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When and where:
temporal and spatial regulation
of biological processes

Abstracts

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Speakers' Abstracts

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

Genome-wide integrative analysis implicates ERBB signaling pathway as a downstream effector of p63 and p73 in skin cancer

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The molecular processes driving the formation and progression of cutaneous squamous cell carcinoma (cSCC) have not been completely defined. Although p53 mutations are known to occur in early stages of skin carcinogenesis, the functional role of the other two members of the p53 family, p63 and p73, in skin cancer biology is still incompletely understood. Combining tissue microarrays and gene expression analyses, we demonstrated that p63 and p73 expression is significantly upregulated already at early stages of cSCC and persists during tumor progression, suggesting that both genes may be required for cSCC formation. The integration of ChIP-seq and RNA-seq data identifies p63 and p73 target signatures and reveals that these two hub genes jointly regulate several genes functionally associated to ERBB signaling pathway. ERBB signaling pathway is commonly hyperactive in human cancers, playing a key role in the regulation of essential biological processes as cell cycle progression, cell survival and cell adhesion, sustaining tumor growth and progression. Accordingly, in vitro and in vivo analyses demonstrated that tumor growth is impaired in the absence of p63 or p73, by inhibition of the ERBB signaling pathway or depletion of specific ERBB ligands. On the other hand, reactivation of ERBB signaling pathway in the absence of p63 or p73 is sufficient to restore tumor cell proliferation. Taken together, our findings provide a rich dataset for investigating additional mediators of p63/p73 driven oncogenesis and implicate ERBB signaling pathway as a downstream effector of p63 and p73 in cSCC, with possible clinical implications.

Non-coding regulation of myogenic chromatin

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Advances in sequencing technologies have revealed that more than half of the transcriptome encodes for several classes of RNA molecules lacking coding capacity. The impact of these species on the control of cell growth, differentiation and development, has been well established, and it is clear that they exert their functions in both nuclear and cytoplasmic compartments (Ballarino *et al*, JCI, 2015). Through RNA-*sequencing of* murine myoblasts and differentiated myotubes, we recently identified novel long non-coding RNAs (lncRNAs) which expression is dynamically ordered during myogenesis (Ballarino *et al*, MCB, 2015). These transcripts were classified on the basis of their expression in proliferating *versus* differentiated conditions, muscle-restricted activation and link with muscle dystrophy. I will describe the characterization of a murine chromatin associated muscle-specific long noncoding RNA, named as *Charme* (for *Ch*romatin *ar*chitect of *m*uscle *e*xpression), which contributes to the robustness of the myogenic programme both *in vitro* and *in vivo*. In myocytes, *Charme* knock-down produced the disassembly of specific chromosomal domains and the down-regulation of myogenic genes therein contained. Notably, several *Charme* -affected genes are associated to human cardiomyopathies, and *Charme* depletion in mice produces a pathologic phenotype consisting in a global alteration of heart architecture. The existence of a homologous human counterpart, which regulates the same subset of genes, suggests an important and evolutionary conserved function for *Charme*. Altogether, our study identifies a novel example of chromatin-associated lncRNA controlling the acquisition of muscle cell identity.

MicroRNAs: gatekeepers of space and time in brain wiring

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Billions of neuronal connections are established during development in vertebrate brains. To create these intricate connections, axons must first navigate a complex environment and find their exact target. Extrinsic chemotropic cues, present in the axonal environment, guide the leading tip of the axon, the growth cone, with exquisite precision. Growth cones can be located hundreds of microns away from the cell body and rely on their local proteome for their directional steering. Newly synthesized proteins are produced at the right time and place in response to cues to ensure highly accurate growth cone steering. However, the nature and mechanisms of action of molecules regulating local protein synthesis (LPS) within axon is largely unknown. I will provide evidence suggesting that axonal microRNAs gate the temporal onset of cue-induced LPS in navigating growth cones and are thereby major players in brain wiring.

HSPB2 forms nuclear compartments that affect lamin A and compromise nuclear function: implications in neuromuscular diseases

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HSPB2 is a small heat shock protein that contains predicted intrinsically disordered regions (IDRs). In cells, IDR-containing proteins drive the assembly of liquid droplets via liquid-liquid demixing. Evidence that HSPBs can phase separate into liquid droplets is lacking. We report that, in cells, HSPB2 phase separates to form nuclear assemblies that behave as liquid droplets. The intrinsically disordered C-terminal domain of HSPB2 is required for phase separation. HSPB2 nuclear droplets sequester lamin A and displace chromatin, with detrimental consequences for nuclear function and integrity. Aberrant phase separation of HSPB2 is negatively regulated by its partner HSPB3. In agreement, HSPB3 depletion in differentiating myoblasts decreases myogenin expression, enhances HSPB2 compartmentalization and leads to nuclear morphological defects. These observations suggest that imbalances of HSPB2-HSPB3 expression and enhanced HSPB2 foci formation may have deleterious consequences on myoblast viability and differentiation.
We also report the identification of two HSPB3 mutations in myopathy patients: A33AfsX50-HSPB3, which is unstable and rapidly degraded and R116P-HSPB3, which can no longer interact with HSPB2. Thus, both HSPB3 mutants lose the ability to control HSPB2 aberrant phase separation. Intriguingly, a muscle biopsy from the patient with the R116P-HSPB3 mutation shows alterations of nuclear morphology with chromatin margination, further suggesting that nuclear defects may be at the heart of HSPB3-linked diseases. In summary, based on our data, we propose that deregulation of HSPB2 compartmentalization, due to decreased HSPB3 expression or HSPB3 mutations, may contribute to muscle aging and disease.

Circadian clocks and seasonality in *Drosophila*

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Diapause is a hormonally-mediated strategy that allows insects to predict and respond to unfavourable environmental conditions by altering their metabolism and behavior to resist the oncoming challenges. In *Drosophila melanogaster*, diapause can be stimulated by reduced temperatures and shorter photoperiods that herald the approach of winter, and lead to reproductive arrest in females. We have genetically manipulated the circadian pacemaker cells, the s-LNvs, which express two neuropeptides, Pigment dispersing factor (PDF) and short Neuropeptide F (sNPF). We observe both reductions and enhancements of gonadal arrest with these interventions, suggesting that PDF and sNPF are diapause antagonists acting in a similar way to the Drosophila insulin-like peptides (dILPs) that are expressed in the insulin producing cells (IPCs). Indeed, we observe that the dendrites from the s-LNvs appear to overlap with those from the IPCs implying that the clock cells signal to the IPCs. We confirm this possible communication by applying the two synthetic peptides to the IPCs and detecting a response in the IPC signal transduction pathway. We conclude that the clock neurons activate the IPCs via PDF and sNPF, which in term release the dILPs, which antagonise diapause and lead to reproductive growth, thereby uncovering a circadian diapause axis.

Transcriptional activation of RagD GTPase primes the response of mTORC1 to nutrients

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The mechanistic Target Of Rapamycin Complex 1 (mTORC1) regulates cellular biosynthetic pathways in response to variations in nutrient availability. mTORC1 is activated by Rheb on the lysosomal surface where it is recruited by nutrient-activated RagGTPases (Rags). Whereas the post-translational control of mTORC1 activity has been extensively studied, little is known about its transcriptional regulation. We found that MiT/TFE transcription factors, master regulators of lysosomal biogenesis and autophagy, are nutrient-sensitive transcriptional activators of mTORC1 signaling. During starvation, they induce the expression of the RagD gene and this enhances mTORC1 recruitment to the lysosome and reactivation when nutrients become available. In vivo, this mechanism mediates adaptation to food availability after starvation and physical exercise and fuels tumor growth. Patient-derived cell lines from melanoma, renal cell carcinoma and pancreatic ductal adenocarcinoma associated with hyper-activation of MiT/TFE factors presented a significant increase in both RagD transcript levels and mTORC1 signaling. Importantly, RagD silencing virtually abolished xenograft tumor growth of melanoma cells in mice, suggesting that RagD is a potent driver of MiT/TFE associated cancers. Thus, we identified a nutrient- dependent transcriptional regulation of mTORC1 that enables cellular adaptation to nutrient availability and may be essential to the energy-demanding metabolism of cancer cells.

Lipid metabolism: connecting the dots

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Lipid metabolism attracts much attention owing to the pleiotropic roles of lipids and their roles in human pathologies. This interest motivates growing efforts dedicated to cataloguing the lipidome. All aspects of lipid function rely on their heterogeneous distribution in living systems and the formation of molecular signatures that define organelle membranes or microdomains. Lipid metabolism – and its associated disorders – needs to be understood in the context of this functional, three-dimensional organization.
We developed systematic approaches for lipid biology, integrating proteomics, lipidomics, microfluidics and bioinformatics. Understanding how cell signaling – and many biological functions – can be modulated by discrete changes in the chemical properties of cellular membranes and how this affects the switching behavior of specific signaling lipids represents our first research interest. Many lipids have signaling functions and form molecular signatures that are read by specialized lipid-binding domains on effector – the so-called (and still elusive) “lipid code”. We discovered that discrete changes in specific lipid concentrations or in the collective properties of all lipids in different membranes affect the functioning and efficiency of downstream signaling. This has broad implications for human health, as 60% of all drug targets reside in biological membranes. Another research interest concerns the study of the mechanisms involved in the creation and maintenance of lipid gradients in eukaryotic cells. An emerging player in these processes is a group of disease-associated proteins known as lipid-transfer proteins (LTPs). They spatially organize lipids and connect lipid metabolic pathways that are distributed across distinct organelles, but our knowledge of these transport mechanisms remains fragmented. I will present a number of new large-scale biochemical methods designed to systematically characterize the pathways of LTP-mediated lipid movement.

PRMT1 regulates microRNA biogenesis through the methylation of the Large Drosha Complex

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MicroRNA (miRNA) biogenesis is tightly controlled and alterations of this process occur in different pathological conditions, including cancer. Hence, dissecting the molecular mechanisms underlying miRNA biogenesis is crucial to understand the causes of aberrant miRNA expression. The Large Drosha Complex (LDC) plays a crucial role in miRNA production, catalyzing the cleavage of the primary miRNAs (pri-miRNAs) into the precursor miRNAs (pre-miRNAs). This step is crucial and rate limiting for the production of miRNAs and occurs in the nucleus. In previous systematic annotation of the human methyl-proteome, we found that the LDC is hyper-methylated. To better investigate the role of protein methylation in the modulation of the LDC and miRNA biogenesis, we characterized in-depth its methylation pattern, through a combination of heavy-methyl SILAC labelling of human cancer cells, immuno- enrichment of the complex, and high resolution MS-analysis. We identified a total of 156 methyl-peptides on the LDC occurring on 15 out of 23 subunits of the LDC with more than 80% of the methylation events present on R-residues which were both mono- (40%) and di-methylated (60%). Moreover, by quantitative methyl-proteomics analysis upon genetic and pharmacological inhibition of PRMT1, we found a strong reduction of the LDC methylation, which correlates with a global decrease of miRNA expression, caused by the specific impairment of the pri-to-pre- miRNA processing step. In particular, RIP experiments carried out with the accessory protein ILF3 in the absence of PRMT1 activity suggest that R-methylation of some LDC subunits may be necessary for the interaction with the pri-miRNA targets.
Overall, this study unravels a mechanism through which mammalian cells modulate miRNA biosynthesis and suggests the possibility, in the future, to modulate LDC methylation via selective PRMT inhibitors in order to limit the aberrant miRNA expression measured in various human diseases such as cancer.

Genetic and epigenetic regulation through the germline

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Mammalian ontogeny is associated with distinct phases of global epigenetic reprogramming – which occur during preimplantation development and in primordial germ cells (PGC) – and comprehensively remodel the epigenome. These events are thought to reset cellular potential for development, and also act as a barrier against the inheritance of epigenetic information between generations. Our studies have investigated the dynamics and mechanisms of epigenome reprogramming in mouse and human PGCs. Whilst erasure of global DNA methylation is comprehensive, we unexpectedly identified extensive genomic regions that escape reprogramming and are consequently transmitted. We have further used unbiased CRISPR screening to understand the regulatory factors that drive PGC development and the associated epigenetic programme. Using this approach, we have traced the genetic determinates that underpin the transitions from naïve pluripotency to germ cell fate. Taken together we have gained insight into both the genetic and epigenetic regulation that underpins mammalian germline development.

A common molecular logic determines pluripotent stem cell self-renewal and reprogramming

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During differentiation and reprogramming new cell identities are generated by reconfiguration of gene regulatory networks. Here we combined automated formal reasoning with experimentation to expose the logic of network activation during induction of naïve pluripotency. We find that a Boolean network architecture defined for maintenance of naïve state embryonic stem cells (ESC) also explains transcription factor behaviour and potency during resetting from primed pluripotency. Computationally identified gene activation trajectories were experimentally substantiated at single cell resolution. Contingency of factor availability explains the counterintuitive observation that Klf2, which is dispensable for ESC maintenance, is required during resetting. We tested 136 predictions formulated by the dynamic network, finding a predictive accuracy of 78.7%. Finally, we show that this network explains and predicts experimental observations of somatic cell reprogramming. We conclude that a common deterministic program of gene regulation is sufficient to govern maintenance and induction of naïve pluripotency. The tools exemplified here could be broadly applied to delineate dynamic networks underlying cell fate transitions.

In situ structural biology and the NPC - a long winding road

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Recent advances in cryo-EM revolutionized the possibilities and capabilities in cryo-EM based structural analysis. It is currently possible to reconstruct macromolecular complexes at atomic resolution even by non-experts. This presents an exciting opportunity to explore the molecular architecture of macromolecular complexes that could not been crystallized at atomic resolution, but also opens a window into *in situ* structural determination by means of cryo-electron tomography (cryo-ET). In particular, large assemblies as the nuclear pore complex (NPC). These macromolecular assemblies fuse the inner and outer nuclear membrane and form aqueous channels for translocation. NPCs facilitate unchallenged passive diffusion of small molecules and control the translocation of larger cargos in a highly selective manner.
We have studied the structure of NPCs using cryo-ET of intact nuclei, across species, and compare the molecular organization of NPCs. This analysis indicated major structural differences that suggests variability in the NPC function, i.e. nuclear transport.

Activating anti-cancer immunity through a novel cell stress mechanism

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High Mobility Group Box 1 protein (HMGB1) is a DNA chaperone that also acts as a damage-associated molecular pattern when released outside the cell. Extracellular HMGB1, together with calreticulin cell surface exposure and ATP emission, is a hallmark of Immunogenic Cell Death (ICD), a process whereby tumor cells treated with certain chemotherapeutics or radiotherapy, enables tumor recognition by T cells and induces immunological memory. We and others have shown that mesothelial cells exposed to asbestos release HMGB1, which in turn promotes tumorigenesis by recruiting pro-tumoral macrophages. In this and other tumors, HMGB1 is pro-tumorigenic. However, it has been shown that HMGB1-deficient tumors have defective ICD.
BoxA is a partial agonist of HMGB1 that has anti-inflammatory properties in many disease models. We set up a novel syngeneic mouse model of MM that recapitulates well several features of human mesothelioma, and we found that BoxA significantly increases survival of MM-bearing mice, as hypothesized, and unexpectedly eradicates the tumor in 50% of mice. Furthermore, we found that surviving mice are protected against a tumor re-challenge, and have a selective expansion of tumor-reactive IFNϒ producing memory T cells. *In vitro*, BoxA induces mesothelioma cells to express ICD hallmarks and activation of the unfolded protein response without affecting cell death.
These preliminary data raise the possibility that BoxA might work in improving the recognition of tumor antigen, through a novel mechanism of cell stress.

H3K9 methylation controls Fibro-Adipogenic Progenitors identity and skeletal muscle repair

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Fibro-Adipogenic Progenitors (FAPs) are crucial regulators of muscle homeostasis as they possess the intrinsic ability to either support muscle regeneration or to contribute to fibro-adipogenic degeneration of dystrophic muscles. Therefore, the elucidation of the molecular mechanisms controlling their phenotypical plasticity holds therapeutic potential. Here we provide evidence that FAPs are particularly enriched in histone H3 lysine K9 methyltransferases (H3K9 KMTs), G9a, GLP and PRDM16. Our data indicate that H3K9 KMTs safeguard FAPs identity by repressing alternative transcriptional programs through deposition of H3K9 di-methylation (H3K9me2). Specifically, we show that PRDM16 controls G9a/GLP's genomic recruitment and H3K9me2 deposition at muscle-specific loci. Of note, PRDM16, G9a and GLP are enriched at the nuclear lamina of FAPs suggesting that they organize heterochromatin at the nuclear periphery to maintain the stable repression of genes encoding alternative developmental regulators. Accordingly, pharmacological inhibition or RNAi-mediated knock-down (KD) of H3K9 KMTs de-repress master myogenic genes in FAPs and induce the muscle differentiation program.
Together, our findings reveal a FAPs-specific epigenetic axis of therapeutic relevance since we show that *in vivo* inhibition of H3K9 methylation in dystrophic mice enhances skeletal muscle regeneration while reducing formation of adipogenic and fibrotic scars.

Role of Polycomb mediated cell memory system and epigenome plasticity in metabolic and circadian clock regulation

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Circadian rhythms have evolved as an adaptive response to the daily light-dark cycles. As a consequence, many aspects of metabolism and behavior in mammals display oscillatory rhythms of 24 hours period. The circadian control of our physiology is achieved through a specific program of gene expression, characterized by a complex interaction between clock-core proteins and chromatin remodelers that allow circadian modulation of gene expression.
In this study, we investigated the role of Polycomb proteins group (PcG) mediated cell memory system and in particular the PRC2-Ezh1 complex in the mouse skeletal muscle. We found that PRC2-Ezh1 pathway involving a novel isoform of Ezh1 (Ezh1b) specifically present in the cytoplasm and controlling stress induced PRC2-Ezh1a activity shows an oscillatory profile consistent with clock genes dynamics and their direct regulation in the nucleus. The circadian pattern of core clock component was impaired in PRC2-Ezh1 depleted cells. Notably, clock gene loss of function affected PRC2-Ezh1 expression, indicating a direct crosstalk between the two systems. Further, mass spectrometry analysis revealed that distinct ubiquitin E3 ligase components interact with cytosolic Ezh1beta and nuclear Ezh1alfa complexes, and their activity appear to modulate PRC2-Ezh1 circadian pattern.
Further, under nutritional challenge such as High Fat Diet (HFD) induced obesity, the circadian transcriptome and metabolome can be remodeled in the mouse liver rapidly. Surprisingly, metabolome and gene expression analysis performed on various tissues revealed that skeletal muscle is the most affected under HFD. Interestingly we found that both HFD and fasting induced metabolic stress affected PRC2-Ezh1 mediated adaptive response pathway but with opposite dynamics.
Our data unveil a novel physiological role of PcG memory system to regulate natural circadian tissue specific transcription programs and adaptive response to diet induced metabolic stress.

Heterochromatin silencing of stemness during CD8$^+$ T cell fate commitment

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Following activation by antigens, naïve CD8$^+$ T lymphocytes establish specific heritable gene expression programs that define the progression to long-lasting memory or to short-lived effector cells. While lineage specification in T cells is critical for protection, the impact of epigenetic silencing on T lymphocyte differentiation is still incompletely understood.
Here, we explore the role of heterochromatin-mediated gene expression silencing by Suv39h1, a histone H3 lysine 9 methyltransferases that plays a critical, evolutionary conserved, role in heterochromatin structure and dynamics. We show that in murine CD8$^+$ T cells activated after *Listeria monocytogenes* infection *in vivo*, Suv39h1-dependent H3K9me3 deposition controls the expression of a set of stem cell-related/memory genes. Single-cell RNA sequencing analysis reveals that the silencing in stem/memory genes selectively affects terminally differentiated effector subsets. The results also show increased proportions of CD8$^+$ T cells with central memory phenotype and the de-repression of stem cell-related genes across different *Suv39h1*-defective CD8$^+$ T cell sub-populations. In line with these observations, *Suv39h1*-defective CD8$^+$ T cells show increased memory potential, including sustained survival and increased long-term re-programming capacity, as compared to *Suv39h1* -proficient CD8$^+$ T cells.
We conclude that Suv39h1 plays a critical role in marking chromatin to silence stem/memory gene expression during CD8$^+$ effector T cell terminal differentiation. In doing so, Suv39h1/H3K9me3 would establish an epigenetic barrier on the stem/memory gene expression program, preventing the effector re-programing into memory cells. These results open new perspectives for the manipulation of epigenetic programming of T lymphocyte identity in the context of T cell-based immunotherapies.

Che-1/AATF-induced transcriptionally active chromatin promotes cell growth in Multiple Myeloma

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Tumor transformation is the result of genetic modifications that alter gene transcription, resulting in a specific oncogenic program. In recent years, several exciting discoveries have shown that aberrant epigenetic modifications play a major role in the genesis and progression of cancer. Multiple myeloma (MM) is a cancerous pathology resulting from a clonal expansion of plasma cells, characterized by abnormal production and secretion of monoclonal antibody proteins. This disease shows a great heterogeneity, caused not only by genetic abnormalities but also by numerous epigenetic aberrations. Here we demonstrate that the RNA Polymerase II (Pol II) binding protein, Che-1 is required for MM cell growth by sustaining genome wide transcription and recruitment of Pol II to the DNA. Notably, we found that Che-1 localizes on active chromatin and that its depletion leads to accumulation of heterochromatin by a global decrease of histone acetylation. Strikingly, transgenic mice expressing human Che-1 in plasma cells develop MM with clinical features resembling those observed in the human disease. Moreover, Che-1 downregulation decreases BRD4 chromatin accumulation to further sensitize MM cells to bromodomain and extra-terminal (BET) inhibitors. In summary, our findings identify Che-1 as a key player for maintaining open chromatin required for sustaining MM growth. These findings support Che-1 as a possible target for MM therapy, alone or in combination with BET inhibitors.

Control of cell identity by chromatin modifiers

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Establishing and then maintaining cellular identity during differentiation requires signalling events to be transmitted to the chromatin level; transcription factors (TFs) and chromatin-remodelling activities work together to orchestrate the transcription programs underlying this transmission. It is now clear that chromatin remodelers play a major role in regulating cellular identity, resulting one of the most mutated pathways among all type of human cancers. In this context, Polycomb proteins (PcG) play a crucial role as regulators in development and differentiation and are frequently mutated or altered in their activity in numerous types of human cancers, via molecular mechanisms that are still poorly understood. At the meeting will be presented the recent advances of our laboratory aimed to dissect the molecular mechanisms underling the activity of distinct PcG activities in establishing and maintaining cell type specific transcriptional identity during both normal homeostasis and pathological conditions.

The importance of being modified: a novel code for RNA function

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In recent years it has become evident that the transcriptome is extensively and dynamically altered by a variety of RNA modifications, which – similar to post-translational modifications - harbor the potential of altering RNA fate and, in turn, regulating cellular physiology. N^6-methyladenosine RNA (m^6A) is among the most abundant mRNA modifications in vertebrates that regulates most mRNA processing steps and is involved in several biological processes, including circadian clock, metabolism and embryonic stem cell differentiation. However, its precise roles during development of complex organisms remain unclear. We carried out a comprehensive molecular and physiological characterization of the individual component of the m^6A methyltransferase complex as well as of the reader proteins in *Drosophila melanogaster*. Components of the complex are ubiquitously expressed with clear enrichment in the nervous system. Consistently, mutant flies for the catalytic subunits suffer from severe locomotion defects due to impaired neuronal functions. Components of the m^6A methyltransferase complex also fine-tune the female-specific splicing of *Sex lethal (Sxl)* transcript and of its downstream targets, revealing a role for this modification in sex determination and dosage compensation. I will present our current data regarding the role of reader proteins in these m^6A-dependent RNA processes in *Drosophila*.

Age-Dependent deterioration of nuclear pore assembly in mitotic cells decreases transport dynamics

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With most of a eukaryotic cell's genetic material being compartmentalized in the nucleus, and with the need for rapid communications and exchange of macromolecules, there is heavy traffic between the cytoplasm and the nucleus. This transport, nucleocytoplasmic transport, is performed by the Nuclear pore complex (NPC), a long-lived and exceptionally large structure. How well is this rapid and selective access to the nuclear compartment preserved in ageing? Previous reports have shown that post-mitotic cells accumulate oxidative damage, resulting in increased permeability of old nuclei. The fate of NPCs in mitotic cells however, is largely unknown. We study the NPC’s structural and functional changes in single replicative aging baker’s yeast cells, kept in well controlled microfluidic environments. We show that the abundance of NPC components and NPC assembly factors decreases in aging and is predictive of a cell’s lifespan while signs of misassembled NPCs appear in old cells. Aged cells show a reduction in nuclear permeability, a phenotype uniquely mimicked in an assembly mutant with a reduced number of functional NPCs. Our work shows that old nuclei are less permeable due to problems in NPC assembly, a weak spot in aging that is specific for replicative aging cells. We think a molecular understanding of nuclear transport in the context of ageing may help unravel the emerging relationship between NPC biology and neurodegeneration.

Poster Abstracts

(presenting authors are shown underlined)

P.2

U1 snRNA-mediated exon skipping to correct Retinitis Pigmentosa GTPase-Regulator (RPGR) splice defects

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Retinal dystrophies are an extremely diversified group of diseases characterized by visual dysfunctions that in the worst cases can lead to partial or complete blindness. As of today, no genetic therapy approaches are available for these diseases, and the most advanced clinical therapies are restricted to treatment of complications (cataract and macular edema) and slowing down of the degenerative process by light protection and vitamin-therapy. Overall, more than 250 genes have been identified as responsible for syndromic and non-syndromic retinal dystrophies, one of these causative genes encodes for the so-called *Retinitis Pigmentosa GTPase-Regulator* (RPGR), involved in a common form of retinal dystrophy: Retinitis Pigmentosa (RP). In particular, it has been shown that a nucleotide substitution in intron 9 of *RPGR* gene causes the increase of a form of the mature mRNA including an alternatively spliced exon 9a (E9a).
The relevance of alternative splicing in the pathogenesis of RP is still poorly understood, but several splice variants of RPGR have been reported. We developed a U1 snRNA-based therapeutic approach for RPGR nucleotide substitution to induce E9a skipping. We designed a set of specific U1 antisense snRNAs (U1 asRNAs), and tested their efficacy, *in vitro*, upon transient co-transfection with RPGR minigene reporter systems (MINI mut and MINI wt) in different cell lines (Human Embryonic Kidney 293 cells (HEK-293T) and rat pheochromocytoma (PC-12)).
We were able to identify the best chimeric U1 asRNAs that can mediate E9a skipping thus correcting the genetic defect. Our data provide a proof of principle for the possible application of U1 snRNA exon skipping-based approach to correct alternative splicing defect in RPGR E9a, thus paving the way for the development of an effective cure for Retinitis Pigmentosa linked to *RP*.

P.3

Epigenetic regulation of eukaryotic DNA replication origins

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In higher eukaryotes, DNA replication initiates at multiple sites distributed along the chromosome, called replication origins. The location and timing of these initiation events are developmentally and epigenetically regulated. In the early *Xenopus* embryos replication origins are more abundant than in somatic cells, or at later stages of development. This particular organization of the replicon ensures rapid and accurate genome duplication during early embryonic cell divisions. Close to the mid-blastula transition (MBT), the density of replication origins declines, initiation of DNA replication becomes restricted to specific sites and correlates with cell cycle lengthening. Intriguingly, in nuclear transfer experiments exposure of somatic nuclei to egg cytoplasm is sufficient to convert the somatic origin density to the embryonic one, suggesting that the replication origin assembly in somatic cells is restrained by epigenetic factors modifiable by the egg cytoplasm. We performed extensive biochemical fraction of egg cytoplasm to identify factors that regulate replication origin density in embryonic and somatic nuclei. This effort led to the isolation of SSRP1 as major factor that stimulates somatic nuclei replication in egg extract. We showed that exposure of somatic nuclei to SSRP1 removes epigenetic determinants that restrain replication origin assembly and restores replication origin density typical of embryonic nuclei on somatic chromatin. Strikingly, SSRP1-mediated regulation of replication origin assembly has a dramatic impact on cell cycle progression and early embryonic development. These results and their implications will be described in details in the context of more general regulation of DNA replication in vertebrate organisms.

P.4

Role of N6-methyladenosine in the translation of circular RNAs in eukaryotes

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Circular RNAs (circRNAs) are covalently closed, single-stranded transcripts produced by exon back-splicing circularization, wherein a downstream 5′ splice site (splice donor) is joined to an upstream 3′ splice site (splice acceptor). Circular RNAs have been shown to be abundant and evolutionarily conserved but their function is still largely unknown.
Expression profiling of circRNAs during in vitro differentiation of murine and human myoblasts allowed the identification of conserved species regulated in myogenesis.
Upon an RNAi-Based circRNA functional screening we focused on circ-ZNF609, which affects myoblast proliferation when knocked-down.
Circ-ZNF609 contains an open reading frame spanning from the START codon, shared with the linear transcript, to an in-frame STOP codon created upon circularization. We demonstrated that circ-ZNF609 is translated in a splicing-dependent and cap-independent manner from two different START codons, providing an example of a protein-coding circRNA in eukaryotes. Moreover, Circ-ZNF609 translation responds to cellular stresses such as heat shock.
N6-methyladenosine (m6A) is the most abundant mRNA modification. It occurs preferentially at the consensus RRACH motif and it is involved in every step of RNA metabolism.
Circ-ZNF609 contains several RRACH motives both in its untranslated and coding region with different functions in circ-ZNF609 metabolism.
In this regard we are currently focusing on the possible regulatory role of m6A in circ-ZNF609 producing vectors able to overexpress the wild type sequence of circ-ZNF609 or mutants which are abolished for the m6A modification.
Furthermore, we are investigating the role of specific readers known to be involved in m6A-mediated translation and the writers and erasers involved in circRNA methylation through a knock down based approach.
This study could increase knowledge in the fields of both circRNAs epitranscriptome and cap-independent m6A-mediated translation.

P.5

R-loops and proteasome activity mediate DNA damage and micronuclei formation due to DNA topoisomerases inhibition

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DNA Topoisomerases 1 and 2 (Top1 and Top2) have an important function in preventing replication/transcription (R/T) conflicts caused by RNA polymerases elongating in the opposite (or same) direction of advancing replication fork. Persistent R/T conflicts can be rescued by overexpression of RNase H1, which specifically degrades the RNA of DNA:RNA hybrids present in R-loop structures, common non-B DNA structure of cellular genomes that mainly occur during transcription. R-loops are important player in several cellular functions but they can also lead to genome instability. With the present work, we have determined the dynamics of cellular R-loops generated by chemical poisoning of Top1 and Top2 and the role of the proteasome in DNA damage induction. Treatment of HeLa and U2OS cells with Topo poisons triggers an immediate increase of nucleoplasmic R-loops after few minutes of treatment, followed by a dramatic reduction of R-loop levels at 1 hour. Concomitantly with the reduction of R-loops structures, we detect an increase of the DNA damage response, visualized as phosphorylation of H2AX histone, a marker of DNA double-stranded breaks (DSB). Pretreating cells with MG132 (an inhibitor of the proteosome), stabilizes the presence of R-loops at longer treatment times in Top1 poisoned cells and concomitantly reduces DSB formation, suggesting a function of nuclear proteasome in generating a free DNA break from a poisoned Top1 that will eventually lead to DSB. Overexpression of RNaseH1 in U2OS cells abolishes the transient increase of R-loops and partially revert the induction of DNA damage. Intriguingly, Top1 and Top2 poisons induce the formation of micronuclei, in an R-loop dependent manner, therefore suggesting that an innate immune system activation can be mediated by the effect of poisons on R-loop formation. The obtained data reveal new cellular pathways activated by topoisomerases inhibitors, that potentially could be useful to develop new anticancer strategies.

P.6

Molecular role of a circular RNA in myoblast proliferation and in rhabdomyosarcoma

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Circular RNAs (circRNAs) represent a class of covalently closed RNAs, derived from a non-canonical splicing (back-splicing), ubiquitously expressed among Eukaryotes and conserved among different species.
We identified several circular RNAs expressed and modulated during myogenesis. Thanks to a knockdown-based phenotypic screening, we identified a circRNA (circZNF609) involved in the regulation of human primary myoblasts proliferation. Upon its depletion, the percentage of proliferating cells is highly reduced. An RNAseq performed on human primary myoblasts revealed that the expression of nearly 300 genes is altered upon circZNF609 specific depletion, of which 60% are down-regulated, and specifically enriched for cell cycle related genes.
To deepen our knowledge about circZNF609 role in proliferation, we are studying it in Rhabdomyosarcoma (RMS), a pediatric muscle malignancy. We found that circZNF609 is strongly up-regulated in biopsies from both the two major RMS subtypes, the embryonal and the alveolar, and we discovered that its knock-down blocks proliferation of an embryonal RMS-derived cell line, promoting an accumulation of cells in G1 cell cycle phase, with a reduction of cells in S phase.
To understand the mechanism through which circZNF609 affects cell proliferation, we compared the different effects of circZNF609 depletion in embryonal and alveolar RMS. In fact, while circZNF609 knock-down induces a proliferation block in embryonal RMS, it has not the same effect in alveolar RMS. Comparing the two transcriptomic responses, we can identify which are the pathways affected by circZNF609 down-regulation.
As we are interested in understanding circRNAs' roles in the onset and the progression of RMS, we are characterizing several circRNAs differerentially expressed among wild-type myoblasts and RMS subtypes, and/or between alveolar and embryonal RMS.

P.7

Genome-wide mapping of 8-oxo-7,8-dihydro-2'-deoxyguanosine shows its enrichment at DNA replication origins of transcribed long genes

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8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) is one of the major DNA modifications. It is a potent premutagenic lesion, due to its ability to pair with both cytosine and adenine residues, thus causing G:C to T:A transversions during DNA replication. 8-oxodG is an effective biomarker of oxidative stress, and its accumulation in the genome has been associated withcancer, aging, and cardiovascular diseases.To date, however, genomic distribution of 8-oxodG remains poorly characterized. We developed an innovative technique, the OxyDIP-Seq, combining single-stranded DNA immunoprecipitation with next-generation high-throughput sequencing to identify and map the oxidized guanine residues on mammalian genomes.Here, we report the genome-wide distribution of 8-oxodG in human non-tumorigenic epithelial breast cells (MFC10A), and mouse embryonic fibroblasts (MEFs). Moreover, comparaing OxyDIP-Seq with gH2AX ChIP-Seq data, we found a distinctive co-enrichment of 8-oxodG and gH2AX within gene bodies of very long transcribed genes in both human and mouse genomes. Our data suggest that accumulation of 8-oxodG at ORIs of active long genes significantly contributes to the inherent instability of these genomic region.

P.8

RNA-mediated correction of aberrant DNA methylation at the *P15* gene locus

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DNA methylation is a key epigenetic signature implicated in the regulation of gene expression that occurs within CpG dinucleotides. Methylation of CpG-rich promoters is carried out by the DNA Methyltransferases (DNMTs) and negatively correlates with gene expression leading to long-term gene silencing. Abnormal DNA methylation is the dominant mechanism for tumor suppressor gene silencing in malignant transformation. We, previously, discovered a class of RNAs, named DNMT1-interacting RNAs (DiRs), able to regulate DNA methylation by binding to and inhibiting DNMT1, supporting the possibility to use RNA molecules to correct DNA methylation in a gene-specific manner.
Short activating RNAs (saRNAs) are a novel non-coding and double-stranded RNA-based platform developed to upregulate transcription at gene-specific *loci*. Yet their mechanism of action remains unknown.
We hypothesized that saRNAs may act as DiR-mimicking molecules and affect DNA methylation patterns**.** To fulfill the goal of the study we focused on *P15*, the tumor suppressor gene most frequently silenced by aberrant promoter methylation in Myelodysplastic Syndromes. Three saRNAs designed against the promoter, first exon and intron of the *P15* gene locus were introduced in the *P15* not expressing and heavily methylated KG1a cell line. Seventy-two and ninety-six hours upon transfection we observed reactivation of *P15* expression, along with changes in DNA methylation profile of the *locus*.
Currently, the most prominent demethylating agents employed to reverse DNA methylation are nucleoside analogs whose lack of specificity accounts for the high toxicity and numerous side effects. RNA molecules offer greater advantages as therapeutic tools as they can provide a strategy to target gene *loci* with high specificity and absence of drug based off-target side-effects.

P.9

Epigenetic changes associated with the expression of Amyotrophic Lateral Sclerosis (ALS) causing genes

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Neurodegenerative disorders, including Amyotrophic Lateral Sclerosis (ALS), have been associated to alterations in chromatin structure resulting in long lasting changes in gene expression. ALS is predominantly a sporadic disease and environmental triggers may be involved in its onset. In this respect, alterations in the epigenome can provide the key to transform the genetic information into phenotype. We investigate two modifications associated with transcriptional activation, i.e. dimethylation of lysine 4 on H3 tail (H3K4me2) and phospho-acetylation of serine 10 and lysine 14 on H3 tail (H3K14ac-S10ph), and two modifications associated to transcriptional repression, i.e. trimethylation of lysine 9 on H3 tail (H3K9me3) and DNA methylation are selectively altered in cellular and animal model of ALS. Our results reinforce the idea that epigenetic therapy may represent a potential and attractive approach for ALS treatment

P.10

The deregulation of specific miRNAs acts as a major non-genomic alteration at the basis of de novo drug resistance in melanoma

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MAPK signaling is the main oncogenic driver in metastatic melanomas bearing mutations in BRAF kinase. These tumors are currently treated with the combination of BRAF/MEK inhibitors (MAPKi), but this therapy is plagued by drug resistance. In this context we recently focused on non-mutational mechanisms contributing to the development of drug resistance and in particular on the role of miRNAs. Indeed, we have firstly identified miR-579-3p as an antagonist of melanoma progression and resistance. However, the great heterogeneity of melanoma tumors renders necessary to conceive a more comprehensive approach. Herein, we assessed through the innovative approach of Nanostring platform the deregulation of the whole miRnome during the development of drug resistance *in vitro* in two different BRAF-mutated melanoma cell lines. This approach allowed us to identify the progressive deregulation (up or down) of a growing number of miRNAs during the selection process. In addition, thanks to bioinformatics analysis we have identified the molecular pathways affected by those deregulated miRNAs which mostly hit on pro-angiogenic and pro-inflammatory cues. Hereafter, we have focused our further studies on a subset of four selected miRNAs, namely miR-4443, miR- 4488, miR-199b-5p and miR-204-5p since they were found to be deregulated in both cell lines analyzed at the highest drug doses tested. In detail, the first two miRNAs are up-regulated in drug resistant cells and act as possible facilitators of this process whereas the second ones, which were down-regulated in the same conditions act as antagonists. Of note, we have demonstrated that the simultaneous targeting of miRNA alterations blunts development of drug resistance. Finally, we demonstrated that significant miRNA deregulation is detectable in tumor biopsies and plasma from patients after disease recurrence. These findings together have important therapeutic and diagnostic implications.

P.11

The “awakening” of LINE-1 retrotransposons is a functional and lineage-specific event during human mesenchymal stem cells (hMSCs) differentiation

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Nearly half of the human genome is composed of sequences derived from mobile elements. 17% is represented by LINE-1 (L1), among which few tens still retain the ability to retrotranspose autonomously using a RNA mediated “copy and paste” mechanism. Although host cells have evolved different epigenetic defense mechanisms to prevent deleterious uncontrolled transposition, evidence indicates that neurons support L1 mobilization, generating mosaic brain, in response to environmental experience. Moreover, recent *in vivo* use of antiretroviral drug Lamivudine as a nucleoside reverse transcriptase inhibitor (NRTI), demonstrated that L1 mobilization enables memory formation in mice developing brain. However the question whether L1 mobilization is part of normal differentiation in other tissues and its function remains open.
Interestingly, clinical observations indicate that NRTIs treatment of HIV-infected individuals strongly correlates with loss of bone density. This made us hypothesize that L1 might be functional players in adult bone homeostasis. Therefore, we drove the *in vitro* differentiation of hMSCs into mature osteoblasts and we found that L1 are transcriptionally activated, translated, and *de novo* inserted in the genome of differentiating cells. By drug inhibiting retrotransposition and knocking-down L1 mRNA, we demonstrated that L1 “awakening” upon osteogenic induction is required to proper accomplish a fully functional differentiated phenotype. Further, we found that another genetic program, adipogenesis, imposed to the same progenitors lacks the reactivation of L1 elements, suggesting that L1 mobilization is a lineage-restricted event, rather than an unspecific, correlative phenomenon broadly triggered by the differentiation process itself. Finally, we show that *in vivo* L1 genomic copy number correlates with bone density and that it is significantly reduced in patients affected by osteoporosis, an *in vivo* model of impaired osteogenesis.

P.12

Functional interaction between MyoD and PARP1 in the regulation of the p57-KvDMR1 locus

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The myogenic factor MyoD, regulates a complex program of gene expression involving a high number of muscle specific genes and cell cycle regulators. The MyoD–dependent induction of the cell cycle inhibitor p57kip2 (p57) presents a complex and multilevel regulation. In particular, we found that MyoD causes the release of a repressive chromatin loop, mediated by CTCF, between the promoter of p57 and a 150 kb-distant regulatory element, KvDMR1[1, 2].
It has been previously reported that CTCF physically interacts with and is a target of the poly(ADP-ribose)polymerase PARP1. Moreover, PARP1 activity is required for CTCF-mediated long-range chromatin insulation[3].
In light of the observation that PARP1 expression decreases during muscle differentiation we wanted to investigate the possible involvement of PARP1 and poly(ADP-ribosyl)ation in mediating the MyoD effects on the architecture of the p57-KvDMR1 locus.
For this purpose, we realized a stable PARP1 knocked-down myoblasts cell line, observing that the downregulation of PARP1 expression increases p57 levels in differentiated cells.
We also demonstrated the physical interaction between PARP1 and MyoD, and PARP1 binding to the same p57 regulatory region within the KvDMR1 bound by both CTCF and MyoD, and involved in the formation of the chromatin loop. In order to characterize the molecular mechanism we are currently investigating the role of PARP1 enzymatic activity in mediating MyoD function.
Interestingly, we observed that PARP1 depletion also causes an increased expression of other MyoD-regulated genes and that PARP1 binds to the promoter of the muscle specific MyoD target Myogenin.
Collectively these results suggest a role for PARP1 in regulating the MyoD-dependent release of p57-KvDMR1 chromatin loop, prospecting the possible extension of the described model to other MyoD targets genome-wide.

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P.13

Dissection of the mechanism of action of LSD1-inhibitors in Acute Myeloid Leukemia by quantitative proteomics

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Lysine specific-demethylase 1 (LSD1) is aberrantly expressed in acute myeloid leukemia (AML) and is emerging as a promising target for the epigenetic therapy of AML subtypes not responsive to retinoic acid treatment. The Experimental Therapeutic Unit at the IFOM-IEO Campus optimized potent and specific LSD1 inhibitors, already characterized *in vitro* for their selectivity and *in vivo* for their anti-proliferative effects on self-renewing AML cells. We are studying the effects of these compounds on the pattern of histone post-translational modification (PTM) and on the LSD1 interaction network in NB4-AML cells, using a panel of quantitative mass-spectrometry strategies. We discovered that a 24-hour treatment with the inhibitors alters the levels of histone modifications (increases H3K4me2, H3K27me2 and H3K27me3, and decreases H3K27me1). LSD1 knock-out NB4 cells display similar changes in histone PTMs, strongly suggesting a specific association with the cellular response to LSD1 inhibition. We identified the complete set of LSD1 interactors in our model using SILAC-based proteomics, most of which are involved in chromatin remodelling and transcription regulation activities. The analysis of the LSD1-interactome after drug treatment identified two LSD1 interactors (GFI1 and GSE1) with decreased binding, further providing new insight into the molecular activity of the inhibitors under study and unravelling novel targetable avenues.

P.14

No evidence of mtDNA methylation by real-time direct Nanopore sequencing

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The occurrence of DNA methylation in the human mitochondrial genome (mtDNA) and its functional role is since long largely debated and diverging results have been obtained so far, also explained by the specific features of mtDNA, such as its circular structure. In any case, when detected, the level of methylation is very low.
The Oxford Nanopore Technologies (ONT) sequencing devices may allow direct real-time DNA methylation analysis, based on the observation of peculiar current signals at level of methylated/non-methylated cytosines when ssDNA is crossing the pore. As the Nanopore based technique resulted rather effective and accurate in detecting the methylation pattern of nuclear DNA, we investigated as well the methylation pattern of cytosines within mtDNA. Basecalling of ONT reads was performed by Albacore v.2.2.4, while read mapping onto the human genome hg38 assembly was performed using Minimap2 v.2.9. Resulting BAM files were processed using samtools v.1.3.1, retaining only reads uniquely mapped to the mitochondrial genome or nuclear chromosomes. Methylated DNA cytosines in ONT data were detected using Nanopolish 0.9.0.
The above described methodology was applied to ONT sequence data obtained through the MinION device, sequencing native and amplified mtDNA from osteosarcoma wild type cybrid (OWTC) cell lines, using the sequencing data from amplified mtDNA as a negative control. Methylated CpG sites were detected by Nanopolish selecting only positions supported by at least 1000 reads. The observed comparable average methylation level for native and amplified mtDNA, 2.9% and 3.8%, respectively, does not provide evidence of mtDNA cytosine methylation above the expected signal noise. On the contrary, a remarkable signal was observed by analyzing the methylation pattern in nuclear DNA from NA12878 benchmark data (coverage > 20) with observed average levels of 60% and 7.7% for native and amplified DNA, respectively.

P.15

Genome-wide RNA editing analysis in human neurological disorders

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RNA editing is a widespread co/post-transcriptional mechanism that modifies RNA sequences by insertions, deletions or base conversions. The most common RNA editing event in humans include the deamination of Adenosine (A) in Inosine (I) catalysed by ADAR enzymes. Inosine is recognized as Guanosine by cellular machineries (PMID: 24165678) and is abundant in the brain, mostly in untranslated mRNA regions. RNA editing deregulation has been linked to several psychiatric, neurological and neurodegenerative diseases (PMID: 17114938).
To better understand the correlation between RNA editing and neurological pathologies, we present here the analysis of more than 830 RNA-Seq experiments from different SRA BioProjects related to Alzheimer, Parkinson, Amyotrophic lateral sclerosis, bipolar disorder, autism and major depression.
To evaluate RNA editing dysregulation in human neurological diseases, a total of 839 RNA-Seq experiments from fourteen BioProjects were downloaded from the SRA repository. In addition, transcriptome data from 30 RNA-Seq experiments from sporadic ALS, Alzheimer and Parkinson diseases were generated in our laboratory and added to final list of samples. For each RNA-Seq, raw reads were quality checked by FastQC and mapped onto the reference human genome using the ultra-fast aligner STAR. RNA editing candidates were detected by REDItools (PMID: 23742983). Next, the RNA editing activity was assayed calculating per each output table the Alu Editing Index (AEI) and the Recoding Editing Index (REI) by means of custom python scripts.
Preliminary results based on AEI and REI metrics suggests that RNA editing is dysregulated in neurological disorders and especially in neurodegenerative diseases such as Alzheimer and Parkinson. Further analyses to identify common dysregulated RNA editing signatures in human neurological pathologies are ongoing, hoping to provide novel insights into the challenging search of biomarker candidates for the design of innovative drugs.

P.16

Variation of microglial phenotype during wake and sleep in mice

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Sleep is an involuntary process observed in all mammals. It is required to sustain good mental and physical health, and it plays a crucial role in learning and memory functions in the nervous system. Indeed, sleep promotes the reestablishment of synaptic homeostasis, which is challenged by the remarkable plasticity of the brain during the wake (Tononi G and Cirelli C, 2014). Moreover, sleep deprived mice show memory impairments (Patti CL et al., 2010). Microglia are the resident immune cells of central nervous system, they actively monitor the tissue and perform different functions, such as the phagocytosis of cellular debris and the release of cytokines to modulate the synaptic transmission. In this context, microglial cells contribute to neural plasticity through the remodeling of brain circuits, including the formation, modification and elimination of synaptic structures. However, the role of microglia in sleep-associated neural plasticity is little known.
The purpose of this project is to study the role of microglia in sleep regulation and sleep-associated homeostatic functions, including neural plasticity.
To this aim, we analyzed the expressions of different genes related to microglial functions during the wake and sleep phase, in C57BL/6 male mice. Furthermore, we analyzed cell morphology and microglia density in specific brain areas.
Understanding the role of microglia in sleep regulation and its homeostatic functions could be a key point to outline the pathologies associated with sleep disorders and thus define a new therapeutic approach.

P.18

Studying the heterogeneity of NF-κB dynamics using clonal populations

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BACKGROUND: The transcription factor NF-κB translocates from cytoplasm to the nucleus upon external stimuli in order to regulate the expression of many target genes fundamental for the cell survival and the inflammatory responses. Therefore, the key role of NF-κB both in inflammation and cancer has been studied extensively. However, the biological role of the translocation dynamics is not still completely understood. Indeed, it has been reported that different cell types display different dynamics [1]. Most surprisingly, it has even been shown that cells of a same population of mouse embryonic fibroblasts expressing a fluorescently labelled NF-κB can present drastically different dynamics [2], and the source of this heterogeneity is still an open question. To address this, we analysed the dynamics of cells obtained from a single cell cloning of this population.
METHOD: Generation of the clones was performed by serial dilution method. Live-cell imaging was performed under different stimuli, also taking advantage of a microfluidic device to compare the response of different clones to time-varying stimuli. Gene expression profiles were checked by RT-qPCR. We used a mathematical model to integrate our imaging data with the gene expression data.
RESULTS: We identified different dynamics of NF-κB in different clones. More specifically, we took into considerations criteria such as timing, degree and damping of the response, that have distinct values in different clonal populations. Our preliminary results suggest that expression profiles in genes involved in the negative feedback regulation of NF-κB differ between the clones.
CONCLUSION: Our results could explain the previously reported heterogeneity of NF-κB dynamics and shed light on how the NF-κB system can produce different dynamic responses to external stimuli depending on the biological context.

1. Hoffman *et al* 2002.Science 298:1241-1245.
2. Zambrano *et al* 2016. eLife. 5:e09100.

P.19

Unravelling new mechanisms required for survival of dormant/indolent breast cancer cells in the lung

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In breast cancer, approximately 20% of disease-free patients relapse many years after surgical removal of the primary tumour. Disseminated cancer cells can enter a state of quiescence, or dormancy, in secondary organs and can re-enter in a proliferative state after several years. Despite several recent papers shed light in the signals that induce a quiescent state, little is known about how the disseminated cells survive during dormancy in the secondary organ. To understand this, we performed an unbiased transcriptomic analysis from *in vivo* derived disseminated cells and identified a distinctive expression profile activated by dormant cells in the lung. Then, we performed a functional shRNA screen to identify which of these genes is required for survival of dormant cells *in vivo.* To better dissect the role of lung microenvironment on the persistence of disseminated metastatic cancer cells we set up a lung organotypic assay where defined stromal populations are cocultured together with cancer cells on an air-permeable surface. Interestingly, transcription of candidate genes identified *in vivo* is triggered *in vitro* by interaction with specific lung stromal populations. Finally, single-cell Mass Cytometry of cells cultivated in our lung-mimicking system revealed that disseminated cells themselves impact on the proliferation of lung stromal cells and highlighted a novel signalling network involved in the survival of disseminated metastatic cancer cells in the context of lung microenvironment.

P.20

Exploiting a PI3Kγ Mimetic Peptide as a standalone drug to restore CFTR function, reduce inflammation and limit obstruction of the respiratory tract in cystic fibrosis

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The underlying cause of cystic fibrosis (CF) is a mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a cyclic AMP (cAMP)-stimulated chloride channel. The consequent CFTR dysfunction results in obstruction of small airways and airway inflammation and eventually leads to respiratory failure. Recently, a number of CFTR correctors and potentiators, improving membrane expression and gating of the channel respectively, have been developed, but their ability to rescue the basic defect of CF is still unsatisfactory. We designed a cell-permeable peptide (Patent n° PCT/IB2015/059880 - WO/2016/103176) able to interfere with PI3Kγ scaffold activity, since it facilitates PKA-mediated phosphorylation and activation of cAMP phosphodiesterases 4 (PDE4). We hypothesize that targeting PI3Kγ anchoring activity enhances cAMP in airway smooth muscle, immune and epithelial cells, leading to concomitant (i) bronchodilation, (ii) anti-inflammatory effects and (iii) CFTR modulation.
We found that, in vivo, the peptide can be efficiently delivered to the lower airways by intratracheal instillation in mice and significantly elevates cAMP in the lungs. Notably, the peptide limits methacholine-induced airway hyperresponsiveness and reduces neutrophilic lung inflammation in OVA mice. In vitro, the peptide potentiates F508del-CFTR currents upon pharmacological correction with VX-809 and, unlike the gold-standard potentiator VX-770, does not interfere with channel stability in the chronic setting. In VX-809-corrected primary cells, the peptide also synergizes with VX-770, by increasing its efficacy by 5 fold and is able to stabilize the CFTR to the plasma membrane. Overall, these results demonstrate that the peptide can be exploited as a new medicinal product that may offer unique advantages over current existing therapies for CF: I) three independent therapeutic benefits in a single molecule (CFTR rescue, anti-inflammatory and bronchodilator effects); ii) high intrinsic specificity of action (due to its peptidic nature) and iii) limited side effects thanks to the delivery by inhalation.
This work was supported by grants from the Italian Cystic Fibrosis Research Foundation and Cariplo Foundation.

P.21

TRAF3 involvement in the NF-κB pathway deregulation mediated by HTLV-1 Tax protein

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The retrovirus human T-lymphotropic virus type 1 (HTLV-1) is the causative agent of adult T-cell leukemia (ATL). The viral genome encodes two main regulatory proteins involved in the cell transformation, Tax and HTLV-1 basic zipper protein (HBZ). Tax is crucial for $CD4^+$ T-cell transformation by constitutively activating both the classical and alternative NF-κB pathways that play a primary role in inflammation, cell survival and cancer. HBZ is essential for viral persistence and T-cell proliferation contributing to ATL development. HTLV-2 is genetically related to HTLV-1 and is not associated with ATL. Comparative studies of Tax-1 and HBZ and HTLV-2 homologous regulatory proteins, Tax-2 and APH-2, are useful to understand the HTLVs pathobiology. The aim of this study is to investigate the role of TRAF3 in the deregulation NF-κB activation mediated by Tax and HBZ. TNF-receptor associated factor 3 (TRAF3) is a negative regulator of the alternative NF-κB pathway that we have previously demonstrated to interact with Tax. To analyze the contribution of TRAF3 in the Tax-induced deregulation of NF-κB, we generated $TRAF3^{-/-}$ cell line using the CRISPR/Cas9 technique. By co-immunoprecipitation and confocal microscopy analyses, we showed that TRAF3 interacts with Tax and APH-2 in the cytoplasm. Performing luciferase assay, we found that $TRAF3^{-/-}$ cells show activation of the NF-κB promoter. Western blot analyses revealed that the processing of p100 is detected in the absence of TRAF3, indicating the activation of the alternative NF-κB pathway. Furthermore, analyzing the localization of the transcription factor p65, we found that p65 is more represented in the nucleus of $TRAF3^{-/-}$ cells, than in the parental line. Moreover, the results indicate that the activation of the NF-κB promoter mediated by Tax-1 is dramatically reduced in the absence of TRAF3. Further analyses will be performed to study the effects of the antisense HBZ and APH-2 proteins on NF-κB promoter activity in $TRAF3^{-/-}$ cells.

P.22

Building non-coding RNA networks in IntAct

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In the last decade, an increasing number of studies have reported on the involvement of ncRNAs in various physiological processes, including cell cycle progression and differentiation, and demonstrated their crucial role as epigenetic regulators in the development of diseases such as cancer. ncRNAs have been suggested as promising targets for the treatment of many human pathologies, therefore the availability of the molecular interactions networks which involve ncRNAs in public repositories in a standardized representation would provide researchers with the opportunity both to design better experiments and to investigate therapeutic interventions. Since 2002, the HUPO Proteomic Standard Initiative (HUPO-PSI), has provided a well-defined annotation system for molecular interactions, to standardize the minimal information requirements to describe an interaction experiment and to define the syntax of terms used for protein interaction annotation, to allow the sharing of data from different resources to build better defined networks. The IntAct team has recently started a project focused on the development of similar standards for the capture and annotation of ncRNAs interactions. In this public resource, the knowledge about RNA, proteins or genes involved in the interaction is integrated with a detailed description of the cell types, tissues, experimental conditions and effects of mutagenesis, providing a computer-interpretable summary of the published data integrated with the huge amount of protein interactions already gathered in the database (database website https://www.ebi.ac.uk/intact/).
This effort will provide high-quality, reliable networks for the advancement of ncRNA research, for example to identify specific hubs to engineer to modulate gene expression or to predict off-target effects.

P.23

Computational approach for the identification of kinomes and kinase-specific phosphorylations in eukaryotic proteomes

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Phosphorylation is one of the main protein post-translational modifications, a fast and reversible regulation mechanism of signaling and metabolic pathways. The intricate network of kinases and target proteins represents a key component of the functional state of a cell and its determination in different contexts (e.g. different cell types or cancer cells) is of crucial importance. Every year tens of thousands of phosphorylation sites are discovered in different organisms, tissues and experimental conditions; however the responsible kinase is known for only a fraction of known phosphosites (~10000). Different methods have been recently developed for the prediction of phosphorylations specific to a selection of kinases or kinase families.
We present a novel approach that is able to i) automatically detect more than 90% of the kinases in different Eukaryotic proteomes, ii) infer the kinases' determinants of specificity (Dos), iii) integrate the DoS with the target peptide to infer a kinase-specific phosphorylation (KsP). The underpinning methodology involve a Hidden Markov Model of human kinases able to correctly detect kinase domains in different Eukaryotes, with a specificity of 0.99 and a sensitivity higher than 0.92. The Kinspect method is then used to detect DoS residues in kinases (Creixell et al., Cell 2015) and a Deep Neural Network trained on experimentally validated human KsP is then tested on different organisms outperforming other available methods (Song et al., Mol. Cell. Proteomics, 2012; Ellis et al., PLoS One, 2011; Horn et al., Nat. Publ. Gr. 2014), with AUC values ranging from 0.78 to 0.81. The methodological novelty of this approach is in the adaptation to different proteomes, not only in terms of variations in the target sequence but also in the DoS of the kinases. This allows a versatile prediction of KsP networks in different contexts: e.g. evolutionary variations in orthologous proteins or missense mutations introduced in different cancer types.

P.24

Axl-148b aptamer inhibits melanoma and breast cancer tumor progression

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MicroRNAs are small non-coding RNAs acting as negative regulators of gene expression and playing a central role in tumor progression. Recently, we showed that inhibition of miR-214 and the simultaneous overexpression of miR-148b successfully reduced metastasis formation due to a synergistic effect on tumor cell extravasation. Therefore, modulation of this miRNA network offers considerable therapeutic potential. To this aim, we explored the use of nucleic acid aptamers as carriers for cell-targeted delivery of miR-148b. Precisely, we used an aptamer that binds to and antagonizes the oncogenic receptor tyrosine kinase axl to deliver miR-148b to breast, melanoma and lung cancer cell lines. The specific delivery of miR-148b to cancer cells was evidenced and the efficacy of the conjugated axl-148b chimeric aptamers was appreciated when migration of tumor cells through a porous membrane or an endothelial cell monolayer or invasion of a matrigel layer resulted impaired compared to controls. In parallel, expression of two known miR-148b direct target genes, ALCAM and ITGA5, was reduced. Relevantly, axl-148b chimeric aptamers were able to significantly inhibit the formation of breast cancer cells-derived mammospheres *in vitro*, to promote apoptosis and necrosis in primary tumors and to block primary tumor cell dissemination and metastasis formation in mice. In conclusion, our data demonstrate that the chimeric axl-148b aptamers allow a specific delivery of miR-148b to cancer cells and are effective in inhibiting malignancy. We believe these aptamers are promising targeted therapy tools that can help to block metastatic traits on their own or in combination with traditional anti-cancer therapies.

P.25

p140Cap affects breast cancer features through the regulation of the tumor microenvironment composition

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Increasing data indicate the key role of the tumor microenvironment in cancer progression, metastasis formation and treatment sensitivity, where the tumor stroma and the immune infiltrate strongly influence tumor features.
Our recent studies indicate that the adaptor protein p140Cap is highly expressed in 50% of ERBB2-amplified breast cancer patients. High levels of p140Cap correlate with increased patient survival and decreased probability to develop metastasis. In the NeuT preclinical mouse model,p140Cap negatively regulates breast tumor growth, Epithelial to Mesenchymal transition (EMT) and spontaneous metastasis formation.
Here we exploit Mock and p140Cap-expressing TuBo cells, as syngeneic ERBB2+ breast cancer models. Weshow that p140Capimpacts on the G-CSF levels (mRNA and protein) and affects theTuBotumorimmune infiltrate composition. Indeed, we observed a reducedmobilization of G-CSF-dependent myeloid-derived suppressor cells (MDSCs-$CD11b^{+}$ $Ly6G^{+}Ly6C^{lo}$) from bone marrow and a decreased level of gMDSCs in tumor, blood and spleen of mice orthotopically injected with p140Cap TuBo cells, compared to controls.This "less" immune suppressive microenvironment associated to p140Cap-expression, was also confirmed by an increase in CD8+T lymphocytes, M1 macrophages and Natural Killer lymphocytes,with a concomitant decrease in M2 macrophages and FoxP3 immunosuppressive regulatory T cells.
Interestingly, p140Cap significantly reduces the fraction ofcancer stem cells (CSC) and their sphere and tumor initiationfrequency. Since that the CSC population exhibits an enhanced G-CSF production, these data indicate that p140Cap, by regulating the CSC compartment, may limit tumor aggressiveness by creating a "less" immunosuppressive tumor microenvironment,adverse to tumor progression.

P.26

Characterization of Retinoic Acid-responsive hPSCs by induced Zinc finger and Master Regulator analysis

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Retinoic acid (*RA*) is a powerful regulator of cellular proliferation and differentiation. The effect of *RA* on target genes is mediated by a family of transcription factors (*TFs*) known as retinoic acid receptors, *RARs*. The nuclear RA-receptors interact with specific RA response elements (*RAREs*) located in the promoters of target genes. *hESCs* and *hiPSCs* have a wide appeal for numerous biology studies because of their potential to give rise to almost any cell type in the human body and ability to self-renew. *RA* is essential on early embryonic development and induces differentiation primarily. *RA* plays a key role in differentiation through its nuclear receptors *RAR,* modulating the expression of target genes. Among them, there are several Zinc-Finger genes, *ZNF*. *RAREs* are composed of direct repeats (*DRs*) of a core motif *(A/G) G (G/T) TC (A/G)* separated by 0, 1, 2 or 5 nucleotides and referred to as *DR0*, *DR1*, *DR2* and *DR5*. Although *RA* induces differentiation and *hPSCs* consist of several cell populations with different degrees of potency, our hypothesis is that it might exist a subpopulation resistant to differentiation. We developed customized algorithm able to find *RAREs*, to distinguish *ZNFs* modulated by *RA*, based on *DRs* classification, in stem cells environment. To achieve a full overview of *ZNFs* regulated by *RA,* we integrated the results with a Master Regulator Analysis, *MRA*. In this analysis, we define *MRs* as genes that are sufficient and/or necessary to induce or describe a cellular state change. Firstly, we reconstruct a cellular network using *ARACNe*. Secondly, we define as target list all *ZNFsDR* previously classified and, thirdly we define *MR* genes. To validate our results, we analysed our ZNF-*MRs candidates* by RT-qPCR. We are presently focusing on silencing and overexpression of ZNF-*MRs*. RA-responsive-ZNF-*MRs* could be important target because they act as ligand-dependent TFs that regulate a variety of gene networks controlling cell differentiation and self-renewal

P.27

Metabolic alterations in a slow-paced model of pancreatic cancer-induced wasting

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Cancer cachexia is a devastating syndrome occurring in the majority of terminally ill cancer patients. Notably, skeletal muscle atrophy is a consistent feature affecting the quality of life and prognosis. To date, limited therapeutic options are available, and research in the field is hampered by the lack of satisfactory models to study the complexity of wasting in cachexia-inducing tumors, such as pancreatic cancer. Moreover, currently used *in vivo* models are characterized by an explosive cachexia with a lethal wasting within few days, while pancreatic cancer patients might experience alterations long before the onset of overt wasting. In this work, we established and characterized a slow-paced model of pancreatic cancer-induced muscle wasting that promotes efficient muscular wasting *in vitro* and *in vivo*. Treatment with conditioned media from pancreatic cancer cells led to the induction of atrophy *in vitro*, while tumor-bearing mice presented a clear reduction in muscle mass and functionality. Skeletal muscle atrophy was associated with several metabolic alterations as increased fatty acid oxidation, succinate dehydrogenase activity and mitochondrial ROS. Intriguingly, a stabilization of the hypoxia-inducible factor 1-alpha under normoxic conditions has also be observed. Taken together, we report a new model of cachexia and we identified metabolic alterations that can potentially be new therapeutic targets.

P.28

Modeling immunoediting in glioma progression

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The occurrence of a mutual reshape of tumor cell and immune system during tumor progression is a widely accepted notion in many different cancers including gliomas. The importance of this phenomenon in shaping glioma progression and the mechanisms governing it, however, are not fully elucidated. We used a well-characterized glioma model, based on somatic gene transfer of PDGF-B. Novel custom image-analysis tools allowed us to define the *in vivo* immune cell composition of gliomas at different stages of progression. Complementing this, genome-wide transcriptomics on purified tumor cells coherently pointed to the progression-related reorganization of glioma-immune system interactions. A set of *in vivo* tumor-propagating experiments in immune-competent or -deficient hosts allowed dissecting stage-specific roles of the immune system in shaping acquisition of malignancy in this model of glioma progression. Specifically, we show that the inability of cells from low-grade gliomas to propagate upon grafting in the brain of syngeneic immunocompetent mice, positively correlates with the abundance of infiltrating CD8+ lymphocytes in donor tumors and correlates with a highly immunostimulatory transcriptional profile, comprising genes typically involved in the immune response and inflammation, cytokine production and lymphocyte chemotaxis. Importantly, during tumor progression glioma cells downregulate these genes and the composition of their immune infiltrate accordingly shifts towards a pro-tumorigenic phenotype. Challenging low-grade and immune-stimulatory gliomas with grafting into immunodeficient (NOD/SCID) hosts revealed the crucial role of the adaptive immune system in constraining glioma progression. Finally, we observed that although progression still takes place in NOD/SCID mice, it is apparently slower, likely due to a far milder selection and reinforcing the view that the immune system constitutes a central player in shaping the rate of glioma progression.

P.29

PKR and GCN2 stress kinases promote an ER stress-independent eIF2α phosphorylation responsible for Calreticulin exposure in melanoma cells

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The immunogenic cell death (ICD) process represents a novel therapeutic approach to treat tumours, in which cytotoxic compounds promote both cancer cell death and the emission of damage-associated molecular patterns (DAMPs) from dying cells, to activate the immune system against the malignancy. Therefore, we explored the possibility to stimulate the key molecular players with a pivotal role in the execution of the ICD program in melanoma cells. To this aim, we used the pro-ICD agents mitoxantrone and doxorubicin and found that both agents could induce cell death and stimulate the release/exposure of the strictly required DAMPs in melanoma cells: i) calreticulin (CRT) exposure on the cell membrane; ii) ATP secretion; iii) type I IFNs gene up-regulation and iv) HMGB1 secretion, highlighting no interference by oncogenic BRAF. Importantly, although the ER stress-related PERK activation has been linked to CRT externalization, through the phosphorylation of eIF2α, we found that this stress pathway together with PERK were not involved in melanoma cells. Notably, we identified PKR and GCN2 as key mediators of eIF2α phosphorylation, facilitating the translocation of CTR on melanoma cells surface, under pro-ICD drugs stimulation. Therefore, our data indicate that pro-ICD drugs are able to stimulate the production/release of DAMPs in melanoma cells at least in vitro, indicating in this approach a potential new valuable therapeutic strategy to treat human skin melanoma malignancy.

P.30

ATM kinase activation phosphorylates HSP90β on T297 residue modulating HSP90-dependent HER2 protein function and stability in breast cancer cells

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ATM kinase plays a central role in the DNA Damage Response (DDR) and ATM heterozygosity has been linked to higher predisposition of breast cancer development, suggesting that ATM may indeed play a role as a tumor suppressor gene, most likely through its ability to control DDR. Nevertheless, more recent evidences suggest a dual role for ATM kinase in cancer raising the question whether ATM may contribute to a more general hijack of signalling networks in cancer.We recently demonstrated that this tumor suppressor gene could act as tumor promoting factor in HER2 positive breast cancer and moreover that ATM kinase sustains the Breast Cancer Stem Cell phenotype. At a molecular level, we have demonstrated that the ATM kinase is activated by HER2 and sustains the interaction between HSP90 and its client protein HER2, promoting its stabilization and therefore sustaining HER2-dependent tumorigenicity. HSP90 is an ATP-dependent molecular chaperone whose expression increases under stress conditions. It is therefore exploited by cancer cells to support the stability and the aberrant activity of oncoproteins either overexpressed or mutated in malignancy including HER2, BCR-ABL and EGFR. In line with this observation, HSP90 is one of the most actively pursued cancer drug targets and several different HSP90 inhibitors have entered clinical trials. Herein we further characterize the interplay between ATM and HSP90. We show that upon its activation, triggered by DNA damage or by HER2 overexpression, ATM kinase forms a complex with HSP90. Moreover, ATM activation drives the phosphorylation of HSP90β isoform on T297, promoting its functionality and preventing HER2 ubiquitination and degradation. Finally, ATM-dependent HSP90 phosphorylation is important to sustain vitality of breast cancer cell lines overexpressing HER2 receptor. Our data emerge a novel molecular mechanism through which ATM kinase sustains HSP90 chaperone activity and may therefore contribute to the control of protein quality and stability.

P.31

Gene regulation of VDAC isoforms in metabolic stress condition: NRF1 and HIFs transcription factors function

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VDAC (voltage dependent anion cannel) is a pore forming protein mainly located in the mitochondrial membrane. It is a channel involved in solutes exchange between mitochondria and the rest of the cell regulating metabolic and energetic mitochondrial function in normal and pathological condition. An intriguing aspect of VDAC expression is the identification in mammalians of three isoforms with differentiated channel properties but still with unclear biological roles. To investigate on VDAC isoforms function, in a contest related to metabolic and energetic mitochondrial dysfunction and adaptation, we analyzed gene expression and regulation inducing cell stress condition by nutrient and O_2 deprivation, common features in pathology as tumors or neurodegenerative disease. Interestingly VDAC1 and VDAC2 expression levels increased in a time related manner while VDAC3 transcript level remained unchanged or was slightly down-regulated.
Using a bioinformatic approach, we performed a prediction analysis of transcriptional factors binding on VDAC promoters. In particular, we addressed our attention on NRF1 and HIFs motifs recognition identification, respectively involved in mitochondrial biogenesis induced by nutrient depletion and in cell response to O_2 deprivation. All three promoter analyzed present NRF1 binding sites but with a major numerical distribution and statistical significance on VDAC3. In contrast only VDAC1 and VDAC2 promoters present HIFs motif recognition. We hypothesize a different transcriptional control of VDAC3 gene expression probably correlated with transcription factor which could repress NRF1 activation or compete with its overlapping binding sites identified on the sequence. In conclusion for the first time we propose a study on regulatory mechanisms controlling VDAC isoforms expression in metabolic stress condition affecting mitochondrial functionality.

P.32

MDM4 function and its prognostic potential in ovarian cancer progression

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Epithelial ovarian cancer (EOC) is the fifth leading cause of cancer death among women and overall prognosis has remained poor over the last 30 years. To date, minimal residual disease is the best available prognostic factor. MDM4 (MDMX) is a well-known inhibitor of p53 with tumour-promoting activities. A recent genome-wide association study of >4000 tumour samples described a SNP in the MDM4 3'-UTR as a susceptibility locus of breast and prostate cancer. This SNPs has been associated with decreased MDM4 C allele expression.
Others reports showed that high MDM4 mRNA and protein levels correlate with chemosensitivity in wild-type p53 EOC tumors and a high proportion of invasive ovarian carcinoma (48%) shows no detectable full-length MDM4 protein expression. Molecular studies evidenced the MDM4 ability to confer cell sensitivity to platinum, the elective treatment for patients with EOC. These data suggest a relevant role of MDM4 in EOC and in tumors treated with platinum based therapies.
To validate these data and get molecular insights on the MDM4 function, we have modulated MDM4 levels by using different EOC cell lines cultured in 2D and 3D systems. Of interest, modulation of MDM4 in EOC cell lines leads to changes in cell migration, invasion and adhesion, all signatures of cancer cell aggressiveness. To further understand MDM4 mechanism of action, we have performed a comparison of EOC cell lines expressing different levels of MDM4 by shotgun proteomic analysis, whose data are under evaluation.
Further analysis of human samples showed that MDM4 is highly expressed in the ovary tissue of healthy specimens whereas its expression is decreased in borderline ovarian tumors and in patients with EOC resistant to chemotherapy, independently of p53 status. Overall, these data suggest that MDM4 may be a candidate marker of good prognosis in patients with EOC with the possibility to tailor patient treatment more precisely.

P.33

Genetic barcoding reveals the clonal dynamics of glioma progression

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During tumor progression, transformed cells accumulate mutations and gain malignancy. Despite this process has been extensively studied, it is still unclear how easily transformed cells can undertake the whole progression and gain full malignancy.
To measure the probability of glioma progression we used a well-characterized murine model of gliomagenesis, induced by overexpressing PDGF-B in embryonic brain to mimic the first hit of gliomagenesis. In order to univocally tag each PDGF-transduced cell, we added to PDGF-transducing vector a degenerated barcode sequence. High-complexity libraries of barcoded retroviruses were injected in mouse embryos, and glioma masses were harvested at different stages.
Next generation sequencing of barcoded gliomas revealed a strong bottleneck in glioma progression, since from thousands of initiated cells, just hundreds of them are able to undertake the first stages and, more strikingly, just few of them gain full malignancy and reach the high grade stage.
To further study the clonal aspects of progression, we collected PDGF-B expressing cells from a low-malignancy tumor and orthotopically transplanted them in multiple mice. Interestingly, we found that the same pool of clones is able to evolve in a non-predetermined manner, indicating progression as a gradual evolution rather than the selection of pre-existing malignant clones

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Different mRNA localization patterns for metallothioneins (MTs) during embryo development

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MTs play pivotal roles in physiological and redox homeostasis. They are also essential during embryo development of *P. lividus* sea urchin. At least five MT genes are expressed in *P. lividus* embryo.
MT7 and MT8 are constitutively expressed; while, MT4, 5, and 6 are considered as metal-induced homologues. Whole mount *in situ* hybridization (WMISH) defined the MT mRNAs localization across the embryo territories of the sea urchin. At the gastrula stage, MT7 is localized principally in the endomesoderm, in the vegetal pole. Progressively, it becomes heavily expressed in the endoderm during archenteron specialization in midgut and hindgut and then in stomach and intestine at the pluteus stage. In contrast, at the gastrula stage MT8 appears strictly localized in the oral ectoderm and in the ventral region of the ciliary band. At the pluteus stage, it is mainly localized in a narrow strip of cells between the anal arms of the larva (the boundary between oral and aboral ectoderm) and lightly in the oral ectoderm.
Inducible MTs are usually undetectable by WMISH albeit MT6 appeared at the pluteus stage only in couples of cells at the tips of the elongating anterolateral and post-oral skeletal rods. After metal exposure, inducible MT expression is detected only in mesenchyme cells, no matter where they were delocalized with respect to their correct arrangement in the blastocoel by the specific metal treatment. Interestingly, hybridisation signals appear punctiform, looking as grouped and anchored in specific structures or accumulated in vesicles. The PMCs indeed contain electron-dense granules (named calcein puncta) which correspond to calcium-rich vesicles that contain nanospheres of calcium carbonate necessary for the larval skeleton formation. A preliminary bioinformatics search for cis-acting localizing elements (zip-codes) and secondary structures recognized by RBPs is giving promising results for the unveiling of mechanisms leading to transcript localization.

P.35

HIPK2 and extrachromosomal histone H2B are separately recruited by Aurora-B for cytokinesis

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Cytokinesis, the final phase of cell division, is necessary to form two distinct daughter cells with correct distribution of genomic and cytoplasmic materials. Its failure provokes genetically unstable states, such as tetraploidization and polyploidization, which can contribute to tumorigenesis. Aurora-B kinase controls multiple cytokinetic events, from chromosome condensation to abscission when the midbody is severed. We have previously shown that HIPK2, a kinase involved in DNA damage response and development, localizes at the midbody and contributes to abscission by phosphorylating extrachromosomal histone H2B at Ser14. Of relevance, HIPK2-defective cells do not phosphorylate H2B and do not successfully complete cytokinesis leading to accumulation of binucleated cells, chromosomal instability, and increased tumorigenicity. However, how HIPK2 and H2B are recruited to the midbody during cytokinesis is still unknown. Here, we show that regardless of their direct (H2B) and indirect (HIPK2) binding of chromosomal DNA, both H2B and HIPK2 localize at the midbody independently of nucleic acids. Instead, by using mitotic kinase-specific inhibitors in a spatio-temporal regulated manner, we found that Aurora-B kinase activity is required to recruit both HIPK2 and H2B to the midbody. Molecular characterization showed that Aurora-B directly binds and phosphorylates H2B at Ser32 while indirectly recruits HIPK2 through the central spindle components MgcRacGAP and PRC1. Thus, among different cytokinetic functions, Aurora-B separately recruits HIPK2 and H2B to the midbody and these activities contribute to faithful cytokinesis.

P.36

Molecular insights on nuclear localization of the FSHD candidate gene, DUX4

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Facioscapulohumeral muscular dystrophy (FSHD) is an inherited myopathy associated to contraction of the D4Z4 repeat array in the subtelomeric region of chromosome 4q35 (FSHD1) or to the presence of SMCHD1 mutations (FSHD2). These events lead to local chromatin relaxation that in presence of a polyadenylation signal (PAS) causes aberrant expression of the DUX4 retrogene. DUX4 exerts its pathogenic activity in the cell nucleus by activating downstream transcriptional targets. The mechanism of DUX4 nuclear entry is still unascertained. A recent report from our group demonstrated that in vitro muscle differentiation of myoblasts from FSHD patients is improved by the presence of estrogens, that are able to promote DUX4 displacement from cell nucleus and impair its transcriptional function and consequent pathogenic activity. Accordingly, FSHD is characterized by extreme variability in symptoms, with females being less severely affected than males and presenting a higher proportion of asymptomatic carriers.
To understand the mechanism of DUX4 nuclear displacement by estrogens, we have performed comparative analysis of the DUX4 interactome in presence/absence of 17β -estradiol (E2) during myoblast differentiation. This analysis showed that DUX4 is associated with transport receptor (importin-α) and members of the nuclear basket (Nups). Of interest, comparison of the interactome of E2-treated vs -untreated cells showed that hormone treatment significantly reduces the levels of these proteins associated to DUX4. Co-immunoprecipitation experiments confirmed the interaction of DUX4 with endogenous Nups and their dissociation upon estrogens treatment. Moreover, exogenous co-expression of DUX4 and Nups is able to increase DUX4 transcriptional activity and estrogen treatment is able to counteract this activity only in the presence of Nup. These data highlight a novel mechanism that underlies DUX4 nuclear import, and support the disease modifying activity of estrogens in FSHD

P.37

A computational approach for reconstruction of human pluripotency gene regulatory network

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Pluripotent Stem cells (PSCs) are characterized by the ability to form all the differentiated cell types found in the adult organism and by the capacity to self-renew *in vitro* virtually indefinitely.
The choice between differentiation and self-renewal is affected by external cues such as the TGFb and FGF signals. Moreover, several pluripotency regulators have been identified but little is known about their interplay with external signals and their role during differentiation. The aim of this project is to understand how human PSCs compute external signals in order to choose between self-renewal and differentiation. To do so I built a computational model of the human pluripotency Gene Regulatory Network (GRN). I exploited the Reasoning Engine for Interaction Networks (RE:IN), a Microsoft Research online tool, to generate a model consisting of functionally validated components and interactions able to recapitulate observed human PSCs behaviour. First, I compared different type of data suitable for the derivation of interactions (e.g. ChIP-sequencing, motif enrichment analysis, co-expression studies) and found those allowing to build a model consistent with a set of experimental constraints with also a good predictive power. Second, I expanded my model by the addition of two novel pluripotency regulators identified in our laboratory. Such expanded model was used to formulate predictions on the effect of combined inactivation of different GRN components. This model will be used to study and optimize the process of induction of pluripotency (reprogramming) and to elucidate how PSCs differentiate toward different germ layers.

P.38

UHRF1 is involved in mono-ubiquitination of H2BK120 in Colorectal Cancer Cells

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UHRF1 is a master regulator of the epigenome as it coordinates DNA methylation and histone post-translational modifications (PTM). In this study, we investigated the role of UHRF1 in the ubiquitination of histone H2B at lysine residue 120 (H2BK120ub), a prominent PTM. Despite the progress in understanding the role of H2B in gene transcription regulation, the function of this specific modification remains poorly defined. Based on chromatin immunoprecipitation followed by DNA sequencing (ChIP-seq) analysis, peaks of H2BK120ub were identified both upstream and downstream of transcription start sites (TSS) and across gene bodies, underpinning a role in transcriptional elongation and/or repression. We correlated H2BK120ub with UHRF1 and showed that modulating UHRF1 affected also H2BK120ub levels. To investigate the possible crosstalks with ubiquitinases (RNF20, RNF40) and deubiquitinases (USP7), we specifically silenced each single protein and evaluated the interactions with UHRF1 in RKO cell line. UHRF1silencing was associated with USP7, RNF20 and RNF40 overexpression and a remarkable decrease of H2BK120ub. Interestingly, silencing of RNF20 and RNF40 was tied to a conspicuous reduction of H2BK120ub, despite that UHRF1 levels remained unchanged. These results suggested that UHRF1 is structurally bound to all these proteins as further confirmed by co-immunoprecipitation experiments. By transfecting specific deletion mutants in cells silenced for UHRF1 we also demonstrated that the RING domain of UHRF1 is capable to rescue the H2BK120ub levels through the recruitment and binding to the proteins of the complex. Finally, H2BK120ub has been associated with chromatin changes occurring at inducible enhancers upon hormone stimulation. Experiments are ongoing to verify whether this hypothesis holds true also for other nuclear receptors and whether UHRF1 plays any role in the chromatin modifications underlying these events in a CRC cell model system.

P.39

Large-scale analysis highlights the role of epigenetics in Facioscapulohumeral muscular dystrophy (FSHD)

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Facioscapulohumeral muscular dystrophy (FSHD), the third most common hereditary muscular disorder, is associated to an epigenetic defect in the chromosome 4q subtelomere. Two clinically indistinguishable forms of FSHD are known, FSHD1 and FSHD2. FSHD1 is an autosomal dominant disorder associated with the contraction of the highly polymorphic D4Z4 macrosatellite repeat array on permissive 4qA (PAS+) chromosomes. FSHD2 is caused by mutations in the D4Z4-binding modifier SMCHD1 gene. Both FSHD forms are associated to D4Z4 DNA hypomethylation on 4qA allelic chromosomes. These epigenetic changes provide a transcriptionally permissive chromatin environment leading to the production of a pathogenic protein DUX4. The analysis of the correlation of DNA methylation levels and genetic features of FSHD has been largely impaired by the presence of multiple non-permissive alleles and by a highly homologous locus on chromosome 10. We investigated the epigenetic changes of FSHD, developing methylation assays specific for permissive chromosomes 4qA (PAS+). The analysis of a large cohort of individuals (consisting of 287 FSHD1, 53 FSHD2 and 165 control subjects) showed highly significant difference of methylation levels between affected and control subjects further supported by strong correlation with the number of D4Z4 repeats both in FSHD1 and FSHD2. Moreover evaluation of intra-D4Z4 methylation and other SMCHD1 target sequences provides an effective approach to evaluate the severity of SMCHD1 mutations.
This study indicates the region distal to the D4Z4 array as a critical region summarizing the effect of multiple factors affecting the epigenetic changes in FSHD.

P.40

Identification and characterization of Lin28 molecular complexes regulating mRNA recognition and translation in embryonic stem cells

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In the last years, the RNA-binding protein Lin28a is emerged as a crucial regulator of embryonic stem cell (ESC) differentiation as well as a key factor in the development of many tumors. Lin28 proteins can act through two main different mechanisms: miRNA-dependent and miRNA-independent. In biological contexts in which the let-7 miRNAs are expressed, Lin28 proteins mainly work to block the biogenesis of these miRNAs, that results in the upregulation of let-7 targets. In absence or low levels of let-7, Lin28a can directly bind hundreds of mRNAs and positively and negatively regulates their translation through a miRNA-independent mechanism. This is particularly relevant in the modulation of ESC differentiation and in some tumors, where Lin28 effects of are not erased by let-7 overexpression thus suggesting the existence of let-7-independent pathways. We have recently demonstrated that Lin28a negatively regulates the translation of Hmga2 mRNA during the first step of ESC differentiation, i.e. the establishment of epiblast-like stem cells (EpiLCs). Interestingly, we have also found that Lin28a positively controls the translation of a key factor of differentiation: the DNA methyltransferase a (Dnmt3a). Lin28a allows the accumulation of Dnmt3a in the establishment of EpiLCs by directly binding at least two different regions on 3'UTR of Dnmt3a mRNA. These observations, coupled with several papers from other groups strongly support our working hypothesis: Lin28 mediates the interaction of many mRNAs with different molecular machineries to enhance or block translation. How Lin28 achieves this incredibly complex orchestration of its targets remains to be determined. To understand the mechanisms through which Lin28a fulfills these different regulation mechanisms we are working to characterize its protein interactome network in the model system of ESC differentiation into EpiLCs.

P.41

New potential circulating microRNA biomarkers for the detection of prostate cancer

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MicroRNAs are short single-stranded non-coding RNAs which can be released in the bloodstream and whose expression can be altered in cancer. In recent years the use of circulating microRNAs as cancer biomarkers has been proposed. In this study we identified circulating microRNAs that could be used as biomarkers for the detection of prostate cancer. We used a public dataset (GEO accession GSE61741) composed of microRNA expression profiles of peripheral blood of patients with various diseases and healthy controls. We extracted from this dataset the expression profiles of 65 prostate cancer patients and 103 healthy patients and we split them in a training set and a validation set. We tested three different methods for the classification of samples: random forest, Lasso regression and SCUDO, a rank-based signature identification method recently developed. The random forest and the Lasso regression performed equally well in classification (AUC = 0.92 and 0.91, respectively). SCUDO was also able to classify correctly most of the samples, with a sensitivity of 0.64 and a specificity of 0.94. Lasso regression produced a signature of 17 microRNAs that act as predictors. To gain insight on the role of these microRNAs in prostate cancer we used the recently proposed web-based tool miRNet to retrieve the validated targets of these microRNAs and we performed a functional enrichment analysis using the pathways in the KEGG database. We identified 23 pathways as significantly enriched (adjusted p-value < 0.05), among which the pathways: Cell cycle, p53 signaling, mTOR signaling, Prostate cancer and Wnt signaling. We performed a similar analysis using a list of 13 microRNA predictors obtained from the random forest, identifying 19 significantly enriched pathways. Interestingly, the overlap between the two lists of pathways (11 pathways) is larger than the overlap between the two lists of microRNAs (6 microRNAs). Our results are promising but warrant further experimental validations.

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The “Charming” regulation of muscle development and heart remodelling by Charme lncRNA

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The current view of coordinated gene expression suggests the existence of chromosome territories acting as discrete regulatory units. By Next-Generation RNA sequencing on murine myoblasts and differentiated myotubes, we recently identified an atlas of novel lncRNAs expressed at different stages during in vitro skeletal muscle differentiation (Ballarino et al, MCB 2015). Within this collection, the attention towards lnc-405, renamed as Charme (for Chromatin architect of muscle expression) was prioritized based on several peculiarities (Ballarino et al., submitted). At variance with most characterized lncRNAs, Charme is an abundant and highly conserved non-coding transcript specifically required for in vitro differentiation of myoblasts into myotubes. Mechanistically, we found that, in the nucleus, Charme acts as a structural RNA, which contributes to the formation of Chromosomal territories where coordinated expression of pro-myogenic genes occurs. In line with this, Charme knock-down produces the disassembly of specific chromosomal loops and the downregulation of myogenic genes therein contained, including genes linked to cardiomyopathies (Ballarino et al., submitted). Indeed, genetic ablation of Charme in vivo produces a macroscopic pathological phenotype in which the global architecture of the murine heart is altered. Moreover, we identified a human highly conserved Charme counterpart which regulates the same subset of target genes. Altogether, these data show that Charme regulates the chromosomal architecture and the expression of myogenic loci controlling muscle differentiation and heart remodelling.

P.43

The nucleoporin RANBP2 regulates protein SUMOylation in time and space during mitosis

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Protein conjugation with SUMO (small ubiquitin-like modifier) peptides is a post-translational modification that modulates protein interactions and localization. Growing evidence implicate protein SUMOylation in control of key processes during mitosis. Many kinetochore proteins are targets of SUMO conjugation in vitro. In addition, several de-SUMOylating enzymes reside at kinetochores, suggesting that cycles of SUMOylation and de-SUMOylation can take place therein.
RANBP2 is a nucleoporin with SUMO-ligase and SUMO-stabilizing activity and, after nuclear envelope breakdown, it is required in several mitotic steps. Here we have sought to identify RANBP2-guided SUMOylation processes in mitosis. We show that:
a) a subtle mechanism of spatial control regulates the localization of RANBP2 in mitotic cells;
b) a fraction of RANBP2 associates with kinetochores and therein regulates Topoisomerase II-alpha SUMOylation, which is required for decatenation of sister kinetochores prior to chromosome segregation;
c) RANBP2 also regulates SUMOylation of Aurora B, a mitotic kinase acting in the correction of erroneous kinetochore-microtubule interactions and therefore essential for balanced chromosome segregation. Indeed, expression of a SUMO-null Aurora B mutant results in uncorrected chromosome segregation errors. RANBP2 depletion inhibits Aurora B activity at kinetochores, placing RANBP2 in this pathway.
These results reveal a key role of RANBP2 in local SUMOylation of mitotic regulators at kinetochores. The failure to ensure spatial and temporal control of this post-translational modification impairs several independent yet converging pathways in mitotic control, and can originate genetic instability in daughter cells.

P.44

A novel potential class of biomarkers for diagnosis and treatment of systemic lupus erythematosus (SLE) using induced pluripotent stem cells

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SLE is an inflammatory autoimmune disease characterized by the production of autoantibodies against nuclear components. The heterogeneous nature of SLE suggests an interplay between genetic susceptibility and environmental factors. It is known that oxidative stress may cause lupus flares by inhibiting Erk pathway signaling in T cells, leading to *DNMT1* reduction and DNA demethylation.
Here, we used SLE and healthy control -derived induced pluripotent stem cells (hiPSCs) whose DNA methyltransferase activity was comparable. Gene expression analysis provides evidences of Erk and Akt pathways as molecular mechanisms involved in SLE pathogenesis. Modulation of these pathways allowed us to the identification of a novel cohort of potential biomarkers for diagnosis and prevention of SLE such as *CHCHD2*, *IDO1*, *S100A10*, *EPHA4* and *LEFTY1*. We further investigated on oxidative stress-related miRNAs, demonstrating a dysregulation of miR-497 and miR-29a in SLE-derived hiPSCs. Interestingly, mimicking SLE oxidative stress conditions *in vitro* by adding peroxide hydrogen, miR-424 was markedly reduced in SLE-derived hiPSCs. Finally, the pre-treatment with the antioxidant ascorbic acid on oxidative stress condition produced a discordant expression trend of miR-192 and miR-152 between SLE- and healty hiPSCs.
Identification of SLE biomarker candidates by gene expression analyses on multiple datasets encourages us to insight in the diagnosis and potential therapeutic applications for the treatment of SLE pathology.

P.45

TRF1 PARylation by PARP1 is required for the accomplishment of telomere replication

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PARP1 is the most abundant chromatin-associated protein (1–2 million copies/cell) after histones. It is activated upon DNA damage and in turn regulate different pathways of DNA repair. PARP1 is also a regulator of chromatin structure being a component of insulator complexes, and consequenty modulating transcription. At telomeres, the current literature highlights a specific role for PARP1 in telomere homeostasis. It binds to the telomeric repeat binding protein TRF2 and its partner RAP1 and this binding was reported to repress homologous recombination (HR) at telomeres to avoid loss of telomere repeats. PARP1 is also activated by replication fork stall upon a replication dependent damage where it promotes HR dependent repair against Non Homologous End Joining (NHEJ) and fork restart. At telomeres, the increased topological stress generated by G-quadruplex stabilization was reported to increase PARP1 recruitment at telomeric sites. Since telomeres and hetherochromatin topology in general represent a source of difficulty for DNA replication, we asked wether PARP1 could play a role in assisting telomeric replication in absence of genotoxic stress. Here, we show that endogenous TRF1 and TRF2 associate with PARP1 in a cell-cycle dependent manner. In particular, since TRF1 is essential for telomere replication and its lack induces telomere fragility, we asked wether TRF1 could be directly parylated by PARP1 and if this modification could impact on TRF1 binding to chromatin during replication. We showed that TRF1 is a direct PARP1 target for heteromodification, with a parylation site different from the Tankyrase I substrate (wich is implicated in telomerase dependent telomere elongation). In addition, PARP1 interference impinge on TRF1 dynamics at replicating telomeres and induces a huge increase of telomere fragility, shown by telo-FISH analysis on metaphase spreads. Interestingly, PARP1 inhibition per se affect telomere stability in a higher extent compared to TRF1 alone, expecially in terms of induction of telomere fusions. This suggests that PARP1 activity could directly or indirectly modulate more than one component of telomere replication machinery to allow the accomplishment of telomere replication.

P.46

PI3K-C2α loss promotes hepatic damage in adult mice

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PI3K signaling axis is involved in several processes such as proliferation, survival and metabolism. Emerging evidences highlight the involvement of class II enzymes in a multitude of different signaling pathways however their specific function in the cell is still unclear. Recent genetic and biochemical studies have pointed out the non-redundant roles of α isoform of class II PI3Ks (PI3KC2α), which produces PtdIns3P and PtdIns(3,4)P2, critical regulators of membrane dynamics and vesicular trafficking. Since we previously demonstrated that deletion of Pik3c2a is embryonic lethal, we systemically targeted its expression in adult mice. CAGGCre-ER mice, carrying a tamoxifen-inducible Cre recombinase, were crossed with Pik3c2afl/fl mice. The systemic deletion of Pik3c2a strongly impaired the survival of Pik3c2afl/fl/CreER mice, which died within 4 days after tamoxifen delivery. We demonstrated that the rapid lethality was caused by a severe hepatic damage, associated with high apoptotic rate and lipid droplets enrichment. This increased hepatic lipid deposition is caused by defects in vesicular trafficking in Pik3c2afl/fl/CreER livers. Given that the pool of PtdIns3P produced by PI3K-C2α is required to induce autophagy, a strategy used by hepatocytes to adjust their cellular metabolic capacity, PI3K-C2α may be a critical node connecting the endocytic and autophagic pathways.

P.47

A CRISPR knockout EGR1 model in the neuronal cell line SH-SY5Y

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The transcription factor EGR1 is an immediate-early gene often used as an indirect marker to measure neuronal activity. It has been hypothesized to have a role in many biological processes in neuronal cells, such as neuronal plasticity and development of learning and memory. The expression of EGR1 is often altered in neurodegenerative diseases and psychiatric disorders including Alzheimer's disease and schizophrenia. However, the role of EGR1 and the transcriptional programs under its control in neuronal development are not fully understood. In this regard, we established a neuronal cell model with a defective *EGR1* gene. We generated a knockout cell (KO) line for *EGR1* gene using one of the most popular neuronal cell line, the SH-SY5Y, that can be differentiated to a more mature neuron-like phenotype using retinoic acid (RA). *EGR1*-KO cells show clear morphological differences compared to WT cells, particularly evident for neurite arborization. In addition, *EGR1*-KO cells show higher proliferation and migration rate compared to WT cells. Under different stress conditions, such as serum withdrawal and H_2O_2-induced oxidative stress, KO cells have higher survival rate than WT cells. A preliminary molecular analysis demonstrated that KO cells react to stress-induced conditions likely involving autophagy process in contrast to WT cells. Surprisingly, *EGR1*-KO cells undergo cell death after RA treatment in contrast to WT cells that undergo neuronal differentiation. Molecular analyses of the *EGR1*-KO model show a dysregulation of many differentiation markers, including those related to neurite maturation and synaptogenesis. In fact, the western blotting analysis shows a higher level of GAP43 and SYT1 proteins in KO cells compared to WT. Therefore, *EGR1* knockout cell model obtained by CRISPR/Cas9 method may represent an interesting model for studying the molecular mechanisms regulated by *EGR1* for the proper development, differentiation, and survival of neurons.

P.48

Transient exposure to the antiangiogenic drug Axitinib induces senescence of endothelial cells

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Therapy-induced cell senescence is a double-edged sword, which restricts malignant cells replication but may promote tumor growth through the senescent associated secretory phenotype (SASP) of transformed or normal cells. Axitinib is an antiangiogenic drug that acts as tyrosine kinase inhibitor targeting VEGF receptors. Recent studies demonstrated that chronic treatment with Axitinib induces senescence of transformed cells *in vitro*. Here we investigated whether a transient exposure to Axitinib similarly induces cell senescence of its principal cellular target, endothelial cells. Early passage Human Umbilical Vein Endothelial Cells (HUVECs) were exposed for 1 hour to a pulse of Axitinib and, 4 days later, senescent cells were quantified by acidic β-galactosidase assay. More than 65% of the cells stained positive in Axitinib as well as Doxorubicin treated HUVECs, used as a reference of senescence induced by genotoxic stress. In tubulogenesis assay, HUVECs treated with both drugs showed a scarce tube formation ability associated to senescence. As revealed by RNAseq analysis, Axitinib- and Doxorubicin-senescent HUVECs share many similarly regulated genes and, at the same time, they express context-specific transcriptome. Gene Set Enrichment Analysis showed a significant enrichment in the inflammatory response and in the p53 pathway hallmark genesets in Axitinib- and Doxorubicin-treated HUVECs, respectively. It is conceivable that Axitinib and Doxorubicin promote different SASPs whose biological consequences need to be investigated.
Moreover, since oxidative stress is both a hallmark and an inducer of cell senescence, we measured ROS levels in Axitinib- and Doxorubicin-treated HUVECs. A significant and long-lasting increase in intracellular ROS follows both treatments. Surprisingly, buffering ROS production during the drug pulse prevents Axitinib- but not Doxorubicin-dependent senescence, both in terms of β-galactosidase positivity and in the gene expression profile.

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Role of cholesterol in caveolin-1 trafficking between plasma membrane and exosomal secretion in melanoma cells

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Caveolin-1 (Cav-1) is a fundamental constituent of caveolae, specialized invaginations of the plasma membrane, rich in glycosphingolipids and cholesterol, involved in different cellular processes. The functionality and structure of the caveolae are strictly dependent on cholesterol. The U18666A inhibitor produces an abnormal accumulation of cholesterol in the endolysosomal compartments due to a blockage in the transport of intracellular cholesterol. The endolysosomal system is characterized by a set of specialized vesicular bodies involved both in the endocytic process and in the extracellular communication through the Multi Vesicular Bodies (MVBs), responsible for the formation and release of exosomes. The exosomes are nanosized vesicles of 30-100 nm that are secreted by all cell types and are implicated in different physiological and pathological processes. In the present work, the U18666A inhibitor was used to study the role of cholesterol in Cav-1 trafficking investigating its implications in the endolysosomal degradative-secretory system in metastatic human melanoma cells. Furthermore, we used an innovative methodology which allows the production of nascent fluorescent vesicles. The results showed, after the blockage of cholesterol, an increase in the production of exosomes with chemical-physical characteristics similar to the control exosomes but with a different protein composition (lower expression of Cav-1, increase of CD63 and LC3II) and an impaired transfer capacity. Finally, functional assays demonstrated a lower migration capacity of the treated cells. We hypothesized that the blockage of cholesterol transport, determining the internalization of Cav-1, is important in the regulation of autophagy and alteration of the exosomes secretory pathways through an increase in fusion between autophagosomes and MVBs, inducing, as a response in cells, a secretion of the contents of these organelles to maintain cellular homeostasis.

P.50

PTBP1 promotes *FOXP2* alternative splicing in Hek293 cells

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"Language" gene *FOXP2* major trascripts are *FOXP2 full-length (FOXP2-FL)*, comprising 17 exons plus 2 alternatively spliced exons (3b and 4a) and a shorter variant ending with an elongated exon 10 (*FOXP2-10+*). Since little is known on the regulation of *FOXP2* alternative splicing, we undertook a study on the ribonucleoproteins involved. *In silico* analysis showed putative binding sites for the Polypyrimidine-Tract Binding protein 1 (PTBP1) in *FOXP2* introns flanking exon 3b but also exon 11. We overexpressed PTBP1 in Hek293 cells and performed a semi-quantitative RT-PCR (RT-SqPCR) on *FOXP2* transcripts. Untransfected cells showed three major transcripts: *FOXP2-10+*, *FOXP2-FL* and a novel low abundant transcript 200 bp shorter than *FOXP2-FL*, missing exon 11 (*FOXP2-Δ11*). Interestingly, overexpression of PTBP1 caused a diminution of *FOXP2-FL* and an increase of *FOXP2-Δ11* transcripts. Sequencing of *FOXP2-Δ11* transcript revealed a premature stop codon within exon 12, questioning its function. To confirm PTBP1 effect on *FOXP2-Δ11* generation, we: 1) created a *FOXP2* minigene by cloning the 7.5 kb genomic sequence between exons 9 and 13 in pcDNA3.0; 2) co-trasfected *FOXP2* minigene together with PTBP1; 3) performed RT-SqPCRs using T7 and SP6 primers amplifying only *FOXP2* minigene transcripts. As a result, we detected almost exclusively the transcript missing exon 11, confirming exon-skipping actions by PTBP1 on *FOXP2*. Of note, protein analysis showed a significant decrease of FOXP2-FL while the predicted protein for translated *FOXP2-Δ11* transcript was not detected. The latter observation together with *FOXP2-Δ11* sequence analysis lead us to the hypothesis of a controlled degradation of *FOXP2-Δ11* transcripts via Non Sense mediated Decay (NMD) that we are now testing. Interestingly, we detected *FOXP2-Δ11* transcripts also in H4, T47D and MDA cells. We propose a regulatory role for the newly identified *FOXP2-Δ11* variant in controlling the levels of FOXP2-FL expression, mediated by PTBP1.

P.51

Caspase-8 expression promotes neo-angiogenesis, tumor progression and chemotherapy resistance in human glioblastoma

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Caspase-8 (C8) is a key player in extrinsic apoptosis and its activity is often downregulated in cancer. However, human C8 expression is retained in some tumors, including glioblastoma, suggesting that it may support cancer growth in these contests.
Glioblastoma is the most aggressive primary brain tumor in nervous system and it is associated with a poor prognosis. Here we present data on a novel signal pathway through which C8 sustains neoplastic transformation in human glioblastoma cellular models. We could show that C8 promotes neo-angiogenesis and tumor growth in vitro and in vivo. C8 promotes the activation of NF-kB transcription factor, which in turn enhances the expression and secretion of a panel of angiogenic cytokines such as VEGF, IL-6, IL-8, IL-1β and MCP-1, leading to neovascularization and increased resistance of glioblastoma cells to temozolomide (TMZ), a chemical compound used in chemotherapy. Importantly, the bioinformatics analysis of microarray gene expression data derived from a set of high-grade human gliomas, showed that high C8 expression levels correlate with increased expression of the aforementioned cytokines and with a worse prognosis.
We previously demonstrated that tyrosin kinase Src is able to phosphorylate C8 on Tyr380 in some tumor contests (such as colorectal carcinoma, hepatocarcinoma and glioblastoma) as a molecular switch to downregulate the apoptotic role and to turn on non-canonical tumorigenic functions of C8. Results obtained through preliminary experiments suggest that C8 phosphorylation on Tyr380 contributes to modulate NFkB localization in glioblastoma, strengthening the idea that C8 and its phosphorylation may be involved in promoting the aggressive glioblastoma phenotype and resistance to TMZ treatment.
The modulation C8 functions may represent a novel target for cancer therapy, so future experiments will investigate the possibility to develop strategies to inhibit C8 cancer boosting activity.

P.52

Regulation of catalase expression in chronic lymphocytic leukemia cells

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Catalase is a well-studied enzyme that plays a pivotal role in protecting cells against the toxic effects of reactive oxygen species (ROS). The main function of catalase is to decompose hydrogen peroxide into water and oxygen. Under physiological conditions, production of ROS is counterbalanced by their elimination avoiding the alteration of cell homeostasis due to excessive ROS levels. Deregulated rates of ROS play an important role in human diseases, including cancer. Cancer cells show elevated levels of ROS probably due to increased metabolic activity and mitochondrial dysfunction. We have recently demonstrated that catalase is differentially expressed in PBMCs derived by indolent or more aggressive subtypes of patients with chronic lymphocytic leukemia (CLL). The molecular mechanism controlling the different catalase expression and activity in CLL still has to be clarified. The aim of this study is to investigate the genetic, transcriptional and post-transcriptional processes that underlie the catalase expression in CLL subtypes. We started to analyze, in DNA extracted by isolated PBMCs from buffy coats of CLL patients, the presence of the single nucleotide polymorphism (SNP) -262 C/T (Id: rs1001179) in the promoter region of the catalase gene. This SNP is known to be associated with different levels of the catalase activity in cancer cells. We will proceed with the expression analyses by Real-time quantification of two transcription factors (Sp1, NF-Y) and selected microRNAs which are known to be involved in the catalase expression regulation in CLL cells. Finally, we will functionally validate the role of these regulatory factors on the catalase expression in MEC1 and MEC2 CLL cell models. This study may contribute to the understanding of the molecular mechanisms that regulate catalase expression and activity in CLL and to the development of therapies targeting redox pathways.

P.53

Neuronal circular RNAs in a mouse model of autism spectrum disorders

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Circular RNAs (circRNAs) represent a class of non coding RNAs characterized by a covalently closed structure, resulting from backsplicing reaction. The development of next generation sequencing (NGS) and new computational tools, has led to the identification of thousands of endogenous circRNAs in mammalian cells. Recent researches have revealed that circRNAs can function as microRNA sponges, regulators of splicing and transcription, modifiers of parental gene expression and scaffolds for RNA binding proteins, indicating a specific role in cellular physiology. circRNAs are highly expressed in the brain and potentially involved in plasticity mechanisms. The autism spectrum disorders (ASDs) are developmental disorders characterized by impairments in social interactions and communication, and by repetitive and stereotyped behaviuors and interests. It has been hypothesized that the deregulation of the activity-dependent signalling network at the synapses could represent the fundamental molecular component of this pathology. In this study we performed circRNA transcriptome analyses by NGS on hippocampal RNA of a well characterized mouse model for ASD (BTBR T+ Itpr3tf/J), compared to a control strain (C57BL/6J). NGS data allowed us to highlight the differential expression of specific circRNAs between the two experimental groups. We selected three ASD-modulated circRNAs for further characterization. Their structure has been validated by RNAse R treatment. Their expression has been confirmed by RT-qPCR in individual animal samples and analyzed in different brain structures implicated in the disease. In silico analysis revealed possible functions of selected circRNAs. Gene expression analysis combined with functional studies will enable us to elucidate the possible partecipation of circRNAs in neuronal signalling pathways, in order to better understand the molecular basis underlying ASDs.

P.54

Development of a screening system to identify phosphoinositide modulators required for hepatitis B virus life cycle

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Chronic infection with hepatitis B virus (HBV) is one of the most important global health problems and one of the main causes of chronic liver disease. 350 million people are affected by chronic hepatitis B and, despite the presence of an effective recombinant vaccine, every year there are about 1 million of new cases of infection.
Phosphoinositides (PtdIns) are phosphorylated derivatives of phosphatidylinositol and they are important phospholipid constituent of cell membranes. The inositol head can be reversibly phosphorylated at the 3, 4, and 5 positions thanks to the activity of kinase and phosphatases, specifically localized within the cell. PtdIns are secondary messengers responsible for transmitting receptor signals to effectors that induce a cellular response. They also act as regulators of several target proteins involved in vesicular trafficking and cytoskeletal \rearrangement, by which they control cell polarity, migration, proliferation and differentiation.
Considering the importance of PtdIns metabolism in cellular signaling and trafficking events, numerous intracellular pathogens modulate and exploit PtdIns in order to ensure their survival and efficient intracellular replication.
Many studies in literature have thus addressed the modulation of cellular phosphoinositides (PtdInsP) by viral infections such Hepatitis C virus (HCV), Dengue virus (DENV) and Ebola virus.
The aim of this project was to develop a high content pilot screening in order to identify PtdInsP modulators that could affect the HBV life-cycle. We took advantage of a library of shRNA-encoding lentiviral vector targeting several PtdIns -modulator genes. In order to both validate the methodology and to preliminarily identify PtdIns-modulators involved in viral life cycle, a pilot screen was performed in HepAD38 hepatoma cell line.

P.55

Epigenetic signature in T helper 17 and T regulatory cells in multiple sclerosis patients during pregnancy

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Relapsing Remitting Multiple Sclerosis (RRMS) shows a protective effect of pregnancy in relapse rates. Immune tolerance associated with pregnancy correlates with high levels of circulating estrogens. Therefore, estrogens may act on CD4+ T lymphocytes dynamics by remodeling chromatin hubs. Here we performed an integrative analysis of human CD4+ epigenomic and transcriptomic data. We identified cell type-specific regulatory regions (CSR) by combining super enhancers' prediction using H3K27ac ChIP-Seq data with a genome-wide chromatin states analysis. Selected CSRs, associated to a set of transcription factors, defined a core regulatory network in Th17 and Treg cells. Thus, estrogen response element found in CSRs, revealed potentially ERα-modulated core genomic regions in these cells. 17β-estradiol induced active histone marks enrichment at FOXP3-CSRs and repressive histone marks enrichment at RORC-CSRs in *in vitro* polarized Th17 cells. We validated this epigenetic profile in peripheral blood mononuclear cells of RRMS patients, suggesting a FOXP3 positive regulation in third trimester of pregnancy while RORC is positively regulated in the postpartum. Altogether these data indicate that estrogens act as immunomodulatory factors on the epigenomes of CD4+ T cells in RRMS; the identified CSRs may represent potential biomarkers for monitoring disease progression or new potential therapeutic target.

P.56

Sequencing human mRNAs from head to toe

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Although isoform choice of eukaryotic mRNAs is highly diverse, functionally important and subject to complex regulation, there is little progress in obtaining isoform information from complex in vivo samples. In addition to the mRNA sequence complexity that is obtained by alternative transcription start, splicing and termination, further diversity resides in the poly(A) tail, which is known to play a role in most of the key steps of post-transcriptional regulation. Here we present data obtained with a new method to sequence full-length mRNAs, including the poly(A) tail. With this method, we are able to observe genome-wide poly(A) length distributions consistent with previous reports, the internal nucleotide composition of the tails and mRNA isoform-specific changes in tail lengths.

P.57

Analysis of translation efficiency of TOP mRNAs in cultured cells during recovery from different kinds of stress

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In vertebrates, all of the mRNAs encoding for ribosomal proteins, elongation factors and several other proteins involved in the translational apparatus, contain a common 5'-terminal oligopyrimidine (5' TOP) tract, which is highly conserved among mammals. The pyrimidine tract starts with a cytosine residue (CAP site) and extends for 5-14 nucleotides; there is a similar proportion of C and U residues within the stretch and a CG-rich region immediately downstream of the 5'-TOP motif.
It has been shown that the perturbation of cellular environment is correlated to specific, rapid and reversible changes in TOP mRNA polysomal association. The TOP sequence has been demonstrated to be essential for translational regulation in response to different growth stimuli. In particular, the first 27 to 35 nt are sufficient to confer translational control on a reporter mRNA.
In general, TOP mRNAs are inefficiently translated in comparison to non TOP mRNAs. For this reason they are sometimes referred to as "weak" mRNAs. It is not clear if the low efficiency is dependent on some unknown specific factor or if it is a structural property of TOP sequence.
In order to investigate this issue, we developed an experimental setup to monitor the change of mRNA polysome association following culture medium alteration. We started with treatment of HeLa cells with an hypertonic medium followed by recovery in regular medium. The hypertonicity caused a ribosome run-off, whereas the recovery from hypertonicity caused a rapid reassociation of mRNA to polysomes. In this experimental setup we could follow the kinetics of polysome association of TOP mRNA with respect to non TOP mRNAs. Preliminary data indicate that TOP mRNA recover their polysome association more slowly compared to controls. We are now further addressing this issue by using other kinds of stress to induce ribosome run-off and recovery and by characterizing the signaling pathways involved.

P.58

Mechanism of translational control of the alternative *D. melanogaster* VDAC1 mRNA

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The eukaryotic porin, also called Voltage Dependent Anion-selective Channel (VDAC), is the main pore-forming protein of the outer mitochondrial membrane. In *Drosophila melanogaster*, a cluster of genes evolutionarily linked to VDAC is present on chromosome 2L. The main VDAC isoform, called VDAC1 (or Porin1), is expressed from the first gene of the cluster. The *porin1* gene produces two splice variants, 1A-VDAC and 1B-VDAC, with the same coding sequence but different 5′ untranslated regions (UTRs). We studied the influence of the two 5′ UTRs, 1A-5′ UTR and 1B-5′ UTR, on transcription and translation of VDAC1 mRNAs. In porin-less yeast cells, transformation with a construct carrying 1A-VDAC results in the expression of the corresponding protein and in complementation of a defective cell phenotype, whereas the 1B-VDAC sequence actively represses VDAC expression. Identical results were obtained expressing in *D. melanogaster* SL2 cells constructs containing the two 5′ UTRs upstream of the luciferase reporter gene. A short region of 15 nucleotides in the 1B-5′ UTR should be able to pair with an exposed helix of 18S ribosomal RNA (rRNA), and this interaction could be involved in the translational repression. Overall, our results suggest that contacts between the 5′ UTR and specific 18S rRNA sequences could modulate the translation of the alternative *Drosophila* 1B-VDAC mRNA. Interestingly, this mechanism is independent of the coding region cloned downstream of the 5′-UTR. Furthermore, our results show that the unproductive 1B-VDAC mRNA is able to respond to 1A-VDAC transcript levels, and thus it might work as a molecule signaling the need for activation of mitochondrial biogenesis.

P.59

Role of the stem cell associated transcription co-factor, ZNF521, in ovarian cancer cells

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The transcriptional co-factor Zinc Finger 521 (ZNF521) is expressed in normal and malignant stem cells, in the hematopoietic and nervous system, in bone and adipose tissues (Bond 2004); it contains 30 zinc fingers and a N-terminal motif of 12-amino acid, shared with a small set transcriptional co-repressors with a regulatory role in different developmental processes (Bond 2008;Spina 2013). ZNF521 gene amplification was found in a significant portion of total ovarian cancers (OCs) and is associated with poor prognosis (Crijns 2009) and suggests that may play an important role in development, maintenance and dissemination of OC. An important therapeutic target in OC may be represented by a subpopulation of cells with stem-like features (CSCs), considered a key factor in cancer initiation, relapse and resistance to therapy. Two OC cell lines, HEY-A8 and OVCAR-8, were used to assess the effects of ZNF521 in vitro. The expression of ZNF521 in these cells, measured by Q-RT-PCR and western blotting, was found to be intermediate levels compared to other cell lines that display high, intermediate or low levels of ZNF521. Lentiviral transduction was used to obtain overexpression of ZNF521 in the OC cell lines studied. Enforced expression of ZNF521 in these cells enhances their growth, sphere-forming efficiency and motility. These results indicate that, as a transcription co-factor, ZNF521 is important in regulation of several different types of stem-like cells. Independent research conducted in our laboratory has highlighted a link between ZNF521 and Hedgehog pathway, that plays an important role in ovarian tumorigenesis. The aberrant activation of the SHH pathway has been described in ovarian tumorigenesis (Bhattacharya 2008). In the OC cell lines studied it was found that the SHH pathway was dysregulated in cells overexpressing ZNF521, which may contribute to the transformed phenotype, potentially rendering the cells susceptible to drugs that target this pathway.

P.60

Quantitative analysis of rRNA and rDNA in cells from DBA patients

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Mutations in any one of sixteen ribosomal protein (RP) genes has been identified as the genetic cause of hematological alterations as well as the physical malformations observed in Diamond-Blackfan anemia (DBA). However, the molecular mechanism that links a defect in ribosome biogenesis and/or function (ribosomal stress) with alteration of hemopoietic cell growth has not been fully clarified. A number of report showed that both in experimental models and in cells from DBA patients, the processing of rRNA is altered, with the accumulation of specific rRNA precursors. Moreover, due to the unbalanced production of ribosomal subunits, the ratio of the mature forms 28S/18S appears to be altered as well. We have developed a simple RT-qPCR method to measure the ratios of precursors and mature forms of rRNA. We are now testing the method to verify it can be used as diagnostic tool.
In the last few years, it has been shown that ribosomal DNA is one of the most variable regions in the human genome with respect to copy number. Despite the importance of rDNA for cellular function, we know virtually nothing about what governs its copy number, due to challenges associated with mapping and analysis. Three of the rRNAs (18S, 5.8S, and 28S) are produced from a single precursor RNA (47S rRNA) that is encoded by a tandemly repeated locus residing on several chromosomes in humans and mice. The fourth rRNA molecule (5S rRNA) is also encoded by a repeated locus that is physically unlinked from the 45S rDNA loci in humans and mice. The 5S and 45S elements do not share segments of homology and are, indeed, transcribed by a different RNA polymerase (the 45S arrays are transcribed by RNA polymerase I whereas the 5S array is transcribed by RNA polymerase III. Recently, it was demonstrated that copy number of the ribosomal DNA can change in human cancer genomes. To address if copy number variation of rDNA genes could play a role in DBA or other ribosomopathies we have developed a qPCR assay.

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Lower UHRF1 overexpression is linked to higher DNA methylation of eighty-three selected genes and associated with worse prognosis in colorectal cancer

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Cancer is a multistep process that involves the acquisition of multiple genetic and epigenetic changes to overcome the barriers that normally restrain cell proliferation. Among the epigenetic changes, DNA methylation has recalled remarkable interest because of the apparent paradox represented by an overall DNA hypo-methylation associated with site-specific DNA hyper-methylation. The molecular bases of this paradox are still unclear. Here, we demonstrate that UHRF1 plays a pivotal role in colorectal cancers (CRC) as it is upregulated and paradoxically associated with a better prognosis in approximately two-thirds of our cohort, with higher expression in MSI than MSS samples. A survey of several human CRC derived cell lines confirmed UHRF1 upregulation in MSI vs MSS cells and correlated with a differential methylation profile. By analyzing RNA-seq and DNA methylation array from the TCGA-COAD dataset, and stratifying patients for UHRF1 expression, we identified 83 differentially expressed genes and 156 differentially methylated cytosines on the basis of which we classified patients in four subsets. The group displaying low UHRF1/high methylation had the worst outcome. The results from our patient cohort and CRC dataset were confirmed in UHRF1 silenced RKO cell line. A detailed survey of the distribution of the differentially methylated cytosines (RRBS analysis) along with differentially expressed genes (RNA-seq), indicated that both hypo- and hyper-methylation may simultaneously occur within promoter and gene body regions. Specifically, massive DNA hypo-methylation variations occurred in up-regulated genes, enriching the WNT, angiogenic, inflammatory and EMT pathways, worsening tumor progression. This might explain also why lower UHRF1 levels found in MSS are associated with a less favourable prognosis than MSI tumors.

P.62

JARID1B expression and its function in DNA damage repair are tightly regulated by miRNAs in breast cancer

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JARID1/KDM5 enzymes are histone demethylases (HDMs) involved in many cellular processes, including transcriptional regulation and DNA damage repair. Their deregulation has been widely reported to significantly contribute to tumor initiation and progression. The mRNA of JARID1B/KDM5B, a member of this sub-family of enzymes, resulted to be markedly over- expressed in breast tumor tissues and cell lines. However, post-transcriptional regulation of JARID1B mRNA in cancer cells is still elusive. By means of a computational analysis of transcriptomic data from a set of 103 breast cancer patients, we identified, along with JARID1B up-regulation, two strongly down-regulated miRNA, mir-381 and mir-486. We demonstrated that both miRNAs can target JARID1B 3’UTR and that MCF7 breast cancer cells over-expressing JARID1B transfected with mir-486 showed a strong reduction of protein levels. This protein’s decrease is related to accumulation of DNA damage, enhanced radiosensitivity of breast cancer cells and increase of BRCA1 mRNA levels. These findings provide insights into the role of miRNAs in regulating JARID1B’s activity and underline the function of this histone demethylase in genome
stability maintenance.

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Conditioned media from cancer-associated fibroblasts induce epigenetic modifications responsible for Epithelial Mesenchymal Transition in colorectal cancer cells

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Epithelial-Mesenchymal Transition (EMT) allows epithelial cells to acquire the ability to migrate and invade neighbouring tissues. Cancer transformed cells loose their epithelial characteristics and acquire features of motile and invasive mesenchymal cells and stem cell behavior, contributing to cancer progression and metastasis. The tumor microenvironment (TME), formed by transformed cells and adjacent stromal cells, among which cancer associated fibroblasts (CAFs) are the predominant type, triggers this event through bidirectional crosstalks. In order to decipher the epigenetic alterations underlying EMT in colorectal cancer (CRC), we cultured DLD1 cells for 72 hr with the conditioned media from CAFs explanted from primary tumors (CAF-PT) of different patients. Upon exposure, cells lost their ability to form aggregates, expression of E-cadherin, CK20 and other epithelial markers while acquired a fibroblast-like phenotype and expressed vimentin and EMT regulators (ZEB1, Snail, GRHL) as documented by qRT-PCR and western blot analysis. All three DNMTs were reduced along with UHRF1. Preliminary data of RNA-seq and Methyl-array experiments performed on DLD1 cells silenced or not for DNMT3B and exposed to CAF-PT conditioned media, show that DNMT3B silencing advances EMT entry, and also point to some specific pathways among which the non-canonical WNT appears the most relevant. In support, an array of cytokines/chemokines secreted by CAF-PT revealed enrichment of molecules stimulating the same pathways as those identified here. On this basis, we mimicked the conditions generated by the CAF-PT by administering DLD1 cells with LiCl (20mM for 24h, 48h and 72h) to stimulate the WNT pathway. The results achieved are parallel to those obtained with the CM-CAF-PT. Finally, experiments are ongoing to silence all DNMTs, to investigate whether these enzymes play key roles in the epigenetic changes and in the tied variations of gene expression occurring during EMT in CRC.

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Silica-induced fibrosis: an ancient response from the early metazoans

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Exposure to crystalline silica particles causes silicosis, an occupational lung disease leading to an overproduction of collagen. The first step of this pathology is characterized by the release of various inflammatory mediators as TNF, one of the pro-inflammatory cytokines directly involved in silica-induced pulmonary fibrosis. Conversely, it has been observed as in nature the marine sponge *Chondrosia reniformis* can incorporate silica sand from the environment and partially dissolve it without apparent toxic effects.
In the present study, *C. reniformis* tissue explants were experimentally treated with fine quartz dust and the expression level of fibrogenic genes was assayed by qPCR, demonstrating an overexpression of a fibrillar and a non-fibrillar collagen and of prolyl-4-hydroxylase enzyme. The deposition of new collagen could also be documented in quartz treated sponge explants. Furthermore, TNF pro-inflammatory cytokine overexpression and involvement in silica-induced sponge collagen biosynthesis was demonstrated in quartz-treated explants as compared with controls by means of specific TNF inhibitors affecting the fibrogenic gene response. As no documentable detrimental effect was observed in treated explants, we conclude that in *C. reniformis* unique quartz engulfment and erosion is physiological and beneficial to the animal, leading to new collagen synthesis and strengthening of the body stiffness. These results, take together, can indicate as an ancient physiological behavior, observed in the lowest of the Metazoa, persisting through evolution via the same molecular mediators such as TNF, may have become the cause of disease in the specialized tissues of higher animals.

P.65

Identification of STAT3 targets mediating the cross-talk between cancer associated fibroblasts and cancer cells

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Recently, interactions between cancer cells and the stroma have attracted considerable attention. In particular, Cancer Associated Fibroblasts (CAFs) are one of the main components of the mesenchymal tumor microenvironment. Many studies have gradually clarified their origins, features, and roles. However, the molecular mechanisms underlying their functions are still poorly understood.
Several studies of the cross-talk between stroma and primary tumor have demonstrated a pivotal role for proinflammatory cytokine resulting in constitutive activation of STAT3, a common converging point of several oncogenic signaling pathways.
Here, we set up an in-vitro model to test STAT3-dependent CAFs activities and we performed gene expression profiling of CAFs silenced or not for STAT3. Importantly, we identified a set of STAT3 targets enriched in secretory pathway proteins. We have tested three of these candidate proteins, Stc1, Mmp13 and Angptl4 by means of shRNA and CRISPR-Cas9 silencing. Indeed, silencing of Stc1 and Mmp13 leads to dramatic reduction of both CAF-induced migration and invasion of 4T1 tumor cells, whereas no significant changes were observed for Angptl4. We are now completing the characterization of the silenced cells including analysis of their in vivo tumorigenic potential.

P.66

Nucleoporin 153 regulates estrogen-dependent nuclear translocation of endothelial nitric oxide synthase and estrogen receptor beta in prostate cancer

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Nucleoporin 153 (Nup153), key regulator of nuclear import/export, has been recently associated to oncogenic properties in pancreatic and breast tumour cells modulating either cell motility and migration or gene expression by chromatin association. In the present work, we have characterized the role of Nup153 in a cellular model of prostate cancer (PCa). The analysis of several immortalized cell lines derived from freshly explants of prostate cancer specimens showed that Nup153 protein was higher and present in multimeric complexes with eNOS and ERβ as compared to normal/hyperplastic prostate epithelial cells. This phenomenon was enhanced in the presence of 17β-estradiol (E_2, 10^{-7}M). Further experiments revealed that eNOS and ERβ were present in a DNA binding complexes associated with Nup153 promoter as demonstrated by ChIPs. Notably, after Nup153 depletion (siNup153), a reduction of migration capacity and colony formation in primary tumor-derived and metastatic PCa cells was observed. In addition, eNOS and ERβ nuclear localization was lost upon siNup 153 regardless of E_2 treatment, suggesting that Nup153 is a key regulator of prostate cancer cell function and of the nuclear translocation of these proteins in response to hormone stimulus. Taken altogether our findings indicate that in PCa cells: i. the expression and function of Nup153 is modulated by estrogen signaling; ii. Nup153 contributes to cell migration and proliferation; iii. Nup153 regulates the nuclear translocation of eNOS and ERβ by forming a multimeric complex. Our findings unveil Nup153 as a novel component of the estrogen-dependent multimeric complex, thus representing a potential therapeutic candidate in prostate cancer.

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miR-27a-3p is a master regulator of metabolic reprogramming in colorectal cancer

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Metabolic reprogramming towards aerobic glycolysis is a frequent event in cancer to support unrestricted cell proliferation and survival. The molecular bases of this phenomenon are still unclear. By *in silico* analysis of the TCGA-COAD dataset, we found that miR-27a overexpression in colorectal cancer (CRC) is associated with gene expression signatures of mitochondrial dysfunction, deregulated oxidative phosphorylation and mTOR signaling activation. Furthermore, we found that miR-27a overexpressing CRCs are more frequently mutated in known cancer drivers and display increased chemoresistance. Based on these premises, we modified miR-27a-3p (the predominant mature form in CRC) in an *in vitro* model system and experimentally observed that its upregulation is tied to impaired tricarboxylic acids cycle, oxidative phosphorylation and mitochondrial activities, consistent with the *in silico* analysis. In addition, miR-27a-3p overexpression enhances mTOR signaling, glutamine uptake, HIF-1a stabilization and acts in concert with key tumor cell metabolic regulators to force an aerobic glycolytic metabolism that supports biomass production, unrestricted proliferation and reduced sensitivity to chemotherapy. In conclusion, we provide the first evidence that miR-27a-3p acts as a master regulator of metabolic reprogramming in CRC that promotes cancer progression and, therefore, miR-27a-3p could represent a novel theragnostic for CRC.

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Mutual inhibition of Lin28b and miR-125a in hepatocellular carcinoma cells

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Increasing evidence indicates that miR-125a plays a suppressive role in several types of tumors, including hepatocellular carcinoma (HCC) where it targets sirtuin-7, matrix metalloproteinase-11, and c-Raf. Another established target of miR-125a is Lin28, a pluripotency factor that is generally undetectable in differentiated cells but is often upregulated/reactivated in tumors where it acts as an oncogenic factor promoting cell proliferation and tumor progression. In this study we show that downregulation of Lin28b by miR-125a partially accounts for the antiproliferative activity of the miRNA toward HCC cells. We also found that Lin28b is able to bind a conserved GGAG motif of pre-miR-125a and to inhibit its maturation in cultured HuH7 cells. Reciprocal inhibition between Lin28b and miR-125a likely yields a positive feedback loop where reactivation of Lin-28b inhibits the expression of both miR-125a and let-7, reinforcing its own expression and leading to a marked overexpression of the mitogenic targets of the two miRNAs. On the other hand, perturbation of these circuits by overexpression of miR-125a suppresses Lin28b leading to a decreased cell proliferation. Overall, these data support an HCC suppressive role for miR-125a and contribute to the elucidation of its molecular targets.

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LSD1 sustains Glioblastoma tumor growth acting at crossroad between autophagy, DNA damage response and senescence

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Epigenetic enzymes are promising targets for cancer therapy due to their involvement in cellular processes leading to oncogenesis; accordingly, a number of epi-drugs are currently under investigations.
Lysine-specific demethylase 1 (LSD1) removes mono- and di-methylated groups from lysines 4 or 9 on H3, as well as non-histone protein targets, via a flavin adenine dinucleotide (FAD)-dependent oxidative reaction. Notably, high-levels of LSD1 expression are the hallmarks in several human solid cancers and leukemia.
We investigated the biological and molecular outcome of LSD1 inhibition in Glioblastoma cell lines. Pharmacological as well as genetics inhibition of LSD1 reduces cell growth and severely affect mitochondrial oxidative capacity. At mechanistic levels we found that LSD1-inhibition induces DNA damage-response, impairs mTOR pathway and induces cellular senescence. Together, our findings demonstrated that LSD1 sustains Glioblastoma cell growth acting at crossroad between autophagy and senescence and highlights the relationship between these two important homeostatic stress responses.

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Network analysis to unravel pathways in triple negative breast cancer

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Breast cancer is an heterogenous disease with several subtypes that have been classified both histologically and molecularly. The most aggressive, recurrent and metastatic form, for which no effective therapy is available, is denominated triple negative, since: it lacks the expression of progesterone receptors, estrogen receptors and HER2, which are main therapeutic targets for other breast cancer subtypes with better prognosis. Here, we sought to study breast cancer expression patterns in an unbiased way to define gene networks specific for different molecular subtypes and groups of patients within each subtype, focusing on triple negative breast cancer. Specifically, we used Weighted Gene Coexpression Network Analysis (WGCNA) on a large primary breast tumors dataset (TCGA) identifying 25 networks that we could relate to subtype, survival, cell type specificity within the stroma, signaling pathways and molecular functions. Moreover, the network approach allowed us to select hubs of potential interest as therapeutic targets. From the integration of our results with publicly available datasets on pathways down- or up-regulation, on the transcriptional effect of a panel of drugs, together with analyses on specific hubs, we expect to identify interesting candidates for therapy in a currently hardly treatable disease.

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An integrated proteogenomic analysis of induced pluripotent versus human embryonic stem cells

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Although human induced pluripotent stem cells (hiPSCs) represent a promising tool to overcome troubles due to research and medical use of human embryonic stem cells (hESCs), it is now well known that the two cell types are very similar but not identical. Therefore, a deeper understanding how these differences can affect pluripotency mechanisms is eagerly needed.
Previous studies focused primarily on diversity at transcriptome or epigenome level and more data are needed for proteins and their post-translational modifications.
Here we used an integrated transcriptomic and a proteomic strategy to compare a commercially available hESCs line (H9) and an hiPSCs line (HL1) obtained in our laboratory by reprogramming of peripheral blood T-lymphocytes with Sendai virus carrying Yamanaka factors *OCT4, SOX2, KLF4*, and *c-MYC*.
Differentially expressed genes, proteins and phosphoproteins were identified using a fold change (FC) cut-off criteria of Log_2 FC ± 0,5 and were analyzed with Ingenuity Pathway Analysis (IPA) software.
The combined analysis highlighted an enrichment of pathways related to different biological functions, such as redox and proteins homeostasis (NRF2-mediated Oxidative Stress Response, HIF1α Signaling, Antioxidant Action of Vitamin C, Protein Ubiquitination), cell adhesion and motility (Integrin Signaling, PCP pathway), cellular cycle (Sonic Hedgehog Signaling, ERK/MAPK signaling) and metabolism (Pyruvate Fermentation to Lactate, Glycolysis I, Superpathway of Cholesterol Biosynthesis).
These results provide novel molecular insights into the induced versus naïve pluripotency.

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Epigenetic role of transposable elements (TEs) in shaping CD4$^+$ T cell identity and plasticity

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CD4$^+$ T cells play a central role in modulating adaptive immune system response principally by mean of CD4$^+$ Naïve cells ability to differentiate into at least six different cell types, depending on the cytokines milieu.
Transposable elements (TEs) are interspersed repetitive DNA sequences that cover 45% of the human genome and growing evidence suggest that TEs exert a crucial function in epigenetic regulation at different level both *in cis* and *in trans*, being a source of non coding regulatory RNAs and participating to chromatin folding.
We aim to find novel molecular mechanisms and players that could explain CD4$^+$ T cell's plasticity in TEs' mediated epigenetic regulation.
We first analyzed the expression of different classes of TEs in *ex-vivo* sorted CD4$^+$T cells (Naïve, Th1, Th2, Th17 and T-reg) finding a specific enrichment for LINE-1 chromatin associated transcripts in Naïve and T-reg cells in respect to T effector subsets (Th1, Th2 and Th17) that are less enriched.
Interestingly, L1 RNAs show a peculiar and timely specific dynamics being rapidly depleted from the nuclei in a TCR activation dependent and replication independent manner.
We also further investigated LINE-1 RNAs' chromatin localization by RNA FISH combined with immunofluorescence, finding a specific enrichment in euchromatic region.
Based on these discoveries we would further dissect the TCR signalling dependency, and, by mean of functional experiments and NGS approaches we would like to highlight the mechanisms by which L1 transcripts might contribute to human CD4$^+$ T lymphocytes cell identity, plasticity and specialization in healthy and disease conditions.

P.73

De novo methylation requirement for CAF-CM induced EMT activation in prostate cancer cells

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The combination of epigenetic modifications and aberrant activation of epithelial-to-mesenchymal transition (EMT) in prostate cancer (PCa) is strictly related to phenotypic changes that promote cancer progression and an increase of the tumor metastatic properties. EMT activation is largely dependent on tumor microenvironment, composed of a heterogeneous population of stromal cells, among which cancer-associated fibroblasts (CAF) are considered central, since they are involved in tumor progression and metastasis.
The aim of the project is to investigate whether EMT activation due to fibroblasts' conditioned media (CM) might depend on *de novo* methylation, namely on DNMT3A methyl-transferase, whose activity is pivotal in PCa and for EMT activation. For this purpose, in PCa cells we intend identifying genes, whose expression is regulated by CAF-CM dependent DNMT3A activity.
We first isolated and characterized CAFs derived from the cultivation of tissue samples from tumor areas of radical prostatectomies and obtained one CAF stable clone. The cells were then plated in serum-free medium to produce CM for a first conditioning experiment of PC3 cells, in which cells seemed to obtain a well-defined mesenchymal phenotype, suggesting a potential EMT activation. To confirm DNMT3A requirement for EMT, we siRNA silenced the relative messenger in PC3 cells before the exposure to CAF-CM. Results confirm that silencing of DNMT3A before CAF-CM treatment prevent activation of EMT markers (VIM and ZEB1) and down-regulation of epithelial markers (CDH1 and GRHL2), strongly indicating hindrance of the EMT phenotype appearance.
We are currently performing RNA-seq and DNA methylation analysis, to identify transcripts dependent or not on DNA methylation changes required for EMT induction following administration of CAF-CM.

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