufficient to induce aggressive features in normal colonocytes, further supporting the hypothesis that ALK may be an attractive target for CMS1 colorectal cancer therapy.

Effectiveness of mesalazine in long-term treatment in patients with Crohn's Disease
A therapeutic agent in patients with gastrointestinal disorders - IBD and CRC -

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The purpose of the study is to assess the long-term efficacy of mesalazine in Crohn's disease and, in particular, to evaluate any differences between patients who maintain remission on mesalazine therapy versus those who require more advanced treatments such as biotechnological drugs, immunosuppressants, or surgical interventions. Patients with a follow-up of at least 1 year and aged over 14 years were included. Patients who could not take mesalazine due to allergy, intolerance, or adverse events related to its administration were excluded. The study included 1000 patients from the IBD Center archive at Sant'Orsola Hospital in Bologna. 12.2% maintained remission on mesalazine monotherapy with an average remission duration of 22.25 years. Among patients undergoing surgery, 65.1% maintained post-surgical remission with mesalazine monotherapy. Real-life clinical data from this study demonstrated the efficacy of mesalazine in maintaining remission in Crohn's disease patients with a specific phenotype: non-smokers, onset of the disease in non-pediatric age, disease localized to the ileum, non-stricturing or non-fistulizing behavior, and no or fewer extraintestinal manifestations. Therefore, the use of mesalazine may be an additional therapy available to specialists in the treatment of mild to moderate Crohn's disease.
The NLRP3 inflammasome regulates innate inflammatory responses, implicated in inflammatory bowel disorders like Crohn's disease and ulcerative colitis affecting the colon and small intestine. In murine models, IBD can be induced by compounds like DSS and AOM, potentially leading to Colon-Rectal Carcinoma. Identifying NLRP3 inhibitors and anti-inflammatory molecules as therapeutic agents presents a promising approach in managing these conditions.

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Expression of YAP target genes is supported by ARID1A through maintenance of an open chromatin state at YAP bound enhancers

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NF1 and ARID1A are vital tumor suppressor genes with distinct roles in tumorigenesis. Loss of NF1 activates oncogenic transcription factors YAP and TAZ, dependent on TEAD1-4. ARID1A is a key component of the remodeling complex SWI/SNF, regulates cell cycle progression and represses epithelial mesenchymal transition (EMT). Recently, it has been reported that at low mechanics ARID1A acts to inhibit YAP/TAZ mediated gene expression. We explored the interplay between these two factors in high mechanics.
Transcriptomic analysis of MCF10A cells knocked-down for either ARID1A or YAP reveals a partial overlap in affected transcripts. Notably, the subset of genes whose expression is significantly affected in both conditions (n=271) is concordantly down-regulated by KD of each of these factors. This data suggests that ARID1A supports the expression of a set of relevant YAP target genes, including the canonical YAP targets AXL and CCN2. Furthermore, ATAC-seq experiments in ARID1A knockout cell lines show a significant and specific loss of chromatin accessibility at TEAD4 bound enhancers, suggesting that ARID1A plays a role in maintaining an open chromatin status at regulatory regions of YAP target genes.
Our data implies that at high mechanics, loss of ARID1A may dump YAP oncogenic activity. In order to better understand the functional consequences of this hypothesis, we took advantage of different breast cancer cell lines displaying distinct degrees of YAP hyperactivation.

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Exploring EGLN1 as a therapeutic target for KRAS mutated lung cancer: insights into mithocondrial modulation

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Despite the introduction of innovative therapeutics, lung cancer is still the leading cause of cancer- related death world-wide. Patients harboring a KRAS mutation, comprising about 30% of lung adenocarcinoma cases, are particularly difficult to treat and often show a worse prognosis. For this reason, KRAS-driven lung cancer still requires deep molecular characterization to identify pharmacological targets.
We identified and validated EGLN1 as a novel druggable dependency gene, associated with KRAS- mutated lung cancer. The EGLN1 gene encodes the PHD2 prolyl-hydroxylase, mainly known to promote HIF-degradation during aerobic condition. EGLN1 is overexpressed in tumor tissue and its high expression correlates with worse prognosis in lung cancer patients. In lung cancer cell lines, EGLN1 supports proliferation, migration, colony formation and 3D growth. Pharmacological inhibition of EGLN1 exerts anti-proliferative effects both in cell lines and patient-derived organoids. EGLN1 promotes cell proliferation through at least two different molecular mechanisms, one HIF1a dependent and one HIF1a independent. To unravel the HIF-independent mechanism, we generated cell lines knockout (KO) for EGLN1, HIF1a or both and analyzed proteomic and transcriptomic profiles. We showed that EGLN1 modulates the level of a set of mitochondrial proteins, through a HIF1a independent mechanism. Some of these proteins are membrane channels involved in solute transport, some are involved in mitochondrial metabolism, some others are required for the maintenance of mitochondrial cristae or for mitochondrial ribosome assembly. These results suggest that EGLN1 regulates a number of processes leading to mitochondrial remodeling. Overall, we identified EGLN1 as a novel therapeutic target in KRAS-mutated lung cancer. EGLN1 has a pro-oncogenic function in lung cancer, in part because of its capacity to control mitochondria through an HIF-independent mechanism.

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Cell-cycle phase specific roles of alternative DNA polymerases and R-loops in genome instability by G-quadruplex structures

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Non-canonical DNA structures, such as G-quadruplexes (G4s) and R-loops, play several important roles, both in physiological and pathological mechanisms. G4s form in G-rich sequences and are composed by stacked guanine tetrads held together by hydrogen bonds, whereas R-loops are a triplex structure composed by an RNA strand annealed to its template and a non-template single strand DNA. The stabilization of G4s, by molecules called selective G4 binders, is known to activate an innate immune response through the formation of micronuclei, a well-known *biomarker* of genome instability. The aim of this project is to determine the molecular mechanisms of micronuclei formation induced by Pyridostatin (PDS), one of the most characterized G4 binders. More in detail, we performed a variety of Immunofluorescence assays to evaluate micronuclei induction after PDS treatment by altering the physiological balance of two alternative DNA Polymerases, Primpol and Pol Eta. Interestingly, Primpol seems to be necessary for micronuclei formation only during late S phase, while Pol Eta appears to be fundamental in all cell cycle phases. Surprisingly, R-loops play a complementary role to Primpol. Hybrid degradation by RNAse H1 overexpression inhibits micronuclei formation during late G1/early S phases, but it does not seem to affect the action of PDS during late S. In addition, by using a Proximity Ligation Assay, we observed that PDS can enhance the interaction between DNA:RNA hybrid duplexes and Pol Eta. Overall, these findings contribute to the elucidation of the mechanism of micronuclei formation by identifying critical factors and highlighting the presence of distinct mechanisms during different phases of the cell cycle.

Three-dimensional environment sensitizes pancreatic cancer cells to the anti-proliferative impact of budesonide through the reprogramming of energy metabolism

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Pancreatic ductal adenocarcinoma (PDAC) is the primary and most highly aggressive form of pancreatic cancer, characterized by late detection, rapid metastasis, and therapy resistance. Thus, the development of new therapeutic strategies is urgently needed. Interestingly, asthmatic patients display a decreased likelihood of developing PDAC. Budesonide, a glucocorticoid commonly used for asthma treatment, was recently identified as a potential antimetastatic agent for triple-negative breast cancer cells. This study aimed to evaluate the potential repositioning of budesonide for treatment of PDAC using 2D and 3D culture both *in vitro* and *in vivo*. We found that budesonide altered PDAC cell colony morphology and reduced invasiveness in 2D cultures without affecting proliferation. Conversely, budesonide significantly suppressed 3D tumor spheroid growth by inhibiting cell proliferation at nanomolar concentrations. Budesonide treatment also reduced PDAC tumor growth *in vivo* by restricting proliferation and promoting apoptosis, consistent with 3D results. Notably, transcriptome profiling analyses of 2D and 3D cultures indicated that PDAC cells underwent a general metabolic reprogramming to meet the energy needs to grow in a 3D environment, with an increase in glycolysis. However, in contrast to the metabolic reprogramming observed in 3D culture, budesonide promoted oxidative phosphorylation metabolism. Collectively, our findings elucidate how growth conditions influence the susceptibility of PDAC cells to budesonide. Our results provide unprecedented insights into the fact that transition from 2D to 3D culture sensitizes PDAC cells to budesonide and add to the emerging evidences that the response and susceptibility of tumor cells to drugs are affected by the culture environment. Our data also reinforce the notion that 3D cultures serve as valuable *in vitro* tools for drug screening to uncover novel anticancer molecules targeting metabolic pathways.

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Tailoring hydrophobicity in human H ferritin for targeted delivery of ellipticine and doxorubicin to tumor cells

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Ferritin, a natural iron storage protein, has gained attention as a promising drug delivery platform due to its ability to encapsulate therapeutic and imaging agents while targeting the transferrin receptor 1 (TfR1) overexpressed in tumor cells. Comprising 24 subunits forming hollow spheres with an 8 nm internal cavity, ferritins provide an ideal cargo space for various therapeutic agents, including conventional antitumor drugs, peptides, proteins, and nucleic acids.
Despite its potential, the hydrophilic nature of the internal surface of ferritin poses challenges in encapsulating hydrophobic drugs, limiting their use despite their efficacy. Consequently, it is worth considering modifying the internal surface of ferritin to enhance its overall hydrophobicity to encapsulate such drugs, allowing their targeted delivery to cancer cells. Human H ferritin was thus engineered by incorporating 4 or 6 tryptophan residues per subunit, strategically oriented towards the inner cavity of the nanoparticle. Comprehensive characterization of the mutants revealed that only the variant with four tryptophan substitutions per subunit retained proper disassembly and reassembly abilities. As a proof of concept, we evaluated the loading capacity of this mutant using ellipticine, a natural hydrophobic indole alkaloid with multimodal anticancer activity. Our data demonstrated a ten-fold higher loading efficiency compared to native human H ferritin.
Furthermore, to assess the versatility of this hydrophobicity-enhanced ferritin nanoparticle, we conducted a comparative study by encapsulating doxorubicin. Ellipticine- and doxorubicin-loaded nanoparticles were tested on a promyelocytic leukemia cell line, showing efficient uptake and eliciting cytotoxic effects. This engineered ferritin platform presents a promising strategy for enhancing the delivery of hydrophobic drugs to cancer cells, thereby paving the way for the development of more effective and targeted cancer therapies.

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ATAT1 silencing affects microtubule cellular function in mitosis and interphase

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Microtubule post-translational modifications (PTMs) can contribute to microtubule diversity and regulate different microtubule cellular functions such as cell division, cell motility and migration. a-tubulin acetylation occurs at lysine-40 (K40) and regulates several cellular functions often deregulated in cancer, as microtubule dynamics, cell migration and cell division. ATAT1 is the enzyme responsible for tubulin acetylation, but little is known about its relevance in cancer, based on bioinformatic data we put forth the hypothesis that ATAT1 may exert a pro-oncogenic role in lung cancer.
Here we show that lung cancer ATAT1-silenced cells exhibit a dramatic downregulation of acetyl-tubulin and similar levels and distributions of other PTMs on a-tubulin. ATAT1-silenced cells show a reduced colonies formation, during mitosis a higher percentage of mitotic defects such as polar and lagging chromosomes, and chromatin bridges, resulting in a delay in mitotic progression and in the activation of mitotic checkpoint. In addition, ATAT1 silencing affects migration rate and focal adhesion signalling in two different non-small cell lung cancer cell lines.
To discriminate the role of K40 tubulin acetylation and ATAT1 in cancer biology, we have induced GFP-tubulin overexpression in either WT or acetylation-resistant K40R configuration. Our results suggest that K40 acetylation orchestrates the progression of mitosis and delays cell migration. Interestingly, focal adhesion signalling was only disrupted in ATAT1-silenced cells and not in the acetylation-resistant mutant, suggesting an ATAT1 function independent of tubulin acetylation.
We believe that the successful completion of this study will provide a detailed mechanistic understanding of the role of ATAT1, tubulin acetylation and microtubule dynamics in lung cancer, besides experiments are underway to identify new selective compounds that inhibit ATAT1 activity.

Targeting of mitochondrial calcium and metabolism to counteract sarcopenia

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Mitochondrial Ca^{2+} ($mitCa^{2+}$) homeostasis links oxidative metabolism to muscle function. Ca^{2+} enters into the mitochondrial matrix through a highly selective channel (the Mitochondrial Calcium Uniporter, MCU) located in the inner mitochondrial membrane. The MCU complex is composed of pore-forming subunits (MCU, MCUb and EMRE) and of regulatory subunits (MICU1, MICU2, and MICU3). Within the mitochondria, Ca^{2+} activates key dehydrogenases of the TCA cycle, i.e. pyruvate dehydrogenase (PDH), isocitrate dehydrogenase and oxoglutarate dehydrogenase. PDH activation occurs upon dephosphorylation by pyruvate dehydrogenase phosphatase. On the contrary, pyruvate dehydrogenase kinase (PDK) phosphorylates and inactivates PDH. Sarcopenia is an age-related loss of skeletal muscle mass and strength. Mitochondria are negatively affected by ageing and the most prominent age-associated mitochondrial dysfunction includes reduced overall volume density, oxidative capacity and ATP production. In skeletal muscle, $mitCa^{2+}$ uptake positively regulates muscle trophism and metabolism by impinging on hypertrophic pathways and on PDH activity respectively. Thus, we decided to target $mitCa^{2+}$ uptake and PDH activity by using the novel RNA-based therapy to prevent muscle loss in sarcopenia. While the effect of MCU overexpression on skeletal muscle trophism is already established, the impact of MICU2 or of PDK isoforms mainly expressed in skeletal muscle (PDK1, PDK2, PDK4) were not investigated. For this purpose, we tested *in vitro* 4 different short hairpin RNA (shRNA) for PDK1, PDK2, PDK4 and MICU2 and we selected the most effective ones in knocking down the protein levels. Then, we showed that there is a significant increase in the cross-sectional area of the fibers upon silencing of *Micu2*, or *Pdk* isoforms, *Pdk1*, *Pdk2*, or *Pdk4* in the tibialis anterior of the 2-month-old CD1 wild type mice.

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Unveiling the role of astrocytes in Autosomal Dominant Leukodystrophy (ADLD)

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Autosomal Dominant Leukodystrophy (ADLD) is an ultra-rare and fatal late-onset neurodegenerative disorder that affects the central nervous system myelination and lacks effective therapy. The disease is caused by lamin B1 (*LMNB1*) gene alteration that leads to demyelination with the disease mechanisms remaining unknown. Although oligodendrocytes are responsible for myelination, astrocytes and ADLD patients' cells overexpressing *LMNB1* have displayed nuclear alterations with activation of proinflammatory and oxidative stress mechanisms that were absent in oligodendrocytes. The present study involved the characterization of astrocytes overexpressing lamin B1 and the elucidation of their role in demyelination. Human astrocytes (HA) were transduced with *LMNB1* and sorted for two assays: 1) characterization of astrocytes for expression of inflammatory markers, and 2) myelination assay on 3D microfiber co-cultures with oligodendrocyte precursor cells (OPCs). For the characterization, immunocytochemical analysis displayed nuclear localization of NFAT4 (nuclear factor of activated T cells 4) and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) suggesting astrocytic activation and inflammation. Additionally, proteome arrays revealed elevated levels of inflammatory cytokines present in the transduced HA supernatants. For the myelination assay the sorted HA were co-cultured with human OPCs on a microfiber scaffold for two weeks. It was displayed that OPCs were unable to produce myelin basic protein when grown with HA overexpressing *LMNB1* indicating the crucial role of astrocytes in supporting myelination. Overall, the study elucidated that *LMNB1* overexpression leads to astrocyte activation that consequently triggers inflammatory states and hinders myelination. Thus, these novel findings could place astrocytes at the epicenter of ADLD demyelination and drug development studies.

Perfect genomic correction of Human iPSCs from Becker Muscular Dystrophy through chromosome transplantation and generation of functional cardiomyocytes

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Dystrophinopathy is a group of X-inherited disorders caused by mutations in the DMD gene (Xp21.2 chromosome) encoding for the dystrophin protein. It features loss of respiratory and cardiac muscle strength and the destruction of nerve tissue. Within this spectrum, there are Duchenne (DMD) and Becker muscular dystrophies (BMD). The worldwide prevalence of BMD is around 1:19,000 and it is results in progressive muscle degeneration and proximal muscle weakness and in 70% of the cases also cardiomyopathy, the leading cause of death. Dystrophin mutations in BMD typically exhibit an in-frame pattern, leading to the production of misfolded or abnormal protein with reduced functionality. Due to the extensive size of this gene and the typical gross mutations associated with the disease, conventional gene therapy approaches are unable to correct the defects. Thus, we propose to correct the molecular defect of the dystrophin gene by using a novel genomic tool developed previously in our lab, the chromosome transplantation (CT) approach, in induced pluripotent stem cells (iPSCs). CT consists of the perfect substitution of an endogenous defective chromosome with an exogenous normal one, resulting in a normal euploid karyotype with a complete resolution of the gross mutation. After performing CT, we conducted genomic stability validation through Karyotype analysis and assessed pluripotency via stemness analysis and differentiation capability. BMD iPSCs and corrected iPSC clones will undergo differentiation into cardiomyocytes (CMs) to assess protein restoration at various levels: transcription and expression (via RT-PCR and WB), localization (via immunofluorescence) and functional recovery (electrophysiological properties using Ion Optix and Patch Clamp, as well as metabolomics and RNAseq profile analysis). This study provides an opportunity to fully correct not only this pathology but also, in the future, other X-linked genomic diseases.

P.65

DEC1 involvement in the adaptive response to respiratory complex I impairment in high grade serous ovarian cancer

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High grade serous Ovarian Cancer (HGSOC) is a deadly female neoplasia with only partially effective treatment options, whereby it is essential to identify new targets to improve current therapies. Targeting mitochondrial respiratory complex I (CI) has been proposed as a promising anti-cancer strategy for HGSOC too. However, cancer cells respond to CI ablation with compensatory mechanisms that allow cells to survive. DEC1 (Differentiated embryonic chondrocyte gene 1) is a transcriptional factor whose over-expression in OC has been described as negatively related to prognosis. The combination of glucose restriction and CI inhibition has been demonstrated to reduce tumor growth in association with the activation of two DEC1 interactors, i.e. glycogen synthase kinase 3β (GSK3β) and PGC1α, of which DEC1 is also a repressor, which prompted us to investigate the potential involvement of DEC1 in OC adaptation to CI ablation. In CI-Knock Out (KO) OC cells, obtained by CRISPR/Cas9 system, DEC1 protein levels are significantly reduced under glucose restriction and show a strong dependence on glucose availability, suggesting a possible role of DEC1 in metabolic stress responses. Furthermore, in our CI-KO models, the reduced DEC1 expression correlates with the dephosphorylation of GSK3β, suggesting a dependance of DEC1 on GSK3β regulation. Moreover, DEC1 transcriptional levels significantly drop in CI-KO xenografts, which may be related to the slow-down in tumor growth *in vivo*. Interestingly, DEC1 has also been described as a Transcription Factor EB (TFEB) target. Such mitochondrial stress-responsive protein translocates to the nucleus when mammalian Target Of Rapamycin Complex 1 (mTORC1) is inactive, a condition occurring in our CI-KO models under glucose restriction. Since TFEB is retained in the cytosol upon phosphorylation by GSK3β, thereby inhibiting its transcriptional function, the interplay between DEC1 and TFEB is being explored.

Dissecting and piloting the intracellular trafficking of adeno-associated viruses (AAVs)

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AAVs are commonly employed vectors for *in vivo* gene therapy. However, high doses are often needed to achieve the clinical goal. These doses induce severe, even lethal, adverse reactions in some patients. Moreover, even though many genomewide screenings have identified host factors needed for AAV transduction, due to different experimental setups, the overlap is limited. In addition, how these factors–such as AAVR–affect transduction remains unknown. It is, therefore, crucial to gain greater insight into the biology of AAVs to develop strategies that allow for safer therapeutic settings. Our goal is to dissect the trafficking pathway of AAV2 via generation of synchronized waves of AAV trafficking. We are currently using two approaches. The first is the retention using selective hooks (RUSH) assay. This system allows for the trapping of a streptavidin-binding peptide (SBP)-tagged protein within an organelle provided with a streptavidin-linked marker. Biotin releases the trapped protein and allows for its exit from the organelle. We have produced a recombinant AAV2 bearing the SBP within the capsid protein VP1. We have also generated HeLa cell lines stably expressing streptavidin-tagged hooks through which we will follow the trafficking of the engineered vector. The second approach relies on temperature synchronization of viral entry at 20°C and subsequent release. From accumulation in the early endosomes, we can track the movement of AAV2 through the endolysosomal system and to the Golgi up until cytosolic escape. This will allow us to test a focused panel of siRNAs targeting hits emerged from the genomewide screenings encoding for components of membrane trafficking machineries. The panel could be then expanded to include hits the role of which in intracellular trafficking is not yet known. These preliminary investigations will be propaedeutic to develop trafficking assays suitable for high-content applications to search for factors that enhance AAV transduction.

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Characterization of extracellular vesicles for early detection and treatment resistance in lung cancer

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Lung cancer is the leading global cause of cancer-related deaths, primarily due to high mortality rates associated with late-stage diagnoses. Treatment approach is dependent on tumor stage and molecular characteristics. Surgery, particularly for non-metastatic cases, is the primary option, followed by combined radiotherapy and chemotherapy. Targeted therapies, enabled by specific mutations, play a crucial role.
Two pilot mutations have been identified: (i) constitutive activation of EGF receptor tyrosine kinase (EGFR), targetable with inhibitors (Gefitinib, Erlotinib, and Afatinib); (ii) ALK gene translocation, leading to fusion protein formation and pro-tumorigenic mechanisms, addressable through ALK inhibitors (Crizotinib, Alectinib, Ceritinib, and Lorlatinib).
Drug resistance is becoming a clinical problem, Therefore, identification of new biomarkers represents an important challenge. Liquid biopsy is emerging as a promising screening tool in oncology research. It involves analyzing specific molecules in biological fluids, such as circulating tumor cells, miRNAs, ctDNA, and extracellular vesicles (EVs) secreted by cells.
The study aims to identify novel circulating biomarkers (proteins and miRNAs) associated with lung cancer EVs, focusing on differences between sensitive and resistant phenotypes to molecular therapies, especially Erlotinib. EVs were isolated from two lung adenocarcinoma cell lines, HCC-827 and ER-3 (sensitive and resistant, respectively), then subjected to proteomic analysis with specific validation.
At the same time, also microRNAs differentially expressed in lung cancer cell lines sensitive and resistant to Erlotinib were evaluated through bioinformatic analyses. The goal is to confirm microRNA expression differences in both cell lines and their derived vesicles, in order to identify specific biomarkers associated with Erlotinib resistance.

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Sudden cardiac death: mtDNA variability in an Italian cohort

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Background: Sudden cardiac death (SCD) is a major health concern with population rates ranging between 50 and 100/100.000. Although SCD can be the onset sign of a latent mitochondrial disease, mitochondrial DNA (mtDNA) contribution is largely unexplored in SCD at population level. Recently, mtDNA variants have been associated with congenital heart disease and may increase the risk of ischemic cardiopathy, suggesting mtDNA variants as potential risk factors also in SCD.
Therefore, we aim to define the mtDNA variation in a pilot Italian cohort of patients died from unexpected cardiac death.
Methods: The cardiac cause of death was confirmed by autopsy and individuals who had a diagnosis of illicit drug abuse or inherited cardiac disease were excluded from the analysis. Whole mtDNA Sanger sequencing was performed on blood samples of 28 unrelated cases referred to the Unit of Legal Medicine in Bologna between 2018 and 2022. tRNA, rRNA and nonsynonymous coding variants were prioritized according to their population and haplogroup frequency.
Results: Out of 28 patients, 32% were diagnosed with coronary heart disease, 43% with structural defects and 25% with unspecified cardiac disease. The overall frequency of top-level haplogroups in our cohort follows the distribution in the European population with the haplogroup H as the most represented in both groups (35.7% vs 43% respectively). No known or novel mtDNA clearly pathogenic variants were found. 2 rRNA variants and 7 missense variants were rarer than polymorphisms as they had a frequency lower than 0.5% in population databases. All variants but 2 were homoplasmic. 5/7 missense variants were clustered in ATP synthase genes and 4/7 missense variants were previously detected in patients with suspected mitochondriopathy.
Conclusion: We conclude that primary mitochondrial disease is not a major cause of SCD, but rare mtDNA variants may occur (32% in our cohort vs 0,52% in the population; $p<0.01$), potentially modifying the risk.

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XMH95: a new apoptotic strategy against aggressive leiomyosarcoma

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New cancer treatments are required for facing the high incidence of tumors. From a virtual screening of 1581 compounds forming the Diversity set of the National Cancer institute (USA), 18 were selected for their prediction to disrupt protein-protein interactions. A screening for the induction of cell death was next performed in a leiomyosarcoma (LMS) cell line. The compound XMH95 resulted the one to be able to induce cell death. LMS is an aggressive form of malignant smooth muscle tumor, where 40% of treated patients develop recurrencies or metastasis. The activity of the compound is selective against highly proliferative cancer cells. XMH95-induced cell death is mediated by Caspase 3 and antagonized by BCL2. To better understand the pathways involved, RNA-seq experiments were performed. The sequencing validated the involvement of apoptosis with the upregulation of different pro-apoptotic BH3-only genes in response to XMH95. The compound exhibits peculiar modifications of transcriptome with the engagement of different antiproliferative responses. The upregulation of BH3-only genes *BIK*, *PUMA* and *NOXA* was verified by qPCR and occurred in SKUT1 LMS cells, but not in normal uterine smooth muscle cells. We also found that XMH95 emits fluorescence and localizes to the nucleus, where it induces chromatin rearrangements with the formation of heterochromatin dots. This suggests a mechanism of action involving the binding of DNA, which is sustained by *in silico* predictions. Furthermore, the mechanism by which it induces growth arrest and apoptosis does not involve the induction of double-strand breaks, as we demonstrated by the detection of γH2AX signals and the lack of upregulation of DNA repair pathways. Our results lead us to investigate XMH95 as an antineoplastic drug in aggressive metastatic leiomyosarcoma.

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The role of lysine histone demethylase KDM5B isoform on cellular migration and transcription regulation in breast cancer

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Histone Lysine Demethylases (KDMs) regulate gene expression and other biological processes as DNA repair and damage checkpoint activation. KDMs deregulation in tumour environments contributes to cancer development and progression. Specifically, KDM5B, acting on H3K4me3, is up regulated in several cancer types, including breast cancer. It is still unknown what contextual factors make the demethylases functioning as oncogenes or tumour suppressors. The presence of different isoforms and their relative amount may define their behaviour in the tumour context. In breast cancer, the existence of a KDM5B-N-terminal truncated (NTT) isoform has been recently demonstrated (Di Nisio et al., 2023). NTT’s truncation affects the whole JmjN and part of the ARID domains. Thus, it lacks the demethylase activity, but it could maintain the ability to bind the H3 tails through PHD domains, maybe acting as a negative dominant competitor for the canonical KDM5B-PLU1 isoform. Since the ratio between PLU1 and NTT is variable in different cancer cell lines, it is relevant to understand its regulatory roles. One of the main processes that involve KDM5B-mediated regulation is cell migration. In this study, we investigated the effects of the over-expression of NTT or PLU1 on migration in breast cancer cell lines. NTT-overexpressing MCF7 cells significantly increased their migratory potential compared to PLU1-overexpressing or empty vector-transfected ones. MDA-MB-231 showed the same trend even if the observed differences were not statistically significant. However, previously performed RNAseq experiments highlighted a strong involvement of epithelial-mesenchymal transition (EMT) pathways in the transcriptome of breast cancer cells overexpressing NTT. Thus, we are further investigating the expression of these differentially expressed genes which could be related to the phenotype.

Detecting microRNAs as laryngeal cancer biomarkers via SERS biosensor based on electrospun nanofibers decorated with in situ synthesized gold nanoparticles

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Laryngeal Cancer (LCa) represents one-third of all head and neck cancers and is characterized by high mortality and morbidity due to late diagnosis. microRNAs (miRNAs) are small non-coding RNAs (18-20 nucleotides) involved in post-transcriptional gene regulation that are emerging as potential biomarkers of several cancers, including LCa [1]. Due to their very low concentrations, down to femtomolar (10^{-15}M) level, it is challenging to detect miRNAs using the old standard molecular techniques (PCR, Northern blot, Microarrays and so on). In recent years, nanotechnology has provided new diagnostic and prognostic strategies for miRNA detection by offering the possibility of constructing nanostructured biosensors [2]. Here, we present a Surface Enhanced Raman Spectroscopy (SERS) [3] biosensor for detecting microRNAs as LCa biomarkers. The proposed biosensor consists of *in situ* synthesized Gold Nanoparticles on electrospun polymeric nanofibers. The immobilization of a molecular beacon DNA probe labeled with Cyanine 3, as Raman Reporter, on the overmentioned polymeric scaffold allows a specific interaction with target miRNA 223-3p, resulting to be overexpressed in affected patients. A Limit of Detection (LOD) of 19.4 fM was achieved. The binding specificity was further tested using a non-complementary miRNA sequence, showing any change in the SERS signal. Results in the characterization and application of this system will be discussed.

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Therapeutic targeting of Glioma Stem Cells in Glioblastoma Multiforme with a promising miRNA-340-5p/A40s aptamer

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Glioblastoma multiforme (GBM) is a highly aggressive brain tumor in adults with poor survival rates despite chemotherapy advancements. Glioma stem cells (GSCs), a small but resistant subset of cells within GBM, contribute significantly to tumor aggressiveness and recurrence. GSCs communicate with the tumor microenvironment (TME), including hypoxic regions. Hypoxia promotes GSC self-renewal, plasticity, proliferation/differentiation, angiogenesis, and invasion.
Aptamers, short synthetic DNA or RNA oligonucleotides offer a promising avenue for targeted therapy due to their low toxicity and ability to penetrate the blood-brain barrier (BBB). A40s, an RNA aptamer sorts GSCs from differentiated GBM cells by engaging the erythropoietin-producing hepatocellular receptor A2 (EphA2) and is rapidly internalized by GSCs. Our study aimed to target the hypoxic niche of GSCs using an aptamer-miRNA based tool. We used A40s as a carrier for the delivery of miR-340-5p in GSCs. Our unpublished data indicate that miR-340-5p targets carbonic anhydrase 9 (CA9), a transcriptional target of HIF-1α, overexpressed protein in GBM and linked to a poor prognosis. We identified a novel molecular mechanism that functionally connects CA9 and HIF-1α in GSCs via miR-340-5p, creating a feedback loop that sustains hypoxia.
We employed non-covalent conjugation of miRNA-340-5p mimic duplex to A40s aptamer. GBM patient-derived GSCs were treated with chimeric constructs, and intracellular accumulation of conjugates was evaluated. The analysis revealed a downregulation of the CA9 protein and EphA2 receptor, after treatment with the chimera. Functional assays were conducted to elucidate the therapeutic features of the A40s-miR340-5p chimera.
These findings suggest that the aptamer-based approach could represent a promising therapeutic strategy to enhance the efficacy of GBM treatment. Further research is needed to clarify the mechanism of action and implement the efficacy of chimeric constructs.

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Investigation of mechanisms regulating AurkA nuclear localization and oncogenic functions and their dependence on TPX2

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AurkA is a serine-threonine kinase involved in mitotic spindle assembly and mitotic progression, frequently overexpressed in cancer. The microtubule binding protein TPX2 is the best characterized AurkA activator, recruiting the kinase to spindle microtubules, stabilizing its active conformation, and protecting it from degradation. Oncogenic kinase-independent functions of AurkA depending on its interphasic nuclear localization have been recently described, particularly in breast cancer, but the mechanisms leading to AurkA nuclear accumulation are still poorly explored. This project aims to investigate mechanisms involved in AurkA nucleo/cytoplasmic trafficking and oncogenic functions related to the AurkA nuclear fraction, focussing on the evaluation of TPX2 contribution. Here we show that in non-transformed hTERT RPE1 cells AurkA nuclear localization is regulated by cell cycle phase, proteasome degradation and nuclear export, but is independent of its kinase activity. Bioinformatic analysis, accompanied by cellular validation, showed no obvious nuclear localization signal (NLS) in AurkA. In the search for factors mediating AurkA nuclear accumulation, we explored the possibility that TPX2 plays a role, given its nuclear localization and the frequent co-overexpression with AurkA in tumours. Using hTERT-RPE1 overexpressing AurkA alone or in combination with TPX2, we show that TPX2 co-overexpression promotes nuclear accumulation of overexpressed AurkA in interphase nuclei. Importantly, TPX2 co-overexpression favours AurkA oncogenic nuclear functions in mammospheres derived from MCF10A cells. Together, these results highlight the TPX2 determinant role in promoting AurkA oncogenic functions dependent on its nuclear fraction. Investigating the mechanisms underlying AurkA nuclear import may pave the way for the development of specific approaches to target nuclear AurkA in cancer, e.g. by targeting the AurkA/TPX2 complex.

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Exploring the synergistic effect of Graminex G96 Pollen and Teupol 25P on human macrophages and prostatic cancer cells

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Benign prostate hyperplasia (BPH) affects 50% of men aged between 51 and 60 worldwide. Inflammation, oxidative stress and androgen activity are reported as key factors in BPH's pathogenesis. The concern about the risks associated with the long-term use of 5α-reductase inhibitors has led to the search of alternative therapies. Considering the increasing interest in the use of phytotherapics and food supplements, the current study aims at investigating the effectiveness of the combination of a pollen extract (Graminex® G96® Pollen - G) with teupolioside (Teupol 25P - T) in terms of anti-inflammatory effect on LPS-stimulated human macrophages and anti-proliferative action on human prostate cancer cells (PC-3). The modulation of the Nrf2-dependent antioxidant response as well as the NF-κB-driven inflammatory pathway were analyzed on inflamed macrophages. In parallel, the inhibitory activity of the GT combination on the 5α-reductase enzyme was checked in PC-3 cells through the measurement of epiandrosterone, a metabolite of dehydroepiandrosterone (DHEA) via the 5α-reductase enzyme. The well-known 5α-reductase inhibitor finasteride was administered for comparison. Our work demonstrates that the GT association promotes Nrf2 and catalase-dependent antioxidant responses and counteracts the NF-κB-driven inflammatory pathway in macrophages. In parallel, PC-3 cell proliferation rate is reduced by GT alone and in association with finasteride (0-5 µM). Moreover, the GT combination shows a statistically significant reduction of the 5α-reductase enzyme activity. According to the results obtained, the GT association could be a suitable tool for the treatment of BPH, alone or in association with clinically used 5α-reductase inhibitors.

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Into the Heart at Single Cell Resolution: Unveiling Transcriptional Changes of Doxorubicin-induced Cardiotoxicity

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Doxorubicin (DOX) is a potent chemotherapy agent used to treat a wide range of malignancies, but its clinical use is hampered by a dose-related cardiotoxicity that can lead to heart failure. The underlying molecular basis of DOX-induced cardiotoxicity (DIC) remains largely elusive as only few studies have explored the molecular events distinguishing an early and reversible phase of the disease. However, unveiling the early transcriptional changes of DIC could be crucial for the development of new cardioprotective agents.
Using our established murine model of AIC, BALB/c mice received either saline or DOX (3 weekly injections of 4 mg/kg). Hearts were collected at 3 days (early) or 6 weeks (late) post-injection. Nuclei isolated from frozen hearts underwent single-nuclei transcriptomic analysis (snRNAseq).
Analysis of snRNAseq data from 12 hearts revealed 8 major cell types, including cardiomyocytes (CM). Unbiased subclustering of CM identified a distinct CM_Stressed population characterized by high expression of cardiac stress markers such as *Nppb* and *Myh7*. Moreover, CM_Stressed was enriched in both 3 days and 6 weeks DOX-treated groups. ScFate trajectory analysis of CM uncovered a progression path to CM_Stressed, suggesting that DOX drives profound transcriptional changes toward a stress status in CM at both early and late stages of DIC. Among the top DEGs, Golgi associated kinase 1B (*Gask1b*) was significantly upregulated at both 3 days and 6 weeks in CM. Intriguingly, preliminary screening in a zebrafish model of DIC showed that *Gask1b* silencing prevents DOX-induced decline of heart function, identifying *Gask1b* as a new potential player of DIC.
We generated a single-nucleus dataset of DOX-treated mouse hearts and identified transcriptional changes driven by DOX in CM at early and late stages of the disease. Furthermore, we identified *Gask1b* gene, whose role in cardiac pathophysiology was previously unappreciated, as a new potential marker and determinant of DIC.

P.76

HDAC1-3 controls WT1 inhibitory effect on H19 expression during MMT induction

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Peritoneal fibrosis is a pathological alteration of the peritoneal membrane occurring in a variety of conditions including peritoneal dialysis (PD), abdominal adhesions and peritoneal metastases. Characteristic of this process is the acquisition of invasive/pro-fibrotic abilities by mesothelial cells (MCs) through induction of mesothelial to mesenchymal transition (MMT), a cell-specific form of EMT.
Histone acetylation and deacetylation, exerted by histone acetyltransferases (HATs) and histone deacetylases (HDACs), play an essential role in modifying chromatin structure and in regulating gene expression. HDAC pharmacological inhibitors are promising tools in the therapy of fibrotic diseases and in cancer.
Aim of this study was to investigate molecular mechanisms linking HDAC1-3 inhibition to the reacquisition of epithelial features in primary fibrotic MCs from PD patients, focusing on the effect on lncRNA expression.
Treatment with MS-275, a HDAC1-3 specific inhibitor previously known to promote MMT reversal in MCs and to upregulate the master gene of mesothelial differentiation Wilm’s Tumor Protein 1 (WT1), downregulated lncRNA H19 expression. The same effect was observed upon WT1 genetic silencing. Based on these results, a bioinformatics analysis of H19 promoter revealed a WT1 specific binding site on H19 promoter, and Chromatin Immunoprecipitation experiments demonstrated that this binding was increased upon MS-275 treatment. Taken together, these results demonstrated an inhibitory effect of WT1 on H19 expression.
We then focused on a functional characterization of H19 role in MMT. Genetic silencing/ectopic expression revealed that H19 promoted the expression of MMT markers such as SNAIL, TGFBRI, SMAD3 and PAI-1, while downregulating the epithelial marker E-Cadherin, and favoured MC migration/invasion on collagen matrix.
Overall, we discovered an HDAC1-3-WT1-H19 axis potentially relevant for the design of new therapies aimed to counteract peritoneal fibrosis.

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In vitro and *in vivo* testing RNA delivery and translation efficiency by novel split-GFP based delivery sensor

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The delivery of nucleic acids (NAs) remains a challenge for the exploitation of this class of therapeutics. Indeed, NAs suffer from biological instability and low target access due to biological barriers. A great effort has been devoted to the development of effective NA delivery systems, and nanoparticles (NPs) appear promising. However, the absence of a systematic and reproducible technique to evaluate the effectiveness and specificity of NPs remains critical. We propose to develop a quantitative assay to detect NAs efficiency delivery and translation *in vitro* and *in vivo*, by exploiting split-green fluorescent protein (splitGFP) technology. SplitGFP is composed of two nonfluorescent fragments, the GFP1-10 and GFP11, able to reconstitute the GFP fluorescence upon self-complementation. We generated GFP11 variants and tested their properties to complement the GFP1-10 fragment expressed stably in HeLa cell clones. We have generated tandem repeats of the β-strand 11 (1X, 3X, and 7X) to develop probes characterized by a proportional enhancement of the fluorescence signal, and found that the GFP11 3X and 7X display an approximately 2-fold improvement in the amount of complemented fluorescence compared to the GFP 1X. We have also set up a protocol to introduce plasmids for the expression of GFP11 variants and to monitor the reconstituted GFP signal in 3D cellular models, *i.e.* spheroids. The detection of splitGFP fluorescence in spheroids transfected after their formation was successful. So far, we have validated the approach by using expression plasmids; the next step will be to test the system by challenging our 2D and 3D GFP1-10 cellular models with mRNA for GFP11 variants encapsulated in NPs of different origin and composition.

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Respiratory complex I deficiency triggers OMA1-mediated integrated stress response and affects lipid homeostasis during glucose restriction

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Respiratory complex I (CI) is a pivotal enzyme for cellular bioenergetics and its functional alterations trigger metabolic and molecular adaptive responses that help cancer cells survive despite the energetic deficit. Since such mechanisms are far from being completely understood, we exploited multiple cancer cell lines of different tissue origin lacking CI in which we knocked out the core subunit NDUFS3 (NDUFS3$^{-/-}$) to elucidate their response to energetic stress. During glucose restriction, NDUFS3$^{-/-}$ cells showed significant mitochondrial network fragmentation and depolarization, that in turns triggered the activation of the mitochondrial protease OMA1, as shown by OPA1 and PGAM5 cleavage and DRP1 dephosphorylation. These mitochondrial alterations were accompanied by a consistent activation of Integrated stress response (ISR) as proved by the phosphorylation of eIF2α, the block of protein synthesis, the nuclear re-localization of ATF4 and the increased expression of CHOP. This molecular mechanism is directed by OMA1 and suppressed when the protease expression is prevented by siRNA. We also found that glucose starvation triggered the accumulation of lipid droplets in close proximity to deranged mitochondria in CI-defective cells both *in vitro* and *in vivo*, accompanied by a profound alteration of lipid content, in particular we observed an increase in triacylglycerols and in cholesteryl esters amount. In this context lipid droplets could sequester misfolded proteins, excess lipids and prevent free calcium overload in the cytosol in order to alleviate endoplasmic reticulum stress. In conclusion, loss of CI and glucose restriction induce a striking mitochondrial distress with depolarization and network fragmentation and trigger OMA1-mediated ISR to possibly overcome the stress status. Further analyses are necessary to dissect the role of ISR in CI-defective cancer cells adaptation and survival.

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FK506 bypasses the effect of erythroferrone in cancer cachexia skeletal muscle atrophy

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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. 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.

Hijacking NRG1/ERBB3 axis to overcome Osimertinib resistance in NSCLC

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EGFR-mutated non-small cell lung cancer (NSCLC) is successfully treated with small molecules TKI inhibitors. However, tumor relapse occurs and the underlying mechanisms of drug resistance remain largely unexplored. The complete abrogation of EGFR axis, achieved through the combination of osimertinib (OS) and cetuximab (CTX), leads to the activation of the parallel pathways HER2 and HER3 along with the upregulation of the HER3 ligand, NRG1. In line,NRG1 when overexpressed increases proliferation and invasion of cancer cells conferring resistance to EGFR-targeted therapy. Interestingly, EGFR resistant patient-derived cell lines, confirm the strong activation and overexpression of HER2 and HER3. The evaluation of NRG1 mRNA levels, unveils a correlation between OS resistance and HER3 activation. In addition, we successfully established ex-vivo cultures of OS resistant patients. A single cell RNA sequencing analysis of OS resistant patient-derived cell lines confirm the abundance of the NRG1 driven bypass pathways activation in response to therapy. Proliferation assays performed both in monolayer and in 3D growing conditions on sensitive cells shown that NRG1 strongly impairs OS response, as tested by cell invasion in gelatin degradation assays. Conversely, employing a monoclonal antibody anti-NRG1 we observed a reduced gelatindegradation in resistant cells, confirming the key role of NRG1 in cell invasion and metastasis. *In vitro and vivo* dataconfirmed that, when combined with OS and CTX, the anti-NRG1 antibody strongly inhibited cells and tumor growth in mice, preventing TKI-induced up-regulation of HER3 and its activation. Collectively, these findings support the hypothesis that NRG1 may represent an escaping mechanism to EGFR inhibition and its neutralization will effectively impair ERBB parallel pathways activation.

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Dissecting tumor microenvironment remodeling triggered by targeting complex I in ovarian cancer

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Mitochondria-targeted anticancer therapy, in particular inhibition of respiratory Complex I (CI) as a key enzyme of the oxidative phosphorylation (OXPHOS), has recently proven to be a promising strategy. This has yet to be demonstrated in the context of High Grade Serous Ovarian Cancer (HGSOC), the most lethal gynecological neoplasm. We aim to demonstrate that targeting respiratory CI decreases tumor progression of HGSOC and to characterize the role of tumor microenvironment (TME) in activating compensatory mechanisms. We generated a functional CI knock out (KO) in OV90 cell lines and evaluated tumor growth in NOD/SCID mice. OV90 CI KO xenografts presented with a lower proliferation index (Ki67) than CI wild-type (WT) tumor masses. Moreover, HIF1a stabilization was lacking in CI KO tumors, paired with the decreased expression of HIF1-targeted genes. Nevertheless, CI-deficient tumors continue to thrive, suggesting a compensatory role of TME in supporting tumor growth, whereby KO cancer cells may rely on protumorigenic immune cell functions. In this context, compared to WT, tumors lacking CI displayed less necrosis and a higher number of vessels positive for Endomucin/Smooth Muscle Actin immunofluorescence staining. Massons' Trichome coloration showed stromal abundance in CI KO xenografts, with tumor infiltrating macrophage (F4/80+) numbers higher than in WT OV90 tumors. To determine whether targeting CI may affect macrophage or endothelial cell phenotype, we are currently investigating *in vitro* M1 versus M2 polarization of THP-1 monocyte model upon CI inhibitors treatment, as well as tube formation potential of HUVEC endothelial cells. We propose that CI inhibition be considered as an anti-cancer strategy in OC, while further studies are ongoing in immunocompetent murine systems, to appropriately characterize the interplay between targeting CI and immune cell remodeling within the TME.

Tackling the invasive properties of drug resistant melanomas using locked nucleic acid (LNA) to inhibit the oncomiRs miR-4443 and miR-4488

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The combinatorial treatments with MAPK inhibitors have improved the survival of BRAF-mutant melanoma patients. However, acquired resistance to target therapy still limits the efficacy of these treatments in time. Recently, many findings have underscored the involvement of microRNAs in this process. In our laboratory we have identified a subset of miRNAs divided into oncosuppressors and oncomiRs strongly deregulated in drug resistant melanomas. Among them, we discovered that the two oncomiRs miR-4443 and miR-4488 govern the migratory and invasive phenotypes, which are hallmarks of drug resistant melanoma cells. This occurs through their ability to target the intermediate filament nestin. Starting from these evidences, the focus of my PhD is to exploit the targeting of miR-4443 and miR-4488 as a novel therapeutic strategy to tackle the invasive and migratory properties of drug resistant melanoma cells and therefore to impair their metastatic potential. This will be accomplished using locked nucleic acid (LNA)-modified antimiRs. In a preliminary set of experiments, we have observed that LNA-miR-4443/miR-4488 reduce migration and invasion of different drug resistant melanoma cell lines. Molecularly, the treatment of LNA- oncomiRs is able to restore nestin levels in drug resistant cells. Our future plan is to assess LNA-oncomiRs activity as anti-metastatic agents in vivo in xenograft models. An open question is related to the molecular regulation of the two oncomiRs in drug resistant melanomas. Bioinformatics analyses allowed to identify the histone H3 lysine 4 demethylase JARID1B as a potential regulator of miR-4443/miR-4488. This observation is of interest given that the global DNA hypomethylation due to the elevated expression of JARID1B is associated to MAPK inhibition. The axis JARID1B/oncomiRs will be investigated by ATAQ- seq and ChIP experiments in drug resistant vs sensitive melanoma cells. Altogether, the outcome of these studies has implication in the attempt to deepen the therapeutic potential of miR-4443/miR-4488 for melanoma treatment and to identify the molecular mechanisms behind oncomiRs' regulation in drug resistant melanomas.

P.83

Understanding the effect of inflammation on mtDNA release under mitochondrial cation aberrations

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As a central hub for a plethora of functions, Mitochondria are responsible for the majority of downstream signaling cascades involved in health and diseases. It has been extensively studied how mitochondria play a pivotal role in innate and adaptive immunity, from induction to maintenance. Notably, maintaining Ca^{2+} and K^{+} homeostasis is one of the crucial tasks carried out by these organelles. Our preliminary results, in agreement with previous works, demonstrated that altered mitochondrial Ca^{2+} or K^{+} fluxes reduce NLRP3 inflammasome induction; however, the precise molecular mechanisms are still under investigation. Here, we examine whether and how mitochondrial Ca^{2+} and K^{+} regulate mtDNA release during NLRP3 inflammasome induction. To this purpose, we developed a fluorescent probe based on GFP-SPLICS technology to follow mtDNA release into the cytoplasm upon inflammasome induction. Finally, to test this probe, we are setting up a cellular system based on HEK293T cells expressing all the components of NLRP3 inflammasome together with the silencing of MCU, the mitochondrial Ca^{2+} uniporter, responsible for Ca^{2+} entry into mitochondria, or MITOK, the mitochondrial ATP-sensitive K^{+} channel, which plays a central role in controlling mitochondrial matrix volume, respiration, and membrane potential. Our results indicate that MCU silencing decreased NLRP3 inflammasome induction, suggesting MCU as a strong candidate to prevent the negative consequences of inflammation.

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Functional loss of HIPK2 alters TDP-43 subcellular localization: a new potential mechanism of amyotrophic lateral sclerosis (ALS) pathogenesis

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Homeodomain interacting protein kinase 2 (HIPK2) is a serine-threonine kinase that phosphorylates various transcriptional and chromatin regulators, thus modulating numerous important cellular processes, such as DNA damage response, cell proliferation and apoptosis. The relevant physiological role of HIPK2 emerged from the phenotypic analysis of *Hipk2* null mice (*Hipk2*-KO). These mice present several neuronal defects associated to psychomotor behavioral abnormalities including dystonia, impaired coordination, reduced motility and clasping of posterior limbs, highly resembling the features of Amyotrophic Lateral Sclerosis (ALS). One of the main proteins associated to ALS pathogenesis is the TAR DNA Binding Protein 43 kDa (TDP-43), whose cytoplasmic aggregates are a hallmark of both sporadic and familial ALS cases. To investigate the possible role of HIPK2 in the pathogenesis of ALS, we analyzed the consequences of HIPK2 dysfunction by using both *in vivo* and *in vitro* models. Interestingly, we found a strong cytoplasmic mis-localization of TDP-43 protein in spinal motor neurons from adult *Hipk2*-KO mice, and the same effect was observed upon HIPK2 silencing in human-derived neuroblastoma SH-SY5Y cells. Strikingly, the re-establishment of the expression of active HIPK2, but not of its kinase-dead mutant, was able to restore the nuclear TDP-43 localization, thus indicating that TDP-43 subcellular distribution may depend on HIPK2 expression level and activity. Altogether, these data unravel the existence of a functional relationship between HIPK2 and TDP-43 proteins that may have a role in the pathogenesis of ALS.

miR-23b-3p, miR-126-3p and GAS5 encapsulated in extracellular vesicles from breast cancer cells treated with sorafenib inhibited the growth of tumor xenografts in zebrafish model

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Extracellular vesicles (EVs) are cell-derived membranous structures secreted by all cell types. Given their cargo, EVs as a mimic of “nature’s delivery system” can be used to transport nucleic acids, peptides, lipids, and metabolites to recipient cells for intercellular communication.
We previously demonstrated that the treatment of breast cancer cells with sorafenib, a multikinase inhibitor, determined the dysregulation of miR-23b-3p, miR-126-3p, and long non-coding RNA (lncRNA) GAS5. These ncRNAs generally act as tumor-suppressor transcripts, being down-modulated in various human cancers. Here, we collected the EVs released by 4 different sorafenib-treated breast cancer cell lines. We quantified the levels of miR-23b-3p, miR-126-3p, and GAS5 encapsulated in the EVs by ddPCR technology, and we found an increased expression level of these ncRNAs. To assess the possibility of using the EVs rich of miR-23b-3p, miR-126-3p, and GAS5 as potential ncRNAs delivery-system, we treated *in vitro* and *in vivo* breast cancer models with the EVs. We successfully used the enriched EVs as vehicles in breast cancer cells that determined increased expression levels of the 3 ncRNAs up to 7.5 times ($p<0.01$) and the inhibition of cellular proliferation *in vitro* (up to 19%; $p<0.01$). Additionally, we injected two breast cancer cell lines into zebrafish embryos and we treated the xenografts with enriched EVs to establish their role as ncRNA carriers *in vivo*. We found the reduction of the xenograft tumor mass (up to 85%; $p<0.0001$ at 24h post-treatment) and the inhibition of angiogenesis, as well as the number of micrometastasis in the tails (99% $p<0.0001$).
Taken together, our findings indicate a new way to selectively enrich EVs using an anti-cancer drug treatment; the great potential of EVs as vehicles of ncRNAs; the combined role of miR-23b-3p, miR-126-3p, and GAS5 in limiting the aggressive properties of breast cancer *in vitro* and *in vivo*.

The impact of mitochondria in the crosstalk between airway and phagocyte cells in cystic fibrosis

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Cystic fibrosis (CF) is a genetic disease caused by mutations of the gene coding for the CF transmembrane conductance regulator (CFTR) channel. In recent years, studies highlight the connection between mitochondria and CF pathogenesis, shed lighting on their role in regulating host responses and inflammation in CF. Mitochondria play a central role as a checkpoints of intracellular downstream signal cascades to pathogen recognition receptor responses, induced by exogenous pathogen-associated molecular patterns. Furthermore, they act as a key source of mitochondrial danger-associated molecular patterns (mtDAMPs), where Radical Oxygen Species (ROS), mtDNA, and Ca^{2+} are released, in part following a vesicle pathway, as a danger signal into the cytosol or in the extracellular milieu, which recognized by specific receptors trigger inflammation and influence the inflammatory responses of recipient cells. It has been demonstrated that the abnormal mitochondrial Ca^{2+} signaling in CF airways, during Pseudomonas *aeruginosa* infection, increased ER-mitochondria juxtapositions favoring inter-organelle Ca^{2+} transfer, via Mitochondria Calcium Uniporter (MCU). This led to mitochondria dysfunctions with persistent mitochondrial Unfolding Protein Response and NLRP3 inflammasome activation and pulmonary damage. The main goal of this project is to dissect the role of mitochondrial Ca^{2+} signal in the intercellular communication, mediated by mtDAMPs and mitochondria-derived vesicles, between CF airway and phagocyte cells during the phases of strong activation of pulmonary infection. Controlling the mitochondrial Ca^{2+}-overload in CF airways, using the newer mitochondrial Ca^{2+}-targeting agents available, we will prevent the exacerbation of inflammation regulating the exchange of mitochondrial content in the cell-to-cell communication indispensable for controlling immune cells responses.

Aptamers: an innovative tool for new biomarkers discovery in Alk+ NSCLC

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Among non-small cell lung cancers, anaplastic lymphoma kinase (ALK) gene rearrangements occur in 3-7% of cases. Although many very effective therapies are now available, they lose efficacy as the tumor progresses over time. Moreover, the current failure of an early diagnosis of these tumors often results in the worst prognosis. Indeed, the discovery of new biomarkers specific for this tumor could greatly improve its diagnosis and therapy.
In this context, we used the SELEX (Systematic Evolution of Ligands by EXponential Enrichment) technology to select for nucleic acid molecules, called aptamers, able to bind proteins exclusively expressed on the surface of cells harbouring ALK rearrangements. This approach is the most powerful at identifying new targets molecules for therapy and diagnosis, as it does not rely on any prior knowledge of the tumor.
Using a cell-SELEX (Systematic Evolution of Ligands by EXponential Enrichment) approach, we first enriched a starting random RNA library for aptamers discriminating between NSCLC (A549) and normal (BEAS-2B) cells. Then, to select only the sequences of the pool able to bind proteins specifically expressed on the surface of cells harbouring ALK rearrangements, we switched A549 for counterselection steps and introduced in selection an isogenic A549 cell line CRISPR modified to express EML4-ALK rearrangement (A549-EML4-ALK), so that the two cell lines only differ for proteins whose expression is driven by ALK oncogene.
The final aptamer pools were tested and showed high affinity and specificity for ALK+ NSCLC cells, thus suggesting that, after further study and optimization, the single sequences could be useful to discover and target new biomarkers for improving ALK+ NSCLC therapy and diagnosis.

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Testosterone depletion drives loss of skeletal muscle mass impinging on intracellular Ca^{2+} signaling

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Testosterone is a hormone with anabolic effects on skeletal muscle. Low testosterone levels are associated with reduced skeletal muscle mass and decreased physical activity. We recently identified testosterone as a positive modulator of the Mitochondrial Calcium Uniporter (MCU), the highly selective channel responsible for mitochondrial Ca^{2+} uptake, which contributes in maintaining skeletal muscle trophism. Thus, we decided to check the effects of either acute or chronic testosterone depletion on intracellular Ca^{2+} homeostasis and oxidative metabolism in skeletal muscle. Short-term castration reduced the mitochondrial Ca^{2+} ($mitCa^{2+}$) uptake and the Oxygen Consumption Rate (OCR) in Flexor Digitorum Brevis (FDB) myofibers. Subsequently we assessed the effects of long-term castration on $mitCa^{2+}$ uptake, cytosolic Ca^{2+} ($cytCa^{2+}$) transients, and mitochondrial membrane potential ($\Delta\Psi m$) in FDB. One week of surgical castration does not affect muscle trophism, calcium homeostasis, and $\Delta\Psi m$. Two weeks after surgery, when muscles are not atrophic yet, castration increases $cytCa^{2+}$ transients and $mitCa^{2+}$ uptake. Three weeks after castration, only $mitCa^{2+}$ uptake is elevated, while $cytCa^{2+}$ returns to control levels. Muscle size is still unaltered. Four weeks after castration, skeletal muscle atrophy, mitochondria depolarization and reduced $mitCa^{2+}$ uptake occur. To investigate whether mitochondrial Ca^{2+} accumulation, occurring as early as two weeks after testosterone depletion, contributes to the consequent loss of $\Delta\Psi m$ and of muscle mass, we performed surgical castration of $MCU^{+/-}$ mice, characterized by reduced $mitCa^{2+}$ uptake. Our results indicate that $MCU^{+/-}$ muscles are protected from castration-induced $\Delta\Psi m$ loss and atrophy. Overall, these data indicate that testosterone depletion affects intracellular Ca^{2+} homeostasis before the occurrence of muscle atrophy, and that reducing $mitCa^{2+}$ uptake can represent a promising strategy to avoid mitochondria depolarization and muscle atrophy.

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BCL2 targeting overcomes the stroma mediated resistance to crizotinib in ALK+ anaplastic large cell lymphoma

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Background: ALK+ Anaplastic T Cell Lymphomas (ALK+ ALCL) represent one of the most common types of Peripheral T-cell lymphomas (PTCL) characterized by aberrant ALK translocation. Despite the efficacy of ALK-targeted therapies (crizotinib), the insurgence of relapsed/refractory phenotypes remains a medical challenge.

Aims: Functionally elucidate, mechanistically define, and pharmacologically overcome the stroma-mediated protection of ALK+ALCL cells exposed to crizotinib.

Results: First, we implemented a co-culture platform demonstrating that stromal cells from different sources (human-derived and continuous lines) mitigated the efficacy of crizotinib on ALK+ALCL cell lines (SUPM2 and L82).

Transcriptionally, co-cultured ALK+ALCL cells challenged with crizotinib displayed the upregulation of signatures promoting the anti-apoptotic response, T-Cell activation, and cell cycle progression. Interestingly, cell lines specific enrichments were detected (SUPM2: PI3K - L82: JAK/STAT3 pathway). In addition, we revealed the upregulation of genes promoting ALCL pathogenesis, such as IL2RA, TNFRSF8 and CCR5, in SUPM2 and L82 co-cultured with MS5 (stroma cell line), elucidating shared stroma-mediated signals.

Further, considering the transcriptomic activation of the anti-apoptotic response in crizotinib-resistant ALK+ALCL cells, increased BCL2 protein levels were validated in crizotinib treated cells in presence of MS5. Lastly, we reported a potent synergistic effect of the combination of crizotinib and navitoclax (BCL2 inhibitor), ultimately overcoming the stroma-mediated protection on SUPM2 and L82 exposed to crizotinib.

Summary: Our findings confirm stromal cells' pivotal role in shielding lymphoma cells from crizotinib through the activation of the anti-apoptotic mechanism. We developed the rationale for the implementation of (pre)clinical studies combining crizotinib and navitoclax to overcome crizotinib resistance, potentially improving ALK+ ALCL patients' treatment.

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Testing a T2T-based diagnostic pipeline to explain clinical variability in a rare hereditary disease: the case of facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD), a hereditary myopathy, is currently described in two distinct forms both associated with the loss of epigenetic silencing of the 4q35 subtelomeric region. FSHD1 is associated with the heterozygous reduction of the number of D4Z4 repetitive elements at 4q35 (DRA, <11 units); FSHD2, accounting for 5% of cases without DRA, results from global demethylation of the locus due to damaging variants in chromatin remodeling factors. These existing models present crucial limitations: i. ~3% of the population bear a DRA; ii. D4Z4 reduced methylation is detected also in cases with no FSHD phenotype; iii. different clinical phenotypes are observed in DRA carriers, including healthy relatives in FSHD families. All this creates a gap between clinical and molecular diagnosis which advocates for a revision of the diagnostic approaches and counseling for people carrying a DRA or presenting a FSHD clinical phenotype.
To this aim, 4 families were selected from the Italian National Registry for FSHD with FSHD phenotype but not fulfilling the current molecular diagnostic criteria and presenting inconsistent genotype-phenotype correlation. A revised diagnostic protocol was applied based on the integration of multi-level data: i. fine phenotypic description of patients through standardized neurological examination; ii. anamnestic/clinical family evaluation; iii. molecular assessment of the D4Z4 size; iv. rationale-guided molecular investigations as meth-seq, WES, Oxford Nanopore long reads technology based WGS. This approach allowed to identify new genetic candidate involved in FSHD pathogenesis at single-family level.
This integrated multi-level data analysis, applied to an expanded number of FSHD families, can enhance diagnostic/prognostic outcomes and counseling opportunities for FSHD patients, duly considering clinical variability, penetrance and genetic/epigenetic background.

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Investigating the Contribution of the Base Excision Repair Pathway to Genomic Instability

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One of the most common DNA base lesions caused by reactive oxygen species (ROS) is the oxidation of guanine to 8-oxo-7,8-dihydroguanine (8-oxodG). In human cells, 8-oxoG is repaired by the base excision repair (BER) pathway. Deficiencies in BER can impair the cell's ability to repair DNA lesions, leading to accumulation of DNA damage, activation of DNA damage response (DDR) pathways, induction of DSBs, chromosomal instability and contributing to several human diseases, including cancer and neurodegenerative disorders. To gain a deeper insight into the involvement of BER in genome stability, we generated APE1- and XRCC1-depleted MC10A cell lines. Here, we report the transcriptome analysis of BER-deficient cell lines, which revealed an up-regulation of the p53 pathway compared to the control cell line. This finding suggests an increase in endogenous DNA damage. Therefore, by monitoring double-strand break (DSB) formation, we found an increased level of γ-H2AX signalling in BER-deficient cell lines compared to the control cell line. Interestingly, we observed a higher frequency of micronuclei in BER-deficient cells, indicating compromised genomic stability. These findings are integrated with the increased 8-oxodG levels observed in APE1- and XRCC1-depleted MC10A cell lines compared to control.
Such insights could help to understand the relationship between BER and genome instability, as well as the implication of targeting this process in human malignancies.

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MiR-181c/d regulates drug-sensitivity by targeting SIRT1 in biliary duct cancer

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Biliary duct cancers (BDCs) comprehend a cluster of rare aggressive tumors, arising from the gallbladder or cystic duct [gallbladder carcinoma (GBC)] or the biliary tree [cholangiocarcinoma (CCA)]. Despite the latest progress, BDC remains one of the most lethal cancers worldwide due to lack of specific symptoms, limited therapeutic strategies and resistance to conventional therapy. Recently, the advent of high-throughput technologies has enabled extensive multi-omics sequencing unveiling that diverse miRNAs are dysregulated in BDC, promoting carcinogenesis and chemo-resistance.
An exhaustive study of miRNA expression profiles of BDC patients and cell lines was performed by SmallRnaSeq, thus identifying miR-181c/d as critically dysregulated microRNAs. Remarkably, miR-181c/d significantly correlated with a worse prognosis and survival. Functional *in vitro* experiments were executed on BDC cell lines transiently transfected with inhibitors and/or mimics of miR-181c/d, showing that overexpression of miR-181c/d decreased cell growth and enhanced sensitivity to chemotherapy. Integrative miRNA-mRNA bioinformatic analysis revealed that the miR-181c/d functional role is determined by binding to their target *SIRT1* (Sirtuin1). BDC patients expressing high levels of miR-181c/d and low levels of SIRT1 showed an improved survival and treatment response. Overexpression of miR-181c/d affected cell viability and increased chemo-sensitivity through the downregulation of SIRT1. Furthermore, an integrative network analysis demonstrated that miR-181c/d considerably had a negative regulatory effect on several important metabolic tumor-related processes.
Our study identified miR-181c/d as potential tumor suppressors in BDC and suggest that miR-181c/d levels might be exploited as an innovative therapeutic tool and a useful biomarker to monitor and predict drug response in BDC patients.

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[WITHDRAWN]

P.94

Unraveling the power of inter-organelle communication: a focus on DJ-1-dependent mitochondria-organelles contact sites remodelling

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Inter-organelle communication is essential to maintain cellular homeostasis. Organelles continuously exchange with each other information, such as metabolites and calcium, through specific regions called membrane contact sites (MCSs). Mitochondria, fundamental for sustaining cellular bioenergetics, are often at the heart of different inter-organelle communication networks. To improve our understanding of mitochondrial MCSs dynamics and regulation, we expanded our set of genetically encoded sensors to detect membrane proximity, the so-called SPLICS, exploiting the split-GFP-based technology. We developed a new probe that specifically reveals lipid droplets (LDs) and mitochondria CSs ($SPLICS_{LD-MT}$). Our results demonstrated the proper localization of the sensor and its efficiency in monitoring changes in LD-mitochondria communication under various conditions, including starvation or LD accumulation, as well as genetic manipulations of tethering proteins, like Mfn2 and PLIN5. Then, we applied our set of SPLICS probes to explore the mitochondrial contact sites network rewiring upon the manipulation of the expression of the multifunctional protein DJ-1 in HeLa cells. Our results indicated that DJ-1 overexpression, in addition to increasing the ER-mitochondria CSs, impacted CSs between nucleus-mitochondria and lysosomes-mitochondria.

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Reduction of oxidative stress and promotion of autophagy by oleuropein aglycone in induced senescent skeletal muscle cells

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Skeletal muscle ageing is characterised by a progressive loss of muscle mass and strength that drastically reduces the quality of life. The aetiology is multifactorial, such as increased inflammatory cytokines, mitochondrial impairment and increased oxidative stress (Riuzzi, 2018). In contrast, autophagy is a protective mechanism that maintains muscle health by eliminating dysfunctional organelles and poorly functioning mitochondria (Lee, 2012). Oleuropein is a natural polyphenol found in olive leaves and oil and has potential health benefits, including antioxidant activity, anti-inflammatory properties and promotion of autophagy (Rishmawi, 2022).
The aim of the study is to investigate the antioxidant and autophagy-inducing effects of oleuropein aglycone (OLE) on human AB1079 cells derived from quadriceps muscle biopsy of a 38-year-old donor as a pretreatment of an H_2O_2-induced senescence stimulus simulating physiological oxidative stress.
AB1079 cells showed morphological differentiation after 7 days (D7) under different conditions. At D7, 25µM OLE treatment for 24h in combination with 300µM H_2O_2 exposure for 2h preserved cell viability by 90%. Pretreatment with OLE reduced the amount of ROS and the X-Gal senescence area induced by H_2O_2, confirming the antioxidant and anti-ageing properties of the bioactive molecule. The increased expression of SESN1 genes, which are involved in defence mechanisms against oxidative stress, in OLE-treated cells also confirmed the antioxidant activity. The increase in the number of autophagic vesicles in OLE-treated cells suggested that the natural compound is an activator of autophagy. Finally, the mitochondrial morphology results indicated mitochondrial fragmentation in the H_2O_2-treated cells, while OLE partially reversed the H_2O_2-induced oxidative stress.
In conclusion, pretreatment with OLE promotes antioxidant activities and the autophagy process in human AB1079 skeletal muscle cells, leading to a preventive anti-ageing effect.

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DNA damage response does affect neither the localization of NBS1 at the centrosome/basal body nor the primary ciliogenesis

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DNA and centrosome integrity is critical to maintain genome stability, avoid cell death and prevent tumour transformation. Therefore, in the presence of DNA or centrosome damage, cells activate the DNA damage response (DDR) or the centrosome damage response, respectively, to block the cell cycle progression. Moreover, DNA damage induces centrosome alterations as a mechanism to ensure the death of cells evading DNA damage checkpoints. Literature highlights a link between DDR and centrosomal proteins, suggesting a potential role of the DDR in regulating cell cycle progression by controlling their activation or localization.
NBS1, a member of the MRE11/RAD50/NBS1 (MRN) complex, plays a crucial role in the DDR, and mutations in its gene cause the DDR-defective Nijmegen Breakage Syndrome. Importantly, NBS1 localizes at the centrosomes, prevents their hyperduplication and regulates centriole separation.
We recently discovered that NBS1 also localizes at the Basal Body (BB) and its depletion lengthens Primary Cilium (PC) and affects its morphology and functionality.
Since MRN proteins form a complex which anchors on DNA in response to DNA damage, we asked whether NBS1 centrosomal/BB localization requires MRN complex formation and if it is affected by the DDR. Further, we also evaluated whether the DDR influences primary ciliogenesis.
We found that MRE11 depletion or pharmacological inhibition does not impair NBS1 localization at the centrosome/BB. The same result was obtained after P53 KO or activation by treatment with clastogenic drugs. Moreover, DNA damage induction does not affect PC frequency, length and morphology.
Our results indicate that NBS1 localizes at the centrosome/BB independently of MRN complex formation and DDR activation. Moreover, they suggest that also the PC regulation by NBS1 is not a mere consequence of the DDR activation, highlighting a possible uncanonical role of DDR proteins in the regulation of centrosome/BB/CP homeostasis.

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Generation and characterization of lung organ-on-chip and organoids to tackle lung fibrosis

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Lung fibrosis, which occurs in a heterogeneous group of lung disorders is characterized by high cellular heterogeneity and plasticity with inflammatory infiltration, fibroblasts proliferation and accumulation of extracellular matrix (ECM), which eventually lead to impairment of pulmonary function. The mechanism underlying the pathogenesis of the fibrotic process is complex and remains still poorly understood. Moreover, the *in vitro* and *in vivo* systems available have limitations in terms of poor reproducibility, highlighting the need to develop new strategies. Here we propose the generation of two different *in vitro* models to study and tackle lung fibrosis. As a first strategy, we generated lung organ-on-chip, made of lung fibroblasts and alveolar epithelial cells[1]. The morphological and molecular characterization showed that this matrix-based model recapitulates the alveolar histology; moreover, the use of the pro-fibrotic cytokine, TGFb, promotes the formation of a collagen-rich tissue. As a second strategy, we generated lung organoids from human pluripotent stem cells (hPSCs), based on a recently published work[2]. Following this protocol, we first generated anterior foregut spheroids, which, once embedded into matrigel and cultured in specific cocktails of growth factors, differentiate into lung (HLO) and bud tip organoids (BTO). Our results showed that both HLO and BTO properly form different cell types of the lung, such as basal stem cells, ciliated, epithelial, mesenchymal, alveolar and goblet cells. Experiments are ongoing to reproduce the fibrotic condition, using TGFb and oxidative stress. The generation of the described models will set the bases to study the lung pathologies and test in the future the effect of drugs with anti-fibrotic activity.

Expression analysis of circulating microRNAs for the identification of clinically relevant biomarkers for oral squamous cell carcinoma

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Oral Squamous Cell Carcinoma (OSCC) represents 90% of oral cancers. Incidence and mortality are increasing, making this neoplasm a serious challenge worldwide. The discovery of molecular biomarkers is crucial as they offer invaluable insights into tailoring individualized strategies for enhancing clinical decision-making in patients with OSCC. Liquid biopsy (LB) is a minimally invasive procedure used to identify molecular biomarkers. In this context, circulating miRNAs (cmiRNAs) have emerged as biomarkers that may be associated with tumourigenesis.
The purpose of this study is to investigate the association of candidate cmiRNAs expression with OSCC onset and progression, in order to identify potential molecular biomarkers.
A series including 20 patients with OSCC, 12 patients with Potentially Malignant Epithelial Oral Lesions (PMEL) and 5 healthy controls was enrolled. All 37 individuals underwent LB of saliva and plasma samples at time of enrolment. The expression profile of six cmiRNAs (-21, -31, -138, -145, -184 and -424) selected on the basis of their role in OSCC development and progression, was analyzed by quantitative Real-Time PCR, using miR-16 as endogenous control. Results were expressed as $2^{-\Delta Ct}$ (Ct target - Ct endogenous control) and compared among the three study groups using the Kruskall-wallis test.
By comparing cmiRNA expression levels, cmiR-138 showed a statistically significant decreased expression in saliva samples from PMEL and OSCC patients compared to those from healthy controls ($p=0.012$). No statistically significant differences emerged in the expression levels of cmiRNAs in plasma samples among OSCC, PMEL and controls.
This study, although with limitations to be addressed in larger studies, provided the first evidence of saliva cmiR-138 as a potential early diagnostic biomarker in OSCC. Saliva LB emerged as a reliable tool for the identification of cmiRNAs in OSCC patients, to be eventually validated and implemented in the clinical setting.

PI4KA early-cytokinetic function prevents tetraploidy induction and breast cancer progression

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Cytokinesis is a spatially and temporally regulated process involving sequential changes in membrane lipids composition. Phosphoinositides are critical regulators of remodeling events which occur during different steps of cytokinesis. We showed that phosphoinositides are physically confined at the plasma membrane surrounding the midbody and that PI4KA locally produces a pool of PI(4)P necessary for midbody attachment at plasma membrane. Loss of PI4KA leads to mitotic cell refusion and tetraploidization, a condition generating aneuploidy and chromosomal instability, which drive breast cancer (BC) progression.
Publicly available databases showed that 39.6% of BC patients display reduced PI4KA mRNA levels with an increased aneuploidy score. In accordance, modulation of PI4KA in BC cell lines generated tetraploid cancer cells marked by DNA damage, micronuclei and chromosomal instability, together tuning tumor microenvironment and BC progression. Drugs enhancing aneuploidy in cancer cells already carrying an abnormal chromosome count can force cells beyond a critical threshold, leading to mitotic catastrophe and cell death. Loss or inhibition of PI4KA in BC cell lines treated with low doses of Spindle-Assembly Checkpoint inhibitors increases sensitivity in cells resistant to Mps1 inhibition, suggesting a therapeutic approach for low-PI4KA tumors.

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Proteome diversity predicts functional differences of micronuclei in human cancer cells

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Micronuclei (MNi) are small cytoplasmatic bodies containing nuclear chromatin separated from the primary nucleus. Recently, they have gained significance especially for their ability to link genomic instability to innate immune response. However, their molecular components are still unknown. Thus, we purified at high homogeneity and purity MNi induced by two small compounds (PDS and RHPS4) able to target G-quadruplex, a DNA non-canonical structure, to determine their proteome and get insights into the mechanism of formation. Through mass spectrometry, we characterized the protein composition of purified micronuclei isolated from U2OS human cancer cells. Our findings reveal a largely similar pattern of proteins between untreated and treated cells, however a subset of protein associated with DNA repair, immunity, and mitochondrion differs among the two G4 binders. Next, we explored if the MNi-protein content results into different cellular responses and cellular fate. Our data indicate that PDS-MNi, specifically enriched with immunity proteins, exhibited the ability to promote the expression of Interferon-B (IFN-B) response in a murine fibrosarcoma cell line, as demonstrated by RNA-seq and ELISA assays. Interestingly, our data indicated that PDS-MNi, unlike to the RHPS4 ones, were recognized by autophagic mechanisms, which leading to their cleavage, are involved in triggering the cGAS-STING pathway. Conversely, RHPS4-MNi lacked immunity-related proteins and failed to activate IFN-B response, probably through an inhibitory effect mediated by mitochondria as suggested by the abundance of mitochondrial proteins in the MNi. Our research sheds light on different effect promoted by G4 binders and reveals that distinct MNi protein content results into different micronuclear fate, localization, and cellular response. These findings contribute can open new opportunities to further exploration concerning their formation, localization, resolution, and immunity in cancer biology.

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Novel therapeutic frontiers for multiple myeloma: exploring the functional implications of NEAT1 and AURKA double-targeting

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Multiple myeloma (MM) is an incurable plasma cell malignancy. It is characterized by the de-regulation of long non-coding RNAs (lncRNA) that influence disease progression and therapy resistance. NEAT1 is a lncRNA essential for nuclear paraspeckles and gene expression regulation. We showed that NEAT1 supports the proliferation of MM cells *in vitro* and *in vivo*, making this lncRNA an attractive therapeutic target. Here, we used a combinatorial strategy that integrates transcriptomic analysis and, computational predictive tools with functional high-throughput (HT) drug screening to identify compounds that synergize with NEAT1 KD reducing MM growth. These approaches converged on identifying Aurora kinase A (AURKA) inhibitors as promising compounds to potentiate the cytostatic effect of NEAT1 KD on MM cells. We showed that the combined inhibition of NEAT1 and AURKA profoundly impaired microtubule (MT) organization and mitotic spindle assembly. Mechanistically, NEAT1 KD leads to the down-regulation of main AURKA interactors involved in MT nucleation, concurring with the observed phenotype. To consolidate our findings, we performed rescue experiments in NEAT1 overexpressing AMO-1 cell line. In line with our previous reports, overexpression of NEAT1 in MM cells increased resistance to AURKA inhibitors confirming the functional interplay between AURKA and NEAT1 in MM scenario. Taking advantage of the large publicly CoMMpass dataset, we also demonstrated that AURKA expression was strongly associated with reduced progression-free ($p < 0.0001$) and overall survival probability ($p < 0.0001$) in MM patients and that patients displaying high expression levels of both NEAT1 and AURKA have a worse clinical outcome. Overall, our work demonstrated for the first time a functional cooperation between AURKA and NEAT1, paving the way for the development of a combinatorial therapeutic strategy targeting AURKAi and NEAT1 in this context.

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New molecular targeted therapies in adult and pediatric osteosarcomas

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Osteosarcoma (OS) is a rare, malignant tumor of bone. Currently, chemotherapy based on antiblastic drugs is the primary treatment due to the lack of targeted molecular strategies. In this context, the aim of this research is to develop innovative therapeutic approaches focusing on well-defined molecular targets. To achieve this goal, RNA-sequencing (RNA-seq) analysis was performed on OS and normal bone specimens. Principal component analysis revealed significant variations between these two cohorts, which were further confirmed by differential gene expression results. We obtained a large list of genes strongly up or down-regulated between OS and normal bone. In addition, the alghoritm CiberSortX was used to perform the deconvolution of lymphocyte populations, contributing to a more comprehensive molecular profile of the tumor microenvironment. To better understand the potential impact of the signaling pathways on OS tumorigenesis and progression, we performed gene ontology analysis. Notably, we found that one of the most significant pathways involved in OS pathogenesis is related to the activity of the transmembrane tyrosine kinase receptors, especially ephrin receptors (EPHA5, EPHA6, and EPHA7). Furthermore, analysis focused on surface molecules revealed Folate Receptor 1 (FOLR1) as particularly expressed in OS samples. RNA-seq data were validated by immunohistochemistry (IHC) in a large cohort of OS specimens, that also includes sequenced samples. To better characterized the role of ephrin receptors and FOLR1on OS progression we are using OS commercial cell lines (MG-63, U2OS, SaOS-2) and primary human fibroblasts as controls (BJ, HF). Overall, our results suggest that an innovative characterization of tumor genetics will provide an opportunity to ameliorate the therapeutic management of OS. Recognizing the promise of membrane molecules as therapeutic targets, we have conceived the idea of formulating a novel therapeutic approach centered around CAR-T cells.

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Embryonic hematopoietic stem and progenitor cells show differential susceptibility to leukemic transformation upon acquisition of JMML-associated Kras^{G12D} mutation

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Juvenile myelomonocytic leukemia (JMML) is a rare childhood myeloproliferative neoplasm, often originating prenatally, typically caused by a RAS pathway mutation. However, underlying causes of clinical heterogeneity remain unclear. Unveiling its cellular origins will allow studying pre-leukemic stages and epigenetic and transcriptional changes. In embryonic hematopoiesis, distinct waves of HSC-dependent and independent progenitors emerge from Cdh5+ hemogenic endothelium. In transgenic Cdh5-CreER^{T2}::Kras$^{LSL-G12D}$::R26-tdTomato mice, we can target the Kras^{G12D} mutation to specific hematopoietic waves by defined pulses of 4-hydroxytamoxifen (4-OHT). Targeting Kras^{G12D} to erythromyeloid progenitors (EMPs) at E7.5 resulted in low-penetrance myeloproliferative disorder, suggesting a marginal involvement in JMML. Unexpectedly, targeting Kras^{G12D} to HSC-independent multipotent progenitors (HSPCs) at E8.5 or HSCs at E10.5 caused lethal fetal liver (FL) anemia, due to defective erythroid differentiation, more severe if targeted to fetal HSPCs. When Kras^{G12D} was activated in HSPCs in a mosaic-like fashion by lowering 4-OHT, we observed a fully penetrant leukemic phenotype in adult mice, with increased myelomonocytic lineage and decreased lymphocytes, lethal by 16 weeks. Moreover, when the mutation was targeted to embryonic HSCs, it resulted in a similar but more aggressive phenotype, with added splenomegaly and lethality by 15 weeks, that could be recapitulated by transplantation of FL cells from HSC-induced Kras^{G12D}. Here, we demonstrate that embryonic hematopoietic progenitors show differential susceptibility to Kras^{G12D}. These results suggest the potential involvement of both embryonic multipotent progenitors and HSCs in the pathogenesis of JMML, with clinical variability being partially explained by the differences in cell origin. We are currently expanding our analysis to other mutations and analyzing the molecular and epigenetic mechanisms underlying this phenomenon.

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RNA BPs role in extracellular vesicles-mediated cellular communication

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In the recent years, extracellular vesicles (EVs) emerged as fundamental mediators in cancer cells communication, but the underlying mechanisms are still to be fully clarified. EVs importance relies mostly on their content, which differs in relation to the pathophysiology of the cell.
In this frame, our research group focused on the study of EV-cargo regulators and disclosed the RNA-binding protein SYNCRIP, which loads miRNAs in the EVs by recognizing the specific hEXO sequence.
Given the key role played by EVs in cancer cells communication and SYNCRIP role in regulating EVs cargo, we argued whether it promotes cancer progression via EVs.
For this purpose, SYNCRIP was silenced in HCC cells through a stable RNA interference approach and its knockdown validated through qPCR and Western Blot.
EVs produced by shCTRL and shSYNCRIP cells were purified and analyzed for size and concentration to perform both structural and functional analyses.
miRNAs from EVs were purified to perform a NGS analysis, determining the structural differences in the EVs content due to SYNCRIP depletion.
Bioinformatics analyses highlighted 14 downregulated miRNAs (validated by qPCR) in shSYNCRIP with respect to shCTRL EVs; notably the analysis of these miRNA targets revealed some mRNAs involved in tumor progression and epithelial-to-mesenchymal transition.
In line with the predictions, functional analyses have been performed on epithelial and mesenchymal liver cells, treated with shCTRL/shSYNCRIP EVs and tested for the predicted genes' expression. Epithelial cells treated with shSYNCRIP EVs showed an upregulation of the epithelial markers CLDN-1, OCC1 and E-CAD respect to shCTRL while hepatic stellate cells treated with shSYNCRIP EVs showed an upregulation of the mesenchymal markers a-SMA, COL1A1 and COL1A2.
These data highlight a role of SYNCRIP in tumor cell communication mediated by EVs and disclose a new field of investigation to be dissected in the light of potential therapeutic strategies.

Extracellular Nucleophosmin1 promotes breast cancer progression

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Nucleophosmin (NPM1) is a multifunctional phosphoprotein that shuttles between the nucleolus and cytoplasm with several cellular functions such as ribosome biogenesis, chromatin remodeling, cell cycle progression and genomic stability. In tumor cells, the level of NPM is often elevated and can result in increased cell growth and proliferation via hyperactivation of the ribosome machinery and in preventing apoptosis.
We found that NPM can be released in the extracellular culture medium of various human and murine cancer cells lines but not from non-tumor cells.We discovered that NPM is able to create supermolecular complexes in the extracellular mileu of these cell lines with the chaperone HSP90 and its co-chaperone Morgana, indicating that the formation of these complexes is a specific feature of the tumor context. While the role of extracellular HSP90 (eHSP90) as a promoter of cancer progression is well established, the involvement of its extracellular co-chaperones remains poorly understood.
We observed that extracellular NPM (eNPM) promotes cancer cell migration *in vitro* without affecting tumor cell proliferation through the binding to cell surface receptors as TLR4 and LRP1. Preliminary result obtained in a syngeneic mouse model indicates that eNPM promotes cancer cell migration also *in vivo*. Indeed, intratumoral injections of recombinant NPM (rNPM) promotes metastasis formation. Interestingly NPM treatments also support primary tumor growth, suggesting a possible activity of eNPM on the tumor microenvironment.
 Define the composition of extracellular NPM complexes and investigate the pro-tumoral activity of eNPM could lead to development of new therapeutic approaches in cancer.

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DNA aptamer-based approach: an emerging tool to fight Huntington's disease

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Aptamers, ssDNA or ssRNA oligonucleotides, are molecular-recognition agents that recently emerged as therapeutic candidates for several human diseases. Despite the advances made in Huntington's disease (HD) research, as of today no definitive treatment is available for this neurodegenerative disease, caused by the expansion of CAG repeats in huntingtin (HTT) gene. Interestingly, guanine-rich aptamers (named MS1 to MS4) able to preferentially recognize mutant HTT with an expanded polyglutamine tract have been identified, suggesting their use as therapeutic tools in HD.
Biological characterization of MS3, which stood out as the best aptamer, and its two truncated variants (named MS3-33 and MS3-17) was performed in SH-SY5Y cells, a human neuronal cell line. By confocal microscopy analysis, we showed a rapid, dose-dependent uptake of fluorescent aptamers in SH-SY5Y cells, proving their effective internalization with no significant cytotoxicity. In addition, they are stable over time, as evidenced by the persistence of the signal even 72 hrs after cell wash-out, further supporting the feasibility of their *in vivo* use. Remarkably, these aptamers are readily taken up and persist even in a murine striatal cell line model of HD, stably expressing human expanded HTT (STHdhQ111/111), and similarly to its wild-type counterpart (STHdhQ7/7). Our next aim will be to investigate whether and how aptamer treatment may restore the functionality of mitochondrial and endolysosomal pathways that are perturbed in HD.
Finally, by employing a transgenic D. melanogaster, stably expressing human expanded HTT, a significant improvement in the motor neuronal function and lifespan of the flies fed with these aptamers was observed, strengthen their efficacy in vivo.
Overall, these data demonstrate the efficacy of targeting mutant huntingtin aptamers both *in vitro* and *in vivo*, thus supporting further aptamer-based approach research for HD.

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Lavender angustifolia essential oil and its terpenic components impair cell proliferation and migration in a cell model of glioblastoma

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Glioblastoma multiforme (GBM) is the most aggressive form of glioma and it is characterized by a highly proliferative and infiltrative behaviour. Due to its aggressive nature and the limitations of current therapies, GBM presents significant therapeutic challenges. One of the goals of the current cancer research is to identify bioactive molecules able to enhance the effectiveness of traditional treatments, and to improve patient outcomes. Promising results have been reported on the ability of lavender essential oil (LO) or its components to inhibit cancer cell growth in some in *vitro* cancer models, such as colon and prostate cancer, among others. Our investigation was aimed at verifying whether LO and its monoterpenes components could influence tumorigenesis in GBM cell models. We found a significant inhibition of cell proliferation in a time- and dose-dependent manner after LO treatment alone or in combination with Temozolomide (TMZ). Moreover, we observed that LO impairs the migration capabilities of GBM cells, in transwell-based chemotaxis experiments. Following the chemical characterization of LO by GC-MS, we analysed the effects of the most represented terpenes on GBM proliferation. In detail, the proliferation rate of cells was analysed following treatment with Linalool, Borneol and Terpinen-4-ol. All of these terpenes, although at different rates were able to induce an impairment of cell proliferation. Ongoing experiments will show if these compounds are also able to mimic the anti-migratory effect of LO. An investigation of the putative molecular mechanisms involved in terpenes effects on cell proliferation and migration is currently ongoing. The research project offers valuable insights into the potential use of terpenes as main bioactive components of LO against GBM aggressiveness. Our study revealed the first approach to assign to terpenes-enriched LO a potential role as a natural adjuvant for GBM therapy.

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Application of CRISPR/Cas9 technology for studying and developing experimental therapy in a mouse model of RRM2B-related disease

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Mitochondrial DNA (mtDNA) depletion syndromes (MDS) are a heterogeneous group of autosomal disorders. They are principally characterized by critical reduction in mtDNA content. One of the enzymes involved in nucleotide salvage pathway is p53-inducible small ribonucleotide reductase subunit (p53R2), encoded by *RRM2B* gene. Mutations in *RRM2B* gene cause a spectrum of clinical and molecular genetic phenotypes: autosomal recessive infantile encephalomyopathy with renal tubular acidosis, multi-system involvement, and mtDNA depletion; autosomal recessive childhood/adult-onset Kearns-Sayre like syndrome with mtDNA multiple deletions; autosomal recessive adult-onset mitochondrial neurogastrointestinal encephalopathy like syndrome with mtDNA depletion; autosomal dominant or recessive adult-onset chronic external ophthalmoplegia with variable degree of multi-system involvement and multiple mtDNA deletions. To date, no therapeutic approaches are available, due to the absence of an *in vivo* model. My project aims to generate and characterize a ko-mouse model exploiting the CRISPR/Cas9 editing, that will be suitable for testing an AAV-based gene. The target region to be knocked out is exon 4, comprehending the catalytic site in position Y138. To remove it, we designed a couple of gRNAs able to recognize the regions flanking exon 4. Guides and Cas9 were delivered to mouse embryonic cells and single clones were screened. We analysed about 200 hundred clones, and we obtained 2 clones presenting precise deletion in homozygosis. We are currently generating the model by injecting the selected clones into the blastocyst of a female pregnant mouse. We will characterize the genetic, biochemical, and behavioural phenotype of homozygous, heterozygous, and wild-type mice. Shall we be successful in reproducing the human phenotype, we will design a CRISPR/Cas9 approach to correct the defect and deliver packaging into an AAV vector. This will be a proof of principle for a gene therapy approach.

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Dissecting the role of corticosteroid receptors in cardiomyocyte proliferative and regenerative ability

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Severe heart injuries induced by myocardial infarction determine an irreversible loss of cardiomyocytes. Our investigation delved into the role of corticosteroids and their receptors, the Glucocorticoid Receptor (GR) and the Mineralocorticoid Receptor (MR), in modulating cardiomyocyte differentiation and proliferation, aiming to identify potential therapeutic avenues for myocardial infarction treatment. Our recent findings demonstrated that corticosterone, the primary glucocorticoid in rodents, prompts cell cycle exit in cardiomyocytes through GR activation. Additionally, we found that cardiomyocyte-specific GR ablation or GR antagonization promotes heart regeneration upon myocardial infarction (*Pianca, Sacchi [...] & D'Uva, Nature Cardiovascular Research 2022*).
In this project, we noted that, upon specific GR genetic ablation (GR-cKO), corticosterone enhances cardiomyocyte proliferation. This response was not recapitulated by the GR specific agonist dexamethasone. We uncovered that this proliferative effect is mediated by MR activation, substantiated by the increased cardiomyocyte proliferation upon administration of aldosterone, the primary endogenous MR ligand. Furthermore, through RNA sequencing analysis of corticosterone-treated control and GR-cKO cardiomyocytes, we identified a pool of MR targets potentially responsible for this pro-proliferative effect, offering novel candidate targets for heart regeneration. Moreover, we observed augmented MR nuclear localization in GR-cKO models compared to controls following myocardial infarction, suggesting MR activation as a contributor to the enhanced heart regeneration observed in absence of GR.
In summary, our study suggests that the balance between GR and MR governs the impact of corticosterone on cell cycle regulation. Consequently, precise modulation of corticosteroids/GR-MR axis emerges as a promising strategy for heart regeneration based on endogenous cardiomyocyte renewal.

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Experimental model for studying clinical variability of Thymidine Kinase 2 deficiency with induced pluripotent stem cells

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hThymidine Kinase 2 is an enzyme encoded by the nuclear gene *TK2* and it is responsible for the conversion of deoxythymidine and deoxycytidine into their monophosphates in the mitochondrial salvage pathway. Autosomal recessive *TK2* variants lead to an imbalance of dNTPs maintenance, and consequently to depletion and/or multiple deletions in mtDNA, which cause clinically heterogeneous diseases. Although several studies *in vivo* and *in vitro* models have been carried out for Tk2 deficiency, several questions regarding the intra-and intrafamilial variability and tissue-specificity are still open. The goal of our project was to develop an experimental model for studying the phenotype of Tk2 deficiency since the early stage of cell commitment in three patients with different clinical phenotypes. Two patients were from the same family and had the same genotype but different phenotypes. The youngest brother presented with infantile-onset and he was still alive at 18 years of age thanks to the nucleosides' supplementation while the 21-year-old sister had an adult onset of mild myopathy. The third patient was a 6-month-old boy presenting the infantile Tk2 disease with severe CNS involvement that was not responsive to treatment for the neurological signs. Preliminary analysis in patients' derived fibroblast cell lines confirmed the presence of reduced levels of TK2 gene expression and mtDNA copy number compared to controls. Then, we carried out the reprogramming of patients and control fibroblast cell lines into pluripotent stem cells (iPSCs) by using the Sendai Virus kit. Confocal immunofluorescent analysis confirmed the pluripotency of our cell lines. Mitochondrial respiration analyzed with Seahorse confirmed a minimum level of oxidative phosphorylation activity in iPSCs. In conclusion, we were successful in generating an experimental model for studying Tk2 intrafamilial variability. Additional studies are ongoing to define the major players in the disease mechanism.

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Impact of ETC complex I dysfunction on mitochondrial Ca^{2+} homeostasis

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Complex I (CI), also known as NADH ubiquinone oxidoreductase, is a ubiquitous enzyme involved in the generation of the proton-motive force exploited for ATP synthesis in the mitochondrial respiratory chain. CI is a central player of cellular bioenergetics, it owns a pivotal contribution in controlling cell metabolism, adaptation to hypoxia, and sensitivity to apoptosis. Indeed, defects in CI are not only the primary cause of inherited mitochondrial diseases, but are also present in a broad spectrum of pathological conditions, such as diabetes and cancer. Our group is investigating whether and how ablation of CI can impinge on mitochondrial Ca^{2+} homeostasis, i.e. a key signal regulating organelle physiology.
We thus analyzed mitochondrial Ca^{2+} handling in WT and KO for NDUFS3 cell line, wherein NDUFS3 being a non-catalytic core subunit essential for CI assembly. The goal is to understand whether a specific dysregulation of mitochondrial Ca^{2+} homeostasis is present in a model of complex I dysfunction. Our results show that genetic ablation of NDUFS3 can induce significant changes in mitochondrial Ca^{2+} fluxes, although sustained by a global rearrangement of Ca^{2+} signaling at whole cell level. Overall, our data suggest that dysregulation of mitochondrial Ca^{2+} homeostasis could play a role on complex I dysfunction, depending on the cellular context.

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Exploring novel therapeutical vulnerabilities in gastric cancer preclinical models

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Gastric Cancer (GC) is one of the most common malignancies worldwide, and it is burdened by high mortality rate. Differing from other solid tumors, GC is traditionally treated with surgical resection and/or chemotherapy due to the lack of efficient targeted and immuno therapies. Also, high molecular heterogeneity fuels therapeutic failure, poor prognosis and variable responses to chemotherapy. In this context, outlier patients generally fall into two classes: long responders (LR) showing durable clinical benefit and fast progressors (FP) experiencing disease progression. Thus, we hypothesize that specific molecular traits dictate resistance/vulnerability to chemotherapy. We took advantage of cell models obtained from FP and LR patients, and we characterized them through whole-exome and whole-transcriptome sequencing (WES and WTS). A specifically designed 600-compound library was designed and tested on cell models, followed by viability assay. Selected drugs were assessed for GC specificity through challenging of primary lung cancer cell lines. WES and WTS confirmed the molecular heterogeneity and absence of canonical tumour drivers in our models. Higher mutational rate was observed in LR-derived cell lines compared to FP-derived ones. In gene expression and pathway-level differential analyses, FP and LR cell populations clusterized in two distinct groups. Similarly, differential transcription factor expression analysis showed the enrichment of tumour-related pathways and immune response-related factors in FP. Drug screening on FP models revealed vulnerabilities in cytoskeletal and cell cycle processes. The same drugs did not affect cell viability in primary lung cancer cell models, confirming GC-selective efficacy. Our preliminary results identified peculiar molecular features in non-canonical responders and novel targetable vulnerabilities in cytoskeletal and cell cycle-related processes. Future validation should open new possible perspectives in GC treatment.

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Lumacaftor (vx-809), the CFTR corrector, reverses the ER-induced stress condition

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Proteins are essential components for cellular functions; their proper folding is the basis of cellular well-being. In an unfavourable cellular scenario, proteins can misfold giving rise to aggregation phenomena. The main processing site for transmembrane and secreted proteins is the endoplasmic reticulum (ER), which is affected by the accumulation of misfolded proteins, leading cells to a stress condition. ER stress activates the Unfolding Protein Response (UPR) in an attempt to restore cellular homeostasis or, otherwise, lead to death. ER stress and conformational disorders are directly related and result in cellular dysfunction causing various diseases. Of the most widespread diseases is cystic fibrosis (CF), a genetic disorder involving misfolding of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein. Recently, new drugs, called "*correctors*," have been synthesized with the potential to modify the expression, function and stability of the CFTR protein. Lumacaftor (Vx-809) is one of the drugs that acts on misfolded CFTR and increases its amount on the plasma membrane. From the experiments conducted by our research group, despite the absence of the target protein, Vx809 demonstrated the ability to act by reducing not only the major ER-stress sensors, but also apoptosis, oxidative stress, and modulating calcium homeostasis too. Since the exact mechanism of action of the correctors has not yet been elucidated and there are no studies demonstrating *in vivo* direct binding of Vx-809 to the CFTR protein; these results suggest that Vx-809 may interact with other protein partners, suggesting *off-label* use for other misfolding pathologies.

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HIPK2 role in the pathogenesis of liver fibrosis

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Fibrosis represents a pathological process of many chronic inflammatory diseases, characterized by excessive deposition and remodeling of the extracellular matrix (ECM) that eventually progress in organ dysfunction and death. In liver, fibrosis is a hallmark feature of chronic liver diseases primarily driven by the differentiation of Hepatic stellate cells (HSCs) in collagen-depositing myofibroblasts. Homeodomain-Interacting Protein Kinase 2 (HIPK2) is involved in different pathways and it has been already found to regulate fibrotic signaling in kidney, pulmonary and cardiac tissues. Here we investigate the role of HIPK2 in hepatic fibrosis employing in vitro, in vivo and ex vivo models. In vivo, Hipk2 was knocked-out in a liver specific manner in a NASH model of fibrosis. Preliminary data indicate that, compared to Hipk2-WT mice, the absence of HIPK2 determines a reduction of collagen expression, as evidenced by both qPCR and fibrosis grading on Picrosirius Red staining. Further characterization comparing steatosis, inflammation, ballooning, fibrosis and transcriptomic analysis is ongoing. In vitro, to assess the contribution of HIPK2 in HSC activation and evaluate HIPK2 inhibition as a feasible approach to reduce fibrosis, we are testing different HIPK2 small molecule inhibitors on LX-2, a human immortalized HSC line that can be activated into myofibroblast through TGF-β treatment. In this cell line, inhibition of HIPK2 can modulate HSC activation by impairing the TGF-β/SMAD signaling pathway. In particular, HIPK2-inhibited LX-2 myofibroblasts show decreased RNA expression levels of Col1A1 and α-SMA, and decrease SMAD2 phosphorylation. Ex vivo, to verify the feasibility of HIPK2 inhibition treatment on human samples, we are establishing human tissue slice cultures from non-fibrotic liver (hLSC). hLSCs will be treated with TGF-β in combination with HIPK2 inhibitor and the activation of fibrosis will be compared to the relative control.

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The new emerging role of NLRP4 inflammasome platform as regulator of TBK1 and AKT in T47D breast cancer cell line

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Introduction: NLRP4 forms inflammasomes and is a member of (NOD)-like receptors (NLRs) family. This protein acts as a negative regulator of TBK1, a serin/threonine protein kinase, by ubiquitination and proteasomal degradation. TBK1 has a critical role in tumor development activating the kinase AKT signaling pathway. This evidence prompted us to investigate the potential protective role of NLRP4 during tumorigenesis, through the degradation of TBK1 as targetable link supporting context-selective mobilization of AKT regulatory network.
Methods: cell culture and treatments**,** transient and stable transfection, western blotting, qRT-PCR**,** focus formation assay, growth curves and statistical analysis.
Results: NLRP4 was highly expressed in control cell line (MCF10A) compared to breast cell lines (MDA, T47D and MCF7). TBK1 protein showed high level in breast cell lines, especially in T47D, while protein as well as mRNA expression ($p<0,01$) of NLRP4 were observed at lowest levels in T47D. Transient NLRP4 transfection in T47D (T47D NLRP4 myc-DDK) lowered TBK1 protein levels ($p<0,05$). The NLRP4 transfection did not change AKT expression, but reduced its phosphorylation, as observed by p-AKT (T308) decreased levels. Stable T47D transfected cells with NLRP4 were treated with cycloheximide to evaluate TBK1 protein degradation in presence of NLRP4. NLRP4 overexpression in transfected cells reduced TBK1 stability supporting the role of NLRP4 to increase TBK1 degradation.
The phenotypic effects of NLRP4 overexpression in stable T47D transfected cells were analyzed to understand the role of NLRP4 in tumor process. The decreased cell growth observed in T47D transfected cells and the focus formation assay suggest the potential tumor suppressor activity of NLRP4 ($p<0,05$).
Finally, NLRP4 has a putative role in the regulation of TBK1, a crucial protein in the tumoral process disclosing a potential protective role of NLRP4 during tumorigenesis.

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Targeting of a novel interplay between tyrosine kinases and NRF2 enhances sensitivity to standard treatment in triple negative breast cancer

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Triple Negative Breast Cancer (TNBC) represents about 10-15% of all breast cancer and it is characterized by high invasiveness, metastatic potential and worst prognosis. The molecular mechanisms that drives TNBC is still poorly understood.
The deregulation of Protein Tyrosine Kinases (PTKs) is a common feature in various type of cancer. Among these, SRC, a non-Receptor Tyrosine Kinases (nRTKs), is the first oncogene ever identified and its activity is often deregulated in cancer. Importantly, SRC gene is rarely mutated or amplified and its aberrant activation is mainly caused by the constitutive activation of Receptor Tyrosine Kinases (RTKs). NRF2, the master regulator of oxidative stress, is frequently activated to counteract cancer initiation.
However, more recently, its prosurvival role in cancer is emerging. Despite in some tumors the aberrant constitutive activation of NRF2 can be caused by mutations in KEAP1 or NRF2 genes, its upregulation can be achieved also independently of these, suggesting that other signalling pathways may impinge on NRF2 modulation.
We have recently shown that the aberrant activity of SRC can enhance NRF2 expression and activity triggering cancer cell resistance to therapy.
Here we provide evidence for a new functional link between RTKs and NRF2 expression and activity in TNBC models and we suggest that the targeting of this axis may ameliorate the sensitivity to standard therapeutic approaches.

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Genetic Vector Control strategy for a major malaria vector, A. arabiensis

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This investigation explores genetic control strategies for A. arabiensis, major malaria vectors in Africa, building upon prior successful approaches demonstrated in laboratory settings. Inspired by the self-limiting strategy targeting A. gambiae X-chromosome by Galizi et al., this study aimed to create a sex distorter strain using a species-specific guideRNA for A. Arabiensis, focusing on unique species-specific single nucleotide polymorphisms within the repetitive sequence of ribosomal genes. The CRISPR-Cas construct, incorporating β2::Cas9 and piggyBac repeats for random genome integration, aimed to induce a male-biased sex ratio. Two potential candidates for A. arabiensis-specific sex distorters, integrated into intronic sequences of autosomal genes (lines A and B), demonstrated significant male bias (72.9% and 99.3%, respectively). Further experimentation involved introgressing the B line into A. gambiae, overcoming hybrid sterility challenges through five generations of interspecific crosses via females. Hybrids males (F6) carrying the distorter construct were tested and confirmed its specificity by showing sex ratio distortion in the presence of A. arabiensis X chromosomes (100%), while maintaining normal ratios in the presence of A. gambiae X chromosomes (avarage 54%). This groundbreaking strategy paves the way for developing self-limiting, species-specific genetic control mechanisms, enhancing the specificity of vector control mechanisms.

Advancing therapeutic interventions for Fabry nephropathy: harnessing the power of organoid models and AAV-mediated gene therapy to tackle kidney pathology in Fabry disease

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Fabry Disease (FD) is a Lysosomal Storage Disorder caused by mutations in the GLA gene, which encodes the lysosomal enzyme α-galactosidase. FD is a multi-systemic disease often emerging as a cardiomyopathy but frequently culminating in end-stage renal disease (ESRD) subsequent to glomerular and tubular injury. Deficiency of GLA leads to lysosomal accumulation of the sphingolipid globotriaosylceramide (Gb3) in kidney cells resulting in Fabry nephropathy. The gold standard therapy for FD is Enzyme Replacement Therapy (ERT), which reduces Gb3 accumulation after biweekly infusions of the a-galactosidase recombinant enzyme. However, ERT shows limited efficiency in less reachable cell types, and not always is sufficient as cure. New therapeutic approaches are thus requested especially for the kidney pathology. Therefore, we are studying Fabry nephropathy using kidney organoids, in a holistic approach, to develop novel therapeutic strategies. We generated GLA-KO hiPSCs via CRISPR/Cas9, alongside a GLA-inducible KO hiPSC line. We successfully differentiated GLA-KO hiPSCs kidney organoids that show marked Gb3 accumulation, especially in the proximal tubules (PT), perfectly recapitulating the hallmark of FD. We are thus using this platform to evaluate the therapeutic potential of adeno-associated virus (AAV) vectors to tackle Fabry nephropathy. We tested 11 AAV serotypes in both wild-type and GLA-KO kidney organoids. Four AAVs resulted very effective in selectively targeting PT cells, opening the possibility to obtain fundamental proof of concept data on the use of AAV-mediated gene replacement to treat Fabry nephropathy and other inherited kidney tubulopathies.

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Therapy-promoted prostate cancer repopulation and acquired cell-resistance (CRAC) implies vulnerability to ferroptosis via lipoxygenase activation

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Cytotoxic anti-cancer therapies reduce tumor size; however, they often promote cancer progression among surviving cells, due to genetic selection and epigenetic reprogramming. We recently set up in our lab a one-pot *in vitro* model of therapy-induced Cancer Repopulation and Acquired Cell resistance (CRAC), in PC3, a castration resistant prostate cancer (CRPC) cell line treated with a pulse of etoposide and left recovering for 20 days. After an initial apoptotic phase, a tiny fraction of surviving cells repopulates, undergoing epigenetic reprogramming and acquiring EMT features and chemo-resistance. Repopulation occurs via Phoenix Rising, a caspase3-cyclooxygenase2-prostaglandin E2 compensatory proliferation signalling axis requiring arachidonic acid (AA) inflammatory metabolism. Notably, also the AA-dependent inflammatory lipoxygenases (LOX) are activated. LOXs produce both cell toxic products (e.g., the pro-oxidant lipid hydroperoxides HpETE), and pro-survival factors such as HETE. HpETEs are major inducers of ferroptosis, a Fe-dependent cell death mode induced by lipid hydroperoxides, presently attracting much attention as a way of eliminating apoptosis-resistant cancer cells. In this context, we hypothesised that LOX activation in CRAC may guarantee acquisition of chemoresistance, but at the expenses of becoming vulnerable to ferroptosis induction. We observed indeed that in PC3 and in an additional CRPC cell DU145, LOX inhibition prevents repopulation, which is restored by exogenous addition of HETE. Strikingly, repopulating cells become vulnerable to ferroptosis, which was induced by inhibiting glutathione (GSH) or GSH-peroxidase4 (GPX4). Notably, untreated PC3 or DU145 are insensitive to induction of either apoptosis by LOX inhibitors or ferroptosis by GSH/GPX4 inhibitors. This vulnerability may constitute a novel target allowing preventing therapy-induced resistance and tumour reoccurrence.

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An oncosuppressive serum miRNA signature as a predictive biomarker and potential therapeutic target in diffuse large B-cell lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is an aggressive hematological malignancy. Despite the first-line R-CHOP immunochemotherapy, ≈35% of patients are refractory/relapsing. Among the prognostic factors there are genomic alterations and gene expression profiles, which define the activated B-cell-like (ABC) and germinal center B-cell-like (GCB) cell-of-origin (COO) subtypes. However, none of these indicators can entirely predict treatment response.
MiRNAs impact on several cancer processes including drug resistance. They are deregulated in tumors and blood, where they circulate in a stable form, thus being interesting biomarker candidates.
In this study on R-CHOP treated DLBCL patients, we identified by small-RNA sequencing five serum miRNAs as differentially expressed according to treatment response. Three were overexpressed and two downregulated in serum of refractory vs responsive subjects. By ROC curves analysis, the combination of the three upregulated miRNAs led to a better predictive performance.
Next we found that the overexpression of these three miRNAs in DLBCL cells induced a proliferation decrease specifically in the GCB-COO subtype. In GCB double-hit cMYC and Bcl2 translocated DLBCL cells, only two out of three miRNAs have an antiproliferative effect. These data suggest an oncosuppressive role of the miRNA signature depending both on the COO and genetic tumor context.
By an in silico target analysis, we found two novel predicted targets of at least two out of three miRNAs of the signature. A protein-protein interaction analysis revealed that they belong to the same redox homeostasis network, frequently altered in DLBCL and related to tumor cells survival and response to treatment.
Altogether, these data show the potential value of a restricted miRNA signature as non-invasive predictive biomarker in DLBCL. Moreover, its oncosuppressive role suggests a possible application for miRNA-based therapeutic approaches, in particular in the GCB-DLBCL subtype.

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SUMO2/3 conjugation of TDP-43 protects against aggregation

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The neurodegenerative diseases Frontotemporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS) are characterized by the accumulation of cytoplasmic inclusion bodies enriched for the RNA binding protein TDP-43. Nuclear depletion of TDP-43 and its aggregation are central to the familial and sporadic forms of ALS and FTD. Thus, understanding the mechanisms that promote TDP-43 aggregation may open new therapeutic opportunities.
How cells control TDP-43 aggregation is only partly understood. Post-translational modifications (PTMs), such as phosphorylation, acetylation and ubiquitination have been shown to influence TDP-43 aggregation. The TDP-43 aggregates found in the inclusion bodies are ubiquitinated and phosphorylated and both modifications have been linked to protein aggregation. By contrast, recent studies showed that phosphorylation can suppress TDP-43 aggregation.
TDP-43 can also be SUMOylated. Yet, how SUMOylation influence TDP-43 stability and function is still unclear. SUMOylation involves the conjugation of a substrate protein with either SUMO1 or SUMO2/3, via mono-, multi-mono or poly-SUMOylation. SUMO2 and SUMO3 are usually conjugated in the form of SUMO2/3 chains (referred to as SUMO2/3-ylation) in response to stress.
In our work we show that upon oxidative stress TDP-43 is efficiently SUMO2/3-ylated and is recruited inside cytoplasmic condensates known as stress granules (SGs). We further confirm TDP-43 conjugation to SUMO2/3 using a reconstitution system. Using FRAP and subcellular fractionation, we find that inhibition of SUMOylation reduces the mobility of TDP-43 inside SGs and promotes its aggregation. We propose that TDP-43 SUMO2/3-ylation is a mechanism that cells activate to maintain the solubility of TDP-43 during stress.

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mTOR triggers STAT3 S-P at the ER inhibiting calcium-mediated apoptosis in TNBC

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STAT3 is a well-known pro-oncogenic transcription factor that plays a plethora of different roles during tumor transformation and progression via both canonical, nuclear functions and non-canonical, non-transcriptional functions. Among the latter, we have recently described its ability to control ER Ca^{2+} release and Ca^{2+}-mediated apoptosis in STAT3-dependent Triple Negative Breast Cancer (TNBC) cells by localizing to the Endoplasmic Reticulum (ER) and Mitochondrial Associated Membranes (MAMs), where it interacts with the Ca^{2+} channel IP3R3 mediating its degradation when phosphorylated on Serine 727 (S-P). Accordingly, STAT3 and IP3R3 protein levels are inversely correlated in the highly aggressive human basal-like breast tumors, where STAT3 is often constitutively activated. In an effort to elucidate the mechanism regulating STAT3 S-P at the ER, we investigated the role of its interactor mTOR, which has also been reported to localize to the ER. We confirmed STAT3-mTOR interaction within the ER by co-IP from purified ER fractions and Proximity Ligation Assay (PLA) in human TNBC cells. Pan-mTOR inhibition by Torin-1 – but not mTOR Complex 1 inhibition via Rapamycin – could prevent both the observed STAT3-mediated IP3R3 degradation and menadione-induced Ca^{2+}-mediated apoptosis, mimicking the effects of STAT3 silencing. Accordingly, mTOR inhibition abolished STAT3 S-P within the ER, increasing intracellular Ca^{2+} concentration. These data demonstrate for the first time localized STAT3 Serine phosphorylation occurring at the ER and establishes an important correlation with mTOR, which leads to enhanced resistance to apoptosis via IP3R3 degradation. Dissecting these molecular details may reveal novel therapeutic targets to disrupt apoptotic resistance in STAT3-dependent TNBC cells.

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IGFL1 is a new ERG induced and secreted factor in prostate cancer with immune regulatory activity and clinical relevance

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TMPRSS2-ERG gene fusion is an initiating genetic event in ≥50% of prostate cancers (PCa) that, in synergy with partial or complete loss of PTEN, leads to aggressive tumours. PCa progression also relies on signals from the tumour environment (TME).
To investigate the crosstalk with stromal cells, we sought to identify novel potentially ERG+ and/or PTEN- expressed and secreted PCa tumour factors with immune modulating activity. In human epithelial RWPE-1 prostate cell line engineerized to mimic ERG over-expression alone or in combination with PTEN downregulation, we identified IGFL1 as a new ERG+/PTEN- induced and secreted protein. ChIP analysis confirmed ERG binding to IGFL1 promoter and proximal and distal enhancers.
By exploiting The Cancer Genome Atlas (TCGA) we found that IGFL1 was overexpressed in PCa tumours, and its expression progressively and significantly increased between Gleason scores 6 to 9. Significant higher expression of the gene was also observed in PCa patients with lymph node metastasis (N1) and higher pathological T staging (T3-T4), and in ERG+ compared to ERG- PCa tumours. Kaplan Meier for Progression Free Interval survival analysis (PFI) and Disease-Free Interval (DFI), but not for Overall survival, showed that higher IGFL1 levels displayed a worst prognosis in all TCGA PCa patients and in those with no lymph node metastasis (N0). A clear worst prognosis trend for IGFL1 was further observed in ERG+ versus ERG- patients.
TIMER2.0 bioinformatic analysis on TCGA-PRAD dataset, prostate tumours & peripheral blood of high-risk patients confirms the epithelial association of IGFL1 and phenotypic presence of IGFLR1 on macrophage membrane. Preliminary data on in vitro stimulation of M0 macrophage from healthy donors with recombinant IGFL1 protein showed that it favours M1 polarization.
Overall, IGFL1 appears to be an ERG induced and secreted factor with an impact on TME; a result that has relevant diagnostic, prognostic, and therapeutic implications for PCa.

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