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Abstracts

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Speakers' Abstracts

in alphabetical order of presenting author
(presenting authors are shown underlined)

Biomolecular condensates at the nexus of cellular stress, disease and aging

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Biomolecular condensates formed by phase separation are membraneless compartments in the cytoplasm and nucleoplasm of cells, which have major roles in cellular organization and physiology. RNP granules are a specific type of condensate that assemble from RNA-binding proteins and RNA. In this talk, I will discuss how the concept of biomolecular condensates has expanded our view of RNP granules and their link to disease, aging and the cellular stress response. I will introduce in vitro reconstitution systems based on the concept of phase separation that now allow us to reconstruct RNP granules in the test tube. Using these reconstitution systems as well as innovative imaging approaches and biophysics, we have gained important insights into the molecular rules of RNP granule assembly, such as the driving forces and amino acids that govern condensation, the conformational changes underlying assembly and molecular mechanisms of condensate regulation and control. I will further discuss how the concept of phase separation has allowed us to dissect the functions of RNP granules, and I will demonstrate how condensate formation is used by cells to sense and respond to changes in the environment and regulate fundamental cellular processes such as protein synthesis.

ZC3H13 is a crucial target involve in the survival of AML

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Post-transcriptional RNA modification has gained ground in recent years and established as a new layer of gene expression regulation. Currently, considerable attention has shifted to the most abundant internal mRNA modification, N6-methyladenosine (m^6A), which involves the methylation of adenosine at nitrogen-6 position of newly transcribed RNAs. m^6A pattern is dynamically controlled by the coordinating activity of writers, erasers, and readers and it governs RNA metabolism, including splicing, translation, stability, decay, and processing of marked transcripts. Aberrations of m^6A machinery has been also found to contribute to cancer progression. However, the underlying mechanisms of m^6A and the roles of its effectors are still largely unknown in the cancer of different origins. In the present study, we identify the m^6A regulator Zinc Finger CCCH-Type Containing 13 (ZC3H13) as a potential therapeutic target in acute myeloid leukemia. We found that ZC3H13 is highly expressed in myeloid leukemia and its expression is negatively correlated with the overall patient survival. By using reverse-genetic approach, we show that ZC3H13 depletion significantly reduced cell proliferation, ultimately induced cytotoxicity and apoptosis in K562 and THP1 cells. Additionally knock down of ZC3H13 abrogated the abundance of m^6A -marked RNAs at bulk levels. Likewise phenotypic results, total RNA-sequencing followed by ZC3H13 silencing revealed an enrichment of apoptotic pathways and downregulation of pathway involving cell proliferation. Taken together our findings imply that ZC3H13 is crucial therapeutic m^6A -target involved in leukemogenesis.

A novel post-transcriptional circuit required for tumor angiogenesis and cancer growth

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Tumor blood vessels differ in both morphology and functionality when compared to normal vessels, which compromises the efficacy of current anti-angiogenic drugs. Thus, a deeper understanding of the mechanisms sustaining cancer vessel growth is crucial to identify better biomarkers and novel therapeutic targets.
Alternative Splicing (AS) is an essential regulator of gene expression, protein activity and proteome diversity. Despite the established role of AS regulation in cancer cells, its impact on tumor vasculature endothelial cells (ECs) is poorly understood, thus limiting the possibility to identify more specific targets for anti-angiogenic therapy.
In the past, we demonstrated that the AS factor NOVA2 is an important regulator of angiogenesis. We recently found that NOVA2 is upregulated exclusively in tumor ECs with a significant prognostic value: high vascular NOVA2 correlates with shorter overall cancer patient survival, whereas NOVA2-mediated AS is associated with increased tumor vascularization.
By generating a cancer mouse model lacking Nova2 selectively in ECs, we found that Nova2 is critical for the structure/functionality of tumor vasculature and cancer growth. In addition, we found that factors released by cancer cells activate Nova2 in ECs through the upregulation of the endothelium-enriched transcription factor ZEB1, which directly binds to the *NOVA*2 promoter. Accordingly, ZEB1 knockdown in ECs reduces NOVA2 expression and impairs angiogenesis, whereas z*eb1* depletion in zebrafish causes vascular defects similar to those observed in zebrafish lacking *nova2*. Importantly, ZEB1 is co-expressed and positively correlated with NOVA2 in cancer patient endothelium, and ZEB1 upregulation is associated with reduced overall cancer patient survival.
Collectively, our data support a novel regulatory circuit whereby cancer cells activate ZEB1/NOVA2 in tumor ECs to sustain their growth and dissemination.

Dysregulated 3D chromatin structure mechanism in congenital heart disease revealed by chamber-specific cardiac organoids

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Congenital heart disease (CHD) is the most prevalent severe birth defect, affecting ~1 in 100 newborns and ~1.4 million adults in the US. Alas, no specific drugs are available for neither children nor adult carriers. CHD is often caused by mutations in key cardiac transcription factors (i.e., GATA4) or chromatin structure regulators (i.e., CTCF). 3D chromatin organization has emerged as an important regulator of gene expression during embryogenesis. Nevertheless, whether altered dynamics of genome architecture are implicated in CHD remains elusive. We deployed a new model of chamber-specific cardiac organoids (cardioids), derived from human induced pluripotent stem cells (hiPSCs), to correlate morphogenetic defects with chromatin structure-function alterations, measured by chromatin conformation capture and single cell genomics. We found that CTCF binding and the resulting intergenic looping poses a repressive barrier to premature upregulation of cardiomyocyte genes that must move from the inactive (B) to active (A) compartment during cardiogenesis. On the other hand, GATA4 acts as a pioneer factor promoting the unwinding of chromatin at the same loci and their B to A transition. The differentiation of pluripotent stem cells into cardiac progenitors and then cardiomyocytes is facilitated by the gradual downregulation of CTCF up to ~50% and requires the progressive transcriptional activation of GATA4. Haploinsufficiency in CTCF or GATA4 biases this finely balanced tug-of-war, leading to early depletion of cardiac progenitors or their delayed specification into cardiomyocytes, respectively. Conversely, cardiac fibroblasts are underrepresented or overabundant in CTCF versus GATA4 haploinsufficient cardioids. In all, modulation of 3D chromatin structure may represent a therapeutic target in CHD.

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A proteomic perspective on the role of protein methyltransferases in cancer heterogeneity and plasticity

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Chromatin is a dynamic nucleoprotein complex controlling various DNA-dependent processes. A large number of site-specific post-translational modifications of histones (hPTMs) contribute to the maintenance and modulation of chromatin plasticity, gene activation, DNA replication and repair, and other processes. The observation of the diversity, frequency and co-occurrence of hPTMs at distinct genomic loci led to the notion that these marks create a molecular barcode, read by effector proteins that translate it into specific functional outcomes. The histone code is often altered in cancer, as a consequence of the aberrant expression, or mutation, of epigenetic modifiers and with a direct effect on transcriptional patterns and cell identity. The molecular details of this epigenetic code are only partially dissected in both physiological conditions and during tumor onset and progression. Quantitative Mass Spectrometry (MS) has become an excellent technology dissecting the histone code in health and disease. Moreover, the unbiased view offered by MS-based modification proteomics allowed gaining a broader perspective on extent and function of epigenetic modifications beyond histones. My group has been pioneering the field by setting-up distinct MS-proteomics strategies to investigate the chromatin plasticity and nuclear dynamics governed by epigenetic modifications, on and beyond histones.
In my talk I present recently developed MS- approaches both for epigenome mapping of cancer patient samples to identify novel PTMs signatures with potential as biomarker and new mechanisms underpinning cancer plasticity, heterogeneity and response to therapy and for the exploration of the methyl-proteome beyond chromatin, its plasticity upon epigenetic therapy and its functional implication in adaptive response to genotoxic stress in ovarian cancer.

Non coding RNAs: dissecting their mechanism of action in physiology and pathology

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High-throughput transcriptome analysis in mammalian cells has revealed the presence of a large number of RNAs which do not encode for proteins (non coding RNAs - ncRNAs). Moreover, it has been recently shown that quite a considerable number of transcripts have an unusual circular form (circRNAs). These molecules originate from the so-called back-splicing event in which a downstream splice donor site is joined to an upstream splice acceptor site, yielding a covalently closed circular RNA. The interest in the study of circRNAs raised because of several peculiar features, such as evolutionary conservation and tissue-specific expression; but above all because, similarly to ncRNAs, they have been shown to control cell differentiation and to be involved in many pathological conditions, particularly cancer.
The aim of our research is to identify the molecular mechanisms through which ncRNAs and circRNAs control gene expression and to understand how their deregulation is linked to the onset and progression of specific diseases, in particular cancer and neurodegeneration. Understanding the function of ncRNAs will certainly help to identify new targets and possibly conceive novel therapeutic strategies for the cure of different pathologies.

Increased genomic instability and reshaping of tissue microenvironment drive ARID1A-dependent cancer formation

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Loss-of-function mutations in chromatin remodelling complexes have been recently characterised in different tumour types. BRG1/BRM associated factor (BAF) core subunit ARID1A is the most frequently mutated and widely recognised as a tumour-suppressor gene. Whether this role is attributed to its enhancers' regulation function or its ability to maintain genomic stability remains unclear. This study demonstrates that ARID1A is dispensable to preserve enhancer-dependent transcriptional regulation, as ARID1B is sufficient and required to compensate for ARID1A deficiency. Our findings provide *in vivo* evidence supporting the role of ARID1A in maintaining genomic stability in adult tissues.
The loss of ARID1A leads to the accumulation of DNA damage, activation of the interferon type-I response, and recurrent inflammation, culminating in tumorigenesis. We advocate that in healthy tissues, ARID1A mutations may prompt increased genomic instability and a gradual immunoediting process, ultimately leading to aggressive, potentially immune-resistant tumours.

From mouse to human: how RNA epigenetic influences cardiomyocytes maturation

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Tissue-specific long noncoding RNAs (lncRNA) play essential functions in the regulation of cell growth and differentiation. Their dysregulation was often linked with neuromuscular and cardiovascular diseases, although the knowledge concerning their mechanisms of action is still far from complete. In mice, we previously identified several lncRNAs that are enriched in myogenic cells (1). In particular, *Charme* is an abundant and highly conserved lncRNA specifically expressed in differentiated myotubes and cardiomyocytes *(2)*. We found that, during embryogenesis, the nuclear intron-retaining isoform of Charme (pCharme) acts as an “architect” by recruiting the RNA binding protein Matrin3 to scaffold the formation of important nuclear condensates (3). Indeed, mice lacking pCharme develop cardiac hyperplasia due to an abnormal expression of genes involved in the regulation of cardiomyocytes proliferation/maturation balance (4). Intriguingly, the orthologous human transcript (*hs-Charme*) displays several features and a similar tissue specificity to its murine counterpart, suggesting an evolutionarily conserved function and offering many interesting possibilities for its future study. By using the highly adaptable IPSCs system, we are starting to dissect hs-Charme role in human cardiomyocytes. Preliminary data suggest that, in the nucleus, the transcript could play a similar role in the regulation of their maturation. Since the World Health Organization classify cardiomyopathies among the most frequent and fatal diseases in human, understanding *hs-Charme* involvement in cardiac development and pathologies could be extremely important, especially for those without a clear genetic cause.

1.Ballarino et al.,*Molecular and cellular biology*, 2015. DOI: 10.1128/MCB.01394-14
2.Ballarino et al.,*The EMBO journal*, 2018. DOI: 10.15252/embj.201899697
3.Desideri et al.,*Cell reports*, 2020. DOI: 10.1016/j.celrep.2020.108548
4.Taliani, Buonaiuto et al.,eLife, 2022. DOI:10.7554/eLife.81360

Efficient RNA polymerase II pause release requires U2 snRNP function

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Transcription by RNA polymerase II (Pol II) is generally coupled to pre-mRNA splicing, but the underlying mechanisms remain poorly understood. Co-transcriptional splicing is based on coupling between transcription elongation and downstream splicing of the nascent RNA, but evidence for coupling from splicing to transcription also exists. Here we demonstrate widespread functional coupling between early spliceosome assembly and release of promoter-proximally paused Pol II into active transcription elongation at human genes. Inhibition of early spliceosome assembly at the step of branch site recognition by the U2 snRNP component SF3B increases the duration of Pol II pausing and impairs recruitment of the pause release factor P-TEFb subunit cyclin T. Pol II elongation velocity is severely impaired in the 5’-region of the first intron. These results suggest the existence of a bidirectional feedback loop that enables efficient RNA production by connecting the early steps of transcription and splicing machinery.

Esrrb guides naive pluripotent cells through the formative transcriptional programme

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During embryonic development, naive pluripotent epiblast cells transit to a formative state. The formative epiblast cells form a polarized epithelium, exhibit distinct transcriptional and epigenetic profiles and acquire competence to differentiate into all somatic and germline lineages. However, we have limited understanding of how the transition to a formative state is molecularly controlled. Here we used murine embryonic stem cell models to show that ESRRB is both required and sufficient to activate formative genes. Genetic inactivation of Esrrb leads to illegitimate expression of mesendoderm and extra-embryonic markers, impaired formative expression and failure to self-organize in 3D. Functionally, this results in impaired ability to generate formative stem cells and primordial germ cells in the absence of Esrrb. Computational modelling and genomic analyses revealed that ESRRB occupies key formative genes in naive cells and throughout the formative state. In so doing, ESRRB kickstarts the formative transition, leading to timely and unbiased capacity for multi-lineage differentiation.

Small HSPs as molecular chaperones at the interface between proteins and lipids

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The aggregation of soluble proteins into amyloid aggregates is associated with a wide range of human disorders including Alzheimer's and Parkinson's diseases (Chiti & Dobson, 2017). Cells are endowed with a sophisticated protein quality control system to limit protein aggregation and maintain a functional proteome (Hartl, 2017). Molecular chaperones, including the small heat shock proteins (sHSPs), are key components of this system, as they survey protein folding, prevent protein misfolding and aggregation, and target damaged proteins to proteasome and autophagy pathways for degradation (Hartl, 2017).
The aggregation of α-synuclein takes place through a complex non-linear network of coupled microscopic processes, including primary nucleation, fibril elongation and secondary nucleation (Buell et al., 2014). Using a recently developed three-pronged approach it is now possible to separately evaluate each step of α-synuclein aggregation, identifying the contribution of specific environmental factors that are biologically relevant, such as lipid composition and pH. Indeed, α-synuclein aggregation can be triggered by the presence of lipid membranes of specific compositions (Galvaignon et al., 2015). This three-pronged approach has been used here to understand the mechanism of action of sHSPs, which have been reported to interact with lipids.
We found that various members of the sHSP family act on different microscopic steps that lead to protein aggregation. sHSPs can interact with various components by binding to α-synuclein monomers or fibrils, as well as by competing with α-synuclein for binding to lipid membranes. Thus, in addition to their canonical protein chaperone activity, sHSPs may act on the aggregation process also as ATP-independent lipid chaperones. These findings underline the capacity of sHSPs to use multiple strategies to block the aggregation process of α-synuclein, modulating the interplay between proteins and lipids.

TFEB is a master regulator of syncytiotrophoblast formation in the placenta

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The placenta is a highly metabolically organ, transferring nutrients and producing hormones to fulfill the bioenergetic requirements for the proper development of the fetus. Fusion of trophoblast into syncytiotrophoblast triggers the acquisition of transport capabilities and endocrine activities, and dysregulation of this process leads to a broad spectrum of pregnancy abnormalities. Interestingly, embryonic lethality due to defects in placentation has been reported in mice lacking the transcription factor TFEB, a renowned global modulator of energy cell metabolism, via the regulation of autophagy and lysosomal biogenesis. The use of multi-omics approaches led us to delineate the molecular pathway linking TFEB to the metabolic properties of the placenta. Here, we showed that syncytiotrophoblast formation is marked by TFEB nuclear translocation and association with the promoters of key placenta-specific genes. Transcriptomic analyses coupled with LC-MS/MS and cell biology approaches in wild-type and TFEB-depleted cells revealed that TFEB triggers the syncytial fusion of trophoblasts by targeting the fusogenic proteins Syncytin-1 and Syncythin-2. Also, we showed that TFEB controls the endocrine properties of the placenta and, in particular, orchestrates the steroidogenic program by regulating CYP19A1, the rate-limiting enzyme for the synthesis of estradiol. Accordingly, the lack of TFEB dramatically reduces estradiol production measured by lipidomics analysis. Most importantly, we observed a reduction in the expression of TFEB and its newly identified target CYP19A1 in the placenta tissues of women affected by pre-eclampsia, explaining the impairment of steroidogenesis reported in pre-eclampsia. Collectively, these findings elect TFEB as a critical determinant of syncytiotrophoblast formation and, more broadly, endows TFEB with unique properties relevant to understanding the biology of intercellular fusion events and regulation of endocrine activity in other tissues.

Functional dissection of the 3D genome

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Elzo de Wit is a group leader at the Netherlands Cancer Institute in Amsterdam. His group uses genomics methods to study different aspects of gene regulation. He is particularly interested in how DNA is organized inside the mammalian nucleus (or '3D genome') and how this relates to genome function. The 3D genome is organized in loops that can measure hundreds of kilobases in size. These loops are formed by the cohesin complex which progressively increases chromatin loops in size in a process known as loop extrusion. The de Wit lab has developed and implemented tools to measure rapid changes in the 3D genome following acute depletion of proteins that are involved in the formation of loops. How changes in the 3D genome are related to changes in transcription will be discussed. Furthermore, data about how active genes cluster together in the space of the 3D genome and the proteins that are important for this process will also be discussed. These results will be placed within the context of the fundamental features and mechanisms that shape the 3D genome.

RNA-RNA interactions at the onset of human motor neurons specification: CyCoNP lncRNA sustains NCAM1 expression by a direct-binding mechanism

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The transition from dividing progenitors into post-mitotic motor neurons (MNs) is orchestrated by a series of events mainly studied at the transcriptional level by analyzing the activity of specific transcription factors while the role of noncoding RNAs in this biological process is still far from being fully clarified. Recently we described paradigmatic examples of long noncoding RNAs (lncRNAs) induced in a MN differentiation system from mouse embryonic stem cells with a prominent role in MNs formation and maturation (Carvelli et al., 2022; Pellegrini et al., 2022). Here we extend the noncoding perspective to human MNs by detailing the molecular and biological activity of CyCoNP (as Cytoplasmic Coordinator of Neural Progenitors), a highly expressed and MN-enriched human lncRNA. We found that CyCoNP sustains a specific neuron differentiation program mainly by establishing RNA-RNA interactions with the mRNA of NCAM1, a key player in neurogenesis involved in the formation and maintenance of neurites and synapses. Specifically, we were able to dissect a dual RNA-mediated mechanism according to which CyCoNP can bind to and sequester a microRNA that targets NCAM1 and synergically stabilizes its mRNA by a direct RNA-RNA interaction. Finally, we found that PTBP1, a protein involved in many steps of RNA metabolism, supervises this process by controlling the loading of microRNAs on CyCoNP transcript. These data highlight novel circuitries involved in the control of human MNs specification and point out as RNA molecules and the complex relationships they can establish may represent the building blocks of sophisticated cellular mechanisms.

Carvelli A, Setti A, Desideri F, et al. A multifunctional locus controls motor neuron differentiation through short and long noncoding RNAs. *EMBO J*. 2022.
Pellegrini F, Padovano V, Biscarini S, et al. A KO mouse model for the lncRNA Lhx1os produces motor neuron alterations and locomotor impairment. *iScience* 2022.

Identification of a novel genome editing approach for the treatment of AEC syndrome

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AEC syndrome is a rare genetic disease mainly characterized by severe skin lesions, defects in the hair, nails, sweat glands and teeth, closure of eyelid margins, and cleft lip and/or palate. Skin erosions usually appear at birth or soon after and are a major cause of morbidity, bleeding and infection. The disorder is caused by specific mutations in p63 exon 13 and 14, which encode for the carboxyl terminal portion of the p63alpha isoform,a crucial pioneer transcription factor required for epidermal development, stemness and differentiation. Exon 13 is an alternatively spliced exon absent in the p63beta isoform; this latter isoform is physiologically expressed in wild type epidermis albeit at much lower levels than p63alpha. We recently generated a conditional knock-out mouse model lacking exon 13, in which p63alpha is replaced by the p63β isoform. While loss of p63alpha was detrimental for palate and limb formation during mouse embryogenesis, no phenotype was observed in the skin during development or after birth, indicating that the p63beta isoform supports normal skin development and maintains skin homeostasis in adult life similarly to p63alpha. Additionally, using genome editing, we deleted p63 exon 13, where the majority of AEC syndrome causative mutations reside, thus generating the conversion of the alpha isoform into beta isoform of p63 gene in human primary keratinocytes. Here, we show that with a highly efficient and non-toxic viral-free method, we can achieve more than 90% correction of p63 protein function in seventy-two hours ex vivo, without affecting the clonogenic potential of human primary keratinocytes.
The proposed state-of-the-art technologies for genome editing in epidermal stem cells and the development of novel approaches for in vivo delivery of editing molecules will greatly contribute to cure untreatable skin erosions in AEC syndrome.

A truncated and catalytically inactive isoform of KDM5B histone demethylase accumulates in breast cancer cells and regulates H3K4 tri-methylation and gene expression

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The histone demethylase KDM5B (also known as JARID1B or PLU-1) is a master regulator of H3K4 methylome and its aberrant expression correlates with many human cancers. KDM5B often behaves as an oncogene. However, its role in cancer seems multifaceted and complex, depending on the cellular context. A paradigm of this opposite behavior is highlighted by the different roles of KDM5B in gene expression regulation in the luminal breast cancer cell line MCF7, where it sustains cell proliferation, versus the basal breast cancer cell line MDA-MB-231, where it inhibits cell migration and invasion. Recently, it has been proposed that the complexity of KDM5B roles in cancer might be the result of the expression of different isoforms. So far, the expression of KDM5B isoforms in breast cancer cell lines was studied considering only the total pool of proteins, therefore we questioned the relevance of KDM5B isoforms in breast cancer. We show here that a previously uncharacterized isoform of KDM5B, namely KDM5B-NTT, accumulates in breast cancer cell lines due to remarkable protein stability relative to the canonical PLU-1 isoform, which shows a much faster turnover. The NTT isoform is the N-terminal truncated and catalytically inactive product of an mRNA with a transcription start site downstream of the PLU-1 isoform, and the consequent usage of an alternative ATG for translation initiation. It also differs from the PLU-1 transcript in the inclusion of an additional exon (exon-6), previously attributed to other putative isoforms. Overexpression of this isoform in MCF7 cells leads to an increase in bulk H3K4 methylation and induces de-repression of a gene cluster, including the tumor suppressor Cav1 and several genes involved in the interferon-alpha and -gamma response. We discuss the relevance of this finding considering the emerging evidence that KDM5B may possess regulatory roles independent of its catalytic activity.

Topoisomerase I-DNA cleavage complexes induce genome instability at early replicating DNA loop anchors

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Topoisomerase I (Top1) is a key enzyme which resolves DNA topological stress. The formation of DNA-Top1 intermediates (Top1ccs) is fundamental for the enzyme to relax negative supercoils thus preventing the formation of unscheduled R-loops, non-B DNA structures composed of an RNA-DNA hybrid and a displaced non-template strand. Top1 poisons cause the trapping of Top1ccs with consequent increase of R-loops and micronuclei (MNi), extra-nuclear bodies containing damaged chromatin not incorporated into the main nucleus after cell division. We have published that MNi formation is R-loop dependent, but molecular outcomes downstream of Top1cc trapping remained to be fully clarified. By performing genome-wide mapping of R-loops and Top1cc-induced DNA double-strand breaks (DSBs), we found that the Top1 poison camptothecin causes both an increase (gain) and a decrease (loss) of R-loops. In particular, gain R-loops are mainly found at highly transcribed promoters and overlap with paused RNA PolII, lamina associated domains (TADs) and Top1ccs which is consistent with R-loop increase in transcribed regions where supercoil local dissipation is mainly dependent on Top1 activity. We found that Top1ccs are directly involved in the formation of the gain R-loops and that their accumulation causes abundant DSBs at early origin initiation zones placed at the loop anchors of TADs. Interestingly, G1-treated cells, which accumulate DNA damage after G1-to-S phase transition, produce R-loop dependent MNi suggesting a new mechanism of genome instability mainly occurring in G1 cells. Overall, our work shows that Top1cc-induced R-loops trigger DNA damage at early replication zones and MNi formation in G1-treated cells. As MNi are important for innate immune gene activation, understanding the molecular mechanism of Top1cc-related instability will help in developing new strategies for effective personalized interventions by using Top1-targeted compounds as immuno-modulators in cancer patients.

UHRF1 is a better prognostic factor in colorectal and gastric cancers and thymoma by negatively regulating EMT factors and cancer stemness pathway genes

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UHRF1 is a key epigenetic factor for maintaining DNA methylation and sustaining gene-silencing inheritance through the recruitment of epigenetic modulators. While UHRF1 is considered an oncogene, including colorectal cancer (CRC), immunohistochemistry (IHC) experiments performed on our CRC cohort show that higher levels of UHRF1 results in a better prognosis. To address this flaw, we exploited the association of UHRF1 expression with the survival status in all 33 tumours of the NCBI-TCGA database.
Expression values of UHRF1 above its median value (UH) in the TCGA CRC cohort display a better prognosis. UH-correlated genes are enriched with the favourable prognostic genes in the Human Protein Atlas (HPA) database. Inversely, UL-related genes are enriched with HPA unfavourable genes and with gene signatures specific for adult intestinal stem cells that predict disease relapse in CRC patients. Unexpectedly, also in gastric cancer and thymoma, UH correlates with better prognosis and relative HPA favourable genes. Contrarywise, a dismal prognosis and unfavourable genes associate with UH in at least 8 other tumours, including liver and renal carcinoma and sarcoma.
Gene Ontology and Metascape analysis reveals that in all three tumours with best prognosis in UH, the genes in the embryonic morphogenesis sub-category, which include EMT factors and cancer stemness genes, are enriched in UL. In contrast, in UH worst prognosis tumours, those genes are enriched in UH.
Analysis of the TCGA DNA methylation data revealed that UHRF1 tightly correlates with high methylation levels at the promoters of those genes only in UH better prognosis tumours. RRBSeq and RNA-seq analysis and ChIP experiments performed on UHRF1-silenced CRC cells confirms that UHRF1 is an important regulator of those genes.
The results suggest that high UHRF1 expression levels might explain the better prognosis of colorectal and gastric cancers and thymoma by directly repressing EMT and stemness genes.

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Providing new insights on non-coding RNAs involvement in Alzheimer's disease through a comprehensive bioinformatic analysis based on Hippocampus and Fusiform Gyrus RNA-seq datasets

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The deregulation of gene expression is associated with several neurodegenerative diseases, such as Alzheimer's Disease (AD), but how or whether it influences their onset is mostly unclear. The present work aims at contributing to the current knowledge on the involvement of non-coding genes (ncRNAs), e.g. long non coding RNAs (lncRNAs) and circular RNAs (circRNAs) in AD. We analyzed, through state of the art bioinformatic tools, a hippocampus (PMID: 29523845) and a fusiform gyrus (PMID: 29346778) RNA-seq dataset consisting of the transcriptomic profiles of AD patients and control subjects. The analysis identified 2,766 differentially expressed (DE) genes (1905 mRNAs, 777 lncRNAs, 74 circRNAs) in AD hippocampus and 444 DE genes (236 mRNAs and 208 lncRNAs) in AD fusiform gyrus. 194 DE genes were found in common between the two datasets. Through a correlation network analysis with WGCNA, two overlapping gene modules (arbitrarily color labeled) of co-expressed DE genes between the two brain regions were obtained. The darkred and brown modules, comprising the most significant DE lncRNAs of the hippocampus dataset were analyzed with Ingenuity Pathway Analysis and were found significantly associated with pathways related to neurotransmission and memory consolidation.
Our data demonstrate the existence of specific deregulation of the expression profile of ncRNAs in the brain of AD patients, providing an important source of information for better understanding the molecular changes characterizing AD progression.

LINE1 expression dynamics in colorectal cancer sensitivity to DNA damage

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Transposable Elements (TEs) are repetitive DNA sequences that cover about 45% of the human genome. Among TEs, LINE1 (autonomous interspersed nuclear elements, 18% of the genome) are reported to exert also crucial epigenetic regulatory functions and their expression is cancer-type specific. In this context, p53 has been found to repress LINE1 transcription and p53 mutations are associated with higher mutation burden and also LINE1 retrotransposition in different tumors with the exception of Colorectal cancer (CRC), which displays low degree of genome instability, even with a microenvironment characterized by high concentrations of free radicals, molecules well known to induce DNA damage. Thus, we have investigated LINE1 expression and possible function in CRC genome stability.
We first observed that LINE1 are expressed specifically in p53 mutated CRCs, by performing RNA ISH on FFPEs of CRC and normal-like colon (n=20 patients). LINE1 are actively transcribed and retained at chromatin in $p53^{mut}$ CRC cell lines. In particular, LINE1 expression anticorrelates with CD3+ T Lymphocytes abundance, and thus tumor immunogenicity. We then hypothesized that LINE1 transcription could promote genome stability in CRCs. We assessed that $p53^{mut}$ CRCs (LINE1 high) are less sensitive to damaging agents compared to wild-type ones and that LINE1 downregulation could revert this phenotype. Therefore, we are investigating LINE1 RNAs’ involvement in protecting the genome from DNA damage.

The accumulation of 8-oxodG at super-enhancers marks fragile CTCF-mediated chromatin loops

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The DNA oxidization process generates a significant amount of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), which has been suggested to have an epigenetic function in gene regulation and has been linked to genome instability. However, the exact role of 8-oxodG in gene regulation and the mechanisms that regulate the accumulation and maintenance of 8-oxodG at the genome level are not well understood.
In our study, we discovered and characterized a group of enhancers that accumulate 8-oxodG in human epithelial cells. These enhancers are primarily super-enhancers and are associated with bidirectional-transcribed enhancer-RNAs and activation of the DNA Damage Response. By analyzing ChIA-PET and HiC data, we also identified specific chromatin loops that are mediated by CTCF and physically link the oxidized enhancers and promoters.
We observed that the oxidized enhancers, along with their associated chromatin loops, tend to accumulate endogenous double-strand breaks (DSBs). These DSBs are then repaired by the non-homologous end-joining (NHEJ) pathway in a transcription-dependent manner. Our research provides new insights into the inherent fragility of chromatin loops containing pairs of oxidized enhancers and promoters and indicates that the accumulation of 8-oxodG in these elements occurs through a transcription-dependent mechanism.

DNA polymerase η, ribonucleotides and Nanopore sequencing: a story of replication stress

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pol η is a Y-family translesion DNA polymerase extensively characterized for its ability to bypass thymine dimers induced by UV light. More recently, pol η has also been demonstrated to perform numerous functions independent of DNA lesions. We have shown that in budding yeast pol η sustains genome duplication when dNTP pools are reduced, but this activity favors the inclusion/stabilization of RNA stretches in DNA that become toxic if not removed by RNase H enzymes. We are investigating whether, upon dNTPs shortage, pol η may act on transcriptional RNA:DNA hybrids, ultimately causing RNA moieties to be embedded in DNA. High-throughput sequencing techniques developed to map ribonucleotides in DNA only allow to indirectly deduce their genomic location and fail to distinguish a single rNMP from a stretch of consecutive rNMPs in a certain position. Additionally, the most common strategies to study RNA:DNA hybrids, based on the S9.6 monoclonal antibody or on catalytically inactive RNase H1, indistinctly bind R-loops, hybrids at Okazaki fragments or double-strand breaks, and eventually RNA stretches embedded in DNA. We are developing a strategy to directly proof the formation of pol η-dependent stretches of consecutive rNMPs inserted in DNA. We assessed the feasibility of directly detecting ribonucleotides embedded in DNA with Oxford Nanopore Technologies (ONT). To this extent, we synthesized DNA molecules containing rNMPs at known positions and we developed appropriate data analysis pipelines for detecting ribonucleotides embedded in DNA by ONT. We report for the first time that ONT can identify all four ribonucleotides incorporated in DNA by capturing rNMPs-related alterations in nucleotide alignment features, current intensities, and dwell times.

VDAC1 gene promoter regulation: exploring transcription factors functionality and genome methylation

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Voltage Dependent Anion-selective Channel 1 (VDAC1) is the most abundant pore-forming protein crowding the mitochondrial outer membrane and ruling the exchange of metabolites with the cytosol. There are three VDAC isoforms in most eukaryotic cells. VDAC1 is considered the principal regulator of mitochondrial bioenergetics and cellular metabolism. Recently we investigated VDAC genes expression regulation under normal condition as well as during stress. We characterized transcription factors involved in mitochondrial biogenesis and in response to hypoxic stress (1,2). In this work we analyse the methylation status of VDAC1 gene. MethBank data indicate that VDAC1 promoter owns the largest CpG island among VDAC isoforms and that it is the least methylated, as seen in different tissues, cell types, and/or developmental stages. According to this information, we characterized by gene reporter assay the reduction of transcriptional activity of VDAC1 promoter methylated *in vitro*. To circumscribe VDAC1 promoter subjected to methylation we performed a ChIP assay and amplified the isolated DNA. The results confirm the potential regulation of VDAC1 promoter by its methylation in crucial sites including NRF-1 binding sites. In conclusion we are understanding the regulatory mechanism of VDAC1 gene expression in various conditions where mitochondrial function needs to be assured.

1) Guarino F et al, BBA Bioenerg. (2020) 1861(12), 148289.
2) Zinghirino F et al, IJMS (2020) 21(19), 7388.

Modulation of vesicle trafficking rescues Parkinson’s phenotype related to LRRK2 mutations

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Parkinson’s Disease (PD) is the second most common neurological disorder affecting roughly 10 million people worldwide. Mutations in LRRK2 gene are the main genetic cause of PD reaching the 30% of cases in specific ethnic groups. Up to date, the LRRK2 patho-physiological function is still cryptic although different experimental evidence underlines a prominent role of LRRK2 in the control of vesicle trafficking, and, interestingly, alteration in synaptic vesicle dynamics seems a common pathological mechanism in both familial and idiopathic PD. We have previously demonstrated that LRRK2 interacts with Sec8, a member of exocyst complex and regulates the exocyst complex formation. The exocyst complex is an evolutionarily conserved multisubunit protein complex mainly implicated in tethering secretory vesicles to the plasma membrane. Extending the mentioned results, we demonstrate that Sec8 over-expression or a specific inhibition of exocyst complex assembly (by endosidin2 treatment) rescue the LRRK2 pathological phenotype in continuous neuronal cell line and drosophila models. Moreover, Levetiracetam, a compound largely used in human therapy for epilepsy treatment, is also able to rescue the LRRK2 pathological phenotypes. Both levetiracetam and endosidin2 act regulating vesicle dynamics strongly suggesting the vesicle trafficking as a possible therapeutic target for PD treatment.

Regulating innate immunity via RNA-editing

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RNA-editing by adenosine deaminases acting on RNA (ADARs) converts adenosines to inosines in highly structured or double-stranded RNAs. In mammals, two active ADARs can be found. ADAR1 is expressed in two isoforms, ADAR1p110 that is predominantly localized to the nucleus and an interferon-induced ADAR1p150 that is mainly localized to the cytoplasm. ADAR2 is mainly expressed in the nervous system, the large intestine and the vasculature and is located in the nucleus.
ADAR1 deficiency leads to embryonic lethality that is accompanied by a high interferon signature. Interestingly, deletion of the cytoplasmic viral RNA sensor MDA5 or its downstream signaling partners can rescue a catalytically dead ADAR1 allele indicating that the inosines in structured RNAs may be critically required to prevent activation of an immune response by endogenous RNAs.
To identify the RNAs and signals that can activate MDA5 we have isolated RNAs that are bound by MDA5 in the absence of ADARs. Here we characterize these RNAs that can activate MDA5 and show how ADARs can prevent their activation of immune signaling.

RNA editing of CYFIP2 regulates actin related cellular migration and neuronal development *in vitro*

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Cytoplasmic FMRP Interacting Protein 2 (CYFIP2) is a Fragile X Messenger Ribonucleoprotein (FMRP) interactor and a component of the Wave Regulatory Complex (WRC), one of the most important players in regulating cellular actin dynamics. Interestingly, CYFIP2 transcript undergoes RNA editing, an epitranscriptomic modification catalysed by ADAR enzymes, that leads adenosine (A) to inosine (I) deamination. CYFIP2 editing in the coding sequence results in a K/E substitution at amino acid 320. The functional meaning of this regulation is still unknown. In this study, we aim at investigating the potential implication of CYFIP2 RNA editing related to actin dynamics during cell migration, axon development and synaptogenesis in neural cells. We have generated SH-SY5Y neuroblastoma cell lines in which CYFIP2 gene has been deleted by CRISPR-Cas9 technology. Overexpression of CYFIP2 unedited (K) and edited version (E) showed differences in the different cell lines ability to migrate but not in their ability to differentiate into a neuronal phenotype. Furthermore, we took advantage of primary neuronal culture knocked down for endogenous CYFIP2 by shRNA technology and overexpressed with CYFIP2 editing variants. During neuronal maturation, morphological parameters concerning axon development and spine frequency has been analysed. We found a statistically significant difference between neurons carrying K and E variants in the number of axon branches, total axon length, axon complexity index and dendritic spine frequency. Our work reveals for the first time a functional significance of CYFIP2 K/E RNA editing process in regulating the spreading of neuron axon during the first stages of in-vitro development and in the subsequent process of spinogenesis. Understanding the function of CYFIP2 editing regulation during in vivo brain development could help in the comprehension of its physiological role throughout the development and activity of the nervous system.

Counteracting G-quadruplex-induced genome instability

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Pathological conditions characterized by the accumulation of various genetic alterations, ranging from point mutations to significant chromosomal rearrangements, are known as genome instability. This condition can lead to cell death and is considered a key feature of cancer cells in higher eukaryotes. All living organisms have developed intricate mechanisms to control DNA integrity and ensure genome stability. To explore new genes and cellular pathways involved in maintaining genome integrity, we utilized Synthetic Genetic Array (SGA) technology to conduct a genetic screen. Through this approach, we identified *VID22* as one of the candidates, a gene previously known to participate in the repair of DNA double-strand breaks. Our investigation revealed that *vid22Δ* cells display genome instability and aberrations in regions with a high likelihood of forming G-quadruplexes, non-canonical DNA secondary structures. Failure to resolve these structures can obstruct the progress of DNA and RNA polymerases and has been associated with chromosome fragile sites. We demonstrated that Vid22 directly binds to and protects DNA at regions prone to G-quadruplex formation. Specifically, the loss of VID22 leads to an increase in gross chromosomal rearrangement (GCR) events that are dependent on sequences capable of forming G-quadruplexes. Additionally, we observed deficiencies in maintaining G4-DNA rich elements, such as rDNA, telomeres, and mtDNA. To unravel the molecular mechanism by which Vid22 regulates G-quadruplexes, we generated and analyzed several mutants with distinct functional properties.

The interplay between miR-579-3p and MITF controls melanoma progression and resistance to targeted therapies

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Therapy of metastatic melanoma has improved dramatically over the last years thanks to the development of targeted therapies (MAPK inhibitors) and immunotherapies. However, drug resistance continues to be a major limitation to the efficacy of these therapies. Our research group has provided robust evidence as to the involvement of a set of microRNAs in the development of non-genetic resistance to target therapy in BRAF-mutated melanoma cell lines. Among them, a pivotal role is played by miR-579-3p, whose role as oncosuppressor was discovered by our group some years ago. Here we show that miR-579-3p and the microphthalmia-associated transcription factor (MITF) influence reciprocally their expression by positive feedback regulatory loops. In particular we show that miR-579-3p is specifically deregulated in BRAF-mutant melanomas and that its expression levels mirror those of MITF. Luciferase and ChIP studies allowed us to show that MITF is a positive regulator of miR-579-3p, which is located in the intron 11 of the human gene ZFR (Zink-finger recombinase) and is co-transcribed with its host gene. Moreover, we report that miR-579-3p, by targeting BRAF is able to stabilize MITF protein thus inducing its own transcription. As a consequence, upon exposure to MAPK inhibitors or, alternatively upon miR-579-3p transfection the activation of this newly uncovered miR-579-3p/MITF axis induces block of proliferation and senescence of BRAF-mutant melanoma cells. We also observed that the long term development of resistance to MAPKi is able to select cells characterized by the loss of both miR-579-3p and MITF. We observed their down-regulation also in patients relapsing after targeted therapies treatments. Altogether these findings suggest that miR-579-3p/MITF interplay potentially governs the balance between proliferation, senescense and resistance to target therapies in BRAF-mutant melanomas.

The ZZZ3, a subunit of the ATAC complex, is important for human ESCs homeostasis, self-renewal, and regulation of ribosome biogenesis

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Embryonic stem cells (ESCs) are pluripotent stem cells that have the ability to self-renewal and differentiate into any of the three germ layers. The transcriptional regulation of pluripotency has been extensively studied. In contrast, the regulation of ribosome biogenesis is a process that is less well understood. Ribosome biogenesis involves the assembly of ribosomal RNA (rRNA) and ribosomal proteins into functional ribosomes responsible for protein synthesis. The regulation of rRNA synthesis, processing, modification, and coordination of ribosomal protein synthesis and assembly are complex processes that are subject to regulation by a variety of factors. Here, we focused on the role of ZZZ3 (Zinc Finger ZZ-Type Containing 3), a subunit of ATAC complex. To understand the function of ZZZ3 we investigated the ZZZ3-interacting proteins by performing ZZZ3 immunoprecipitation followed by mass spectrometry in wild-type hESCs. ZZZ3 interacting proteins are mostly ribosomal proteins and nucleolar proteins. GO analysis of the ZZZ3 interacting partners revealed that these interactors are mostly enriched in ribosome biogenesis, rRNA processing, and ribonucleoprotein complex biogenesis. To further investigate the global regulatory roles of ZZZ3 in hESCs, RNA-Seq was used to compare the transcriptomes in ZZZ3-KD cells and control hESCs. Downregulated in ZZZ3 KD cells are enriched in processes such as ribosome biogenesis, rRNA processing, and translation. These data are further confirmed by polysome profile analysis showing a reduced amount of monosomes and polysome, indicative of low ribosome production and poor translation in ZZZ3-KD hESCs compared to control cells. Interestingly, the mTOR pathway and its downstream targets are significantly reduced and inactive in ZZZ3 KD ESCs suggesting that ZZZ3 downregulation negatively acts on mTOR signaling pathway. Furthermore, we show that ZZZ3 KD ESCs are less proliferative suggesting that ZZZ3 KD impairs the self-renewal of ESCs.

Extracellular vesicles as carriers of RNA therapeutics: possible role of the p75NTR-NGF-TrkA ternary complex to support targeted delivery

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Extracellular vesicles (EVs) are promising tools for therapeutic RNA delivery, and the EV surfaceome is a recognized mediator of their interaction with cells. Thus, current efforts are focused both in the identification of surfaceome components and in the membrane engineering to achieve targeted delivery.
In this study, the possibility that two neurotrophic receptors, namely the TrkA and $p75^{NTR}$ receptors, and their shared ligand nerve growth factor (NGF), can mediate a targeted interaction between EVs and recipient cells was investigated. TrkA and $p75^{NTR}$ synergistically contribute to NGF signaling. Interestingly, co-crystal structures showed that NGF binds to TrkA and $p75^{NTR}$ extracellular domains in an antiparallel manner. This prompted us to hypothesize that a ternary $p75^{NTR}$-NGF-TrkA complex may be formed in trans, when a receptor is present on the EV surface and the other one on recipient cells. To verify this hypothesis, we exploited three CRISPR-Cas9 edited PC12 cell lines, that were engineered to knock-out only the TrkA or the p75NTR or both. These were first characterized for EV production along with wild-type PC12. The abundance of TrkA and $p75^{NTR}$ in EVs was investigated by western blot and Stochastic Optical Reconstruction Microscopy (STORM). Then, EVs carrying either receptor or both were investigated for their ability to potentiate NGF induced neurite outgrowth in wild-type PC12 cells. Administration of TrkA, but not necessarily $p75^{NTR}$, via EVs increased neurite outgrowth induced by saturating NGF dose, suggesting that TrkA carried by EVs may engage NGF and interact with recipient cells bearing $p75^{NTR}$. We are currently setting-up an internalization assay to assess the role of the putative $p75^{NTR}$-NGF-TrkA complex in the EV uptake. These results may inspire the rational design of a targeted delivery strategy for pathological conditions in which $p75^{NTR}$ is overexpressed, with the aim of re-equilibrating the homeostatic ratio of neurotrophic signaling streams.

Transcriptional, epigenetic and genetic determinants triggering distinct cancer phenotypes coexist in the same cancer population

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Cancer is a highly heterogeneous disease. Single-cell lineage tracing has recently shown that clones often reinitiate a tumour non-randomly, suggesting that cancer cells can be primed to different fates. Yet the molecular features of tumour clones that are primed to aggressive phenotypes remain still elusive and therefore we are unable to reconstruct the molecular basis of transcriptional reprogramming typical of cancer evolution. We investigate the multiple predictive features of two aggressive cancer phenotypes — tumour initiation and drug tolerance — within the same breast cancer population. Using single-cell multi-omic lineage-tracing, we detect distinct clonal subpopulations with stable gene expression profiles and link them to pre-encoded fates. Clones primed to tumour re-initiation *in vivo* display two distinct transcriptional state at the baseline, but share a predictive epigenetic state marked by DNA accessibility, suggesting that tumour initiation is epigenetically driven. The drug-tolerant pool is largely pre-encoded, but only partially overlaps the tumour-initiating niche, and is organised in two genetically and transcriptionally distinct lineages. Our approach highlights genetic, epigenetic and transcriptional determinants coexisting in a tumour population which pre-encode different fates, unravelling the complexity of cancer evolution.

Inspecting roles of 3D chromatin organization in transcription by multiplexed imaging

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The last two decades have seen a meteoric revolution in fluorescence microscopies, with the advent of super-resolution, single-molecule and light-sheet techniques. The number of molecular species visible in a single specimen remains, however, an intrinsic limitation, linked to the spectral overlap of available fluorophores. Recently, this limitation has been uplifted by multiplexing methods. These methods rely on the combination of microfluidics with conventional microscopes to perform sequential and combinatorial acquisition of tens of different molecular species in single cells. Our multiplexing method (HiM) is able to trace chromatin and transcriptional status in single cells within complex samples, such as embryos or tissues, with nanometer resolution. We applied HiM to investigate the regulation of transcription during the early stages of differentiation in Drosophila embryonic development and between different mouse tissues. We revealed that enhancer-promoter proximity can provide a scaffold for both activation and repression in early fly development. In mice, however, EP proximity is modulated amongst cell-types and is affected during physiological perturbations.

A paracrine HGF-MET-STAT3 axis oversees cellular transition towards human pluripotency

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The route to induced pluripotency via human cellular reprogramming is characterized by plastic intermediate stages whose features are still largely unknown. This has long hindered the efficiency of human reprogramming. By taking advantage of a microfluidic approach, we perform reprogramming with high efficiency, thus highlighting an active and pivotal role of a confined microenvironment. To profile the dynamic reciprocity between reprogramming cells and their microenvironment, we profile the time-course of secretome, single-cell transcriptome and single-cell chromatin accessibility. This reveals the functional role of extrinsic pathways across subpopulations emerging along reprogramming trajectories. This protein communication dynamically shapes a permissive extracellular environment resembling primitive node formation during embryo development. Among other factors, we identify HGF as a potent modulator of human reprogramming targeting its receptor, MET, transiently expressed in an intermediate cell population. Further investigations described that HGF-MET activates non-canonical STAT3 pathway in cells along the reprogramming trajectory. Through the perturbation of this pathway, we validated the HGF/MET/STAT3 axis as a crucial signalling in reprogramming. Collectively, we highlighted a finely regulated extracellular context that serves as a developmental nest for transitioning toward pluripotency. These findings, which role has always been overlooked in the human reprogramming process, reveal the influence / lead of population crosstalk for the efficiency of TF-driven cellular reprogramming.

Targeted RNA editing as an enhancer of immunotherapy

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Responsiveness to immunotherapy is associated with (a) a high tumor mutational burden (TMB) (Samstein et al. 2019) and (b) a high T-cell infiltration in the tumor microenvironment. The assumption is that a high TMB results in a higher amount of immunogenic neoepitopes presented by cancer cells that can induce a T-cell-mediated anti-tumor immune response. Indeed, different methods have already been attempted to increase the availability of neoepitopes in tumors (i.e. adjuvant radiation therapy, cancer vaccines, etc.). At the same time, recent data demonstrate that immunogenic neoepitopes are naturally generated by RNA editing, a mechanism mediated by Adenosine Deaminases Acting on RNA (ADAR) (Zhang et al. 2018). This observation has led us to envision a strategy to specifically alter tumor epitopes, while maintaining genomic integrity, based on targeted RNA editing. Specifically, we have applied targeted ADAR-mediated RNA editing to introduce, rather than correct, point mutations in specific mRNA transcripts. The locations we chose within those transcripts are those that encode epitopes that are presented on the surface of tumor cells in the context of MHC. Doing so we could modulate cellular antigenicity and recognition by T cells. Specifically (and as proof of concept) we genetically modified a well-known tumor antigen, Melan-A, such that its processing would result into a peptide (Melanoma antigen recognized by T-cells-1 or MART-1) no longer recognised by T cells. We then used targeted RNA editing to restore the original MART-1 sequence and thus rescue antigen-specific T-cell activation. Our work shows the potential of RNA editing as a precision tool to generate neoepitopes (which we have termed “editopes”) as a method to improve tumor recognition and clearance by T cells.

A human pan-genomic analysis provides insights into the genetic and epigenetic complexity of phenotypes associated with facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is characterized by a wide phenotypic variability in terms of muscle involvement and disease severity. FSHD1 is associated with reduced number of D4Z4 tandem repeats located at 4q35 associated with specific distal polymorphism (4qA-PAS) that is considered the disease molecular signature. FSHD2 comprehends 5% of FSHD clinical cases carrying D4Z4 alleles with more than 10 repeats and is associated with mutations in chromatin-remodeling factors. It has been hypothesized that FSHD results from chromatin changes determining D4Z4 reduced methylation and 4q35 gene anomalous expression.
Analysis of the T2T genome assembly revealed hundreds of D4Z4-like elements scattered throughout 1.2Mb. We evaluated the sequence organization of the FSHD locus in 87 haplotypes from the human pan-genome project. In comparison with GRCh38 assembly, that includes 86.5Kb, 4q-specific elements resulted more variable and abundant (median number: 22), than the 10q-specific (median number: 16). In 5.6% of cases 4q alleles with ≤8 D4Z4 units, associated to the 4qA-PAS haplotype permissive for FSHD, were identified.
Considering the T2T, we investigated the D4Z4 epigenetic setting running a high-depth CpG methylation assay spanning the D4Z4 repeat. Our test revealed that D4Z4-like sequences on the acrocentric chromosomes and chromosome 1 are highly methylated. We identified two 4q and 10q D4Z4 regions that allowed the clustering of samples in three groups on the basis of different CpG methylation levels. Our analysis also revealed that reduced D4Z4 methylation at 4q and 10q has no strict correlation with the clinical phenotype but is associated with the presence of *SMCHD1* variants.
Our work demonstrates the value of the T2T genome assembly for investigating the structure and the functional role of repetitive elements; the assemblies from the pan-genome project provide essential elements for genotype-phenotype correlation studies in FSHD.

Ribosome readthrough over premature termination codons: molecular mechanisms and pathophysiological implications for genetic disorders

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Nonsense mutations, by introducing premature termination codons (PTCs) and leading to the synthesis of truncated proteins and/or nonsense mediated mRNA decay, are a relevant cause of genetic disease.
However, nonsense triplets might, albeit at low frequency, undergo a recoding process named ribosome readthrough, which insert specific amino acids, or subset(s) of, depending on the stop codon. This could account for residual expression of full-length (FL) protein in patients and have pathophysiological implications.
Many efforts have been also made to develop readthrough-inducers for therapeutic purposes. In this view, the dissection of the molecular determinants shaping the outcome of either spontaneous of induced readthrough, namely nucleotide and protein contexts as well as their interplay and impact on protein structure/function, is crucial to identify responsive PTCs resulting in functional FL proteins.
The deficiency of coagulation factor VIII or IX (Hemophilia A or B) represent ideal models to address these issues since nonsense mutations are relatively frequent and even low expression levels might influence the clinical phenotype.
Through the characterization of a large panel of HA/HB-causing mutations we dissected the determinants shaping the outcome of functional readthrough, with the protein context having the pivotal role. This led to classify nonsense mutations and explain a differential association with the development of inhibitory antibodies following replacement therapy, one of the most serious complication. Moreover, the mechanistic findings with readthrough-inducers defined readthrough-favorable features useful to achieve rescue profiles compatible with therapeutic thresholds.
Data help interpreting the variable efficiency of readthrough-inducers in patients for different genetic disorders, and assessing the potential translatability of readthrough into a personalized and mutation-specific, and thus patient-oriented, therapeutic strategy.

Axl-miR-214sponge chimeric aptamer reduces breast cancer and melanoma dissemination

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microRNAs (miRNAs) are important regulators of gene expression and are frequently deregulated in tumor progression. We previously showed that the inhibition of the pro-metastatic miR-214 is able to inhibit metastasis formation. In order to selectively hit tumor cells and reduce miR-214 levels, we generated a chimeric aptamer called axl-miR-214sponge. In detail, we linked GL21.T (axl), an aptamer that specifically binds to AXL, an oncogenic tyrosine kinase receptor abundantly expressed on cancer cells, but poorly present on normal cells, to miR-214sponge, an oligonucleotide sequence able to block miR-214 functions. AXL-expressing breast cancer and melanoma cells treated with axl-miR-214sponge conjugates showed reduced migration, invasion and mammosphere formation compared to controls. In parallel, expression of miR-214 direct and indirect targets resulted affected. Notably, no effect was detected in cells that did not express AXL, demonstrating the chimeric aptamer selectivity. Importantly, axl-miR-214sponge conjugates induced necrosis and apoptosis in primary tumor masses and reduced breast cancer and melanoma metastatization in mice carrying xenotransplants, following systemic treatments. Our data evidence that axl-miR-214sponge chimeric aptamers are specific and are able to reduce metastatic traits and cancer spreading, thus representing new therapeutic tools for the treatment of malignant breast cancers and melanomas.

Epigenetic priming of an epithelial enhancer by p63 and CTCF controls expression of a skin-restricted gene *XP33*

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Skin is the largest organ which helps our body to withstand external stress and injuries. The outermost part of the skin, epidermis, is a stratified epithelium developing from epidermal stem cells in exquisitely organised fashion. The transcription factor p63 is a renowned master regulator of gene expression of epidermal cells. Fine-tuned cooperation between p63 and its cofactors such as CTCF enable spatiotemporal organisation of chromatin architecture and interaction between regulatory elements essential for epidermal differentiation. Non-coding RNAs have an important role in regulation of tissue-specific processes. However, the transcriptional control of epidermal non-coding RNAs by p63 remains poorly studied. Here, we identify a skin-specific non-coding RNA *XP33* whose expression is regulated by p63 during keratinocyte differentiation in vitro. By analysing chromatin state of the *XP33* locus, we show that CTCF and p63 establish an epithelial enhancer to prime *XP33* transcription in tissue-restricted manner. p63 is the pioneer factor which identifies *XP33* enhancer prompting histone acetylation within this region in squamous epithelia. *XP33* promoter and enhancer form a chromatin loop exclusively in keratinocytes but not in other cells types. Altogether, we identify a tissue-specific non-coding RNA whose expression is epigenetically regulated by p63 and CTCF.

Inhibition of METTL3 and m^{6}A induces accumulation of endogenous double-stranded RNAs and innate immune response

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N6-methyladenosine (m^{6}A) is the most abundant mRNA modification and plays multiple roles, yet to be fully characterized, in RNA biology. We have recently found that conditional deletion of the m^{6}A writer METTL3 in murine fetal liver activates an aberrant innate immune response, mediated by the formation of endogenous double-stranded RNAs (dsRNAs)[1]. Here, we show that pharmacological inhibition of METTL3[2] induces an innate anti-viral immune response in human and murine leukaemia cells, with strong activation of the OAS sensors, normally tasked with detecting foreign dsRNAs. Although aberrant endogenous dsRNAs are specific to each of the systems studied, they share common properties: they are long, highly m^{6}A modified in their native state and characterized by low folding energies. Collectively these results suggest that inhibition of METTL3 can be used to modulate dsRNA formation, as potential immunotherapy against leukaemia and other tumours characterized by high METTL3 activity.

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Low endogenous levels of heat shock protein beta-1 impair antioxidant response in patients carrying SCN1A mutations

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Oxidative stress and ROS production prompt different cellular mechanisms to prevent or repair cellular damage. Febrile seizures (FS) and mesial temporal lobe epilepsy (MTLE) have been linked to oxidative stress and increased ROS production because of the brain experiences a sudden increase in metabolic activity. This increase in ROS production can cause damage to cellular structures in the brain, leading to inflammation and neuronal dysfunction. Here, we investigate the role of ROS production in neural stem cells (NSCs) differentiated from iPSCs generated from a patient with loss-of-function mutation in the *SCN1A* gene, causing childhood FS and development of MTLE. Treatment with H_2O_2 induces high levels of ROS production, autophagy deficiency, and increased apoptosis in patient NSCs compared with the healthy control. The HSPB1 (heat shock protein beta-1), also known as HSP27, is a small heat shock protein involved in many protective mechanisms, including the antioxidant response, autophagy, and apoptosis. HSPB1 is a target of the p38-stress kinase pathway, which is activated in response to oxidative stress, inflammation, and other cellular stressors. We found significantly reduced levels of HSPB1 mRNA and protein in levels in patient NSCs, suggesting that patient's cells are more susceptible to oxidative stress. We are currently performing experiments to rescue the HSPB1 levels via inducing nuclear localization of the HMGB1 (high-mobility group box 1) transcription factor. Altogether, although still preliminary, our results suggest that low endogenous levels of HSPB1 might be responsible for defective antioxidant processes and impairment of neurogenesis leading to MTLE.

Poster Abstracts

(presenting authors are shown underlined)

P.1

Dissecting the relationship between DNA repair and autophagy to improve the efficacy of anticancer treatment

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It is becoming increasingly clear that, despite being a cytoplasmic process, autophagy plays a key role in maintaining genomic stability. Many studies provided evidence that autophagy may modulates DNA repair pathways, with major implication for therapy-induced responses and acquired resistance in cancer, although the exact mechanism behind this connection remains a matter of debate. To investigate how autophagy modulates DSB damage response, we have generated a MCF10A cell line expressing the fusion protein consisting of the AsiSI restriction enzyme and a modified hormone-binding domain from the estrogen receptor. Cell exposure to 4-hydroxytamoxifen results in nuclear accumulation of the AsiSI-ER protein and in the rapid induction of ~150 sequence-specific DSBs across the genome. This cellular system enables us to investigate recruitment of DNA repair factors at specific DSBs by using ChIP-based approaches. To investigate how autophagy modulates DSB damage response, we employed CRISPR/Cas9 genome editing to establish a ATG7 KO MCF10A-AsiSI-ER cell line. Our goal is to investigate how autophagy regulates DNA repair proficiency and pathway choice throughout the cell cycle and the molecular events underlying these regulatory mechanisms.

The study of cross-kingdom regulation of plant microRNAs with possible anti-inflammatory and anti-tumor activity in human

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MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by promoting translation inhibition and/or mRNA degradation.
In humans, miRNAs control a wide range of biological processes including carcinogenesis, by acting as either oncogenes or tumor suppressors during cancer development and progression.
Therefore, therapeutic strategies based on the use of engineered miRNAs to restore tumor suppressive miRNAs (mimic miRNAs) or to suppress the expression of oncogenes (anti-miRNAs) have been developed in the past decade. These miRNAs, are often chemically modified to decrease their degradation and to increase their stability.
However, endogenous miRNAs are chemically modified, some specific modification increasing miRNA stability. In fact, plants miRNAs are methylated at the 3’-end, which protect them from degradation by exonucleases and make them more stable than human ones. Recently, it has been shown that plant-derived miRNAs can specifically bind and regulate the expression of human mRNA. Therefore, plant miRNAs provide potential anti-viral, anti-inflammatory and anti-tumoral functional activity, in a cross-kingdom regulation.
This implies that plant microRNAs could represent potential natural drugs for new therapeutic strategies in the treatment of cancer and inflammation. Despite the role of human endogenous miRNAs has been extensively investigated, the role of plant-derived miRNAs in human gene expression regulation needs more investigation.
To this aim, in this study we have identified some plant miRNAs with putative anti-inflammatory and anti-tumor activity. Moreover, to study their effect on human gene regulation we have designed and tested different overexpression strategies to identify a heterologous plant miRNA expression system in human cancer cell lines.

METTL16 is a key target for chronic myeloid leukemia survival

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The field of epitranscriptomics (i.e., RNA modifications) has seen a growing interest in the latest years from the discovery that RNA marks can control the transcriptomic profile. RNA methylation at N6-adenosine (m6A) is dynamically controlled by the coordinating activity of writers, erasers, and readers. It also governs RNA metabolism, including splicing, translation, stability, decay, and processing of marked transcripts, representing an additional layer of gene expression regulation. Despite, several pieces of evidence pointing to the fact that m6A plays a key role in cancer, the underlying mechanisms by which m6A and its effectors are involved in tumors have not yet been fully elucidated. METTL16 has been recently identified as an independent m6A methyltransferase and previous findings have suggested its possible role in contributing to cancer progression. Here we shed light on the oncogenic activity of METTL16 in chronic myeloid leukemia (CML). We found that METTL16 is overexpressed in leukemia compared to its normal counterpart. Additionally, we observed that high METTL16 expression levels in AML patients correlate with reduced overall survival. Next, by performing METTL16 knockdown (KD) in K562 cells we showed that METTL16 suppression strongly inhibits CML cell proliferation and clonogenic potential. Interestingly, we observed that METTL16 silencing in K562 cells induces a decrease in cell metabolic state followed by apoptosis induction. Accordingly, transcriptomic profiling by total RNA-seq in METTL16 KD cells showed deregulation of proliferation-associated transcripts and an enrichment of cell death-related pathways. Collectively, our findings reveal METTL16 as a crucial proliferation regulator of CML cells that may be advanced as a novel therapeutic target in leukemia.

P.4

Human thioesterase ACOT8 has an impact on *in vitro* HIV-1 infectivity

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Introduction. HIV-1 establishes a broad spectrum of interactions with host proteins, including the cellular thioesterase ACOT8, presumably mediated by interactions with the HIV-1 Nef protein. This work investigates the impact on HIV-1 infectivity of ACOT8 depletion during HIV-1 viral particle production.
Methods. CRISPR/Cas9 technology was used to knock-out the ACOT8 gene in HEK293T and TZM-bl cells. HEK293T wild type and knock-out cells were used to produce HIV-1 pseudotyped viruses with different HIV-1 envelopes (the CCR5 tropic QHO, AC-10 and pRHPA and the CXCR4 tropic LAI). Virus titers were assessed by quantification of HIV-1 p24 and the same input was used for infections on TZM-bl wild type and ACOT8 knock-out cell lines; infectivity was evaluated as Relative Luminescence Units (RLU). The Mann-Whitney test was applied to detect statistically significant differences between the infectivity of pseudotyped virus produced in the presence or absence of ACOT8, and in the presence of absence of Nef.
Results. The different HIV-1 envelopes tested showed higher infectivity when pseudotyped viruses were produced in the presence of ACOT8 rather than when they were produced in its absence (QHO, $p= 0.0011$), even when Nef was not present (QHO, p-value<0.0001, AC10, $p=0.0019$, pRHPA, $p<0.0001$, LAI, $p<0.0001$). In contrast, no difference was observed with the VSV envelope control. No differences were found by infecting TZM-bl target cells with or without ACOT8. This suggests that ACOT8 might play a role in increasing HIV infectivity at the pre-entry level.
Conclusions. Our preliminary data suggest that expression of ACOT8 in cells producing HIV-1 pseudotyped virus is associated with increased viral infectivity. Further experiments are needed to better define the mechanisms that modulate virion infectivity and any other factors involved in this interaction.

Functional characterization of lncRNAs involved in epigenetic regulation of human neural development and neurological disorders in human brain organoids

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Neural development is a sophisticated process in which the transcription and repression of specific genes must be tightly regulated. Moreover, neurological and neurodegenerative diseases are gradually becoming the primary burden of society, for most of which there are no effective treatments. In this context, long non-coding RNAs (lncRNAs) have attracted considerable attention due to their prominent expression in the nervous system and the involvement of their misregulation in neural diseases. For this purpose, human brain organoids (hBOs) represent a fascinating approach mimicking the key features of the human brain system, for the study of neural development and disease onset. To investigate the role of lncRNAs in epigenetic regulation during neural differentiation, we performed bulk and single-cell RNAseq (scRNA-seq) of *in vitro* human brain development from H9-human embryonic stem cells (hESCs) through all the crucial neural stages. By analyzing differentially expressed genes and RNA fractionation sequencing data, we selected the 12 most promising candidates based on their chromatin enrichment score and higher expression levels in more committed stages versus hESCs, and prioritized their misregulation in a neurological disease context. We then knocked out candidate lncRNA-genes by inserting a polyA signal using CRISPR/Cas9-mediated genome editing. The role of mutant lncRNA-genes in cell commitment, 3D self-organization, and pathogenesis elucidated by scRNA-seq and phenotypic characterization of human brain organoids will be discussed. Molecular mechanisms of lncRNA-dependent transcriptional regulation will be investigated to prove their role in neural differentiation and disease onset.

8-oxodG accumulation within human mitochondrial genome associates with transcription

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Mitochondrial DNA (mtDNA) can suffer from intrinsic and environmental stressors that lead to oxidative damage and the accumulation of 8-oxo-7,8-dihydroguanine (8-oxodG) in mtDNA. This accumulation of 8-oxodG has been linked to degenerative and aging diseases, as well as cancer. Despite the well-described implications of 8-oxodG in mtDNA for mitochondrial function, there has been no prior mapping of 8-oxodG in mtDNA. To address this, we used OxiDIP-Seq and mapped 8-oxodG in the mitochondrial genome of human MCF10A cells. Our findings indicate that, under steady-state conditions, 8-oxodG is distributed evenly throughout the mitochondrial genome, except for the longer noncoding region (NCR), which seems to be protected from 8-oxodG accumulation. However, when cells were exposed to oxidative stress caused by UV irradiation, 8-oxodG accumulated within the highest transcribed region, which includes the 12S and 16S genes. Therefore, our data suggests that the accumulation of 8-oxodG in the mitochondrial genome is associated with mitochondrial transcription.

De novo transcriptome assembly and annotation for gene discovery in *Salamandra salamandra* at the larval stage

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Dispersal is a key process in ecology and evolutionary biology, as it shapes biodiversity patterns over space and time. Attitude to disperse is unevenly distributed among individuals within populations, and that individual personality can have pivotal roles in the shaping of this attitude. Here, we assembled and annotated the first *de novo* transcriptome of the head tissues of *Salamandra salamandra* from individuals, representative of distinct behavioral profiles. We obtained 1,153,432,918 reads, which were successfully assembled and annotated. The high-quality of the assembly was confirmed by three assembly validators. The alignment of contigs against the *de novo* transcriptome led to a mapping percentage higher than 94%. The homology annotation with DIAMOND led to 153,048 (blastx) and 95,942 (blastp) shared contigs, annotated on NR, Swiss-Prot and TrEMBL. The domain and site protein prediction led to 9850 GO-annotated contigs. This *de novo* transcriptome represents reliable reference for comparative gene expression studies between alternative behavioral types, for comparative gene expression studies within Salamandra, and for whole transcriptome and proteome studies in amphibians.

MiR-148b regulates cell metabolism and affects tumor progression in melanoma and breast cancer

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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 breast carcinoma and melanoma, where it inhibits malignancy by impairing tumor cell dissemination. Since a complex interplay occurs between tumor progression and metabolic rewiring, we aim at understanding the impact of miR-148b on cell metabolism. We characterized the cell metabolic profile upon miR-148b modulation and investigated its intervention on the main players of glycolysis and mitochondrial functions in vitro and in vivo. We evidenced decreased glucose uptake, glycolysis and lactate production in miR-148b-overexpressing cells, while the opposite was found in miR-148b depleted cells. High miR-148b levels led to reduced activity and expression of specific glycolytic players, among them, the glucose transporter 1 (GLUT1), a predicted direct target of miR-148b. In parallel, PI3K/AKT and WNT/β-catenin pathway downmodulation was observed. Importantly, miR-148b-dependent glycolysis modulations could affect metastatic traits suggesting a direct link with tumor dissemination. Decreased mitochondrial oxidative phosphorylation was also found upon miR-148b overexpression, as revealed by decreased electron transport chain activity and mitochondrial ATP production. In parallel, alterations in mitochondrial morphology and function were detected. miR-148b overexpression also led to the downregulation of mitofusin 1 and OPA1, essential for mitochondrial fusion. Some preliminary data suggest that the impairment of miR-148b-dependent mitochondrial function could depend on the downregulation of peroxisome proliferator activated receptor-gamma coactivator-1alpha (PGC1α), a master regulator of mitochondrial biogenesis and a predicted target of miR-148b. Altogether, these data suggest that miR-148b affects glycolysis and mitochondrial functions, which can contribute to metastasis inhibition.

Tau exon 6 alternative splicing is regulated by the RNA-binding proteins PTBP1 and RBM20

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Tau is a microtubule-binding protein, encoded in humans by the *MAPT* gene. *MAPT* transcripts undergo alternative splicing, producing a plethora of different Tau protein isoforms with potentially different functionality. The gene is organized into 16 exons, and at least 6 of them are alternatively spliced during development and tissue differentiation. The alternative splicing of exons 2, 3 and 10 has been extensively studied over the years and, in particular, the imbalance in the expression of exon 10 isoforms has been associated with the development of Alzheimer's disease. Recently, Tau isoforms containing exon 6 have attracted research interest due to their ability to i) block Tau polymerization and ii) promote neural differentiation *in vitro*. Therefore, understanding the Tau exon 6 splicing mechanisms could contribute to the identification of potential therapeutic strategies to fight neurodegenerative disorders. To study the exon 6 expression regulation, we used bioinformatic analysis to predict putative *cis*-acting sequences in the exon 6 intronic flanking regions recognized by splicing factors. We then produced a minigene vector containing this region and used it in conjunction with RNA-binding proteins (RBPs) expressing vectors to characterize their role in determining the exon 6 transcript patterns. We focused our attention on the ubiquitous RBP PTBP1, a known modulator of Tau splicing, and RBM20, an heart- and skeletal muscle–enriched RBP that controls isoform expression of genes essential for cytoskeletal function. Preliminary data confirmed the action of PTBP1 in promoting exon 6 exclusion and highlighted a possible role of RBM20 in determining the tissue specificity of Tau isoforms expression. These results may help shed further light on the complex mechanisms regulating Tau alternative splicing and isoform aggregation.

Development of a pipeline for identifying mito-nuclear variants in patients with amyotrophic lateral sclerosis using whole genome and exome sequencing data

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Many diseases share the feature of having dysfunctional mitochondria, which are under a dual genetic control: mitochondrial DNA (mtDNA) and nuclear DNA (nDNA). Our goal was to create an easy-to-use pipeline for the detection of variants in nDNA and mtDNA using whole-genome and exome data from patients with mitochondrial related neurodegenerative diseases.
Given that mitochondrial dysfunction is a recognized trait in Amyotrophic Lateral Sclerosis (ALS), the pipeline was developed on ALS datasets. ALS dataset 1 consisted of Whole Exome Sequencing (WES) data, while ALS dataset 2 consisted of Whole Genome Sequencing (WGS) data; both datasets were from spinal cord tissue.
Briefly, the pipeline involved the following steps: quality trimming of FASTQ files, independent processing of nDNA and mtDNA, alignment of reads, variant calling, variant filtering, categorization of variants into functional impact categories, and annotation using population databases.
For nDNA variant calling a total of 31,134 and 301,241 variants were called in MitoCarta3.0 genes with an average of 11,976 and 107,560 variants per individual, respectively in ALS dataset 1 and 2. In both datasets, after prioritization steps, a rare missense variant c.785C>A in SIRT3 gene was identified.
For mtDNA variant calling, off-target WES reads were aligned to the mtDNA. After filtering, 10 rare variants were detected in ALS dataset 1, including a never described damaging high-impact variant (7410C>T) in the MT-CO1 gene. For ALS dataset 2 we detected 50 variants particularly enriched in MT-ND5 and MT-CYB genes.
Overall, exome and whole genome sequencing data led to the identification of candidate pathogenic variants in ALS patients in the nDNA and mtDNA. Additionally, this completely automated protocol has the potential to be employed in clinical settings to identify disease-associated variants in mitochondrial-based neurodegenerative disorders.

Effects of cyanotoxins triggers in neurodegenerative cellular models

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Cyanotoxins and, in particular, β-N-methylamino-L-alanine (L-BMAA), a modified aminoacid, have been hypothesized to play a significant role in slow-developing neurodegenerative diseases (ND) such as Amyotrophic Lateral Sclerosis/Parkinsonism Dementia Complex (ALS/PDC), Amyotrophic Lateral Sclerosis (ALS), Alzheimer and dementia. We used pure L-BMAA and Microcystis aeruginosa crude extracts (LCS-MaCe), a cyanobacterial strain isolated from Coghinas' lake (Italy, Sardinia) as environmental toxicity triggers on both neuronal (human neuroblastoma SH-SY5Y cells) and non-neuronal (patient's fibroblast) ND cellular models. By using a wide range of techniques, we evaluated different cellular parameters. Moreover, we demonstrate a dose-dependent toxicity upon L-BMAA or LCS-MaCe exposure, exacerbated by over-expression of A382T mutation in ALS causing gene TDP-43. Our results add new pieces to the molecular mechanisms of ND pathogenesis.

Evaluation of ion channel activity of envelope protein E2 from SARS-CoV-2

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The envelope protein E2 of SARS-CoV-2 is an integral membrane protein crucial for virion assembly, release and immunopathology of the viral infection. It consists of 75 amino acids distributed in three distinct domains: a short hydrophilic N-terminus, a large hydrophobic transmembrane domain (TMD) and a long hydrophilic C-terminus. According to previous studies, E2 assembles in a multimeric structure with ion channel activity [1]. To investigate about this feature, preliminary electrophysiological experiments were performed. First of all, E2 gene sequence from a Covid-19 obtained by a patient's specimen was cloned into pET21b vector, expressed as recombinant His-tagged protein in *E.coli* and subsequently purified by affinity chromatography. Pore-forming ability was tested into artificial membraned at +100mV in symmetrical $CaCl_2$ and KCl solutions. E2 inserted as channels that close by steps, as expected by a multimeric assembly. Moreover, Molecular Dynamics (MD) simulations were performed after rebuilding the entire 3D E2 structure by homology modelling. MD trajectory files suggest that, after total removal of spatial restraints, the multimeric structure of E2 collapses in a non-conducting conformation. These results prompted us to speculate that reference structure was inaccurate. A new channel structure was obtained by inverting the orientation of the hydrophilic C-terminus. Consistent with electrophysiological results, new MD trajectory files showed that more ions penetrate through the pore. MD simulations also suggested the presence of a potential Ca^{2+} binding site located within the pore side exposed to ER lumen. Preliminary analysis performed with MIB2 software identified a series of putative calcium-binding residues, mainly located at the N- and C-termini. Further work will be aimed to define the activity of E2 and the role of calcium in gating mechanism.

[1] W.Surya et al. BBA-Biomembranes 2018, 1860, 1309-1317

Circ-Dlc1 KO affects long-term potentiation in cortical-striatum circuitry *in vivo* and *in vitro*

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Circular RNA (CircRNA) are covalently close RNA molecules which arise following an alternative splicing event termed backsplicing. Circ-Dlc1 was found highly expressed in motoneurons (MNs) in vitro derived from both human and mouse stem cells.
In order to study the function of circ-Dlc1, a knock-out (KO) mouse model has been generated through CRISPR/Cas9 strategy designed to target the intronic sequences responsible for the biogenesis of the circRNA. A primary phenotypic screening has comprised a treadmill exhaustion test to assess the possible presence of neuromuscular defects. Notably, the KO mice present clear defects in the completion of the test if compared to syngeneic WT animals but no molecular and structural alterations were identified at the motor unit level.
The expression of circ-Dlc1 was also explored in different districts of the brain via qRT-PCR and highly sensitive circRNA FISH, and it was found to be enriched in the deep layer of the cortex and in the striatum, indicating that the observed phenotype might be due to alterations in striatal-cortical circuitries, which is involved in motor behavior and control. Indeed, proteomic analyses of the striatum revealed deregulation of circ-Dlc1 KO mice in glutamatergic-associated pathway including long-term potentiation (LTP), a process that involves post-synaptic strengthen via an increased recruitment of AMPA receptor.
Alteration related to glutamate response and LTP has been then validated in *in vitro*-terminal differentiated neurons, obtained from mouse embryonic stem cells WT and circ-Dlc1 KO. Through Calcium imaging it has been observed an increased post-synaptic activity of circ-Dlc1 KO neurons in response to AMPA stimulus.
Moreover, preliminary results show that circ-Dlc1 interacts with mRNAs of glutamatergic receptors and regulates their translation.

Analysis of the expression profile of genes regulated by treatment with glucorticoids and retinoic acid in lung cancer

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Different therapies are available for the treatment of lung cancer, among which the use of glucorticoids (GC) and retinoic acid (RA). However, their role in the treatment of this specific cancer type is not well established.
The aim of this project was to identify differences in expression patterns in both small lung cell cancer (SCLC) and non-small lung cell cancer (NSCLC) when subject to treatment with GC and RA, both alone and in combination with Suberoylanilide hydroxamic acid and azacitidine (AZA/SAHA). With this purpose, a subset of the public dataset (GEO accession GSE66245) was used for the analysis, focusing on data derived from a MYC-amplified cell line in which the BRG1 gene was genetically inactivated.
Classification was performed using four supervised learning methods -Random Forest, LDA, LASSO and SCUDO- and the comparison of performance, quantified through the accuracy, suggested that Random Forest was the best performing algorithm. Gene set enrichment analysis was performed with g:Profiler on the 100 most relevant genes by importance value as computed by the Random Forest. The higher ranked KEGG pathway was the retinol metabolism hsa00830 ($p = 2.026 \times 10^{-2}$). The most significant GO term about the molecular function was retinoic acid 4-hydroxylase activity GO:0008401($p = 3.706 \times 10^{-3}$). Negative regulation of retinoic acid receptor signaling pathway GO:0048387 ($p = 7.417 \times 10^{-3}$) was the most significant GO term concerning the biological process. Additional biological insights were gained from network-based analysis carried out using PathfindR, identifying an up-regulation of Cytochrome P450 26A1 and B1 (CYP26A1 and B1) in cells subject to treatment compared to the non-treated ones. The CYP26-mediated destruction of retinoic acid, suggested by the obtained results, provides a possible explanation for the limited clinical efficiency of RA in the treatment of many solid types of tumor, as previously reported (V. Hunsu et al., Int. J. Mol. Sci. 2021).

RNA regulatory networks governed by miR-125a in hepatocarcinoma cells

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MiR-125a is emerging as an important player in the pathogenesis of hepatocellular carcinoma (HCC), acting as an oncosuppressor: it has antiproliferative activity; it mediates the activity of the antitumoral drug Sorafenib; it is downregulated in HCC biopsies in comparison to normal tissues; it can limit tumor growth; it is involved in hepatitis B virus infection. Different miR-125a oncotargets have been identified and some regulatory circuits based on coding and non-coding RNAs have been unveiled. This work aims to gain a genome-wide perspective of the whole miR-125a targetome with the ambitious goal to piece together the RNA regulatory networks governed by the miRNA impacting on hepatocarcinogenesis. HepG2 and HuH-7 cells have been transfected with miR-125a mimic to boost its intracellular level in comparison to cells transfected with a control molecule and RNA seq experiments have been performed. Multiple data comparisons identified coding and non-coding RNAs whose expression levels resulted changed in HepG2 and/or HuH-7 because of miR-125a increased level. Two candidate miR-125a targets were further validated, NR6A1 and NUP210, encoding for nuclear proteins, overexpressed in HCC tissues in comparison to normal livers with inverse correlation to miR-125a expression. Functional studies on the identified RNA regulatory networks and impact on HCC hallmarks are in progress.

P.16

Analysis of Concentrated Growth Factors (CGFs): cell population and angiogenic potential

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In the field of regenerative medicine there is growing interest in the potential of Concentrated Growth Factor (CGF), an autologous and biocompatible blood derivative. Among the processes involved in tissue regeneration, angiogenesis plays a key role, but the role played by CGF in vessel formation is not clear. Therefore, the focus of this work was to study the contribution of CGF on neovascularization in an in vitro model of angiogenesis, evaluating the effects of its components such as growth factors and cellular components. CGF clots were cultured for 14 days in cell culture medium, then CGF-conditioned medium (CGF-CM) was collected and soluble factors and cellular components were separated and characterized. The angiogenic properties of CGF were analyzed by stimulating cultured human endothelial cells with increasing concentrations (1%, 5%, 10% or 20%) of CGF-CM, and its effect on cell migration and tubule formation was evaluated by wound healing and Matrigel assay, respectively. The expression of endothelial angiogenic mediators was determined using qRT-PCR and ELISA assays. CGF-derived cells were characterized by immunostaining, qRT-PCR and Matrigel assay. Our results show that CGF-CM contains pro-angiogenic factors, such as VEGF, TGF-β1, MMP-9 and MMP-2, which promote: i) the migration of endothelial cells, ii) the formation of the structure of the tubules, iii) the endothelial expression of several angiogenic mediators. Furthermore, CGF-derived cells expressed the stem cell marker CD34, characteristic of endothelial progenitor cells, as well as endothelial markers participating in the neo-angiogenic process. In conclusion, our results suggest that CGFs can promote endothelial angiogenesis and therefore can be used for therapeutic applications in the field of tissue regeneration.

P.17

LINE1 elements are novel epigenetic players of T cell quiescence through phase separation

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Naïve CD4+ T cells when stimulated through antigen recognition exit from quiescence and polarize toward effector cells. Recently, we discovered that alternatively spliced transcript isoforms of T-cell activation genes retain LINE1 elements – the most abundant transposable elements in the human genome (~18%) – and sustain T-cell quiescence. LINE1-transcripts form a complex with KAP1 and NCL and keep the respective canonical genes paused until cell activation. The exit from quiescence occurs through an epigenetic reorganization that is still unexplored. Pieces of evidence showed that repetitive non-coding transcripts can contribute to the assembly of condensates involved in several cellular functions including genome organization and transcriptional control.
We hypothesized that LINE1-transcript/KAP1/NCL complex could exert a novel epigenetic function to maintain T lymphocyte quiescence through the assembly of phase-separated chromatin bodies. To address the epigenetic function, we used super-resolution microscopy and ChIP-seq to show that KAP1/NCL/LINE1-transcript complex occupies megabase-scale domains that anti-correlate with heterochromatin. By using ChromHMM – a machine learning approach for chromatin state discovery – we found out that KAP1/NCL complex establishes a novel chromatin state associated with an intermediate-active transcriptional level. We are integrating these data with RADICL-seq and Hi-C datasets to infer how LINE1-transcript presence affects inter- and intra-chromosomal contacts between T cell-specific genes. To further corroborate our hypothesis, we demonstrated that KAP1 can phase separate via its intrinsically disordered region (both *in vivo* and *in vitro*) and that the concentration of LINE1 transcripts can control condensate formation.
Overall, we aim to unveil the quantitative contribution of LINE1-transcripts in defining a novel epigenetic state and to expand these findings in pathological contexts i.e. tumor-infiltrating lymphocytes.

P.18

NOVA2 upregulation plays a pervasive role in alternative splicing changes of gastric cancer endothelium

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Angiogenesis is crucial for cancer progression and metastasis formation. Although anti-angiogenic drugs are currently in use for cancer treatment, their clinical benefits are unsatisfactory. Consequently, a deeper comprehension of the mechanisms supporting the development of tumor blood vessels is necessary to both understand tumor vasculature aberrancies and to identify novel biomarkers and therapeutic targets.
Aberrant alternative splicing (AS) regulation is an important molecular modifier of oncogenesis and a hallmark of cancer. However, its contribution to tumor endothelial cells (ECs) biology is poorly known.
In the past, we found that AS factor NOVA2 is a key post-transcriptional regulator of the vascular development.
Here, we report that NOVA2 is selectively upregulated in tumor ECs of gastric cancer (GC), one of the most frequent malignancies worldwide. High NOVA2 expression correlates with tumor features associated with invasiveness and metastasis and with poor overall survival of the GC patients. Intriguingly, RNA sequencing supports a pervasive role of NOVA2-driven splicing changes in endothelium identifying novel AS transcripts generated upon NOVA2 upregulation in ECs. Finally, we show that the AS profile of *RapGEF6*, a newly identified NOVA2 target, is altered in GC patients, where it is correlated to NOVA2 expression, tumor angiogenesis, and poor patient survival. Collectively, our results promote NOVA2 as a novel player in GC, offering opportunities for the discovery of novel biomarkers and therapeutic targets.

P.19

Deciphering the role of the PR-Dub complex during liver homeostasis and regeneration

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Increasing evidence demonstrated that mutations altering the activity of chromatin-modifying complexes significantly contribute to liver tumour formation and progression. Inactivating mutations of BAP1, the catalytic subunit of the Polycomb Repressive Deubiquitinase Complex, have been found in a subset of aggressive HCCs, mainly affecting women, and in more than 20% of CCA. Despite BAP1 is unequivocally considered a tumour suppressor, its role in preserving tissue homeostasis is still largely unexplored.
To clarify the role of BAP1 in liver, inducible wild type and knockout mice were kept under chow or DDC diet triggering the activation of a regenerative program characterized by the amplification of a pool of SOX9+/HNF4a+ liver progenitor-like cells (LPLCs) derived from hepatocytes or cholangiocytes.
Our data provided *in vivo* evidences that BAP1 severely alters the epigenetic landscape of liver cells compromising the ability of transcriptionally activate key regenerative programs, such as the YAP/TAZ pathway, affecting cell plasticity and ultimately favouring tumor formation.
Here, we shed light on the mechanisms that could favour liver tumour formation possibly identifying novel diagnostic or prognostic markers and pharmacological targets.

P.20

SFPQ controls genomic stability by suppressing RNA:DNA hybrids in human cancer cells

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R-loops are triple-stranded nucleic acid structures containing an RNA moiety paired with its antisense DNA template strand and the loop of the displaced, single-stranded DNA (ssDNA). RNA:DNA hybrids form under normal physiological conditions and exert multiple biological functions. However, persistent R-loops have been shown to mediate replication stress, DNA damage and drive genomic instability.
R-loops have particular relevance at vertebrate telomeres. Telomere R-loops are formed by pairing of the long, telomere-repeat noncoding RNA (TERRA) with CCCTAA containing leading telomere strand. Replication stress induced by these structures is thought to generate recombinogenic DNA substrates mediating recombination dependent telomere maintenance in telomerase negative tumors.
We recently found that the RNA-binding protein SFPQ has a critical role in limiting R-loops formation at telomeres of human cancer cell. In line with this, loss-of-function SFPQ cells show increased levels of RNA:DNA hybrids, replication stress and DNA damage markers and subsequent genomic instability at telomeres level.
Here we show that SFPQ function is not limited to telomeres, but expands to other repeat regions in the human genome to ensure correct chromosome structure and genomic stability to suppress innate immunity pathways.

P.21

Retargeted or attenuated Herpes Simplex Virus 1 genomes as a platform for arming strategies in cancer therapy

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Oncolytic viruses are immunotherapeutic agents that can be engineered to encode payloads of interest within the tumor microenvironment, thus enhancing therapeutic efficacy. *Herpes simplex* Virus (HSV-1) is widely used as oncolytic because of its safety, availability to host exogenous genes, and easy manipulation. It can be attenuated to target selectively tumor cells through the elimination of the 34.5 gene involved in HSV-1 virulence. Furthermore, engineering of viral glycoproteins allows to detarget HSV-1 from natural receptors and to retarget it to tumor-associated antigens. Ex-vivo and in vitro analysis of the tumors allowed us to observe the remodeling that followed treatments with the oncolytic viruses where secretion of immunogenic cell death mediators, infiltration with T lymphocytes and general inflammation occur. Applying system biology to oncovirotherapy, we identified those pathways involved in tumor immune evasion mechanisms and that were amenable to targeting by payloads. We selected STING, ICOSL, and ADA as three promising payloads for cancer treatment. STING expression leads to immunogenic cell death with activation of systemic antitumor immunity; ICOS Ligand allows the triggering of ICOS co-stimulatory signal in tumor infiltrating lymphocytes, increasing cytotoxicity of antitumoral T cells. On the other hand, the ADA enzyme naturally catabolize the immunosuppressive adenosine into the corresponding inosine derivative, devoid of the immunosuppressive function. HSV-1 genomes, attenuated or retargeted towards HER2, were used as an arming platform to generate three armed oncolytic viruses with ICOSL, STING, and ADA. The preliminary evidence for their suitability as oncolytic vectors will be discussed.

Detecting C-to-U RNA editing by direct RNA sequencing

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In mammals, the hydrolytic deamination of Cytosine (C) in Uracil (U) by the APOBEC enzymes family has important biological functions. Nowadays, genome-wide detection of C-to-U editing events by Illumina RNA-seq is not straightforward because these events occur at a very low frequency in eukaryotic organisms. Oxford Nanopore sequencing Technology (ONT) offers the unique opportunity to detect Us due to the deamination of Cs through the direct RNA sequencing. However, we found a systematic base calling noise that prevented calling the deaminated Cs. To unveil reliable C-to-U editing events, we have developed a novel machine-learning strategy based on the isolation Forest (iForest) algorithm in which C-to-U changes are treated as sequencing anomalies and discriminated from error noise. Basecalling features were extracted from ONT reads of *in-vitro* transcribed synthetics constructs and APOBEC1-knock-down murine cell lines and used to train the iForest model. In parallel, REDItools was used on Illumina data to retrieve a “ground-truth” list of C-U editing sites. Applying our model to independent synthetics data as well as to ONT reads from APOBEC1 overexpressing HEK293T cells, we found that our iForest-based approach strongly improved the signal-to-noise ratio improving the detection of C-to-U editing sites, with accuracy above 90% in all tested samples. Our results, therefore, support the anomaly detection iForest algorithm as a promising tool to denoise direct-RNA reads and improve the detection of other RNA modifications.

P.23

DUX4-r exerts a neomorphic activity that depends on a novel co-factor in acute lymphoblastic leukemia

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B-cell acute lymphoblastic leukemia (B-ALL) is the most common pediatric malignancy and the most frequent cause of death from cancer at young age. B-ALL is a highly heterogeneous disease driven by specific chromosomal alterations giving rise to multiple disease subtypes. Therefore, the characterization of the leukemic pathways associated to specific B-ALL drivers could allow the discovery of new therapeutic vulnerabilities in a personalized medicine perspective.
Translocations producing rearranged versions of the transcription factor DUX4 (DUX4-r) are one of the most frequent B-ALL causes. DUX4-r retains the DNA binding domain of wt DUX4, but is truncated on the C-terminal transcription activation domain. The mechanism through which DUX4-r causes leukemia is unknown, and no targeted therapy is currently available. By combining transcriptomics, genomics, and proteomics with functional assays in cellular and animal models of leukemia, our results elucidate the molecular mechanism of B-ALL caused by DUX4-r and identify a possible therapeutic approach for the disease.
We found that the rearrangement leads to both a loss and a gain of function in DUX4-r. Loss of CBP/EP300 transcriptional co-activators interaction leads to inability to bind and activate repressed chromatin. Concurrently, gain of interaction with a novel transcriptional co-activator redirects DUX4-r toward leukemogenic targets. Importantly, this neomorphic activity exposes an Achilles' heel whereby DUX4-r positive leukemia cells are exquisitely sensitive to genetic or epigenetic drug targeting of the co-activator, which inhibits DUX4-r leukemogenic activity.
Our work elucidates the molecular mechanism through which DUX4-r drives leukemia and identify the first epidrug tailored to this B-ALL subtype.

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Meta-analysis of senescent cell secretomes to identify common and specific features of the different senescent phenotypes: a tool for developing new senotherapeutics

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Genotoxic injury can damage DNA and this occurrence may trigger the cellular senescence. Onset of senescent phenotype is associated with changes in cellular metabolism, lysosomal activity and secretion of factors, collectively indicated as senescence-associated secretory phenotype (SASP). Senescence may have beneficial effects on our organisms, such as anti-cancer properties, wound healing, contribution to tissue development. These effects are due to SASP produced by senescent cells not into their final stage. On the other side, senescence may promote cancer and aging. These occurrences are mainly due to pro-inflammatory SASP activity.
The study of senescence is not trivial, since it relies upon several factors. Genotoxic stimuli induce random damage to cellular macromolecules and hence, in spite of a common executive program, the senescent phenotype may vary cell by cell. Another puzzling issue must be added to the above complex phenomenon: senescence is a dynamic process and cannot be analyzed as a static endpoint.
The study of SASP is particularly intriguing since through it, a senescence process triggered in a few cells can spread to many other cells and produce either beneficial or negative consequences for health. We performed a meta-analysis on the protein SASP content obtained by studies of different research groups, including our team. The collected omic data were grouped according: i) cell type; ii) noxious agent; iii) senescence stage (early and late senescence).
Gene Ontology and Network analysis of the omic data allowed us to identify common and specific features of the different senescent phenotypes. This study could pave the way to development of new senotherapeutics drugs to fight negative consequences of senescence process.

The pancancer overexpressed *NFYC* antisense 1 controls cell cycle mitotic progression through *in cis* and *in trans* modes of action

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Antisense RNAs (asRNAs) represent a new layer of gene expression regulation, generally acting *in cis* on sense genes due to sequence complementarity or by their act of transcription. Besides this, evidence is accumulating regarding the existence of *in trans*-acting asRNAs, which leave their site of transcription to act on different molecular targets, both in the nucleus and in the cytoplasm. In this context, we studied *NFYC Antisense 1* (*NFYC-AS1*), an asRNA transcribed head-to-head to *NFYC*, the subunit C of the trimeric proliferation-associated NF-Y transcription factor. We showed that *NFYC-AS1*, but not its sense gene *NFYC,* is overexpressed pancancer with preferential association with *RB1* mutation. *NFYC-AS1* transcript characterization revealed that it is a nuclear asRNA, with a dominant long isoform, the expression of which peaks early in the cell cycle. Knockdown by gapmer antisense oligonucleotides in lung squamous cell carcinoma and small cell lung cancer cells impairs cell growth, a phenotype recapitulated by CRISPR/Cas9-deletion of *NFYC-AS1* transcription start site. Surprisingly, expression of the sense gene is affected only when endogenous transcription of *NFYC-AS1* is manipulated, suggesting that regulation of cell proliferation is at least in part independent of the *in cis* transcription-mediated effect on *NFYC* and is possibly mediated by RNA-dependent *in trans* effects, which converge on the regulation of G2/M cell cycle phase genes. Accordingly, *NFYC-AS1* depleted cells are stuck in mitosis, suggesting defects in mitotic progression.
Overall, we report *NFYC-AS1* as a cell cycle regulating lncRNA with dual mode of action, endowed with potential as therapeutic target in different cancer types, including the very aggressive *RB1*-mutated tumors.

Role of quercetin in the NAFLD treatment: reduction of lipid accumulation and inhibition of de novo fatty acid synthesis

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In the assessment of non-alcoholic fatty liver disease (NAFLD) the dysregulation of the *de novo* lipogenesis (DNL) is deeply involved. NAFLD is frequently associated with dyslipidemia and type 2 diabetes, so there could be a link between high hematic free fatty acids and glucose excess in the DNL dysregulation. Here we used a cellular model of NAFLD in which elevated levels of glucose and FFA caused high cellular lipid accumulation. This condition led to an increased expression of mitochondrial citrate carrier (CiC), cytosolic acetyl-CoA carboxylase 1 (ACACA), and diacylglycerol acyltransferase 2 (DGAT2), involved in fatty acids and triglycerides synthesis. The transcription factors connected to the DNL activation were XBP-1, an endoplasmic reticulum stress marker, and sterol regulatory element binding protein-1 (SREBP-1). Quercetin (Que) is a flavonoid with antioxidant properties that downregulates the lipid accumulation and the expression of SREBP-1 and XBP-1 in steatotic cells, through the phosphorylation of ACACA. The high level of ACACA phosphorylation in Que-treated cells is caused by the increased AMPK activity together with the reduction of of PP2A phosphatase enzymatic activity. Our study shows a direct anti-lipogenic effect of Que, which acts on ACACA/AMPK/PP2A axis, leading to the inhibition of the DNL pathway. This flavonoid, in conclusion, could be a useful ally for the NAFLD treatment.

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Mining common genetic variants impacting on allele-specific translation and cancer risk

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Single nucleotide polymorphisms (SNPs) can contribute to inter-individual variations in disease phenotypes, including cancer. Despite many studies exploring the effect of SNPs on transcription regulation, relatively few have looked into their impact on mRNA translation. We aim to identify SNPs that regulate protein expression and their impact on mRNA translation, which could provide insights into the relationship between genomic variations and disease risk, particularly cancer.
We developed a new approach to identify SNPs that regulate protein expression by performing RNA-seq on total and polysome bound mRNA fractions followed by computing delta Allelic Fraction between the two compartments. The computational approach led to the identification of a list of candidate SNPs, located in UTRs or coding regions and associated with allele-specific translation, named *TranSNPs*.
We experimentally validated some candidates, by performing Sanger sequencing and AMPLI-seq on freshly prepared samples. We performed cloning and gene reporter to further validate the functional distinction between two alleles and determine if the TranSNP alone was sufficient to affect translation efficiency. Moreover, we developed CRISPR/Cas9- mediated models to study the effect of the TranSNPs and better characterize mechanisms and study their outcomes without the need for single nucleotide resolution technics. We are also performing SNP phasing and allele-specific proteomics to gather further mechanistic insights. We are also working on producing ex-novo data using a non-cancer cell line.

Assessing gut microbiome health using integrative omics data analysis with machine learning predictions

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The human gut microbiome plays a crucial role in maintaining physiological homeostasis, and its dysbiosis is associated with several pathologies. Metagenome-wide association studies have been used to explore microbiome-host interactions within a disease context. However, challenges in microbiome research include inconsistent findings, limited accuracy, and lack of validation. With the aims to develop a Machine Learning supervised model to predict microbiome status by using publicly available and ad hoc generated metagenomic data and related metadata through standardized bioinformatic approaches. Here, I will present the results regarding the data selection. This currently includes 6,737 stool sample data obtained from MGnify, those available in literatures (Gupta et al.2049, contain 4347 samples), and any additional metagenomic data ad hoc generated. The dataset is currently being finalized, with ongoing literature searches and updates to include data from 2020 to 2023. However, it should be noted that the availability of
data published this year is limited in the current databases and literature. Taxonomic classification will be performed by assigning taxonomic labels using both Kraken2 and MetaPhlAn. A combination of feature selection and ML approaches will be employed to perform classification tasks. Evaluation metrics such as overall accuracy, precision, recall, F1, and area under the curve will be utilized to assess classification performance. Furthermore, the performance of the classifier will be compared with established indices such as the GMHI and metaml to validate the classifier's efficacy. The results of this study have the potential to improve our understanding of microbiome-host interactions and contribute to the development of new diagnostic tools for disease.

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Development of a gut microbiome eubiosis/dysbiosis index based on DNA metabarcoding data by using Machine Learning approach

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The human microbiome, particularly that hosted in the gut, is a complex ecosystem of trillions of microorganisms that plays a critical role in maintaining human health. The physiological balance between the gut microbiome and the host is characterized by high diversity (eubiosis), while its perturbation (dysbiosis) is associated with reduced diversity and a pro-inflammatory microenvironment. Metagenomics, particularly DNA-metabarcoding (also known as amplicon-based metagenomics), provides an optimal compromise in term of reduced sequencing and computational costs, to investigate microbiome composition. However, defining a healthy microbiome is not straightforward. In 2020, Gupta et al. developed the Gut Microbiome Health Index (GMHI) to predict eubiosis/dysbiosis state using Shotgun Metagenomics data achieving an overall accuracy around 69%. This study presents preliminary results based on a machine learning approach using DNA-metabarcoding data. Ten studies comparing amplicon sequencing data in an overall dataset of 560 healthy and 749 diseased subjects respectively representing eubiosis or dysbiosis status were selected. Sequence data were analyzed using a common framework relying on Amplicon Sequence Variant (ASV) inference and taxonomic classification. We initially analyzed the obtained data using the GMHI index and achieved an overall accuracy of 70% at the ASV level. To further improve accuracy, we employed a machine learning framework based on Random Forest and feature selection, achieving an overall accuracy of 76% improving the performance obtained with the GMHI index.
Our study highlights the potential of machine learning techniques in predicting host phenotypes and disease states based on taxonomy-informed feature selection. By integrating ML models with microbiome analysis, it is possible to establish significant associations between microbial communities and host health, leading to personalized treatments.

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Tumor neoantigens shape the immune microenvironment of panNETs: an in silico analysis

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Background: The neoantigen landscape of pancreatic neuroendocrine tumors (panNETs) has never been characterized.
Methods: We explored the neoantigen landscape of 29 samples of primary panNETs already subjected to whole-genome sequencing and matched RNAseq (ICGC) through a sequential in silico approach of i) HLA prediction (Optitype and HLAscan for class I and II HLA aplotype respectively); ii) HLA-neoantigens interaction evaluation (NetMHCpan). Candidate neoepitopes were assumed as highly immunogenic when their predicted IC50 to HLA was <50 nM. Neoepitopes possibly undergoing central tolerance and variants showing a non-sense-mediated decay <0.80 following RNAseq analysis were filtered out. RNAseq data were then deconvoluted through the CIBERSORT computational platform to reveal the composition of the tumor microenvironment.
Results: PanNETs harbor a median of 6±9 and 4±11 highly immunogenic neoepitopes for HLA class I and II molecules respectively. There is no apparent relationship between the neoantigen load and the tumor molecular subtype (MEN1/DAXX/ATRXmt vs MEN1/DAXX/ATRXwt; DAXX/ATRXmt vs DAXX/ATRXwt) or the alternative lengthening of telomeres status. An increased neoantigen burden is positively associated with features of malignancy including tumor grade ($p<0.05$), vascular invasion ($p<0.01$), perineural invasion ($p<0.05$), ENETS stage ($p<0.01$) and extra-pancreatic spread ($p<0.01$). By CIBERSORT algorithm, a significant association between the number of neoantigens presented by HLA class I and II molecules and the degree of infiltration by dendritic cells and T cells was found. PanNETs mutated for MEN1/DAXX/ATRX exhibited a significantly higher intratumor infiltration of dendritic cells (but not T cells) as compared with their wild-type counterparts.
Conclusions: PanNETs are characterized by a limited neoantigen load. Tumors harboring an elevated number of neoantigens show features of malignancy. PanNETs with a high neoantigen load may recruit and prime T cells.

The interaction between TBX1 and VEGFR3 in cardiac development

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Background/Introduction. Tbx1 is the major gene involved in 22q11.2 deletion syndrome (22q11.2DS), the most common known genetic cause of congenital heart disease (CHD). The clinical phenotype is well recapitulated in *Tbx1* mouse mutants, including cardiovascular abnormalities affecting the aortic arch, ventricular septum and cardiac outflow tract (OFT). Recent studies reported that in humans, rare variants of the VEGFR3 gene, cause cardiac OFT abnormalities, including Tetralogy of Fallot, the most common CHD found in 22q11.2DS patients, suggesting a previously unknown role for the gene in early cardiac development. In mice, *Vegfr3* is regulated by Tbx1 in endothelial cells and we have shown that the two genes interact strongly in brain vascularization and during cardiac lymphangiogenesis.
Purpose. We hypothesize that Tbx1 and *Vegfr3* is required for OFT development in mice. To test this, we inactivated *Vegfr3* conditionally using the $Tbx1^{Cre}$ and Mef2C-AHF-Cre drivers. *We* then analyzed the hearts of conditional *Vegfr3* homozygous $Tbx1^{Cre/+}$; $Vegfr3^{flox}/^{flox}$ and Mef2C-AHF-Cre; $Vegfr3^{flox}/^{flox}$ embryos at E18.5.
Results and Conclusions. *Tbx1*-driven inactivation of *Vegfr3* caused a variety of intracardiac anomalies, including morphogenesis defects of the OFT in $Tbx1^{Cre/+}$;$Vegfr3^{flox/flox}$ embryos, while homozygous inactivation of *Vegfr3* with Mef2C-AHF-Cre had no effect on heart development, suggesting that *Tbx1* haploinsufficiency might contribute to the cardiovascular phenotype in *Tbx1* conditional *Vegfr3* homozygotes. Our preliminary results suggest that Tbx1-*Vegfr3* interaction is required in OFT development. Future studies using time- and endothelial-specific gene mutations will define the critical tissue domain were Vegfr3 is required for OFT development.

Detecting A-to-I RNA editing by Convolutional Neural Networks

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In mammals, the main non-transient RNA modification involves the conversion of Adenosine (A) into Inosine (I), referred to as A-to-I RNA editing, carried out by ADAR enzymes. To identify genuine A-to-I RNA editing sites, we have developed a novel Convolutional Neural Networks (CNN) algorithm, in which sequencing data have been processed and treated like audio data, according to highly performing neural networks architectures successfully used in the field of speech synthesis. Known RNA editing events as well as RNA variants not due to ADAR enzymes have been obtained from REDItools tables used to populate the specialized REDIportal database. For each site, we extracted an interval of 101 bases, centered at the putative editing position, and collected the corresponding RNAseq and WGS/WES (if any) read frequencies. After the encoding and several preprocessing standardization steps, we trained 30 CNNs, one for each human body site stored in the REDIportal database. An additional model based on all REDIportal human tissues was also generated and trained. A tenfold cross-validation was performed to assess the specificity, sensitivity, precision, F1 score and accuracy of each model. Depending on the number of available RNAseq data per tissue, we obtained an overall accuracy ranging from about 95% to 99%. We further tested our new CNN machine learning architecture in ten brain datasets with a growing percentage of known RNA editing events (from 20% to 100%) and found a mean detection accuracy of 0.99. Next, we applied our classifier trained on all tissues to RNA sites from a wild type and ADAR knockout human kidney cell line and reached an average accuracy of about 0.98. Our preliminary results clearly prove the predicting power of our machine learning approach for unveiling transcriptome-wide A-to-I editing events.

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Analysis of human VDAC pseudogenes highlights an important role of VDAC1P8 pseudogene in acute myeloid leukemia (AML)

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Voltage-dependent anion selective channels (VDACs) are the most abundant mitochondrial outer membrane proteins, encoded in mammals by three genes, *VDAC1*, *2* and *3*, mostly ubiquitously expressed [1]. As 'mitochondrial gatekeepers', *VDACs* control organelle and cell metabolism and are involved in many diseases [2]. Despite the presence of numerous *VDAC* pseudogenes in the human genome, their significance and possible role in VDAC protein expression has not yet been considered.
In this work, we investigated the relevance of processed pseudogenes of human *VDAC* genes, both in physiological and in pathological contexts. Using high-throughput tools and querying many genomic and transcriptomic databases, we show that some *VDAC* pseudogenes are transcribed in specific tissues and pathological contexts. Experimental data obtained in different AML cell lines confirm the association of the *VDAC1P8* pseudogene with acute myeloid leukemia (AML).
Overall, our *in-silico* comparative analysis between the *VDAC1* gene and its *VDAC1P8* pseudogene, together with experimental data produced in AML cellular models, indicate a specific over-expression of the *VDAC1P8* pseudogene in AML, correlated with a downregulation of the parental *VDAC1* gene [3].

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The Sp1-miR-27a-ZBTB10 axis mediates the molecular effects of quercetin in colorectal cancer cells

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Quercetin (Q) belongs to the flavonol subclass of flavonoids, a family of compounds ubiquitously found in plants, plant food sources and in the human diet**.** Q inhibits cell proliferation and angiogenesis while induces apoptosis in a variety of cancer cells; however, the molecular mechanism of action is not fully elucidated. The interplay with some miRNAs has been reported, specifically with miR-27a, an onco-miRNA overexpressed also in colorectal cancer.
In this study, we provide evidence that Q reduces cell viability and induces apoptosis in HCT116 and HT29, colon cancer derived cells, by inducing PARP cleavage and upregulating negative effectors of proliferation pathways as SPROUTY2, PTEN and SFRP1. These are targets of miR-27a, so we checked its expression and found it was reduced by Q. Interestingly, also miR-23a and miR-24-2, the other two components of the gene cluster to which miR-27a belongs, and the pri-miRNA transcript were reduced, suggesting a transcriptional control of the entire cluster. Analysis of the promoter region identified GC-rich elements, binding sites for Sp1. We report that Q reduces both Sp1 protein and mRNA implying a role as a crucial transcription factor for miR-27a but also for its own gene expression.
We identify a regulatory circuit that involves Sp1-miR-27a and ZBTB10, a Sp1 competitor for DNA binding, another miR-27a target that increases following Q treatment. Sp1 is further reduced as it interacts with Q in the nucleus and directed to the proteasome for degradation. Finally, Sp1 knockdown impairs transcription of the miR-27a cluster and upregulates the targets, phenocopying the results obtained by Q. In conclusion, Q exerts its effects through the Sp1-miR-27a-ZBTB10 axis that appears to be its molecular mediator, opening the way for novel anticancer therapies based on the association of therapeutic regimens with Q as neoadjuvant.

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Analysis of the role of the nuclear lamina in genomic stability

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The importance of genomic stability in preventing the development of human diseases, such as cancer and neurodegeneration, has received increasing attention in recent years. Lamina is one of the major nuclear scaffolding proteins critical for maintaining the shape and architecture of the nucleus. Moreover, lamina can also act as a chromatin binding site. In addition, lamina is involved in essential nuclear functions. It plays a significant role in influencing the DNA repair pathway, as demonstrated by the accumulation of DNA damage in laminopathies caused by mutations in LMN genes. This role could, therefore, influence therapy-induced responses and acquired resistance in cancer. To study how lamina modulates the response to DSB damage, we have generated an MCF10A cell line expressing a fusion protein consisting of the restriction enzyme AsiSI and a modified estrogen receptor hormone domain. Exposure of the cells to 4-hydroxytamoxifen results in nuclear accumulation of the AsiSI-ER protein and rapid induction of approximately 150 sequence-specific DSBs throughout the genome. In this cell line, we have generated a knock-out of LMNA gene and a control cell line. Thanks to the possibility of generating damage in a site-specific way, this cellular system allows us to study and compare the recruitment of DNA repair factors at specific DSBs using ChIP-based approaches on two lines, with and without lamina.

miR-214 mediated stroma-tumor cell crosstalk during tumor progression

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Cancer and stroma cells continuously interact during tumor progression and influence each other. Secreted microRNAs (miRNAs) have recently been implicated in the tumor-stroma crosstalk. Here, we show that miR-214 is highly expressed in stromal cells and that it correlates with stromal signatures in human breast cancers and melanomas. Upon tumor cell signals, stroma miR-214 is released *via* Extracellular Vesicles (EVs) and is instrumental for cancer cells to promote metastasis formation through the activation of a pro-metastatic pathway which involves the protein-coding genes TFAP2C, ITGA5 and ALCAM and the anti-metastatic small non-coding RNA, miR-148b. Moreover, we also observed a metabolic rewiring linked to stromal miR-214. In fact, glycolysis enhancement and Oxidative Phosphorylation (OXPHOS) impairment were observed in tumor cells following increased miR-214 expression in stroma cells. Our results underline the relevance of "stroma miR-214" for tumor dissemination and metastasis formation and suggest the possibility of a double-edge therapeutic approach based on the targeting of miR-214 not only in tumor cells but also in the stroma counterparts.

Integration of transcriptomic data to dissect the role of long non coding RNAs in amyotrophic lateral sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder characterized by the progressive loss of motor neurons in the brain and spinal cord. It is associated with protein aggregation and cellular stress,as well as inflammation and oxidative damage, ultimately leading to the death of motor neurons and muscle weakness[1].Understanding the cellular and molecular mechanisms underlying ALS is critical for the development of effective treatments for this devastating disorder.
To make a comprehensive characterization of ALS at molecular level,we integrated publicly available human-derived bulk[2] and single-cell RNAseq[3] data from ALS and normal patients.We identified several excitatory neuron subpopulations characterized by distinct molecular features that make them functionally different from their normal counterparts.A particular attention has been given to long non-coding RNAs (lncRNAs).In our lab, we previously identified several lncRNAs that are enriched in the nucleus of myogenic cells[4].Some of the lncRNAs found in the analysis revealed to be biomarkers of distinct neuronal subpopulations,and are uniquely deregulated in ALS patients,hence representing new potential therapeutic targets that worth further attention.
We have started identifying lncRNAs that might play a role in regulation of gene expression of excitatory motor-neuron in ALS patients.We will also try to validate the results on cell lines carrying the different ALS genetic background by RNA-mediated knock down and co-cultures of iPS-derived neurons with myoblasts.This project is part of PNRR CN “National Center for Gene Therapy and Drugs based on RNA Technology CN3 Sviluppo di terapia genica e farmaci con tecnologia a RNA” Spoke 3,CUP B83C22002870006.

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FGFR3-G375C associated with achondroplasia shows a novel kinase active isoform of 50 kDa

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Fibroblast Growth Factor Receptor 3 (FGFR3) is a tyrosine kinase receptor acting as a negative regulator of bone growth. Binding to FGFs enables FGFR3 dimerization and activation of the MAPK/ERK1/2 pathway. In this study, we present data on the rare FGFR3-G375C mutant causing achondroplasia (ACH) in comparison with FGFR3-G380R mutant, more frequently associated with ACH (incidence 1:25000). A third mutant, FGFR3-R248C, causing the lethal disease Thanatophoric Dysplasia Type I (TDI) was also considered. The project was carried out working with the corresponding murine mutations. The experimental approach was based on: 1) introduction of point mutations by site-directed mutagenesis in pRcCMV vector containing a cDNA encoding wild-type (WT) FGFR3; 2) liposome-mediated transfections in HEK293 cells of WT and mutant FGFR3 molecules; 3) Western Blot assays to analyse protein profiles of the different receptors and activation of downstream signalling pathways. By using specific glycosidases, we found that none of the mutations changed the glycosylation profile of 120 and 130 kDa FGFR3 isoforms, indicating a correct receptor biosynthesis toward the cell surface. Nevertheless, the G375C mutant appeared to express an additional FGFR3 isoform of about 50 kDa, containing the entire kinase domain and still anchored to the cell membrane, as observed by subcellular fractionation. Interestingly, abolishing receptor kinase activity by introducing a second mutation, K508A (kinase-dead mutation), resulted in the disappearance of the 50 kDa isoform, suggesting a role played by FGFR3-G375C kinase activity in the generation of the 50 kDa isoform. In agreement with the latter observation, FGFR3-G375C mutant caused constitutive activation of the ERK1/2 pathway. Ongoing experiments aim to clarify whether the 50 kDa FGFR3-G375C isoform may interfere with receptor activation or delayed downregulation.

A feature-based meta-analysis approach to unravel the pathophysiological landscape of autoimmune diseases

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Autoimmune diseases (ADs) are complex conditions and since many of the aspects of their etiology are still unclear, nowadays ADs represent a field of dynamic research, and an ever-greater number of studies are conducted to uncover their unknown hallmarks. This project aims to characterize the molecular and cellular landscape of 11 ADs, to identify similarities and differences between the different pathologies that are related to their etiology and clinical presentation. To this end we integrated a large and heterogeneous amount of publicly available gene expression data using a meta-analysis framework. We downloaded all the available bulk gene expression case-control studies from Gene Expression Omnibus for a set of 11 ADs. In order to derive more interpretable features from the raw gene expression data we also adopted three different featurization strategies: 1) *cell-mixture deconvolution*: inferring cell proportions estimates from bulk data, employing *immunoStates* basis matrix; 2) inference of *pathways activity scores* using the *GSVA* R package; 3) *Transcription factor master regulator analysis* using *corto*. When expression data is used to infer the proportion of immune cell types, juvenile idiopathic arthritis clusters separately from other diseases due to lower abundance of NK cells in blood, an observation on which conflicting reports exist in the literature. Crohn’s disease, ulcerative colitis, and idiopathic pulmonary fibrosis (IPF) cluster together based on the inferred abundance of different cell types, as they did when analyzing raw gene expression data. A decreased abundance of B cells in blood defines this cluster, a well-known characteristic of inflammatory bowel diseases. Our analysis identifies lowered amounts of B cells in the blood as a defining characteristic of IPF patients. Conversely, meta-analysis of IPF lung tissue samples showed increased levels of B cells, suggesting that infiltration of B cells may contribute to the pathogenesis of the disease.

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Modulation of miR 146b-5p expression during the aging

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The finding of molecules associated with aging is important for prevention of chronic degenerative diseases and for longevity strategies. MicroRNAs (miRNAs) are post-transcriptional regulators involved in many biological processes and miR 146b-5p has been demonstrated to be involved in different degenerative diseases. However, miR146b-5p modulation has not been evaluated on the mesenchymal stromal cells (MSCs) commitment and during the aging.
Therefore, with the aim of exploring the miR 146b-5p modulation, circulating miR146b-5p has been evaluated in human male and female subjects as well as in human females at different age ranges. To deeply evaluate the role of miR 146b, we analyzed its expression in the commitment and differentiation of mesenchymal cells. In zebrafish model, miR 146b-5p together with osteogenic and chondrogenic gene expression and age-related features levels have been analyzed as well.
Our findings show the miR 146b-5p expression is higher in females compared to males and it is associated with aging in humans. In addition, miR 146b-5p mimic drives MSCs to adipogenic differentiation and its expression increases during zebrafish maturation and aging.
In conclusion, miR 146b-5p can be considered an age-related marker and can represent a useful target for identifying strategies aimed at counteracting the degenerative processes of aging.

P.41

8-oxodG and G4: deciphering the crosstalk between DNA damage and transcription regulation

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Reactive oxygen species can cause the oxidation of guanine to 8-oxo-7,8-dihydroguanine (8-oxodG), a common DNA base lesion. 8-oxodG is repaired by the base excision repair (BER) pathway, but incomplete repair can lead to mutagenesis and genome instability. 8-oxodG is involved in the epigenetic control of gene expression through interactions with G-quadruplex (G4) structures. Putative G4 forming sequences (PQS) are prone to 8-oxodG formation. Therefore, 8-oxodG, and the associate BER process, control the stability of the G4 structure, thus affecting gene expression. BER proteins can be targeted pharmacologically and/or genetically to manipulate the levels of 8-oxodG and G4. A normal and a cancerous BER-deficient human mammary epithelial cell line has been established in our lab. Immunofluorescence assay highlighted an increased number of y-H2AX foci in BER protein-deficient cells. These data suggest that BER deficiency results in the accumulation of endogenous DNA damage and in the formation of double-strand breaks (DSBs). This may promote genome instability. However, further experiments are needed to confirm these results.
Finally, BER-deficient cell lines will be useful in future to investigate the crosstalk between 8-oxodG and G4 and identify transcriptional alterations in human diseases such as cancer.

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Alternative splicing and cancer epigenetic: BCLAF1 role in colorectal cancer

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Bcl-2-associated transcription factor 1 (BCLAF1) is known to influence several cellular processes, including cell development, differentiation, and immune function regulation.Previous studies highlighted a distinct splicing isoform of BCLAF1 in CRC and indicated SRSF10 as a key regulator of BCLAF1 splicing isoforms.
Our aim is to get the comprehensive vision over the importance of BCLAF1 in CRC the molecular and characterization of BCLAF1 full length and exone5 truncated isoforms in two CRC modals and furthermore, we investigate the epigenetic regulation of epi-drugs on the BCLAF1 alternative splicing machinery.
We employed techniques such as Quantitative real-time PCR and Gel electrophoresis of PCR products, as well as bioinformatic analysis of meta-data to gain a comprehensive understanding of BCLAF1 in CRC.
Our results confirmed the overexpression of BCLAF1 in CRC cell lines and primary samples compared to healthy controls. We found that the mRNA expression of BCLAF1 in CRC could be downregulated by SAHA, particularly affecting the full-length isoform and increasing the exone5 truncated isoform of BCLAF1. Our evidence reveals that in our methylation deficient system, DKO, SRSF10 was not affected by the drug. We hypothesized that BCLAF1 gene expression can be regulated by other molecular regulatory mechanisms instead of SRSF10 in the absence of methyltransferases.
Our findings propose a novel link between alternative splicing and cancer epigenetics in CRC, suggesting the need for further investigation into BCLAF1 and its regulatory mechanisms.

A multi-omics approach to shed light on the pathogenetic mechanisms of Marinesco-Sjogren's syndrome

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Marinesco-Sjögren syndrome (MSS) is an autosomal recessive neuromuscular disorder that arises in early childhood and is characterized by congenital cataracts, myopathy associated with muscle weakness, and degeneration of Purkinje neurons leading to ataxia. In addition to Purkinje neurons and skeletal muscle cells, several other cells show pathological markers that, however, do not translate into overt clinical phenotypes. About 60% of MSS patients have loss-of-function mutations in the SIL1 gene. Sil1 is an endoplasmic reticulum (ER) protein required for the release of ADP from the chaperone Bip, which in turn will release the folded proteins. The expression of non-functional Sil1 leads to the accumulation of unfolded proteins in the ER and this triggers the unfolded protein response (UPR), which consists of a complex signalling and transcription programme aimed at restoring ER homeostasis. Indeed, a dysfunctional UPR could be a key element in the pathogenesis of MSS. To shed light on the pathogenesis of MSS, a multi-omics approach was implemented in the present study. Proteomic and transcriptomic analysis, performed on patient-derived fibroblasts, showed alterations in the splicing machinery and a downregulation of ribosomal proteins, as well as a reorganisation of several metabolic pathways. Specifically, these fibroblasts showed an activation of lipid and amino acid catabolism and reduced levels of enzymes involved in the synthesis of arginine, serine, glycine and cysteine. Furthermore, changes in the cytoskeleton and a reorganisation of the extracellular matrix were observed. These altered molecular pathways could help explain the compensatory mechanisms put in place by MSS fibroblasts to escape death.

FRG2A is part of a novel family of lncRNAs affecting nucleolus function in FSHD cells

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Facioscapulohumeral muscular dystrophy (FSHD), a common hereditary myopathy has been associated with reduced copy number of the D4Z4 macrosatellite at chromosome 4q35. Deletion of D4Z4 repeats leads to the inappropriate expression of 4q35 genes resulting in disease. Among these, *FRG2A* is selectively overexpressed in FSHD muscles and is stabilized upon genotoxic noxa with an extent which inversely correlates with the number of D4Z4 repeats. We found that *FRG2A* is not a protein coding gene but a heterochromatin-associated lncRNA. We identified thousands of *FRG2A* target sites in the human genome. We also defined a large network of interacting proteins, including factors involved in nucleolus function and ribosomal biogenesis. We showed that *FRG2A* transcripts are specifically associated with rDNA and localise within the nucleolus in the compartment responsible for rDNA transcription and early processing of pre-rRNA. Both intergenic spacer sequences and rDNA promoters were enriched in heterochromatin-associated histone marks in FSHD cells in respect to controls, suggesting that FRG2A increased expression might alter the expression of rDNAs. Consistently, FSHD samples show significant impairment in pre-rDNA transcription and reduction in protein synthesis which are both reverted upon *FRG2A* silencing. Interestingly, there are 14 *FRG2* paralogs interspersed within the human genome annotated as coding genes (n=4) or as pseudogenes (n=10) that we found differentially expressed in muscle cells in a subject-specific manner. Based upon these results, we propose that *FRG2A* belongs to a family of lncRNAs that function as epigenomic modulators impacting on 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.

Dissecting DNA methylation patterns at single-molecule level through next-generation sequencing using *EpiStatProfileR*

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DNA methylation is a well-studied epigenetic modification that plays a pivotal role in major biological mechanisms, such as gene regulation, genomic imprinting and genome stability. Different combinations of methylated/unmethylated cytosines for a given DNA segment (referred to as *epialleles*) may generate epigenetic heterogeneity, which magnitude has also been reported as a contributor of intratumor variability and predictor of disease outcome. Traditionally, computational methods and statistical tests relevant to DNA methylation analysis have been based on average methylation levels of CpG sites.
Most recent tools are attempting to extract the critical information of epigenetic heterogeneity from available bulk bisulfite sequencing data profiling CpG methylation states at single read level.
We have recently developed a novel R package named EpiStatProfileR, which is capable of analyzing CpG and non-CpG based epialleles by applying a collection of dedicated statistical functions. EpiStatProfileR has additional features, such as customizable user-driven analysis, strand-specific heterogeneity assessment, locus annotation and gene set enrichment analysis.
Lately, third-generation sequencing techniques, such as Oxford Nanopore Technology (ONT), are emerging as valuable strategies to profile large-scale DNA methylation on the same DNA molecule. Moreover, compared to bisulfite sequencing techniques, the nanopore technology overcomes some technical drawbacks, being able to discriminate between 5mC and 5hmC and to profile genomic regions which cannot be mapped by short reads, such as highly repetitive DNA segments.
Therefore, we propose to extend the same workflow for the extraction and the analysis of DNA methylation patterns from nanopore sequencing experiments, as it is likely to provide new insights into the basis of epigenetic heterogeneity under normal and pathological conditions, foremost cancer.

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CNRBiOmics and ELIXIRxNextGenIT: establishment and enhancement of the Integrative Omics platform of ELIXIR-IT, the Italian Node of the European Research Infrastructure for Life Sciences

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The ELIXIR-IT integrative Omics platform (iOm) has been established and is being enhanced by the Italian Node of ELIXIR - the European Research Infrastructure for Life Science Data - through cumulative funding of around 33 M€ granted by the MUR (CNRBiomics and ELIXIRxNextGenIT).
iOm is equipped with some of the most advanced technologies available on the market with complementary capabilities that make cutting-edge applications possible. Those include second (Illumina) and third-generation (Oxford Nanopore and Pacific Bioscience) high-throughput sequencing platforms supported by devices for automated liquid handling, optical mapping, single-cell molecular characterisation, and proteomics and metabolomics facilities with high-resolution mass spectrometers and chromatographs.
Such a broad range of technologies facilitates comprehensive and multi-perspective molecular fingerprinting of samples. It further empowers researchers to pursue high-resolution characterisation and validation of novel biomarkers for applications spanning diagnostics, prognosis, and therapeutics.
The iOm platform's extensive data output is accommodated by a robust ICT infrastructure with GPUs, over 20,000 CPU cores, and storage capacity exceeding 20 Pbs. This infrastructure provides an optimised environment for data storage, management, analysis, and FAIRification.
The resources offered by the novel Integrative Omics platform complement the existing ELIXIR-IT services portfolio, which encompasses state-of-the-art databases, bioinformatics software and libraries, ICT platforms, and a training programme providing a variety of basic and advanced courses.
Currently, a program that will allow researchers from both public institutions and private companies to access iOm services is in development. Upon completion, this program will be made accessible via a web portal.

Cellular and molecular component of concentrated growth factor induced osteogenic differentiation and improved the osteointegration of dental implants

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Concentrated growth factors (CGF) is an autologous blood-derived biomaterial, the latest generation of platelet derivatives, produced by centrifugation of the whole blood sample. Many studies reported the ability of CGF to induce osteogenic differentiation, indeed it contains growth factors, platelets, white blood cells and stem cells that play an important role in the processes of tissue regeneration and differentiation. During the last decade the need of implant services in edentulous patients was dramatically increased. In this study, the structural and biological characterization of CGF was carried out and its role in the process of osteointegration of CGF-permeated dental implants has been evaluated. The CGF structural characterization was carried out by SEM analysis, whereas the molecular characterization was performed by ELISA to measure growth factors and matrix metalloproteinases (MMPs) release. CGF primary cells were isolated and their osteogenic differentiation was evaluated through matrix mineralization by alizarin red staining and through mRNA quantification of osteogenic differentiation markers by Real-Time PCR. Likewise, the effects of CGF-permeated implants on human BMSC was evaluated. We found that CGF has a complex inner structure capable of influencing the release of growth factors and cells. These cells had the capability to differentiate into osteoblasts. The CGF-permeated implants showed a kinetic of biomolecules release similar to CGF, this could be due to the growth factors released by CGF primary cells. We demostrated the ability of CGF-permeated implant to induce osteogenic differentiation in vitro. Finally, data obtained from surgical interventions showed that CGF-permeated implants improved osseointegration respect to control implants (without CGF). These data highlight new interesting perspectives in the use of CGF in regenerative medicine and, particularly, in the dental implantology field.

Metabolic adaptations driving 5-Fluorouracil resistance in colorectal cancer: PHGDH heterogeneity as a key driver

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Phosphoglycerate dehydrogenase (PHGDH) is the rate-limiting enzyme in the *de novo* serine synthesis pathway (SSP), a highly regulated pathway overexpressed in several cancer types. It has been shown that high PHGDH levels correlates with accelerated proliferation of different tumour cells , while its depletion shows selective toxicity. Recently, it has emerged that PHGDH expression is dynamically regulated during different stages of tumor progression, promoting cancer aggressiveness. In our laboratory, we demonstrated that high serine availability, due to both increased exogenous uptake or activation of SSP, supports resistance of colorectal cancer (CRC) cells to 5-Fluorouracil (5-FU) administration.
The aim of the present study is to investigate whether PHGDH heterogeneity could be a critical determinant of 5-FU response in human CRC.
Here, by means of distinct colon cancer cell lines, we show that high PHGDH expression correlates with reduced 5-FU sensitivity with respect to low expression of the enzyme. Moreover, we highlighted a great inter-tumor heterogeneity (western blot analysis of samples from different CRC patients), as well as a great intra-tumor heterogeneity (immunohistochemical analysis of PHGDH expression in biopsies from CRC patients), suggesting PHGDH as possible determinant of different response to 5-FU treatment.
In agreement, the modulation of PHGDH expression levels, with a specific inhibitor or through protein overexpression, correlates with altered sensitivity to 5-FU treatment in CRC cells.
Finally, we demonstrated that different levels of PHGDH interfere with metabolic intermediates of the Krebs Cycle, particularly succinate and α-keto glutarate, two known epigenetic regulators. Consequently, alteration in the repressive methylation of K27 of histone 3 (H3K27me3) has been detected. Further studies will be focused in clarifying the PHGDH-dependent epigenetic regulation of genes involved in 5-FU resistance.

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RUNX2 promotes metastatic ability of melanoma cells by increasing CXCR4 expression

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RUNX2 overexpression has been reported in breast cancer, pancreatic cancer, prostate cancer, lung cancer, ovarian epithelial cancer and melanoma. RUNX2 can be considered a cancer stemness marker and it appears to be involved in the osteolytic process. Importantly, the bone sialoprotein (IBSP) and osteopontin (SPP1) coding genes, which are regulated by RUNX2, play important roles in metastases derived from osteotropic cancers.
Therefore, to understand the molecular mechanisms underlying the ability of RUNX2 to promote metastasis, we used melanoma cell lines and analysed the association between RUNX2 and the C-X-C Motif Chemokine Receptor 4 (CXCR4), which is part of the human chemokine system and is involved in tumour progression and metastasis. The CXCR4 inhibitor WZ811 was used to evaluate the osteotropism of melanoma cells in microfluidic co/cultures cells.
Our preliminary results show that melanoma cells express both RUNX2 and CXCR4, whereas CXCR4 is downregulated in RUNX2 KO melanoma cells obtained with CRISPR/Cas9 genome editing. Restoration of RUNX2 in RUNX2 KO cells induces re-expression of CXCR4 and other genes related to the metastatic process. Importantly, the CXCR4 inhibitor WZ811, reduces the osteotropism of RUNX2 restored-melanoma cells.
In conclusion, our data suggest that RUNX2 promotes the metastatic capacity of melanoma cells by upregulating CXCR4, which in turn plays a key role in increasing metastatic progression.

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Loss of PML nuclear bodies in familial Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

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TDP-43, FUS and the C9orf72-dipeptide repeat proteins, accumulate in form of cytoplasmic inclusions in Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). Although the origin of TDP-43 and FUS pathological inclusions is still debated, their protein aggregation is considered a key pathogenic event.
The majority of the studies focus on understanding how cells control TDP-43 and FUS aggregation in the cytoplasm, overlooking how dysfunctions in the nucleus may influence the maintenance of protein solubility outside of the nucleus. However, protein quality control (PQC) systems that recognize and deal with aggregation-prone proteins simultaneously operate in the cytoplasm and nucleus and share players, including chaperones and proteasomes. Thus an impairment of the nuclear arm of the PQC may have a negative impact on the cytoplasmic arm of the PQC, contributing to the formation of cytoplasmic pathological inclusions.
Cells transiently store aggregation-prone proteins in specific deposition sites that are formed in the cytoplasm and nucleus, such as Promyelocytic leukemia protein (PML) nuclear bodies (PML-NBs) in the nucleus and stress granules (SGs) in the cytoplasm. PML-NBs compartmentalize misfolded proteins, including defective ribosomal products, and recruit chaperones and proteasomes to promote their clearance. Cytoplasmic SGs sequester aggregation-prone RNA-binding proteins linked to ALS-FTD and mRNAs to attenuate their translation. We show that dysfunction of the nuclear PQC due to PML depletion promotes the accumulation of misfolded proteins in SGs, impairing their disassembly. We also report that PML assembly is impaired in the human brain and spinal cord of familial C9orf72 and FUS ALS-FTD cases. We propose that altered PQC in the nucleus due to PML-NB loss represents a novel pathomechanism in ALS-FTD that can contribute to SG accumulation and cytoplasmic protein aggregation.

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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 ingegnerized 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 dataset correlates IGFL1 expression with immune infiltration level of myeloid dendritic and resting cells and macrophages M0 and pro-inflammatory M1 cells. Preliminary data on in vitro stimulation of M0 macrophage from healthy donors with purified 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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Mechanisms of pathogenicity in OPA1 deficient fibroblasts

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Mutations in the OPA1 gene, encoding a mitochondrial inner membrane protein that plays an essential role in mitochondrial dynamics, are responsible for Autosomal Dominant Optic Atrophy (ADOA; OMIM # 125250), an inherited form of optic atrophy. ADOA cellular and animal models have shown severe mitochondrial alterations including abnormalities of mitochondrial morphology and distribution, defects in the maintenance of mitochondrial DNA and bioenergetics, impaired calcium homeostasis, increased susceptibility to apoptosis, impairment of mitophagy [1, 2]. We studied mitochondrial function, autophagy and senescence through functional and transcriptomic analysis in skin primary fibroblasts from an ADOA patient. We showed that the mammalian target of rapamycin complex (mTORC)/AKT pathway is involved in determining autophagy and senescence. Genetic and pharmacological modulation of the mTOR/AKT pathway corrects several of the phenotypes observed at the cellular level. ADOA fibroblasts confirm to have potential as an easily accessible and maintainable disease model recapitulating at least some of the pathological dysfunction observed in neurons and helpful to deciphering the underlying molecular mechanisms of the disease. We will move on iPSC-derived neuronal cells.

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P.53

Physical activity modulates miRNA expression in serum extracellular vesicles

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The sedentary lifestyle produces various alterations and physical activity represents a useful tool for counteracting the risk of chronic diseases. MiR-146b-5p has been demonstrated to be involved in different degenerative diseases. Extracellular vesicles (EVs) are gaining increased importance in biomedical research and miRs constitute a high percentage of nucleic acids in the EVs cargo. The evaluation of EVs, circulating in the bloodstream constitutes a non-invasive method to investigate several conditions, including the effects of physical activity. Therefore, in this study we aimed: 1) to identify the modulation of miR-146b-5p expression both in the free circulating form and within EVs in the serum of subjects subjected to physical activity; 2) to investigate the effects of sera of subjects performing physical activity in differentiation of mesenchymal stem cells (MSCs) and 3) to evaluate the ability of EVs to be incorporated into MSCs.
Samples from subjects performing physical activity were used. Circulating miRNAs were extracted by using the miRNeasy Serum Advanced Kit and EVs were isolated with the qEVoriginal-70 nm Gen 2 column. EVs were characterized by Nanoparticle tracking analysis. MicroRNAs were extracted and miR146b-5p expression was analyzed by Real time PCR. Preliminary results showed that free circulating miR146b levels increased with the aging, that physical activity is able to downregulate miR-146b-5p expression both in the free circulating form and within EVs and that EVs are incorporated in MSCs.
In conclusions, miR-146b-5p-EVs can be considered a useful marker to evaluate the effects of stimuli during physical activity.

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