Associazione di Biologia Cellulare e del Differenziamento

Cell Biology of Disease: Cancer

Organisers

Giorgio Scita (Chair) - Fondazione IFOM

Gabriella Minchiotti (vice-Chair) - Institute of Genetics and Biophysics "A. Buzzati-Traverso", CNR

Programme & Abstracts

Parma, 28-29 November 2014

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PROGRAMME

Friday, 28 November

13:00 **Registration**

14:00-14:15 **Welcome Addresses**

Session I: Stem cell and cancer stem cells

Chair: Gabriella Minchiotti

14:15-14:45 **Keynote Lecture**

Enza Lonardo (Barcelona, Spain)

TGF-beta signaling in Metastatic Stem Cells

14:45-15:00 *Michela Lupia (Milan)*

Ovarian cancer stem cells: characterization and potential clinical use

15:00-15:15 *Silvia Restelli (Milan)*

Regulatory mechanisms implicated in the control of Numb asymmetric partitioning in normal mammary stem cells

15:15-15:30 *Cristina D'Aniello (Naples)*

A novel autoregulatory loop between L-Proline metabolism and the Gcn2-Atf4 stress pathway controls the embryonic stem cell-to-mesenchymal-like transition

15:30-15:45 *Marta Peretti (Milan)*

Metformin repositioning as antitumoral agent: selective antiproliferative effects in human glioblastoma cancer stem cells, via inhibition of CLIC1-mediated ion current

15:45-16:00 **Coffee break**

Session II: From cancer medicine to cell biology

Chair: Alberto Bardelli

16:00-16:30 **Keynote Lecture**

Alberto Bardelli (Turin)

From cancer medicine to cell biology

16:30-16:45 *Federica Fusella (Turin)*

Morgana: a novel player in apoptosis resistance and metastasis progression in breast tumorigenesis

16:45- 17:00 *Caterina Ieranò (Naples)*

CXCR4 cyclic peptide antagonist (PepR) – conjugated liposomes (PL–PepR): efficacy and specificity improvement

17:00-17:15 *Antonino Colanzi (Naples)*
JNK2 phosphorylates GRASP65 to control fragmentation of the Golgi complex and G2/M transition in normal and cancer cells

17:15-17:30 *Miriam Martini (Turin)*
Loss of PI3KC2A promotes tumorigenesis and aneuploidy in breast cancer

17:30-19:45 **Poster session**

20:00 **Dinner**

Saturday, 29 November

Session III: Cell metabolism/autophagy in normal and cancer cells

Chair: Francesco Cecconi

8:30-9:00 **Keynote Lecture**
Francesco Cecconi (Rome/Copenhagen, Denmark)
The autophagy signaling network in the coordination of a cell's response

9:00-9:15 *Monica Nanni (Rome)*
HPV16 E5 deregulates autophagy in human keratinocytes

9:15-9:30 *Martina Chiu (Parma)*
L-Asparaginase impairs the growth of liver cancer xenografts

9:30-9:45 *Thomas Vaccari (Milan)*
Multiple functions of the SNARE protein Snap29 in autophagy and trafficking during epithelial formation in *Drosophila*

9:45-10:00 *Giulia Santinon (Padua)*
Identification of a novel feedback mechanism linking aerobic glycolysis to oncogenic transcription

10:00-11:30 **Poster session & Coffee break**

Session IV: Migration and invasion from cell model systems to human cancer

Chair: Giorgio Scita

11:30-11:45 *Veronica Astro (Milan)*
Elucidating the mechanisms of ERC1a/liprin-α1 complex mediated cell polarization and migration

11:45-12:00 *Matteo Biancospino (Milan)*

Myosin VI bridges ubiquitin signaling and cell migration

12:00-12:15 *Chiara Recchi (London, UK)*

The tumour suppressor protein OPCML prevents Axl-mediated EMT and motility in ovarian cancer cells

12:15-12:30 *Gema Malet-Engra (Milan)*

Collective chemotaxis of malignant B cells

12:30-13:00 **Keynote Lecture**

Philippe Chavrier (Paris, France)

Mechanism of matrix metalloproteinase secretion during breast tumor cell invasion

13:00 **Lunch**

Oral Presentations

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

TGF-beta signaling in Metastatic Stem Cells

E. Lonardo, E. Batlle
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The inner layer of the intestinal tube, the intestinal epithelium, is in a constant process of renewal. Hundreds of millions of terminally differentiated intestinal cells are replaced by new cells every day during the life of an adult organism. This tremendous regenerative power is ultimately sustained by a small population of intestinal stem cells (ISCs). We have recently discovered that most human colorectal cancers (CRCs) are constituted by cell populations with phenotypes similar to either ISCs or intestinal differentiated cells organized into well-defined compartments. ISC-like cells purified from primary CRC samples generate tumors in immunedeficient mice with high efficiency and display both self-renewal and differentiation capacity whereas differentiated CRC cells are not capable of propagating the disease. Our observations imply that CRC shares a common hierarchy with the intestinal mucosa and that the acquisition of an ISC-like gene program is a central process in the development of metastatic and recurrent CRC. Here I will present our latest data on the mechanisms employed by CRC stem cells to regenerate a new tumor at the metastatic site. We have demonstrated that metastasis relies on a tumor cell non-autonomous program driven by TGF-beta in the microenvironment. This stromal program confers a survival advantage to the Metastatic Stem Cells (MetSCs) during the initial phase of organ colonization. We have now investigated the dichotomy of TGF-beta signaling in epithelial versus stromal cells during CRC progression and interrogated mouse models about the efficacy of anti-TGF-beta therapies for CRC treatment.

Ovarian cancer stem cells: characterization and potential clinical use

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Epithelial Ovarian Cancer (OC) is one of the leading causes of death for women, and no significant therapeutic progress has been made in the last decades.
Recent data suggest that one of the mechanisms accounting for resistant and/or relapsing disease is a subpopulation of cells in human tumors with stem-like characteristics (cancer stem cells, CSCs). CSCs are defined as a small subpopulation of cells within the tumor bulk that possess the capacity, on one hand, to self-renew and, on the other hand, to give rise to all heterogeneous cancer cell lineages that compose the tumor of origin. The CSC hypothesis provides an attractive cellular mechanism to explain the therapeutic refractoriness, dormant behavior, and relapse of the disease. Our study aims at assessing OCSC as causal players in OC etiology and progression and at defining their molecular and functional profile. Specifically, we are pursuing the following objectives through the accomplishment of these milestones: 1) collection of normal and pathological samples, 2) isolation and functional characterization of OCSCs, 3) comparison of gene expression profiles between cancer stem cells and their normal counterpart.
A preliminary analysis of the transcriptome revealed a number of genetic networks differentially modulated in primary stem/progenitor cell-enriched cultures derived from OC as compared to normal tissue. These data are currently being validated through *in vitro* and *in vivo* assays and are expected to reveal genes and/or pathways that specifically drive OCSC function. Our study might set the stage for innovative therapeutic approaches aimed at the selective elimination of CSC, thus preventing both tumor recurrence and chemoresistance.

Regulatory mechanisms implicated in the control of Numb asymmetric partitioning in normal mammary stem cells

S. Restelli[1], D. Tosoni[1], S. Zecchini[1], M. Coazzoli[1], I. Colaluca[1], S. Pece[1,2,3], P.P. Di Fiore[1,2,3]
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We recently evidenced that the cell fate determinant Numb, by ensuring the asymmetric outcome of self-renewing divisions in normal mammary stem cells (MaSC), safeguards against the emergence of cancer stem cells (SC) with unlimited self-renewal potential. This function is linked to the ability of Numb to asymmetrically partition, at MaSC mitosis, into the progeny that retains the SC identity, where it sustains the tumor suppressor activity of p53. However, Numb is typically distributed in a symmetric fashion in the progeny of SCs with impaired p53 function, such as ErbB2 or p53-/- SCs, arguing that p53 might also act upstream of Numb in the control of MaSC asymmetric cell division (ACD). To investigate whether Numb and p53 might be engaged in reciprocal regulation towards the establishment of the correct pattern of asymmetry in MaSCs ACD, we started from the analysis of the Par/PKC polarity complex, an evolutionarily conserved machinery that directs Numb polarized distribution and ACD in different cellular contexts. Studies of pharmacological activation or inhibition of PKC in MCF10A and Comma-Dβ cells, two models of quasi-normal human and murine mammary epithelial cells (MEC), respectively, and in primary murine MECs showed that PKC-mediated phosphorylation of Numb controls its cytoplasmic vs. plasma membrane localization in the mammary gland. This regulatory mechanism was also found to be relevant to Numb asymmetric distribution during ACD of MaSC, with dynamic variations in Numb phosphorylation influencing the asymmetric *vs.* symmetric outcome of Numb distribution into the sibling cells. In ErbB2 or p53-/- mammary SCs, the default mode of Numb symmetric distribution, due to impaired p53 function, was reverted to an asymmetric one upon restoration of p53 function or PKC inhibition, indicating that p53 also acts upstream of Numb in a pathway that, directly or indirectly, couples control of asymmetry of self-renewing division and of Numb distribution at MaSC mitosis.

A novel autoregulatory loop between L-Proline metabolism and the Gcn2-Atf4 stress pathway controls the embryonic stem cell-to-mesenchymal-like transition

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The process of Epithelial to Mesenchymal transition (EMT), through which epithelial/compact cells acquire mesenchymal/motile characteristics, underlies normal development, as well as embryonic stem cell (ESC) differentiation *in vitro*. Many of the key regulators of embryonic EMT are reactivated in the adult only under pathological conditions, such as tumor progression. The reverse process, the Mesenchymal to Epithelial transition (MET) has also been implicated in tumorigenesis, when cancer stem cells (CSCs) migrate and start metastasis. Thus, the EMT/MET are the bases for normal stem and CSC plasticity. We recently described the role of the non-essential aminoacid L-Proline (L-Pro) in inducing the embryonic stem cell-to-mesenchymal-like transition (esMT), in which ESC compact/adherent colonies are converted into mesenchymal-like/motile and metastatic stem cells.
Here we sought to investigate the early molecular events underlying L-Pro-induced esMT, as a powerful tool to elucidate the crucial mechanisms for the acquisition of the motile/metastatic properties of CSCs.
Our results indicate that ESC behaviour relies on a feedback loop between L-Pro and Gcn2-Eif2α-Atf4 aminoacid starvation response (AAR) pathway that in turn regulates L-Pro biosynthesis. This loop generates a highly specific intrinsic L-Pro shortage in ESCs. Indeed, addition of exogenous L-Pro relieves this nutrient stress condition, induces proliferation and modifies the ESC phenotypic and molecular identity towards that of mesenchymal-like, invasive pluripotent stem cells. This process is accompanied by macroautophagy and apoptosis. The effects of L-Pro are antagonised by either pharmacological inhibition of the prolyl-tRNA synthetase by halofuginone or forced expression of Atf4. Our data reveal a novel regulatory axis between L-Pro and the AAR pathway in the control of esMT, providing also potential novel mechanisms that can be activated/deregulated during cancer progression and underlie CSC plasticity.

Metformin repositioning as antitumoral agent: selective antiproliferative effects in human glioblastoma cancer stem cells, via inhibition of CLIC1-mediated ion current

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Chloride Intracellular Channel 1 (CLIC1) is a protein that shuttles between the cytoplasm and cell membranes upon different intracellular conditions. Once inserted in the plasma membrane, CLIC1 is able to form a chloride selective ion channel. We have previously demonstrated that blocking CLIC1 ionic current in human glioblastoma cancer stem cells (CSCs) impairs the proliferation ability, the motility and, as a consequence, the tumor development (Setti at al., 2013) . Unfortunately the only known CLIC1 specific blocker is IAA94 at doses below 100 μM. IAA94 has been classified to be toxic for humans due to its multiple side effects and for this reason excluded from any therapeutic treatment.
Epidemiological and preclinical studies propose that metformin, a first-line drug for type-2 diabetes, exerts direct antitumoural activity. Although several clinical trials are ongoing, the molecular mechanisms of this effect are partially unknown. Our work shows that CLIC1 is a direct target of metformin in human glioblastoma (GBM) CSCs. Metformin exposure exerts antiproliferative effects in CSC-enriched cultures, isolated from WHO grade IV human GBMs. These effects phenocopy metformin-mediated inhibition of chloride current, which is specifically dependent on CLIC1 functional activity. Metformin inhibition of CLIC1 ion channel activity during its transient membrane insertion slows the transition from G1 to S phase. Furthermore, point mutation of the putative CLIC1 pore region impairs metformin modulation of channel protein activitv.
In conclusion our findings highlight the specific role of CLIC1 in GMB CSCs' proliferation and the ability of metformin to directly inhibit CLIC1 channel protein activity, paving the way for novel and needed pharmacological approaches to GBM treatment.

From cancer medicine to cell biology

A. Bardelli
Univ. of Turin, Candiolo Cancer Institute IRCCS

It is now evident that colorectal cancers (CRC) indistinguishable by light microscopy are actually distinct diseases requiring unique therapeutic approaches. Tissue and liquid biopsies can be used to define CRC molecular subtypes and to monitor response and resistance to therapy. Using these approaches, CRC patients were found to respond selectively to targeted agents interfering with oncogenic nodes of the EGFR signaling pathway. Notably, the patient-specific responses can be recapitulated and paralleled in cellular and mouse clinical proxies (CRC-avatars). The inevitable development of acquired resistance to inhibitors of the EGFR signaling pathway presently limits further clinical advances. Strategies to prevent or overcome resistance are therefore essential to design the next generation of molecularly-driven clinical trials for CRC patients.

Morgana: a novel player in apoptosis resistance and metastasis progression in breast tumorigenesis

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Morgana is a ubiquitously expressed protein that forms a complex with Hsp90, acting as a co-chaperone, and Rho kinases I and II, inhibiting their activity. We previously demonstrated that morgana haploinsufficiency leads to multiple centrosomes, genomic instability and higher susceptibility to tumor development. While a large fraction of human cancers showed morgana down-regulation, a small subset of tumors expressed high morgana levels. Here we show that high morgana expression in different breast cancer subtypes correlates with high tumor grade, mitosis number and lymph node positivity. Moreover, morgana overexpression induces transformation in NIH-3T3 cells and strongly protects them from various apoptotic stimuli. From a mechanistic point of view, we find that morgana destabilizes PTEN by inhibiting ROCK activity, hence triggering the PI3K/AKT survival pathway. In turn, morgana downregulation in breast cancer cells expressing high morgana levels, increases PTEN expression and sensitizes cells to chemotherapy. Furthermore analyzing the effects of the downregulation of morgana in breast cancer cells in which the protein is expressed at higher level, MDA-MB-231, we find that morgana is important not only for chemoresistance, but also for metastatization. Indeed MDA-MB-231 cells, when morgana is downregulated, lose their ability to migrate, invade and, more importantly, to metastasize. In addition morgana downregulation caused a significant reduction in MMP9 transcription explaining the incapacity of cells to metastasize.

CXCR4 cyclic peptide antagonist (PepR) – conjugated liposomes (PL–PepR): efficacy and specificity improvement

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CXCR4 is overexpressed in multiple tumors regulating metastatic dissemination. A new class of cyclic peptides antagonist for CXCR4 receptors was recently developed. To improve peptide efficacy and increase its delivery to target cancer cells the most active antagonist, PepR, was coupled to PEGylated liposomes (PL).
PepR conjugated to the liposomes (PL-PepR) were prepared starting by athiolated derivative of antiCXCR4 peptides coupled to the pre-formed PL. Doxorubicin (DOX) was then encapsulated by remote loading method. PL-PepR was evaluated through migration assay in A498 human renal cancer cell line in vitro and in an experimental animal model of pulmonary metastasis development in vivo. DOX-encapsulating PL-PepR was evaluated in CXCR4 positive cells A498 and HT29 (colon cancer cell line) versus negative CXCR4 expressing cells FB-1(human anaplastic thyroid cell line), as mean cellular fluorescence. The cytotoxic effect of the PL-DOX-PepR was examined in A498 and in HT29 cells. Finally, PL-DOX-PepR was evaluated in vivo polmonary metastasis formations in C57/BL mice injected with B16-CXCR4 cells.
In vitro studies, PL-PepR inhibited migration CXCL12-induced in A498 human renal cancer cells-CXCR4 expressing more efficiently than PepR alone. A significant reduction in lung metastases was detected in mice treated with PL-PepR even with lower dose of the PL-PepR (0.1mg/kg) compared to the usually used (2mg/kg). Moreover, a CXCR4 dependent higher DOX accumulation was registered in CXCR4 positive cells, A498 and HT29 resulting in a specific higher cytotoxicity. This was confirmed in vivo experiments, in which PL-DOX-PepR reduced lung metastases compared to PL-DOX treated mice.
Liposomes conjugated-rationally designed CXCR4 antagonist were more efficient in inhibiting CXCR4 in vitro and in vivo. Moreover, PL-PepR loaded with a chemotherapeutic drug, such as DOX, demonstrated an enhanced drug accumulation to CXCR4 expressing tumor.

JNK2 phosphorylates GRASP65 to control fragmentation of the Golgi complex and G2/M transition in normal and cancer cells

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In mammalian cells, the Golgi complex is organised in the form of stacks interconnected by membranous tubules. Cleavage of the tubules during G2 is necessary for entry into mitosis, indicating that the correct inheritance of the organelle is monitored by a 'Golgi mitotic checkpoint'. However, the regulation and the molecular mechanisms underling this Golgi disassembly are still poorly understood.
Here, we show that JNK2 has a crucial role in the G2-specific separation of the Golgi stacks through phosphorylation of Ser277 of the Golgi-stacking protein GRASP65. Inhibition of JNK2 by RNA interference or by using three unrelated JNK inhibitors causes a potent and persistent cell cycle block in G2. The JNK requirement for mitotic entry is abrogated if the Golgi complex is previously dispersed by treatment of cells with brefeldin A or by GRASP65 depletion. Finally, Golgi fluorescence recovery after photobleaching analyses demonstrates that JNK is crucial for the cleavage of the tubules connecting Golgi stacks. Our findings reveal that a JNK2/GRASP65 signalling axis has a crucial role in coupling Golgi inheritance and G2/M transition.

Loss of PI3KC2A promotes tumorigenesis and aneuploidy in breast cancer

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PI3K signaling axis is one of the most frequently deregulated pathways in human cancer impacting on cell growth, survival and metabolism. Emerging evidences highlight the importance of class II enzymes in cell proliferation and survival. To evaluate the oncogenic role of PI3KC2A in cancer, we targeted its expression in a breast cancer mouse model (NeuT). The heterozygous loss of *Pik3c2a* initially delays tumor onset and in the long run, leads to a faster growth rate compared to wt. We found that Pi3kc2a loss causes an aberrant microtubule (MT) spindle organization that, in turn, promotes genomic instability. In line with this, Pi3kc2a is specifically enriched at the metaphase spindle, interacting with transforming acidic coiled-coil protein 3 (TACC3)/colonic, hepatic tumor overexpressed gene (ch-TOG)/clathrin complex to stabilize K-fibers during mitosis. Despite the aberrant MT organization, we demonstrate that tumors bypass the requirement of Pi3kc2a through a common mechanism of progression. Multiple genes involved in the spindle-associated checkpoint (SAC), such as Bub1, Bub3 and APC/C genes, resulted either amplified or lost in fast growing (hare) compared to slow growing (turtles) tumors. Moreover, we report that tumors with low Pi3kc2a showed increased sensitivity to anti-MT agents, like Paclitaxel. Finally, we identify a significant correlation between overall survival and reduced PI3KC2A expression in breast cancer, suggesting that this pathway is related to aggressiveness.

The autophagy signaling network in the coordination of a cell's response

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The *a*ctivating *m*olecule in *B*eclin 1-*r*egulated *a*utophagy (Ambra1) is an intrinsically-disordered protein, playing multiple roles as a scaffold factor in autophagy control. Indeed, Ambra1 is a member of the autophagy core complex, favouring Beclin 1/Vps34 interaction and docking them, when autophagy is *off*, at the cytoskeleton; it buffers Bcl-2 at the mitochondrial outer membrane, regulating Bcl-2 inhibitory interaction with Beclin 1; it controls the Ulk1 kinase stability, dimerization and function by regulative ubiquitylation, in a positive feedback loop; it is one of the few known downstream targets of the metabolic switcher mTOR, that inhibits its activity.
For all these reasons, Ambra1 may be considered as a crucial upstream autophagy signaller. However, besides these roles on the direct control of autophagy, Ambra1, due to its numerous post-translational modifications and to its capability to change its conformational status, mediates and coordinates autophagy cross-talk with other cellular processes, such as cell growth modulation, mitochondrial homeostasis and differentiation. This network of regulations is of the highest importance in controlling the fate of a cell and in determining its death and survival. The molecular interactions by which this network acts and its relevance in biomedicine will be discussed.

HPV16 E5 deregulates autophagy in human keratinocytes

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Autophagy plays a crucial role during host defense and many oncogenic viruses have evolved strategies to block the process or to exploit it for replication. Since the E5 early protein of the human papillomavirus (HPV) type 16 (16E5) perturbs epithelial cell homeostasis and differentiation also through down-regulation of the keratinocyte growth factor receptor (KGFR/FGFR2b), the exclusive epithelial splicing variant of FGFR2 whose signaling induces both differentiation and autophagy in epithelial cells, we investigated the possible effects of the viral protein on the autophagic process in human keratinocytes. Biochemical and quantitative immunofluorescence approaches showed that 16E5 expression strongly inhibited the triggering of autophagy induced by either KGF/FGF7 stimulation or serum starvation. The forced expression of KGFR and its activation counteracted the inhibitory effect of 16E5 on KGF-triggered autophagy, demonstrating that the viral protein and the receptor exert opposite and interplaying roles in the control of autophagy. Quantitative real-time RT-PCR showed that 16E5 down-regulates both p53-independent and p53-regulated autophagy genes, suggesting that the viral protein interferes with the transcriptional regulation of autophagy also through the functional inhibition of p53. Our results indicate that HPV16 E5 might use parallel mechanisms, including both KGFR down-regulation and functional repression of p53, for impairment of autophagy.

L-Asparaginase impairs the growth of liver cancer xenografts

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Cancer cells show alterations in their metabolism to balance increased energy requirements and active macromolecular synthesis. Aerobic glycolysis and enhanced glutaminolysis are the most common cancer-associated metabolic changes and constitute potential therapeutic targets. Liver cancer, the third leading cause of cancer death in the world, is usually resistant to chemotherapy. About 30% of hepatocellular carcinomas (HCC) and over 70% of hepatoblastomas (HB) display gain-of-function β-catenin mutations that lead to overexpression of targets, such as two key enzymes of glutamine (Gln) metabolism, glutamine synthetase (GS) and glutaminase 1 (GLS1). The β-catenin-mutated HepG2 cells, which express high levels of GS, were treated with the enzyme L-asparaginase (from *Erwinia chrisanthemi*, ASNase, 1U/ml) w/wo the GS inhibitor methionine-L-sulfoximine (MSO, 1mM). In parallel, nude mice were subcutaneously engrafted with HepG2 cells and treated with ASNase (5 IU/ml, i.p.) w/wo MSO (10 mg/kg, i.p.), three times a week for three weeks. ASNase depleted intracellular Gln both *in vitro* and *in vivo*. In HepG2 cultures ASNase caused proliferative arrest, mTOR inhibition, eIF2α phosphorylation and, in the presence of MSO, caspase-3 activation and massive cell death. When ASNase effects were studied on HepG2 xenografts, the single treatments with either ASNase or MSO significantly delayed tumor growth, which was completely suppressed by the combined treatment with ASNase and MSO. These results were extended to other cell models of liver cancer, carrying different β-catenin mutations and transcriptional activity, indicating that ASNase effects are not specific for the HepG2 model. Whereas these results represent the first evidence of antitumor activity of a GS inhibitor, they point to glutamine-depleting treatments for a metabolic approach to liver cancer therapy.

Multiple functions of the SNARE protein Snap29 in autophagy and trafficking during epithelial formation in *Drosophila*

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How autophagic degradation is linked to endosomal trafficking routes is little known. Here we screened a collection of uncharacterized Drosophila mutants affecting membrane transport to identify new genes that also have a role in autophagy. We isolated a loss of function mutant in Synaptosomal-associated protein 29 kDa (Snap29), the gene encoding the Drosophila homolog of the human protein SNAP29 and have characterized its function in vivo. Snap29 contains two soluble NSF attachment protein receptor (SNARE) domains and a asparagine-proline-phenylalanine (NPF motif) at its N terminus and rescue experiments indicate that both SNARE domains are required for function, whereas the NPF motif is in part dispensable. We find that Snap29 interacts with SNARE proteins, localizes to multiple trafficking organelles, and is required for protein trafficking and for proper Golgi apparatus morphology. Developing tissue lacking Snap29 displays distinctive epithelial architecture defects and accumulates large amounts of autophagosomes, highlighting a major role of Snap29 in autophagy and secretion. Mutants for autophagy genes do not display epithelial architecture or secretion defects, suggesting that the these alterations of the Snap29 mutant are unlikely to be caused by the impairment of autophagy. In contrast, we find evidence of elevated levels of JAK/STAT ligand, receptor and associated signaling, which might underlie the epithelial defects. In summary, our findings support a role of Snap29 at key steps of membrane trafficking, and predict that signaling defects may contribute to tissue alterations associated to loss of SNAP29.

Identification of a novel feedback mechanism linking aerobic glycolysis to oncogenic transcription

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One of the distinctive features of cancer cells is the reprogramming of their glucose metabolism from oxidative respiration to aerobic glycolysis, in the process known as the "Warburg effect". In these conditions, increased glucose uptake meets the metabolic needs of cancer cells for sustaining abnormal cell proliferation. Metabolic reprogramming is usually considered as a simple consequence of tumor development and oncogene activation; a newly emerging paradigm is that oncogenic signaling can also be regulated downstream of metabolic pathways. In this way aerobic glycolysis assumes causative roles in controlling cancer cell behavior in addition to its core biochemical function. Starting from this idea, we explored new possible links between glucose metabolism and gene transcription by performing genome-wide microarray expression profiling in cells treated or not with glucose uptake inhibitor. Through bioinformatic GSEA analyses we found a surprising link between glucose metabolism and key transcription factors regulating cell proliferation, organ growth, tumor cell survival and aggressiveness. In particular, we found that when cells actively incorporate glucose and route it through the glycolytic cascade, the transcriptional activity of these pro-tumorigenic factors is increased; on the contrary, when glucose uptake is blocked, or glycolysis is reduced, the transcriptional activity is decreased. More in detail, we propose that Phosphofructokinase (PFK1), the enzyme regulating the first committed step of glycolysis, has an important role in promoting gene transcription through its physical interaction with transcription co-factors.

Elucidating the mechanisms of ERC1a/liprin-α1 complex mediated cell polarization and migration

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Cell migration during metastatic invasion requires the coordination of actin and adhesion dynamics to promote the protrusive activity at the cell front. We have identified the widely expressed adaptor protein liprin-a1 and its binding partner ERC1a as part of a molecular network linked to cell migration and invasion. Altering the levels of expression of either protein affects key steps of the migratory/invasive circuit: silencing of either liprin-a1 or ERC1a hampers the morphology, speed and directionality of cells within a two- and three-dimensional extracellular matrix (ECM). These effects correlate with the enhanced instability of lamellipodia observed in the silenced cells. Live cell imaging demonstrated a similar spatio-temporal localization of ERC1a and liprin-a1. The two proteins are dynamically concentrated behind the protruding lamellipodia, next to the zyxin-positive focal adhesions (FAs) that turnover rapidly at the front of the migrating cell. Interestingly, the lack of liprin-a1 or ERC1a decreases the internalization of active b1 integrins and FA dynamics. These results suggest that liprin-a1/ERC1a proteins promote cell migration by influencing the internalization of active integrins required for FA turnover at the cell front, and sustain a key role for the liprin-a1/ERC1a complex in the trafficking required for lamellipodia stability and cell locomotion. In support of a role of these proteins in tumour progression, we found that liprin-a1 and ERC1a depletion inhibits cell invasion and ECM degradation. Furthermore, we observed high levels of expression of liprin-a1 in several cases of human breast cancer, while results from xenograft experiments indicate a role of liprin-a1 in tumor cell invasion also *"in vivo".*

Myosin VI bridges ubiquitin signaling and cell migration

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Myosin VI is an unconventional myosin that, unlike all other myosins, moves towards the minus end of actin filaments rather than to the plus end. This unique characteristic enables it to carry out specific, distinct functions, such as endocytosis, maintenance of stereocilia of the inner ear and of the Golgi, cell adhesion, migration and metastasis. In spite of this, the current understanding of the myosin VI protein does not explain how it carries out these diverse processes, as functional mechanistic studies that could be translated into the in vivo context of myosin VI are lacking.
We have previously shown that Myosin VI harbours a functional MIU domain, able to bind Ub chains in vitro (Penengo et al., Cell 2006). During structure-function analysis, we realized that the C-terminal portion of myosin VI harbours a second ubiquitin binding domain (UBD) that lacks sequence similarity with any previously described UBD, which led us to coin the term MyUb (Myosin VI Ubiquitin Binding) domain. This structural domain includes the ^{1116}RRL^{1118} sequence that is required for myosin VI recruitment to autophagosomes and our structure-function analysis revealed an unexpected regulation of this interaction surface mediated by a splicing region positioned between MIU and MyUb.
These findings prepared the ground to elucidate the role of Ub and MIU-MyUb domains in the physiological regulation of Myosin VI. We focused on cell migration as Myosin VI is believed to play an explicit role in this process under normal and pathological conditions. As a consequence of our study we identified an unexpected
role for Myosin VI in the methylation of cytoplasmic proteins during cell migration.
Results will be presented.

The tumour suppressor protein OPCML prevents Axl-mediated EMT and motility in ovarian cancer cells

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Ovarian cancer is a lethal gynecological malignancy that spreads to vital organs before any symptoms appear. The Opioid-binding Protein/Cell adhesion Molecule-Like (OPCML) is a glycosylphosphatidylinositol (GPI) anchored protein belonging to the IgLON family. OPCML is silenced in over 80% of ovarian cancer patients but also in lung, colon and other cancers. OPCML is a tumour suppressor that inhibits tumour cell growth *in vitro* and *in vivo* by negatively regulating a spectrum of Receptor Tyrosine Kinases (RTKs) such as EPHA2, FGFR1, FGFR3, HER2 and HER4. However, the role of OPCML in tumour biology remains to be fully elucidated. AXL, a member of the TAM family of RTKs, is a clinically relevant target in ovarian cancer and is involved in cross talk between oncogenic signalling and Epithelial to Mesenchymal Transition (EMT). Here we show by mammalian 2-hybrid, co-immunoprecipitation and proximity-ligation assay that OPCML interacts with Axl. Upond stimulation by the Axl ligand Gas6, OPCML recruits and concentrates Axl in detergent resistant membranes, where Axl is poorly phosphorylated and non-competent for signalling. As a consequence, sustained phosphorylation of ERK1/2 becomes blunted and the EMT transcriptional regulator Slug is not transcribed. As a result, even though cells stimulated by Gas6 presents a robust acceleration in motility parametres, cells expressing OPCML show a strongly reduced motility in both single and collective cell migration assays. Also, silencing AXL in control cells reduces migration to the same level of OPCML expressing cells. Furthermore, silencing AXL in OPCML-expressing cells does not further reduce migration, thus suggesting that OPCML and AXL function in the same pathway. This and our previous work show that OPCML can interact with numerous cell surface receptors to alter signal transduction cascades and potentially exert its tumor suppressor effects at a system level, thus making it a promising clinical therapeutic agent.

Collective chemotaxis of malignant B cells

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Collective cell migration is a widespread biological phenomenon, whereby groups of highly coordinated, adherent cells move in a polarized fashion.
This migration mode is a hallmark of tissue morphogenesis during development and repair, and of solid tumor dissemination. In addition to circulating as solitary cells, lymphoid malignancies can assemble into tissues as multicellular aggregates. Whether malignant lymphocytes are capable of coordinating their motility in the context of chemokine gradients is, however, unknown. Here, we show that malignant B and T lymphocytes, in response to chemokines, assemble into clusters that migrate directionally along CCL19 or CXCL12 gradients and display a wider chemotactic sensitivity than individual cells. Physical modelling, considering clusters as solid objects, recapitulates cluster motility statistics and predicts that intracluster cell cohesion reduces noise and enhances directionality compared to single cells.
Quantitative image analysis verifies this prediction. In addition we reveal that forward migration runs of clusters are periodically interrupted by transitory rotation and random phases allowing tip cell recycling. Strikingly, cell clusters are much less sensitive to chemorepulsion, a feature of individual cells exposed to steep gradients of CCL19 or CXCL12. Individual cell chemorepulsion depends on endocytosis of CCR7. In cluster, however, CCL19-induced internalization of CCR7 in leader cells, is accompanied by protrusion retraction, loss of polarity, and the ensuing replenishment of leaders by follower cells, in a process that allows clusters to resist chemorepulsion. Thus, coordinated cluster dynamics confers distinct chemotactic advantages, highlighting unexpected features of lymphoid cell migration relevant for tissue homing an dissemination.

Mechanism of matrix metalloproteinase secretion during breast tumor cell invasion

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The trade-mark of a metastatic tumor cell is its ability to disseminate from the primary tumor by degrading the extracellular matrix and basement membranes that form a barrier around the tissue. In particular, in breast cancer the transition of ductal carcinoma in situ (DCIS, *i.e.* epithelial cell proliferations that spare myoepithelial cells and basement membrane) to invasive ductal carcinoma (IDC) is a poorly understood, yet key event as perforation of the basement membrane by carcinoma cells correlates with poor prognosis. Remodeling of the extracellular matrix by metastatic cells requires formation of actin-based protrusions of the plasma membrane called invadopodia, where the trans-membrane matrix metalloproteinase MT1-MMP accumulates. The nature of the carrier(s) that mediate(s) plasma membrane delivery of MT1-MMP, the mechanism underlying MT1-MMP exocytosis in the biogenesis of mature invadopodia and how it is possibly influenced by the composition and biophysical properties of the matrix remain poorly understood.I will present evidence that MT1-MMP is required for the transition of in situ carcinoma to invasive breast cancer lesions based on immunohistochemistry analysis of a large cohort of breast tumors and on the intraductal xenograft model. At the mechanistic level, I will present data supporting a general exocytic mechanism of MT1-MMP used by tumor cells to breach the basement membrane and for invasive migration through fibrous type collagen-enriched interstitial tissues surrounding the primary tumor.

Posters

(presenting authors are shown underlined)

P1

Breast cancer cell responsiveness to Tamoxifen: the potential role of glucose-induced gene expression changes

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Background and aims: Epidemiological data support the hypothesis that type 2 diabetes (T2D) is an independent risk factor for multiple types of cancer. The presence of T2D, as cancer-associated comorbidity, is linked to poorer prognosis and survival, particularly in breast cancer post-menopausal women. Therefore, T2D-induced metabolic derangements must be considered for a correct evaluation of cancer outcomes, especially to tailor novel targeted strategies. Hyperglycemia, the most important feature of T2D, is known to affect breast cancer cell proliferation. Conversely, little is known about the contribute of glucose to a more malignant phenotype of breast cancer cells and particularly to drug resistance.
Thus, we investigated the effect of glucose on breast cancer cell sensitivity to 4-hydroxytamoxifen (4-OHT), an antagonist of the estrogen receptor (ER), widely used in breast cancer treatment.
Methods: MCF7 breast cancer cells (ER^+) have been treated with 4-OHT in high (HG-25 mM) or in low (LG-5.5 mM) glucose medium. Cell viability and cell cycle have been assessed by sulforhodamine and cytometric assays, respectively. The transcriptome of MCF7 cells has been characterized by RNA-Sequencing on a Next Generation Sequencing (NGS) platform.
Results: In LG, 4-OHT reduced breast cancer cell viability by about 50%. At variance, in HG, sensitivity to 4-OHT was 2-fold reduced. Consistently, in HG, a reduced amount of cells in G1 phase was observed, whereas a clear G1/S transition block was induced by 4-OHT in LG. Interestingly, RNA-Seq revealed that glucose significantly deregulates gene expression and that 70 cell cycle-related are significantly down-regulated only when breast cancer cells were shifted from HG to LG.
Conclusions: Glucose exposure may shape the transcriptome of breast cancer cells, affecting their responsiveness to chemotherapy.

P2

Role of the phosphatase Shp1 in the anti-invasive activity of the glycerophosphoinositols

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The glycerophosphoinositols are biologically active metabolites deriving from the activity of the phospholipase A_2IVα on the membrane phosphoinositides (Corda et al., 2012). Three possible phosphorylation sites on the inositol ring give rise to several phosphorylated forms of GPIs but glycerophosphoinositol (GroPIns) and glycerophosphoinositol 4-phosphate (GroPIns4*P*) are the most abundant and extensively studied. Importantly, GroPIns and GroPIns4*P* inhibit the extracellular matrix (ECM) degradation in human mammary carcinoma and melanoma cells by reducing the number of invadopodia, the plasma membrane structures responsible for the ECM degradation, but not affecting their structural organization. This suggests that GPIs might act inhibiting the formation of invadopodia at an early level (Buccione et al., 2005).
In our laboratory, the tyrosine phosphatase Shp1 has been recently shown to be a direct target of both GroPIns and GroPIns4*P* and its enzymatic activity is required for GroPIns-, and not GroPIns4*P*-, mediated inhibition of the ECM degradation in melanoma cells. Moreover, preliminary data suggested that Shp1 negatively regulates the invadopodia formation as the number of cells forming invadopodia is reduced in Shp1 *wild type* overexpressed conditions and increased in Shp1 *knock-down* conditions.
Based on this, the molecular mechanism of GroPIns activity on the ECM degradation was investigated through the identification of Shp1-interacting proteins. In particular, we looked for structural and functional proteins involved in invadopodia formation since complementary experiments showed the localization of Shp1 within invadopodia. The results of Shp1-immunoprecipitation experiments showed that Cortactin binds to Shp1 and GroPIns treatment increased this interaction.
These data together contribute to define the molecular mechanism of the GroPIns-mediated inhibition of ECM degradation and also provide the first evidence of the involvement of Shp1 in cancer invasion.

P3

L1: a new player and target in tumor vasculature

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Anti-angiogenic therapy for tumor treatment, best exemplified by the anti-VEGF drug bevacizumab, has displayed a remarkable potential in certain cancer types. However, it is clear that novel vascular targets are needed to improve the efficacy of current anti-angiogenic strategies as well as to circumvent the resistance/evasion mechanisms that have emerged in different experimental models and in cancer patients.
We have found that the neural immunoglobulin-like cell adhesion molecule L1, that plays a crucial role in CNS development and plasticity, is aberrantly expressed in cancer-associated vessels, while it is not found in normal vasculature. Based on this observation, we have set up a number of *in vivo* and *in vitro* models to investigate the functional role of the neural Ig-CAM L1 within the tumor vasculature.
Our results revealed that L1 orchestrates several functions of endothelial cells and plays a major role in the aberrant pathophysiology of cancer vessels. Such a pleiotropic effect was dependent on L1-mediated control of gene networks and biochemical pathways that, in turn, govern vascular function.
Finally, we demonstrated that the inactivation of L1 in tumor pre-clinical models represents a viable option for novel anti-angiogenic treatments.
Our data may pave the way to innovative vascular targeting strategies in the context of tumor therapy.

P4

Alternative splicing in adhesion and motility processes by RNA-Seq in MCF-7 cells: identification and characterization of a new transcript of SEMA3F

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Breast cancer is the most common tumor among women and the second leading cause of cancer death, mainly caused by metastatic spread. Tumor cells invasiveness is due to an alteration of cell-cell and cell-matrix connections. Thus, an altered expression of adhesion and motility-related molecules is a crucial event in this process. Among these molecules, semaphorins, a large family of transmembrane or secreted molecules, that regulate cell migration and adhesion, are of peculiar interest. A growing number of studies, and our recent work on *SEMA6B* in breast cancer, demonstrated their involvement in cancer progression, often with divergent functions. Moreover, it's known that cancer cells manipulate the alternative splicing (AS) pattern of adhesion/motility genes to escape immune system cells and to initiate epithelial-mesenchymal transition. To determine gene expression and AS of all adhesion/motility encoding genes (including semaphorins, their receptors and co-receptors) we used MCF7 cells, a well-established model of breast cancer. By RNA-Seq, we studied MCF7 transcriptome and found interesting preliminary results, particularly for semaphorin3F gene (*SEMA3F*). We identified, and experimentally confirmed, a novel *SEMA3F* transcript generated by AS, predicted to encode a truncated semaphorin, which lacks Ig-like and R/K rich domains. The expression of the new transcript was analyzed in a panel of breast cancer biopsies and in their healthy counterpart. Interestingly, the new *SEMA3F* transcript is expressed only in tumor cells in all analyzed samples, whereas the canonical transcript is expressed in both tissues. However, by quantitative Real-Time PCR we detected a significant up-regulation of the canonical *SEMA3F* transcript in cancer vs healthy. These findings indicate that both the canonical and the new *SEMA3F* transcript are a potential biomarker for onset/progression of breast cancer, suggesting that semaphorin 3F may have a specific role in breast cancer tumorigenesis.

P5

Life at the leading edge: membrane-actin cytoskeleton interface regulates filopodia formation

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The dynamic assembly and disassembly of actin filaments must be coupled with membrane dynamics, in order to allow cell movement and the formation of different structures, such as lamellipodia or filopodia. In this context, some proteins are of outstanding interest, for example IRSp53 (Insulin Receptor Substrate protein of 53 kDa), a scaffolding protein that sits at the actin:membrane interface.
We found out that IRSp53 is a weak capper, able to prevent uncontrolled filament growth; this effect is relieved by GTP-bound CDC42, that binds IRSp53 partial CRIB domain. Moreover, the SH3 domain from IRSp53 interacts with VASP proline-rich region; VASP localizes to sites of active actin assembly, such as filopodia tips, and possesses a processive elongation activity over actin filaments. Using TIRF microscopy, we demonstrated that IRSp53 was able to recruit VASP over functionalized beads, promoting its clustering and subsequent processive elongation activity. Of note, in presence of active CDC42, IRSp53:VASP interaction is strengthened.
In order to analyze the effect of IRSp53:VASP interaction in cells, we used MEF cells derived from IRSp53 null mice and reconstituted with pBABE-IRSp53 vector. VASP forms bright foci at the plasma membrane, preluding to filopodia formation, but in absence of IRSp53 foci formation is affected. Moreover, in absence of IRSp53, we observed a lower number of CDC42-induced filopodia. Finally, lacking of IRSp53 impairs wound healing, cell migration and invasion.
In summary, we think that in resting conditions IRSp53 exerts a weak capping activity over actin filaments, when signaling is activated GTP-bound CDC42 binds IRSp53, so this latter protein is able to recruit and clusterize VASP at the plasma membrane. At this point, both the membrane bending properties of I-BAR domain from IRSp53 than VASP processive elongation activity cooperate in filopodia formation.

P6

Effect of specific PI3K/mTOR inhibitors on squamous lung cancer cells carrying PI3K/PTEN alterations

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Background A prominent role in the pathogenesis of SQCLC has been attributed to aberrant activation of the phosphoinositide 3-kinase (PI3K) signaling pathway, due to amplification (25-40%) or oncogenic mutations of the PIK3CA (3-10%) and mutation or loss/reduced PTEN levels (10-70%). The aim of this study was to analyse the effect of three inhibitors, NVP-BEZ235, NVP-BKM120 and NVP-BYL719, showing different specificity towards members of the PI3K/AKT/mTOR axis, on SQCLC cell lines with PI3K or PTEN alterations..
Material and Methods SKMES-1 cells were stable transfected with plasmids containing wild type, or mutated E545K or H1047R p110α subunit of PI3K. To generate clones with reduced PTEN levels, SKMES-1 cells were stably transfected with BLOCK-iT POL II miR RNAi Expression Vector containing miRNA directed to PTEN.
Results Alterations of PI3K/PTEN axis caused an increased growth rate both in 2D and 3D cultures. Drugs treatment affected cell proliferation in a similar extent in PI3K mutated/amplified clones compared to SKMES-1 cells whereas PTEN deleted clones showed an increased sensitivity to BKM120. The clones with alterations of PI3K or PTEN showed increased migration and invasion properties associated with increased activity of RhoA, CDC42, Rac1 and MMP proteins. PI3K inhibitors significantly reduced migration/invasion capability, MPPs production, RhoA family activity and EMT only in cells carrying PI3K gain of function. In vivo experiments confirmed that tumors from PI3K mutated clone retained the mesenchymal phenotype and proved the ability of BYL719 in reducing the expression of vimentin in cytokeratin 7 positive cells only in PI3K mutated tumors.
Conclusion The data confirm that specific PI3K inhibitors reverted the invasive phenotype and inhibited the EMT in the presence of PI3K gene alterations warranting further clinical development of agents targeting this pathway.

P7

Interfering with proliferation and survival of tumor initiating cells by targeting phosphatidylcholine metabolism

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It is increasingly evident that tumors comprise hierarchically organized heterogeneous populations of cells, sustained by a limited number of self-renewing cells. Identification of these tumor initiating cells (TIC) and the definition of factors that sustain TIC proliferation could eventually result in more efficient anti-cancer therapies. Metabolic alterations were recently identified as hallmark of tumor cells. We contributed to this field identifying an abnormal choline phospholipid metabolism, describing an up-modulation of a phosphatidylcholine-specific phospholipase C (PC-PLC) in breast and ovarian cancer (Iorio et al, 2010; Paris et al, 2010; Abalsamo et al, 2012). However, no direct correlation between TIC and altered metabolism has been yet reported. To this purpose we investigate the role of PC-PLC in TIC using as models human squamous carcinoma cell lines, A431 and Caski, grown both in adherent (AD) conditions and as spheres (Bortolomai et al, 2010). Compared to non-tumoral keratinocyte HaCaT cell line, analyses of PC-PLC expression and activity showed that A431-AD cells expressed high levels of PC-PLC, while Caski cells had a 3-fold lower overall PC-PLC content. PC-PLC was expressed at high levels only in a subset of cells inside the A431 spheres. The enzymatic activity was higher in A431-AD cells than in A431 spheres. A PC-PLC inhibitor (D609) in a micromolar range was able to reduce to a different extent the proliferation rate of all the analyzed cancer cell lines. Furthermore, A431- and Caski-AD cells lost more than 50% of their sphere forming efficiency. In agreement with a selective effect on TIC, a 50-fold lower dose of the inhibitor strongly reduced the proliferation rate of A431spheres and the expression of stemness markers *Nestin* and *Nanog*. Altogether these results suggest a role for PC-PLC in stem cells maintenance and may open the way to new molecular therapeutic strategies aimed at selective interference with TIC proliferation and survival.

P8

ERK8 (MAPK15) mediates BCR-ABL-induced autophagy and cellular transformation

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ERK8 is the last identified member of the MAP kinase family of proteins. Its activity is modulated by nutrient deprivation and important human oncogenes. We have previously shown that BCR-ABL stimulated ERK8 activity and that the ABL1 proto-oncogene interacted with this MAP kinase and mediated its activation by upstream stimuli. Also, we have recently described a role for ERK8 in the regulation of the autophagic process, and demonstrated the feasibility of pharmacologically interfering with autophagy by modulating the activity of this kinase.
Autophagy has been demonstrated as necessary for BCR-ABL-induced leukemogenesis as well as to protect cancer cells from apoptosis induced by antineoplastic drugs. Based on these evidences, an inhibitor of autophagy is being tested for its ability to potentiate tyrosine kinase inhibitors (TKIs)-induced cell death, in CML patients.
The objective of our research has been to investigate a role for ERK8 in BCR-ABL-dependent autophagy. Indeed, while the use of imatinib and of related 2nd generation TKIs has clearly revolutionized the therapy of chronic myeloid leukemia (CML), these treatments face important problems of primary and secondary resistance. Consequently, there is still need for alternative options to "integrate" current pharmacological approaches.
We demonstrate that BCR-ABL stimulated autophagy in our cellular model system, and that ERK8 was able to mediate this effect. Interestingly, ERK8 was able to physically recruit the oncogene to autophagosomal vesicles. Moreover, not only artificial depletion of the endogenous MAP kinase inhibited BCR/ABL-dependent autophagy but we also show that it was possible to pharmacologically interfere with this process. Ultimately, based on the role of autophagy in BCR-ABL-dependent transformation, we show that ERK8 is required for cell proliferation and transformation induced by this oncogene, therefore establishing this MAP kinase as a novel feasible therapeutic target for human CML.

P9

Deregulation of a c-myc-miR34a circuitry in tumorspheres from *in vitro* transformed cell lines

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According to the cancer stem cell (CSC) hypothesis, only a subset of tumor cells, sharing features with normal stem cells, are endowed with tumorigenic potential. The origin of CSCs is elusive; however, evidence has been reported that they can originate form bulk tumor cells through a dedifferentiation process or, as shown in *in vitro* transformed cell lines, during neoplastic transformation of differentiated cells.
To investigate the possible generation of CSCs during propagation of *in vitro* transformed cell lines, we exploited a cellular system derived in our laboratory from telomerase immortalized human fibroblasts, which recapitulates fibroblast neoplastic transformation. To isolate potential CSCs, we used the tumorsphere approach. Growing cells in the absence of serum and in the presence of growth factors, we obtained spheres with a frequency ranging between 2-10%. Sphere cells could be replated for at least six times and showed increased *Sox2* expression, suggesting that they are endowed with self-renewal potential and stemness features. However, preliminary *in vivo* tumorigenic assays failed to show an increased tumorigenicity of sphere cells. Moreover, compared to adherently growing cells, sphere cells were characterized by a reduced expression of genes involved in tumorigenesis and stemness, as c-*myc*, *GNL3* and *Notch*, as well as an increased expression of the tumorsuppressor microRNA miR34a. These observations suggest that tumorsphere formation might not always be an effective methods to isolate highly tumorigenic cells and that CSC properties and tumorigenicity could be dissociated features. Experiments are in progress to determine the c-myc downregulation mechanism and its possible functional meaning.

P10

Identification of molecular determinants of tumor invasive programs by RNAi-based phenotypic screening

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Cell migration is a prominent feature of cancer cells dissemination and metastasis formation. The precise molecular mechanisms triggering plasticity of tumor cell migration remain largely unresolved. To this end, we characterized novel key regulators of migratory modes by a multi-step medium-throughput approach in which RNAi targeting of the Rab GTPase family is combined with high-content imaging analysis for the identification of trafficking hubs required for the formation of Circular Dorsal Ruffles (CDR), apically restricted actin-rich protrusions, that have emerged as surrogate markers of a mesenchymal mode of migration. We selected Mouse Embryonic Fibroblasts (MEFs), which form efficiently CDRs upon PDGF stimulation, as cellular model. MEFs were reverse transfected, serum-starved and stimulated with PDGF before processing them for immunofluorescence analysis. We developed a dedicated-image analysis pipeline to quantitatively assess CDR formation across the different experimental conditions and generated a list of putative candidate genes altering CDR response. We further characterized RAB35 the knock down of which resulted in the strongest inhibitory phenotype, to validate our experimental strategy. To this end, we showed that loss of RAB35 impairs CDR formation of different cell lines in response to a variety of growth factors, further inhibiting chemotactic single cell migration. Noteworthy, in vitro invasion assays and 3D culture systems demonstrated that the ablation of RAB35 affects the ability of DCIS.COM cells to move in a geometrically defined 3D environment and reduces the number of 3D acinar structures forming invasive outgrowths, phenotypic alterations similar to those associated with tumor progression. Collectively, our data provide evidence for a key role of RAB35 in migratory and invasive processes that might promote cancer cell dissemination.

P11

Mutant cohesin drives chromosomal instability in early colorectal adenomas

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Chromosome missegregation leading to chromosomal instability, is thought to play a pivotal role in cancer development. As major cohesin function is to assure correct chromosome segregation, increasing data suggests its involvement in tumorigenesis. Colorectal cancer (CRC) is a useful model for investigating the role of cohesin in carcinogenesis. CRC develops over the course of many years as a consequence of the accumulation of specific mutations in both oncogenes and tumor suppressor genes. These mutations arise in a characteristic sequence leading to early adenoma/dysplastic crypt, late adenoma and carcinoma. Two types of genomic instability have been identified in CRC: chromosomal instability (CIN), is present in around 85% of colorectal cancers, while the remaining 15% shows microsatellite instability (MSI). CIN was proposed as the major cause of cancer development more than 100 years ago, but its molecular mechanisms have not yet been completely defined. In this regard, the identification of gene(s) that gives rise to a CIN phenotype at an early stage of CRC development has been challenging. To this aim, we analyzed colorectal early adenomas and identified eleven mutations in *SMC1A* core cohesin subunit. Transfection of the *SMC1A* mutants identified in early adenomas and wild-type *SMC1A* gene silencing in normal human fibroblasts led to chromosomal instability. Since *SMC1A* is an X-linked gene, our finding suggests that a single allele mutation is enough to trigger chromosomal instability and tumorigenesis.

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P12

Structure/function analysis of the Numb/Hdm2/p53 circuitry in the perspective of therapeutic applications in cancer

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The cell fate determinant Numb orchestrates tissue morphogenesis and patterning in developmental systems. In the human mammary gland, Numb is a tumour suppressor. The protein is lost or reduced in ~30% of breast and lung cancers and correlates with a more adverse prognosis.
Loss-of-Numb causes increased Notch signaling and decreased p53 tumor suppressor signaling. This latter effect depends on the loss of the inhibitory function of Numb on Hdm2, the E3-ligase mediating p53 ubiquitination/degradation. The downregulatory function of Numb over Hdm2 is a function of the interaction between these proteins in the context of a Numb/p53/Hdm2 tri-complex.
The restoration of Numb levels in Numb-defective primary tumor cells reverts p53 dysfunction. Therefore, gaining insights on how Numb functions to prevent the Hdm2-mediated ubiquitination of p53 could be relevant for the rationale designing of molecules to inhibit Hdm2 and to restore p53 function in Numb-defective tumors.
We set out to identify the structural determinants responsible for the Numb/Hdm2 binding and we restricted the interaction surface of Hdm2 with Numb to the acid domain, the Hdm2 region involved in p53 ubiquitination. In Numb, the adaptor PTB domain appears to be necessary and sufficient for binding to Hdm2, being also required for Numb/p53 interaction. An 11 amino acid insert in the PTB domain, which is present only in Numb isoforms 1 and 2, is critical for Numb/Hdm2 binding. NMR studies point to a key role for polar and hydrophobic residues present on the interaction surfaces of either Numb or Hdm2.
Ongoing work is aimed at further characterizing the structural determinants of the complex with the aim to provide structural knowledge for the identification of new Hdm2 inhibitors. We are also addressing whether the Numb isoforms have distinct roles in stem cells physiology, by controlling the functional asymmetry of the Numb-p53 circuitry at mitosis, and the contribution of their deregulation in cancer.

P13

Crosstalk between c-Myc and/or V12Ras expressing epithelial cells and normal fibroblasts

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It is increasingly evident that cancer cells and their surrounding microenvironmental cells co-evolve, ultimately sustaining neoplastic transformation. A key component of tumour stroma are cancer-associated fibroblasts (CAFs), but is unclear how cancer cells drive the transition from normal-associated fibroblasts to CAFs. In our model, the first stages of tumourigenesis are exemplified by the crosstalk between epithelial cells expressing single or combination of oncogenes (c-Myc and/or V12Ras), and normal fibroblasts. Conditioned media from hT-RPE expressing c-Myc, stimulate normal fibroblast migration. In secretome studies we showed that among the most down-regulated factors by c-Myc are IGFBP6 and IGFBP7 (insulin-like growth factor binding protein-6 and 7), able to sequester IGFs (insulin-like growth factors), thus preventing binding and signaling through their cognate receptors IGFRs. In hT-RPE expressing c-Myc, we have observed also a remarkable increase of IGF2 trascription. Unless pre-exposed to anti-IGF-1R blocking antibodies, TIG-3 fibroblasts are chemoattracted by the CM of hT-RPE-MycER cells. Accordingly, fibroblasts exhibit an increased chemotactic response to CM of hT-RPE-MycER silenced for IGFBP6, suggesting that the IGF-2/IGFR system is involved in the stimulation of fibroblast migration by oncogene-expressing epithelial cells. Also, the expression of urokinase (uPA) and its receptor uPAR is upregulated in TIG-3 fibroblasts, following exposure to IGF-2 or to hT-RPE-MycER CM. Furthermore, uPAR silencing in fibroblasts prevents IGF-2-dependent migration, showing that the uPAR is required for IGF-2 dependent response. Similar experiments are carried out using MCF10A-MycER and human primary breast fibroblasts, as representative of the mammary gland context.

P14

The CDC42-interacting protein 4 controls epithelial cell cohesion and tumor dissemination

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The role of endocytic proteins and the molecular mechanisms underlying epithelial cell cohesion and tumor dissemination are not well understood. Here, we report that the endocytic F-BAR-containing CDC42-interacting protein 4 (CIP4) is required for ERBB2- and TGF-β 1-induced cell scattering, breast cancer (BC) cell motility and invasion into 3D matrices, and conversion from ductal breast carcinoma in situ to invasive carcinoma in mouse xenograft models. CIP4 promotes the formation of an E-cadherin-CIP4-SRC complex that controls SRC activation, E-cadherin endocytosis, and localized phosphorylation of the myosin light chain kinase, thereby impinging on the actomyosin contractility required to generate tangential forces to break cell-cell junctions. CIP4 is upregulated in ERBB2-positive human BC, correlates with increased distant metastasis, and is an independent predictor of poor disease outcome in subsets of BC patients. Thus, it critically controls cell-cell cohesion and is required for the acquisition of an invasive phenotype in breast tumors.

P15

UV irradiation inhibits Ewing Sarcoma cell growth by modulating alternative pre-mRNA processing

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Ewing Sarcoma (ES) is a highly aggressive pediatric malignancy characterized by the fusion between *EWSR1* gene on chromosome 22 and the *FLI1* gene on chromosome 11. This translocation generates the chimeric fusion protein EWS/FLI1 at the expense of one *EWSR1* allele, which encodes the Ewing Sarcoma protein EWS. Both EWS and EWS/FLI1 are involved in the modulation of gene expression and alternative splicing (AS). Moreover, EWS protein displays a pivotal role in the DNA damage response by regulating DNA damage induced alternative splicing. In cancer, AS determines a great variability of proteome thus impacting apoptosis, cell cycle transitions, invasion and allowing environmental adaptation and tumor growth. Chemotherapeutic drugs and irradiation affect directly the RNA polymerase II (RNAPII) phosphorylation and reduce its transcription elongation rate, thus modulating AS choices. It has been described that UV light irradiation induces hyper-phosphorylation of RNAPII and its subsequent ubiquitin-mediated degradation. We characterized here the response to low doses of UV light irradiation in two ES cell lines (SK-N-MC and LAP-35) characterized by the same chromosomal translocation. Clonogenic assay and propidium iodide staining highlighted a higher sensitivity of SK-N-MC than LAP-35 cells to UV insult. RNA profiling revealed genes differentially regulated by UV light irradiation in the two cell lines. Mechanistically, we found that UV light irradiation leads to enhanced phosphorylation and decreased processivity of RNAPII in SK-N-MC cells, which in turn causes different processing of pre-mRNAs. Interestingly, some chemotherapeutic drugs, such as etoposide, also affect RNAPII phosphorylation thus determining a fine-tuned regulation of AS.
Collectively our results suggest that differences in RNAPII phosphorylation upon UV light treatment trigger a specific modulation of AS in two ES cell lines thus contributing to cell survival and tumor growth.

P16

Correlation between the expression of sphingolipid metabolism enzymes and onco-hematological disease markers

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Sphingolipids represent a class of bioactive molecules capable of modulating the destiny of many cell types, including leukemia cells (1). Notably, sphingosine 1-phosphate (S1P) is implicated in survival, cell growth and migration whereas its precursors, namely ceramide (Cer) and sphingosine (Sph), are considered pro-apoptotic and anti-mitotic agents. Since S1P, Sph and Cer are interconvertible, it has been proposed that it is not the content of each but the balance of this "sphingolipid rheostat" to determine cell death or survival. S1P is formed by the phosphorylation of Sph by sphingosine kinase 1 (SK1), the isoform of SK that plays a key role in cancer (2). On the other hand, the level of Cer are mainly regulated by Ceramide synthase and CerK that phosphorylate the lipid to ceramide 1 phosphate (C1P). Many studies reported that SK1 expression is associated with increased disease progression, chemo-resistance and reduced patient survival. In the present study we evaluated the expression of sphingolipid metabolism enzymes, SK, CerK and CerS in a small cohort of chronic myeloid leukemia-, myeloproliferative diseases- and lymphoma affected patients.

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P17

Epistatic regulation of oncogene-induced responses by the COP9 signalosome, a modifier of cullin-ring E3 ubiquitin ligases

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Hepatocellular carcinoma (HCC) is one of the most frequent tumors worldwide. The genetic changes that cause hepatocyte transformation are not yet discovered. Aberrant activation of oncogenes can lead both to tumorigenic transformation and to cell cycle arrest due to an activation of DNA damage and stress pathways (defined as oncogene-induced responses, OIR). OIR acts as a barrier against tumorigenic transformation. The purpose of this work is to investigate the role of the COP9 signalosome (CSN), a modifier of Cullin-Ring ubiquitin Ligase (CRL) complexes whose substrates are key regulators of cell cycle progression and DNA repair, during the OIR. This activity is carried out by COPS5/JAB1, the catalytic subunit of the complex, which controls CRL activities by removing the modifier Nedd8 from the Cullin component of the complex. A recent work from our group demonstrated that, in regenerating hepatocytes, the CSN is a repressor of replicative stress responses. Moreover, the CSN was found overexpressed in a subset of HCC, suggesting that this complex may have a permissive role towards the overcoming of cell cycle checkpoints in tumorigenic transformation. To test this hypothesis we generated a cellular model of OIR in which hepatocyte progenitors cells were manipulated to obtain the inducible overexpression of c-myc, an oncogene that is frequently overexpressed in HCC. This causes an increase of DNA damage markers, such as γ-H2AX, and activates DNA repair pathways, as demonstrated by the activation of CHK1. These results indicate that high levels of c-myc can lead to an OIR in diploid liver cells. Furthermore we have manipulated these cells to obtain combined overexpression of c-myc and COPS5/JAB1. Increased levels of JAB1 dampen the effects of c-myc, reducing the expression of γ-H2AX and phospo-CHK1. The underlying molecular mechanisms are currently under investigation.

P18

Rab2A is a potential novel predictor of metastatic ability in human breast cancers

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The mechanisms by which tumor cells metastasize and the role of membrane trafficking in this process are not well understood. We screened human Rab GTPases to identify endocytic/exocytic molecules involved in invadosomes using in situ matrix-degradation assays. Here, we report that Rab2A, which is essential for protein transport from the ER to the Golgi, is significantly elevated in human primary breast tumors, and its elevated expression correlates with poor prognosis in breast cancer patients. Rab2A is necessary and sufficient to promote not only matrix degradation but also invasion into a 3D matrix. Surface expression of membrane-type 1 matrix metalloprotease (MT1-MMP), secretion of various MMPs, and formation of invadopodia are not impaired upon loss of Rab2A in MCF10A.DCIS.com breast cancer cells. Conversely, silencing of Rab2A augment cell compactness by increasing surface localization of E-cadherin, ZO-1, and beta-catenin, but not P-cadherin. The opposite is observed upon elevation of Rab2A levels. The phenotypes due to loss of Rab2A are rescued by the expression of a Rab2A RNAi-resistant mutant. Notably, the loss of Rab2A leads to a reduction of N-Cadherin protein level, under conditions in which total E-cadherin is unaffected. Collectively, these results suggest that Rab2A is implicated in mesenchymal-epithelial transition programs. Interestingly, knockdown of GRASP55, a Rab2A effector involved in protein glycosylation in the Golgi stack also impairs matrix degradative activity. Thus, a Rab2A/GRAPS55 axis may be required to control EMT ultimately regulating a proteolytic, mesenchymal invasive program via intracellular glycosylation.

P19

Protein-protein interaction inhibitors: small molecule screening for cancer immunotherapy

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Enhancing human immune system responses to produce effective treatments has long been thought a promising approach to fighting cancer. Though a number of immunotherapeutic strategies have been shown to increase the immune system's ability to control cancer, immunomodulatory antibodies that directly enhance the function of T cells have been garnering significant recent attention. Antibodies, interfering with immune-modulating receptor ligand interaction such as the inhibitory complexes CTLA-4/CD80 and PD1/PDL1or the co-stimulatory complex CD40/CD40L, have entered clinical trials for the treatment of different malignancies. Combination immunotherapy, targeting distinct inhibitory receptor pathways (e.g. PD1/PDL1 and 2B4/CD48), can synergistically enhance T-cell immunity. However, no active ("interaction-disrupting") small molecule has been reported so far that could overcome the drawbacks associated with immunotherapy.
To this end, we set up a newly developed yeast bioluminescence resonance energy transfer (yBRET) bioanalytical platform, a cutting-edge high-throughput technology for monitoring protein-protein interactions (PPIs) in vivo and for screening potential PPIs inhibitors. The extracellular domain of receptors (PD1, 2B4 and CD40) and of their ligand partners (PDL1, CD48 and CD40L) were fused in-frame to a high-efficiency donor luciferase (NanoLuc) and to the acceptor yellow fluorescent protein (YFP), respectively. Both a standard ("intracellular") BRET assay as well as a modified, surface-exposed BRET assay (seyBRET) mimicking the extracellular environment of naturally occurring interactions have been developed.
A preliminary screening of 6,500 compounds performed by yBRET on the 2B4-CD48 interaction has led to the identification of hits, belonging to three classes of chemical compounds, capable of interfering with at micromolar concentrations.

P20

RAB5A in the control of mammary epithelial morphogenesis and motility

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RAB5A, a master regulator of endocytosis, promotes a tumor mesenchymal invasive program. In *Drosophila*, however, loss-of-function of RAB5 leads to hyperproliferation, pointing to a tumor suppressor function.
To rationalize the complex role of RAB5A in tumor development, we investigated its impact on MCF-10A, an immortalized non-transformed mammary epithelial cell line that mimics morphogenesis of mammary gland when culture on 3D reconstituted basement membrane. We generated inducible MCF-10A cells expressing either RAB5A-WT or its dominant negative form (RAB5A-S34N). We found that the expression of RAB5A-S34N is sufficient to sustain MCF-10A cells proliferation in the absence of EGF, through the secretion of a diffusible growth-promoting factor. Conversely, the expression of RAB5A-WT delayed cell cycle progression of cells grown in 2D, albeit it promoted the formation of hyperproliferative acini when grown in 3D. Thus, RAB5A may either be implicated in growth factor independent growth or promote proliferation in 3D. In keeping with these latter findings, clinical data and *in vitro* studies demonstrated that RAB5A is required for invasion and metastasis, suggesting its involvement in tumor progression. To further explore this latter role, we tested MCF-10A cells motility. We demonstrated that RAB5A-WT expression does not affect single cell migration, but specifically enhances collective locomotion. Indeed, RAB5A expressing cells showed increased coordination and coherence of epithelial cell sheet motility, probably related to both a tightening of cell-cell contact and an increase in the area and persistence of cell protrusions at the leading front.
We are currently dissecting the molecular mechanisms through which RAB5A altered function or expression impact on both mammary gland morphogenesis and tumor progression.

P21

The architecture of the Par3:Inscuteable:LGN complex uncovers a novel role of LGN in stabilizing the apical site during Asymmetric Cell Divisions

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In multicellular organisms stem cell asymmetric divisions sustain tissue morphogenesis and homeostasis. Asymmetric divisions are attained by unequal segregation of cell-fate defining components, and by differential positioning of siblings within the tissue. They require a tight coordination between cellular polarity and the division plane, and hence the mitotic spindle axis. Core components of the spindle orientation machinery have been found evolutionary conserved. They consist of LGN, NuMA and Dynein/Dynactin, whose motor activity pulls on astral microtubules to orient the spindle. In stem cells, the adaptor molecule Inscutable (Insc) has been reported to act as a molecular bridge coupling the asymmetric distribution of fate determinants with spindle orientation. Insc interacts directly with the polarity protein Par3 at the apical site, and with LGN. Intriguingly, Insc and NuMA are competitive interactors of LGN (2). I will present the crystallographic structure of Drosophila LGN^{TPR} in complex with the asymmetric domain of Insc, and the biochemical characterisation of the Par3:Insc:LGN assembly. The structure reveals a stable tetrameric arrangement of intertwined molecules, and is compatible with the concomitant binding of LGN to Insc and Par3. Based on this evidence we hypothetise a new function of the Par3:Insc:LGN complex in stabilizing the apical site, and sustaining unequal partitioning of fate determinants during asymmetric cell divisions.

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P22

Ultrastructure of STEM cells

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Today, the concept of STEM cancer cells is one of the main hypotheses of the tumorigenesis. Therefore, it might be useful to cover with fresh eyes the concept of STEM cells per se. It is considered that STEM cells are cells present in rare quantities in tissues at the low differential state tissue and can transform into any somatic differentiated cell from any tissue. However, in spite of dozens years of the examination of STEM cells, well-established markers of STEM cells are not established.
In order to study the morphological features of STEM cells, mammal glands of mice were isolated and dispersed (Tosoni et al., 2012; Methods Mol Biol. 916:59-79.). Cell suspension was incubated in culture medium preventing attachment of cells to the substrate. Under these conditions, STEM cells propagated and formed mammo-spheres where in the centre there was the cell with characteristics of large lymphocytes or similar to embryonic STEM cells. These features include round shape, large and slightly rugged nuclei almost complete absence of heterochromatin, low density of organelles in the cytoplasm, narrow cisternae of the endoplasmic reticulum.
In order to isolate STEM cells initially STEM cells were incubated with a lipophilic fluorescent dye, PKH26, which stained the plasma membrane and due to the asymmetric division was present in the central STEM cells in higher concentration than in other cells. Spheres were dispersed and PKH26-positive and negative cells were purified using FACS sorting and fixed with the standard fixative, prepared for electron microscopy (EM), and examined under EM. The ultra-structure of isolated cells was similar to that observed in the central cells of mammo-spheres. However, in many cells there was no any sign of heterochromatin. Most of cells contained autophagosomes, P-bodies and S-granules. The surface of isolated cells contained a lot of processes. The new hypothesis of STEM cells nature will be discussed.

P23

WNT/β-catenin pathway regulates μ-protocadherin expression through the activity of the CDX2 transcription factor

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Intestinal mucosa is the tissue with the highest self-renewal capacity in human body and its proliferation is mainly supported by the WNT/β-catenin signalling pathway. Activating mutations in one of the members of this pathway are essential for the development of colorectal cancer (CRC).
μ-protocadherin (MUCDHL) is an adhesion molecule member of cadherins family, and is regulated by the CDX2 transcription factor (Hinkel I. 2012). In our previous work we assessed this protein through immuno-histochemical analysis, disclosing an inverse correlation between β-catenin activity and MUCDHL expression, both in normal colon mucosa and in CRC tissue (Losi L. 2011). To better characterize this observation, we modulated β-catenin activity with different approaches. To achieve the inhibition of this pathway we down-regulated the β-catenin transactivation partner (TC4) using a compound called FH535 and siRNA technique in two CRC cell lines. In both cases we found an up-regulation of MUCDHL, and consistently, of CDX2 expression. Unfortunately, since standard CRC cell lines exhibit a constitutive activation of the β-catenin pathway, we couldn't assess the effects of stimulating agents in this context. For this reason, we reproduced a primary culture of colonic epithelium in which this pathway is in a near-physiological condition (Jung P. 2011). In this experimental model the stimulation with LiCl, which determines a nuclear accumulation of β-catenin, induced a strong down-regulation of MUCDHL and CDX2 expression, while the withdrawal of WNT in culture medium resulted in the opposite effects.
In our opinion these data allow us to conclude that MUCDHL expression is regulated by the β-catenin proliferation pathway and could be considered as a putative biomarker to evaluate its activity.

P24

Identification and characterization of the pathophysiological role of nicotinic receptors in lung cancer

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Lung cancer is the leading cause of cancer death worldwide and smoking accounts for approximately 70% of non– small cell lung cancer (NSCLC) and 90% of small cell lung cancer (SCLC) cases, although there is a subset of patients who develop lung cancer without a history of smoking.
Tobacco smoke contains multiple classes of carcinogens, and although nicotine, the addictive and most active component of tobacco smoke, is unable to initiate tumorigenesis in humans and rodents, by binding to cell-surface neuronal nicotinic acetylcholine receptors (nAChRs), it promotes tumour growth and metastasis by inducing cell-cycle progression, migration, invasion, angiogenesis, and evasion of apoptosis in a variety of systems.
In this work we have analysed lung cancer cell lines and establishing at molecular level which nicotinic receptor subtypes are expressed in lung cancer cell lines and lung cancer tissues. Moreover we have investigated the effects of nicotine in regulating cell proliferation and cell migration in lung carcinoma derived cell lines and studied the intracellular signalling of nicotine.

P25

MiR-214 and miR-148b: a targetable miRNA network to control tumor metastatization

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MicroRNAs (miRNAs) are small non-coding RNAs that act as negative regulators of gene expression and play a central role in tumor progression. Aberrant miRNA levels influence gene networks contributing to tumorigenesis and metastasis formation. Therefore, therapeutic modulation of miRNAs might result in an effective targeted therapy. We demonstrated that miR-214 is upregulated in malignant melanomas and coordinates metastasis dissemination by increasing migration, invasion, extravasation and survival of melanoma cells via a novel pathway involving TFAP2A and TFAP2C and the adhesion molecule ALCAM. More recently, we proved that downregulation of miR-148b by miR-214, via TFAP2, contributes to miR-214-induced metastatization by regulating ALCAM. Based on these results, we expanded our analysis and unravelled a regulatory network where miR-214 modulates other miR-148b target genes. In fact, we showed that the overexpression of miR-214 causes a reduction of miR-148b expression level and a consequent de-repression of miR-148b target genes such as PI3KCA, ITGA5 and ROCK1, whereas the opposite was observed following miR-214 downregulation. Moreover, we demonstrated that simultaneous inhibition of miR-214 and overexpression of miR-148b results in a strong reduction of the metastasis formation via the regulation of the extravasation process. Our study demonstrates that miR-214 and miR-148b regulatory loop can be exploited for the development of a new miRNA-based targeted therapy.

P26

A RAB5/RAB4 recycling circuitry induces a proteolytic invasive program and promotes tumor dissemination

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The mechanisms by which tumor cells metastasize and the role of endocytic proteins in this process are not well understood. We report that overexpression of the GTPase RAB5A, a master regulator of endocytosis, is predictive of aggressive behavior and metastatic ability in human breast cancers. RAB5A is necessary and sufficient to promote local invasion and distant dissemination of various mammary and non-mammary tumor cell lines, and this pro-metastatic behavior is associated with increased intratumoral cell motility. Specifically, RAB5 is necessary for the formation of invadosomes, membrane protrusions specialized in extracellular matrix (ECM) degradation. RAB5A promotes RAB4- and RABENOSYN-5-dependent endo/exocytic cycles (EECs) of critical cargos (MT1-MMP and ß3 integrin) required for invadosome formation in response to motogenic stimuli. This trafficking circuitry is necessary for spatially localized HGF/MET signaling that drives invasive, proteolysis-dependent chemotaxis in vitro and for conversion of ductal carcinoma in situ to invasive ductal carcinoma in vivo. Thus, RAB5A/RAB4 EECs promote tumor dissemination by controlling a proteolytic, mesenchymal invasive program.

P27

SHMT1 knockdown induces apoptosis in lung cancer cells by causing uracil misincorporation

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Reprogramming of cellular metabolism towards *de novo* serine production fuels the growth of cancer cells, providing essential precursors such as amino acids and nucleotides and controlling the antioxidant and methylation capacities of the cell. A key role in this metabolic shift is played by the enzyme serine hydroxymethyltransferase (SHMT), which directs serine carbons to one-carbon units metabolism and thymidilate synthesis. While the mitochondrial isoform of SHMT (SHMT2) has recently been identified as an important player in the control of cell proliferation in several cancer types and as a hot target for anticancer therapies, the role of the cytoplasmic isoform (SHMT1) in cancerogenesis is currently less defined. In this paper we show that SHMT1 is over-expressed in tissue samples from lung cancer patients and lung cancer cell lines, suggesting that, in this widespread type of tumor, SHMT1 plays a relevant role. Our experiments demonstrate that SHMT1 knockdown in lung cancer cells leads to cell cycle arrest and, more importantly, to p53-dependent apoptosis. Moreover our data clarify that the apoptosis does not depend on serine or glycine starvation, but is due to increased uracil accumulation during DNA replication.

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The interplay between Rab5 and Kinesin-II in mitosis

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Rab5 is a small GTPase involved in the early steps of endocytosis. It is mainly localized to early endosomes, and controls their docking, fusion and movement on microtubules. In its active GTP-bound form, it recruits downstream effectors that, in turn, are responsible for distinct aspects of early endosome function from signal transduction to selection and transport of cargoes. We found that during mitosis, a pool of Rab5-positive vesicles move within the spindle. Pull down assays on purified proteins revealed that KIF3A, one of the two motor subunit of the Kinesin II complex, binds to the GTP-bound form of Rab5. Silencing of Kinesin II affects the localization of Rab5-positive vesicles within the spindle. Similarly to Rab5, KIF3A appears to be involved in the initial steps of mitosis as its depletion affects the duration of prophase. These results suggest that Kinesin II might participate to the function of Rab5 at the onset of mitosis.

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Blimp1 and miR-23b as new collaborators of p130Cas/ErbB2 breast cancer

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The ability of 3-dimensional ErbB2-positive human mammary epithelial cells to invade is strictly dependent on the overexpression of the adaptor protein p130Cas. To identify the changes of gene expression between non invasive p130Cas overexpressing and invasive p130Cas/ErbB2 cells microarray analysis of coding and non coding genes were previously performed.
PRDM1 (also known as BLIMP1) was found to be upregulated both at RNA and protein level in p130Cas/ErbB2-dependent invasion. Few data are available on the involvement of PRDM1 in cancer progression therefore, we investigated the mechanisms underlying its expression, regulation and functional role in breast cancer.
Loss of function experiments pointed out that Blimp1 is necessary to drive invasion in p130Cas/ErbB2-dependent MCF10A cells. Moreover, Blimp1 overexpression alone is sufficient to trigger invasion in non-invasive MCF10A mammary epithelial cells. Given the importance of PI3K/Akt and Erk1/2 MAPK signalling pathways downstream p130Cas/ErBb2 cooperation, we demonstrated that PRDM1 is preferentially expressed downstream Erk1/2 MAPK-mTOR pathway.
Since we already described an altered small noncoding microRNAs (miRNAs) expression in p130Cas/ErbB2 invasive cells, we checked if a miRNA-dependent PRDM1 regulation occurred. Indeed, miR-23b was found to be downregulated in p130Cas/ErbB2 invasion and its overexpression leads to PRDM1 protein and RNA level decrease. Moreover, transient miR-23b overexpression in p130Cas/ErbB2 cells strongly reduces their ability to migrate. Preliminary data also suggest a direct binding between miR-23b and PRDM1 3'UTR.
These data highlight an important role of PRDM1 in mediating p130Cas/ErbB2-dependent invasive behavior, getting new insights into the role of PRDM1 in mammary carcinoma.

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Targeted inhibition of surgery-induced responses prevents breast cancer local recurrence

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For early breast cancer (BC) patients, local relapse represents what mostly influences disease outcome. Multifocality is a hallmark of most BC, however 90% of local recurrences occur at or close to the same quadrant of the primary cancer. Surgery itself and the consequent process of wound healing have been proposed to stimulate local recurrences via pathway(s) still to be clarified. We used wound drainage fluids (WF) collected from BC patients as surrogates of the stimuli present in the post-surgical setting, to characterize *in vitro* BC cell response to post-surgical inflammation. Our results indicate that surgery-induced inflammation promotes the release of factors that, in turn, induce the activation of p70S6K and STAT3 pathways in BC cells. We found that, following WF stimulation, p70S6K activity is required for the survival of BC cells challenged in "hostile" microenvironments, while STAT3 induces the enrichment of BC cells with stem-like phenotypes and promotes their tumor-initiating abilities.
We next designed an *in vivo* experimental model resembling the course of human BC, to investigate and monitor in mice the appearance of local relapse. Interfering with p70S6K or STAT3 activity using a "3-days peri-surgical treatment protocol" strongly impaired BC local relapse, *in vivo*. These results strongly suggest that activation of these pathways in the post-surgical context is critical for the survival and growth of BC cells, eventually leading to BC recurrence.
In conclusion, we investigated a new crosstalk, taking place in the post-surgery mammary microenvironment, therapeutically exploitable to restrain recurrence in BC patients. Our findings suggest that to identify the most effective treatment we will need not only a proper selection of the "right" targeted drug for the "right" BC patient, but also that choosing the appropriate window of administration during the course of the disease will be crucial for the success of the therapy.

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Role of the endocytic protein Epsin3 in the breast tumorigenesis

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Epsin3 (Epn3) belongs to the Epsin family of endocytic adaptors. While the other family members Epn1 and Epn2 are ubiquitously expressed, Epn3 is exclusively expressed in gastric cells and in wounded or altered tissues, suggesting that Epn3 might exert a specific function.
Recent data from our lab pointed to a novel oncogenic role of Epn3, as 30% of human breast tumors overexpresses Epn3 (byHIC) and its upregulation positively associates with markers of aggressive disease and poor prognosis. Moreover, alterations in Epn3 expression levels in breast cancer cell lines influence their tumorigenic potential *in vitro* and *in vivo.* To finally validate the tumorigenic potential of Epn3 overexpression in vivo we are generating an Epn3 Knock-In (Epn3-KI) mouse model, in which Epn3 is specifically expressed in the mammary tissue. This system will be helpful also to establish wether Epn3 cooperates with known oncogenes involved in breast carcinogenesis.
At the cellular level, overexpression of Epn3, but not of Epn1, activates the epithelial-to-mesenchymal transition (EMT) and confers a more invasive phenotype to normal mammary cell lines. Epn3, but not Epn1, enhances TGF-β-dependent E-cadherin internalization and co-localizes with E-cadherin all along the endocytic pathway. Thus, Epn3 seems to contribute to EMT by acting on E-cadherin endocytosis and turn over at the PM. Importantly, this leads to the accumulation of active β-catenin in the nucleus and activation of the EMT cellular program, which includes transcription of EMT inducers, as well as TGF-β receptors ad ligands, further sustaining the EMT conversion. To gain a better characterization of the molecular mechanism involved, we will: i) perform structure:function studies, by exploiting an Epn3/Epn1 chimeric approach, based on the fact that Epn1 overexpression does not phenocopy Epn3 upregulation in triggering EMT; ii) investigate the Epn3 vs.Epn1 comparative interactome taking advantage of a SILAC-based strategy.

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Cellular senescence induced by oncogenic H-Ras prompts exosome release

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Cell proliferation induced by oncogene activation is restrained by cell senescence, which act as barriers in pre-neoplastic lesions. Senescent cells show proliferation arrest, increased size and activation of senescent associated β-galactosidase (SA-β-Gal). Exosomes, 30 - 100 nm extracellular vesicles derived from the endosomal system, have been recently implicated in cell-to-cell communication in a variety of biological processes; in particular exosomes secreted by senescent cells have been hypothesized to transfer senescence signals to surrounding cells. We investigated the role of exosomes in an experimental model represented by human fibroblasts transfected with oncogenic Ras (H-RasV12). We purified exosomes from cell medium by ultracentrifugation or precipitation, and observed that in H-RasV12 expressing cells oncogene-induced senescence is associated with an enhanced release of exosome-like microvesicles. In addition, immunoblotting demonstrated that proteins involved in exosome biogenesis, such as Alix, or in exosome interaction with recipient cells, like the tetraspanins CD9 and CD63, are over-expressed not only in microvesicles secreted by senescent cells but also in H-RasV12 expressing cells. These results indicate that H-RasV12 induces an increase in the biogenesis of exosome-like vesicles and affects their composition. Exosome-induced senescence in recipient cells is under investigation.

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The role of the endocytic protein Numb in tumor cell migration

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NUMB is a cell fate determinant that controls signaling output by intervening in the context of asymmetric cell division. NUMB, however, was originally identified as an adaptor protein regulating signaling emanating from various plasma membrane (PM) receptors, including NOTCH, Receptor Tyrosine Kinases (RTKs), such as EGFR and c-MET, and integrins, acting at different trafficking levels. Moreover NUMB binds to key players of Clathrin-mediated endocytosis, regulating the internalization of various receptors and it has been found to localize in recycling endosomes where it may regulate the delivery of cargos back to the PM. We identified Numb as a negative regulator of Circular Dorsal Ruffles (CDRs) formation downstream of c-MET and PDGFR. This is accompanied by increase in mesenchymal mode of motility and cell invasion. CDRs formation depends on the recycling back of active Rac to spatial restricted sites of plasma membrane via Arf6. We found that Numb is enriched in Arf6 recycling compartment and inhibits MHC I and Rac recycling. These evidences suggest that Numb might act as a negative regulator of Arf6 dependent recycling. Since, as for most of small GTPases, GEFs are the primary regulatory target, we focused our attention on ARF6 GEFs and in particular on EFA6 A-D and Cytohesin (Arno) subfamilies, reported to control actin remodeling. Among these GEFs, only EFA6 B binds Numb via its PTB domain. Numb may regulate Arf6 dependent recycling by interacting and possibly modulating its GEF EFA6B. If it were true the down-regulation of EFA6 B would abrogate the increase in CDRs formation induced by down-regulation of Numb. Preliminary experiments demonstrated that removal of EFA6 B by itself does not alter CDRs formation while it inhibits the increase of CDR brought by Numb down-regulation. These results further suggest that EFA6 B may be the candidate GEF interacting with Numb during the process of CDR formation.