Meeting ABCD

Riunione Nazionale Dottorandi

Gubbio
10-12 giugno 2010

Organizzatori

Stella Zannini
Consiglio Nazionale delle Ricerche,
Istituto di Endocrinologia e Oncologia Sperimentale (IEOS)

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PROGRAMMA

Giovedì, 10 giugno

13:00-14:00 **Pranzo**

14:00-15:00 **Registrazione e affissione posters**

Chair: Silvia Soddu

15.00-15:30 Zinc finger protein 521: a candidate regulator of normal and malignant haematopoiesis
Michela Lupia (Catanzaro)

15:30-16:00 Beta1 integrin is required for EGFR activity and tumorigenic properties of A549 lung cancer cells
Virginia Morello (Torino)

16:00-16:30 A Pin1/mutant p53 axis fosters aggressiveness in breast cancer
Marco Napoli (Trieste)

16:30-17:00 **Coffee break**

17:00-19:00 **Sessione Poster (numeri pari)**

Chair: Cecilia Bucci

19.00-19:30 Overexpression of small heat shock protein Hspb8 enhances the degradation of misfolded proteins in models of amyotrophic lateral sclerosis and spinobulbar muscular atrophy
Elena Bolzoni (Milano)

19:30-20:00 Chromatin dynamics and epigenomics of tubulin PlTα2 promoter gene in *Paracentrotus lividus*
Marco Emanuele (Palermo)

20:30 **Cena**

Venerdì, 11 giugno

8:00-9:00 **Colazione**

9:00-10:00 **ABCD Lecture**

Giulio Cossu (Milano)

Chair: Paolo Pinton

10:00-10:30 New insight into myomiR function
Alessandra Alteri (Roma)

10:30-11:00 Hematopoietic lineage specific transcriptional regulation of miR-223
Laura Vian (Roma)

11:00-11:30 **Coffee break**

11:00-13:30 **Sessione Poster (numeri dispari)**

13:30-14:30 **Pranzo**

Chair: Antonio Musarò

14:30-15:00 *In vivo* induction of satellite cell proliferation by CKI removal
Maria Grazia Biferi (Roma)

15:00-15:30 Citron-k controls the completion of cytokinesis functionally interacting with Anillin and RhoA
Marta Gai (Torino)

15:30-16:00 Ataxia Telangiectasia Mutated (ATM) interacts with Tankyrase-1 and regulates Tankyrase-1-dependent Poly(ADP-ribosyl)ation of TRF1 and NuMA1 in human cells
Luca Palazzo (Napoli)

16:00-16:30 The Rho GTPase RhoU is a transcriptional target of Stat3 and Wnt-1 pathways and is implicated in cellular migration and proliferation
Davide Schiavone (Torino)

16:30-17:00 **Coffee break**

Chair: Ruggero Pardi

17:00-17:30 Hyperglicaemia induces insulin resistance through ER stress activation in L6 skeletal muscle cells
Claudia Iadicicco (Napoli)

17:30-18:00 Role of scaffold molecule KSR2 in immune cells deficiency associated to obesity
Valentina Gubitosi (Siena)

18:00-18:30 Novel control mechanism of hormone action
Luca Lignitto (Napoli)

18:30-20:00 **Assemblea dei Soci**

20:30 **Cena**

Sabato, 12 giugno

8:00-9:00 **Colazione**

Chair: Carlo Tacchetti

9:00-9:30 Role of the Tpf1, a protein of *Treponema pallidum*, in inducing the production of Treg cells and in driving inflammation via the activation of inflammasome
Chiara Babolin (Padova)

9:30-10:00 Role of IL-6 in skeletal muscle growth and atrophy
Maria Grazia Berardinelli (Roma)

10:00-10:30 Gliadin peptide P31-43 enhances IL15 activity by interfering with its intracellular trafficking
Sara Santagata (Napoli)

10:30-11:00 **Coffee break**

11:00 **Proclamazione miglior poster e miglior tesi di dottorato**

PRESENTAZIONI ORALI
(in ordine cronologico)

Zinc finger protein 521: a candidate regulator of normal and malignant haematopoiesis

Michela Lupia[1*], Tiziana Mega[1], Maria Mesuraca[1], Sarah Horton[2], Daniela Pelaggi[1], Raffaella Spina[1*], Lars Bullinger[3], Malcolm A.S. Moore[4], Jan J. Schuringa[2], Heather M. Bond[1], Giovanni Morrone[1]

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The early hematopoietic zinc finger protein/zinc finger protein 521 (EHZF/ZNF521) is a 30-zinc fingertranscription co-factor originally identified for its selective expression in human CD34$^+$ cells compared to mature leukocytes. Its mRNA levels are particularly abundant in the most immature CD34$^+$ cell subsets, rapidly decline during differentiation and become undetectable in precursors and terminally differentiated cells. ZNF521 shares high sequence homology with OAZ/ZNF423, another 30-ZF protein implicated in the maintenance of immaturity in olfactory epithelial progenitors and in development of cerebellum in mouse. Like OAZ, ZNF521 inhibts the transcriptional activity of EBF1, a master factor in the specification of the B-cell lineage. ZNF521 interacts with components of the NuRD complex through a 12-AA N-terminal repressor motif conserved in other transcriptional co-repressors such as friend FOG-1 and 2 and SALL family members. This motif is required for some, but not all, the biological actions of ZNF521.

Enforced expression of ZNF521 inhibits the differentiation and enhances the clonogenicity of human myeloid cell lines; its RNAi-mediated silencing in HSCs and early progenitor cells results in progenitor depletion in long-term stromal co-cultures and considerbly enhances B-lymphoid differentiation in B-cell culture conditions.

The *ZNF521* transcript is present in most AMLs, and higher amounts are detected in putative leukeamic stem cells compared to LSC-depleted fractions. Particularly high expression is observed in AMLs carrying the MLL-AF9 fusion gene generated by the t(9;11) translocation. *ZNF521* silencing in MLL-AF9+ cells strongly inhibits their growth and clonogenicity.

Taken together, these data delienate a relevant role for ZNF521 in the control of the homeostasis of the immature cell compartment in normal haematopoiesis and suggest its implication in the development and/or maintenance of myeloid malignancies.

Beta1 integrin is required for EGFR activity and tumorigenic properties of A549 lung cancer cells

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Increased levels of EGFR are observed in many types of cancer, including lung cancer, making EGFR a good therapeutic target. However recurrence and resistance to therapy require novel treatment approaches. Our previous work demonstrates a tight co-operation between beta1 integrin and EGFR, required for signalling from the membrane to the nucleus. To validate this model in the context of cell transformation, here we silenced beta1 integrin in human NSCLC A549 cells, that express high levels of EGFR and depend on EGFR activation for their growth. Silenced cells are impaired in EGF-dependent activation of EGFR and downstream signalling such as ERK1/2 and Akt, leading to a decreased *in vitro* proliferation both in normal culture conditions and in anchorage-indipendent soft agar assay.Moreover, when cultured in 3D matrix, silenced cells show a less invasive behaviour. Interestingly, *in vivo,* silenced cells gave rise to a 50% reduction of tumour size in SCID mice. Resulting tumours showed defective EGFR signalling. These results show that beta1 integrin silencing affects EGFR activity and cell growth, thus providing evidence for the relevance of the beta1 integrin/EGFR cross-talk in lung cancer.

A Pin1 / mutant p53 axis fosters aggressiveness in breast cancer

Javier E. Girardini[1,#], Marco Napoli[1,2], Silvano Piazza[1], Carolina Marotta[1,2], Alessandra Rustighi[1,2], Valeria Capaci[1,2], Enrico Radaelli[3], Alastair Thompson[4], Miguel Mano[5], Tim Crook[4], Eugenio Scanziani[3], Giannino Del Sal[1,2]

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Alterations in the p53 gene are found in approximately 50% of human tumours. More than 75% are missense mutations, leading to the expression of mutant proteins defective for wild type function, which accumulate to high levels in tumour cells. Furthermore mutant p53 proteins acquire novel gain of function properties and influence the progression of malignancy in a different manner than loss of p53 does. Despite compelling evidences showing that mutant p53 promotes tumour aggressiveness and metastasis, the mechanistic basis for this remains poorly understood.

Here we report that the oncogenic role of mutant p53 is aggravated by the prolyl-isomerase Pin1 and that these proteins contribute to develop tumour aggressiveness by promoting cell migration and invasion. We found that Pin1 enhances tumourigenesis in a Li-Fraumeni mouse model affecting both tumour-free survival and tumour spectrum and cooperates with mutant p53 in Ras-induced cell transformation. In human breast cancer cells, we observed that the ability of mutant p53 to promote migration and invasion is amplified by the catalytic activity of Pin1 and the concerted action of Pin1 and mutant p53 regulates reprogramming of gene expression to favour a specific transcriptional program that supports tumour aggressiveness. In breast cancer patients, we found that the prognostic value of p53 mutation results markedly influenced by Pin1 levels. Cases stratification on the base of p53 status and Pin1 levels allows identification of patients with different clinical outcome and response to adjuvant chemotherapy. Collectively, our results demonstrate the existence of a Pin1 / mutant p53 axis which has a profound effect on tumour aggressiveness in breast cancer.

Overexpression of small heat shock protein Hspb8 enhances the degradation of misfolded proteins in models of amyotrophic lateral sclerosis and spinobulbar muscular atrophy

Elena Bolzoni[1*], Valeria Crippa[1*], Paola Rusmini[1], Francesca Simonini[1], Elisa Onesto[1], Alessandra Boncoraglio[2], Serena Carra[2], Angelo Poletti[1]

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[*]Equally contributed to this work

Small heat shock proteins (sHsps) are molecular chaperones that protect cells against stress and assist the correct folding of native proteins. Mutations in two members of the sHsp superfamily, HspB1 and HspB8, are linked to peripheral neuropathies. The aim of this work was to investigate the role of HspB8 in two motoneuron diseases: familiar amyotrophic lateral sclerosis (fALS) and spinobulbar muscular atrophy (SBMA). fALS is often associated to mutations within the Superoxide Dismutase1 (SOD1) gene, while SBMA is caused by a polyglutamine tract expansion (polyQ) within the Androgen Receptor (AR). Although SOD1 and AR do not share structural or functional domains, both proteins, when mutated, form aggregates that perturb several motoneuron functions. In our studies, we transiently transfected NSC34 cells with wild type SOD1 or AR, or the mutant forms of the proteins (SOD1G93A, ARQ46). Both SOD1G93A and ARQ46 formed intracellular aggregates, visible at fluorescence microscope and detectable in western blotting analyses and in filter retardation assays. They also caused proteasome impairment indicated by YFPu (a proteasome activity reporter) accumulation. Overexpression of HspB8 in NSC34 reduced the levels of the soluble portion of SOD1G93A and ARQ46 and decreased PBS-insoluble and SDS-resistant forms of mutant proteins. HspB8 also decreased the levels of YFPu, indicating that HspB8 mediated a proteasome desaturation. Hence, HspB8 displayed chaperone activity towards mutant SOD1 and AR and enhanced the mutant proteins clearance even when the proteasome was blocked (by the inhibitor MG132), suggesting that an alternative degradative pathway, such as autophagy could mediate its activity.

Telethon Italy #GGP06063 #GGP07063, FOND. CARIPLO 2008.2307; Ministero della Salute #2007-36 #2008-15; Convenzione Fondazione Mondino/CEND-UNIMI

Chromatin dynamics and epigenomics of tubulin PlTα2 promoter gene in *Paracentrotus lividus*

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Expression of PlTα2 gene during sea urchin *Paracentrotus lividus* development, is spatially confined to the neural territory of the embryo and temporally activated from the blastula stage. Regulatory elements of this gene are restricted in 1,8 Kb. Microinjecting experiments shown upstream cis-acting elements involved in α2 enhancement expression and an intron element probably involved in triggering α2 transcription starting from blastula stage. To evaluate a possible involvement of chromatin modifications in regulation of PlTα2 gene expression we first searched for DNaseI hypersentive sites. We found four sites, three localized in the first intron of the gene and one in the second, when we used chromatin extracted from embryo at gastrula stage but not from morula stage. This result suggests a possible functional role of the introns in the activation of the expression of PlTα2 gene. Moreover, we used specific antibodies for RNA polymerase II and for different modified form of lysine 9, lysine 27 and lysine 4 of the H3 histone in quantitative chromatin immune-precipitation (qChIP) experiments to emphasize the different state of chromatin during embryos development. Our analysis show high H3K9 acetylation and H3K4 trimethylation degree in nucleosomes located at α2 promoter region in *P. lividus* embryos at gastrula stage, when the PlTα2 expression level is high. This observation agree with conventional positive role assumed by these post-translational modifications in chromatin remodeling leading to increase promoter accessibility to the transcriptional machinery apparatus. Furthermore ChIP analysis show also high H3K27 dimethylation degree during all development stage but interestingly SeqChIP analysis show no co-occupancy of this modification with RNA polymerase II in promoter region in embryos at gastrula stage. This observation is consistent with the hypothesis of a general repressive role of this modification in the not neural territory of the embryo.

New insight into myomiR functions

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Skeletal muscle cell differentiation involves irreversible withdrawal from cell cycle, expression of a number of muscle-specific-genes and fusion of differentiated myocytes into multinucleated myotubes. Several microRNAs are specifically expressed or enriched in skeletal muscle, particularly three of them, miR-1, miR-206 and miR-133 are induced during differentiation of C2C12 myoblasts. The function of miR-133 in myogenic differentiation appears controversial, as, although expressed in adult muscle and terminally differentiated cells, it seems to have a pro-proliferation activity. To asses the activity of miR-133 in myogenic differentiation, we analysed in more details the expression pattern of miR-133 in C2C12 and the effect of its inhibition and overexpression in proliferating and differentiating C2C12. We found that miR-133 is mainly expressed in myotubes and that its inhibition interferes with the differentiation ability of C2C12. Specularly, its overexpression enhances myoblast differentiation, suggesting that miR-133 may also play a pro-differentiative role in myogenesis. The role of miR-206 in skeletal muscle is well established: miR-206 levels increase during myogenic differentiation and the forced expression of miR-206 enhances myoblasts differentiation in vitro. Although miR-206 expression in other tissues is very poor, if not absent, some authors have reported that it is differentially expressed in normal and neoplastic tissues, particularly it has been proposed that miR-206 may have a role in breast carcinogenesis. We have identified a new target for miR-206 that reinforces its function of cell cycle regulator. Our data suggest that miR-206 may contribute to maintain the post-mitotic state in terminally differentiated myoblasts. Moreover, we found that miR-206 over-expression limits cell growth in normal fibroblasts and some transformed cells, indicating that miR-206 has a broader physiological function than originally anticipated.

Hematopoietic lineage specific transcriptional regulation of miR-223

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MicroRNAs (MiRs) are 22-nt long noncoding RNAs acting as critical mediators of key biological processes such as development, cell differentiation, proliferation and apoptosis by degradation of target mRNAs and/or repression of their translation. During development, the restrict cell type specific expression signature of miRs is modulated by the action of unique combinations of cis-acting regulatory factors. In hematopoietic cells, miR223 acts as essential regulator of myelopoiesis. MiR223 expression/activity appears regulated by two putative promoter regions. Both these regulatory regions present DNA binding sites for hematopoietic lineage-specific transcription factors including CEBPa, NFIA, PU.1, CEBPb, TAL1, GATA1 and LMO2, whose activity is required for the correct execution of the hematopoietic program. Here we investigated the transcriptional regulatory circuits modulating miR223 during lineage specification and terminal differentiation of primary human hematopoietic stem cells and myeloid cell into the monocytic, granulocytic and erythroid lineages. Northern blot and qRT-PCR showed that the expression of miR223 increased during monocytopoiesis, strongly up-regulated during granulopoiesis and negatively regulated during erythroid differentiation. Immunoblot, ChIP and promoter assay suggest a monocytic-dependent recruitment of myeloid CEBPb and PU.1 factors on the distal promoter region of miR223 gene. Equally the recruitment of CEBPa to the proximal region may be related to its strong induction during granulopoiesis, while the binding of TAL1-LMO2 and GATA1 transcription factors at their sites on this region repressed the expression of miR-223 during erythroid differentiation. Thus, the coordinated recruitment and action of lineage specific transcription factors at the two promoter regions of miR223 gene occurring during hematopoietic lineage differentiation, further underlies the key role of miR223 for a correct execution of the hematopoietic program.

In vivo induction of satellite cell proliferation by CKI removal

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We have recently shown that non-proliferating cells possess functionally relevant levels of pre-assembled cyclin-cdk complexes that are held inactive by the presence of cell cycle inhibitors (CKIs). We have found that the cell cycle can be reactivated in all kinds of cells, including terminally differentiated, senescent, and quiescent ones, by simply removing appropriate CKIs, most importantly p21. In principle, the ability to accelerate, promote, or induce cell proliferation would find wide therapeutic application whenever cell proliferation constitutes a limiting factor.
In order to exploit such approach in regenerative medicine, it is necessary to demonstrate that CKI removal causes no harm to the reactivated cells. Quiescent fibroblasts have been reactivated by p21 RNAi and analyzed for histone H2AX phosphorylation, DNA breaks, cytogenetic damage, and HPRT mutagenesis. None of these assays revealed any damage in the reactivated cells.
To verify whether cell cycle reactivation can be obtained in vivo, we suppressed p21 expression by injecting a recombinant AAV9 carrying four copies of a shRNA to p21 into the tibialis anterior muscles of C57BL mice. p21 knockdown induced a strong proliferative response with a three-fold increase in cellularity and BrdU incorporation in a large proportion of the cells. The p21 knockdown muscles, compared with controls, showed a two-fold increase in Pax7+, satellite cells, and comparably higher numbers of CD31+, endothelial cells. In spite of increased proliferation, there were very few CD45+, inflammatory cells.
Similar experiments were performed with des/NLS-LacZ mice, which express LacZ only in activated satellite cells. We observed a 10-fold increase in LacZ-positive nuclei vs. control, 10 days after infection. The presence of many small-caliber muscle fibers and centronucleated fibers was strongly reminiscent of muscle regeneration.
Thus, CKI knockdown can induce satellite cell proliferation in the context of living tissue. This method might be employed to accelerate muscle repair processes.

Citron-k controls the completion of cytokinesis functionally interacting with Anillin and RhoA

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Cytokinesis is the process by which a cell divides after the completion of mitosis. It requires the actomyosin ring contraction that leads to the cleavage furrow formation and ingression. The final irreversible step occurs during abscission when the ring breaks down and the membrane is sealed in its place.
The small GTPase RhoA is well known to play a critical role in cytokinesis initiation, progression and completion through its binding to a complex network of specific effectors. Citron kinase (Citron-k) is a conserved RhoA-binding protein required for cytokinesis from insects to mammals. However, it is still not well understood in which stages of cytokinesis and by which mechanisms this protein deploys its function.
In this study, we demonstrate that both Citron-k depletion and overexpression specifically affect abscission. Moreover, we show that these defects can be explained mechanistically by the functional interaction of Citron-k with RhoA and Anillin, a scaffold protein that links RhoA, Actin, and Myosin during cytokinesis.
Although Citron-k is commonly considered a downstream effector of RhoA, our data are rather consistent with the idea that Citron-k could be an upstream regulator of RhoA during late stages of cytokinesis. Indeed, Citron-k depletion leads to a loss of both Anillin and RhoA from the midbody. Conversely, Citron-k overexpression leads to an increase of RhoA at the midbody and also to a general increase of RhoA total levels at the end of cytokinesis. In addition, while RhoA activity is required to keep Anillin at the midbody, Citron-k localizes properly to this structure in a RhoA independent manner. Finally, we demonstrate that the inhibition of RhoA in cells that are completing cytokinesis is sufficient to reverse the Citron-k dependent abscission delay phenotype.
In conclusion, we propose that Citron-k stabilizes Anillin and RhoA at the midbody and that, by doing so, it plays a very important role in the regulation of abscission timing.

Ataxia Telangiectasia Mutated (ATM) interacts with Tankyrase-1 and regulates Tankyrase-1-dependent Poly(ADP-ribosyl)ation of TRF1 and NuMA1 in human cells

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Ataxia Telangiectasia (A-T), an hereditary syndrome caused by loss of function mutations in the Ataxia Telagiectasia Mutated (ATM) gene, is characterized by cerebellar neurodegeneration, telangiectasia, precocious aging, immunodeficiency, cancer predisposition and type II diabetes. ATM is a Ser/Thr kinase that plays a crucial role in the DNA damage response. ATM is also involved in telomere length maintenance, in chromosome segregation and in the insulin-dependent transport into plasma membrane of the Glut-4 glucose transporter. Telomere length control, spindle assembly and Glut-4 regulation are functions requiring poly-ADP-ribose-polymerase Tankyrase-1 (TNKS1)-dependent poly(ADP-ribosyl)ation. Searching for a mechanistic link between chromosome segregation and ATM function, we found that human cells in which ATM activity was chemically or genetically down-regulated were delayed in assembling bipolar mitotic spindles. The phenotype resembled that of reduced poly(ADP-ribosyl)ation of the mitotic spindle pole organizer NuMA1, that induces high frequency of multipolar spindles, by TNKS1 knock-down. In addition, we found that ATM physically interacted with TNKS1, through the ankyrin domain repeats of TNKS1. ATM down-regulation impaired TNKS1-dependent poly(ADP-ribosyl)ation of NuMA1 explaining the defects in bipolar spindle assembly. In addition, ATM activity was required for TNKS1-dependent poly(ADP-ribosyl)ation of TRF1, a crucial protein whose TNKS1-dependent poly(ADP-ribosyl)ation is required to prevention cellular senescence by telomere attrition. Our preliminary data indicate that ATM did not significantly stimulate TNKS1 activity but, rather, ATM-dependent phosphorylation, in the absence of DNA damage, of both TRF1 and NuMA1 were required for their poly(ADP-ribosyl)ation. We propose that A-T features like neuonal aneuploidy, precocious aging and, possibly, insulin-resistant diabetes could in part be explained by the ATM-dependent regulation of TNKS1 function.

The Rho GTPase RhoU is a transcriptional target of Stat3 and Wnt-1 pathways and is implicated in cellular migration and proliferation

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STAT3 is a transcription factor activated by cytokines, growth factors and oncogenes, whose activity is required for cell survival/proliferation of a wide variety of primary tumours and tumour cell lines. Prominent among its multiple effects on tumour cells is the stimulation of cell migration and metastasis, whose functional mechanisms are however not completely characterized.
RhoU is an atypical Rho GTPase constitutively bound to GTP in an active conformation and its activity is regulated mainly transcriptionally. RhoU was first identified as a Wnt-1-inducible mRNA and subsequently shown to act on the actin cytoskeleton by stimulating filopodia formation and stress fibre dissolution. In our work we have showed that Wnt-1-mediated RhoU induction occurs at the transcriptional level. Moreover, we demonstrated that RhoU can be induced by gp130-cytokines in a strictly STAT3-dependent manner. Two functional STAT3-binding sites were identified on the mouse RhoU promoter, which are required for gp130-mediated induction. Interestingly, Wnt1-induced RhoU activation does not involve STAT3 and is independent of β-catenin, but it is mediated by the Wnt/planar cell polarity pathway through the activation of JNK.
A positive correlation between STAT3 activation and RhoU expression levels was found in a panel of human tumor cell lines. Moreover, treatment of some of these cells with a specific STAT3 inhibitor drastically reduced RhoU expression levels, confirming the functional correlation between STAT3 activity and RhoU expression. RhoU silencing in tumor cell lines with constitutively active STAT3 and high RhoU expression levels, showed a drastic effect on cell migration and proliferation. Furthermore, RhoU overexpression confers an increased migratory capacity to STAT3-/- cells in a strictly dose-dependent manner. These data identify RhoU as a main effector of both the Wnt-1 and STAT3 oncogenic pathways, mediating at last some of their pro-migratory, pro-metastatic effects.

Hyperglicaemia induces insulin resistance through ER stress activation in L6 skeletal muscle cells

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The endoplasmic reticulum (ER) is responsible for the quality control of proteins. When the productive folding process in the ER is perturbed by environmental stress conditions, eukaryotic cells activate the unfolded protein response (UPR). Recently, several studies reported that ER stress is involved both in pancreatic β-cell dysfunction and in peripheral insulin resistance, the two main features of Type2 diabetes. Hyperglycaemia causes insulin resistance and this process appears to be linked to UPR activation. Even glucosamine (GlcN), generated during hyperglycaemia, causes insulin resistance and glucose toxicity in muscle. In this work we investigated the possible role of high glucose (HG)- and glucosamine-induced ER stress on insulin-resistance in L6 skeletal muscle cells. L6 cells were incubated either with 7.5mM GlcN or with 25mM glucose, in presence or absence of insulin (100nM). Upon either GlcN or HG treatment, the ER stress markers BiP, Xbp1 and ATF6 mRNA levels were increased by 6, 3 and 2 fold respectively, indicating UPR activation. Moreover both GlcN and HG blunted insulin induced 2-deoxy-D-[14C]-glucose (2DG) uptake, Akt and GSK3β phosphorylation. Furthermore both GLUT4 mRNA and protein levels decreased by 70% upon GlcN and HG treatment. Interestingly, the use of the chemical chaperon 4-Phenyl Butyric Acid (PBA) prevented the induction of ER stress by GlcN and HG and their effects on 2DG uptake, Akt phosphorylation and GLUT4 levels. In conclusion, we show for the first time that GlcN and HG are able to activate the UPR in skeletal muscle cells, and they may play a role in insulin resistance through the inhibition of Glut4 expression and/or translocation.

Role of scaffold molecule KSR2 in immune cells deficiency associated to obesity

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Obesity is a major risk factor for development of cardiovascular pathologies as well as metabolic disorders. It was also observed that obese subjects have higher incidence of infections and impaired immune response. Studies in obese mice showed cell-mediated cytotoxicity reduction, impaired dendritic cell functions, reduced antigen presentation, altered proliferation of splenocytes, and diminished NK cell functions. However, the underlying causes of these immunological alterations have not been fully identified.

Recent studies have identified several signaling pathways that, linking inflammatory and metabolic signalling, have a role in causing obesity and insulin resistance. One of these pathways includes the mitogen-activated protein kinase (MAPK) pathway which are regulated by specific scaffold proteins. Molecular scaffold proteins coordinate the interaction of signaling molecules for efficient signal transduction. One of the most studied scaffold molecules is the Kinase suppressor of Ras (KSR) that acts as a regulator of the Raf/MEK/ERK kinase cascade. There are two ksr genes in mammals, ksr-1 and ksr-2. Interestingly, while ksr1-/- mice shows no overt metabolic defects, ksr2-/- mice are obese. Our preliminary results show a significant reduction of circulating T and B cell subset in peripheral blood. Furthermore, similar alterations in the proportion of T and B cells were observed in the spleen of ksr2-/- mice. In addition, KSR2 appears involved in insulin signaling, since we found that KSR2 deficient mice are hyperinsulinemic.

Thus, KSR2 might play a critical role in metabolic pathways required to support immune cells response and could represent a new therapeutic target for immune disorders associated to obesity, type 2 diabetes and metabolic syndrome.

Novel control mechanism of hormone action

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Ligand-mediated activation of G-Protein Coupled Receptors (GPCRs) induces the production of second messengers like cAMP. The principal cellular effector of cAMP is the Protein Kinase A (PKA) which is assembled as inactive holoenzyme consisting of two regulatory (R) and two catalytic (PKAc) subunits. cAMP binding to the R subunits promotes the dissociation of the holoenzyme and the consequent release of the catalytic moiety, which induces the phosphorylation of a wide array of cellular proteins1. Re-assembling of the holoenzyme terminates the signal transmission. The rate of association and dissociation of regulatory and catalytic subunits determines the strenght and the duration of the catalytic activity of PKAc, and influences complex biological processes such as differentiation, long term memory and apoptosis.Proteolytic degradation of R subunits has been proposed as mechanism to enhance signalling downstream of PKAc, although so far the enzymes involved in this process are still unknown. Here we report that praja2, a RING E3 ligase widely expressed in mammalian cells, controls the stability of R subunits. Praja2 forms a stable complex with and is phosporylated by PKAc. Elevation of cAMP levels promotes Praja2 activation and subsequent ubiquitination and proteolysis of R subunits. Functional experiments in neuroblastoma cells and rat brains show that praja2 is required for efficient cAMP mediated signalling to the nucleus.These findings indicate that praja2 regulates the total amount of R subunits, constituting an important positive feed-back mechanism that controls the rate and magnitude of cAMP•PKA signalling.

Role of the Tpf1, a protein of Treponema pallidum, in inducing the production of Treg cells and in driving inflammation via the activation of inflammasome

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Syphilis, a sexual transmitted disease of humans caused by spirochetal bacterium *Treponema pallidum,* remains a global public health problem with an estimated 12 million new cases annually (1). Syphilis is a chronic disease that, if not treated with an antibiotic therapy, can persist for several years leading to cardiovascular and neurological complications. The local and systemic cellular immune responses elicited by the bacterium have not been well studied in humans. T helper cells are believed to mediate bacterial clearance primarily through the production of cytokines, such as IFN-γwhich activates macrophages. The latter then engulf and kill opsonised bacteria (2). However, several years ago it was suggested that other lymphocytes may act to suppress the effect of macrophages, and this strategy has been indicated as a possible mechanism for *Treponema* to escape the elimination by the immune system (3). Based on these premises, we were interested to verify whether TpF1, a protein produced by *Treponema*, homolog to HP-NAP of *Helicobacter pylori* and to NapA of *Borrelia Burgdoferi*, both endowed with immunomodulatory properties, might be crucial in facilitate the survival of the microorganism thus contributing to the chronicity of the disease. We found that TpF1 triggers the release of IL-1β, IL-6, TGF- βand IL-10 from monocytes. Such a cytokine milieu is expected to trigger the differentiation of Th cells towards the T reg phenotype. Accordingly, we found that a significant proportion of T cells isolated from syphilis patients are T reg (CD25+ FoxP3+ TGF-β+ cells) and an high percentage of these are specific for TpF1. In parallel, we are now considering the molecular mechanism by which TpF1 promotes the secretion of IL-1β, an event known to be the result of the activation of the caspase-1-activating complex termed "inflammasome".

Role of IL-6 in skeletal muscle growth and atrophy

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IL-6 cytokine is involved in several pathologies characterized by a progressive loss of muscle tissue such as aging, cachexia and muscular dystrophy, revealing an important role of this cytokine in skeletal muscle homeostasis. (Naka et al, 2002)

Recent studies have demonstratedthat the NSE/h-IL-6(+/+) transgenic mice show growth defects associated with high circulating levels of IL-6 expressed since birth. The mechanism by which IL-6 causes growth defect appears to involve a decrease in circulating IGF-I levels, suggesting a block of Igf-1-anabolic-signaling.(De Benedetti et al, 1997; Haddad et al, 2005). Although IL-6 seems also necessary for satellite cells proliferation (Serrano et al, 2008), the specific physiopathological role exerted by this cytokines on muscle growth and in the maintenance of differentiated phenotype remain to be addressed.

To better define the role of IL-6 on muscle homeostasis and growth, we performed molecular and histomorphometric analysis on a transgenic animal model (NSE/hIL-6) expressing high plasma levels of IL-6. We observed a defect in muscle growth during the early phases of postnatal growth (1.5 and 3.5 months of age) in NSE/hIL-6 transgenic mice compared to wild type, and the induction of muscle atrophy in adult (6 months of age) transgenic mice. These results suggest an important role of IL-6 in postnatal muscle growth and identify IL-6 as potential biomarker of skeletal muscle atrophy.

Gliadin peptide P31-43 enhances IL15 activity by interfering with its intracellular trafficking

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Background: We previously showed that gliadin peptide P31-43 induces proliferation of cell lines and celiac enterocytes by delaying degradation of the active Epidermal Growth Factor Receptor (EGFR). P31-43 exerts this effect by delaying maturation of endocytic vesicles. IL-15 is increased in the intestine of patients affected by celiac disease (CD) and has pleiotropic activity that ultimately results in immunoregulatory cross-talk between cells belonging to the innate and adaptive branches of the immune response. *Aim:* The aims of this study were to investigate whether P31-43 is involved in the increased IL15 levels and to determine the mechanisms underlying this process. To this aim, we studied the effects of P31-43 on IL15 transcription, translation and intracellular trafficking, and the role of IL15 in P31-43-induced cell proliferation. *Methods:* We used real time PCR to evaluate IL15 mRNA levels, FACS ELISA and Western Blot to analyze protein levels and distribution. Cell proliferation was evaluated by bromodeoxyuridine incorporation (BrdU). *Results:* P31-43 increased IL15 mRNA levels in CaCo-2 cells only after overnight treatment whereas it increased IL15 protein level on the cell surface already after 3 h of treatment. The increased IL15 protein was linked to IL15 Receptor (IL-15R) alpha, did not require new protein synthesis and functioned as a growth factor. Anti-IL15 blocking antibodies prevented the P31-43-induced proliferation of CaCo-2 cells and of enterocytes from celiac intestine. *Conclusion:* P31-43 enhances presentation of IL15/IL15 R alpha in *trans* to neighboring cells, interfering with its vesicular trafficking. Juxtacrine signaling of IL15/IL15R alpha contributes to both cell proliferation and activation of innate immunity.

POSTER ABSTRACTS

P1. Differential expression of ErbB family tyrosine kinase receptors and sensitivity to letrozole in breast cancer cell lines *in vitro*

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Estrogens induce breast tumour cell proliferation by directly regulating gene expression via the estrogen receptor (ER) transcriptional activity and by affecting growth factor signalling pathways such as mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase PI3K/Akt/mTOR pathway. Combining endocrine therapies for breast cancer with various targeted biological therapies has become a very active area of clinical research aimed at overcoming or preventing endocrine resistance. In previous studies we demonstrated that the combination of the aromatase inhibitor letrozole associated with sorafenib, a multi-kinase inhibitor, produced a synergistic anti-proliferative effect on MCF-7/Arom-1 breast cancer cells.

There is evidence that crosstalk between the ErbB-family receptors and ER by increasing ligand-independent phosphorylation of ER, possibly as a result of an overexpression of EGFR, Her-2/neu (HER2) and the IGF-IR pathways, is one of the mechanisms for resistance to endocrine therapy.

The aim of this project is to analyze the effects of letrozole on a panel of ERα positive human breast tumour cell lines (MCF-7, T47D, BT474, ZR75), stably transfected with the aromatase gene, showing a different expression of EGF family receptor. We will characterize the baseline activity of the PI3K/Akt/mTOR and MAPK pathways and we will evaluate whether alterations in the expression and/or phosphorylation state of the multiple components of these pathways can affect the sensitivity to letrozole. We will modulate the expression of EGFR family members (over-expressing or down regulated using siRNA) to evaluate their role on responsiviness to letrozole.

Our target is to combine inhibitors of ErbB-family receptors and letrozole to improve therapeutic efficacy.

P2. New insights on PTEN subcellular localization and its control of cell fate

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The tumour suppressor PTEN (phosphatase and tensin homolog deleted on chromosome 10), the second most frequently mutated gene in human cancer after p53, is a dual-specificity phosphatase, which antagonizes phosphoinositide 3-kinase (PI3K)-dependent signalling by hydrolyzing phosphatidylinositol 3,4,5-trisphosphate (PtdInsP3) and may also dephosphorylate specific protein substrates. PTEN regulates a variety of cellular processes including cell proliferation, growth, migration and death. Loss of function of this tumour suppressor is associated with inherited cancer predisposition syndromes (such as Cowden's disease, Bannayan–Zonana syndrome and Lhermitte–Duclos disease) and with sporadic cancers, including gliomas, melanomas, prostate, endometrial, lung, renal, ovarian and breast cancer. The mechanisms of PTEN functions in tumor suppression are currently under intense investigation. Many of the effects of PTEN on cell growth, proliferation and survival are believed to be mediated through its inhibition of the PtdInsP3-dependent protein kinase Akt, however, it cannot be ruled out that PTEN might also modulate cell fate even through other signaling pathways. Recent studies indicate that under some circumstances PTEN translocates to mitochondria and acts as acrucial mediator of mitochondrial-dependent apoptosis. Aim of this study is to elucidate the subcellular localization of PTEN. Interestingly, we found PTEN localized at mitochondria, endoplasmic reticulum (ER) and Mitochondrial Associated Membrane (MAM), sites known to play a key role in the participation of mitochondria to calcium (Ca^{2+}) signaling and apoptosis. Moreover, we also found out that PTEN silencing affected mitochondrial and cytosolic Ca^{2+} responses after ER Ca^{2+} release, evoked either by agonist stimulation or by apoptotic stimuli. Studies of these new pathways involving PTEN may provide new insights into the mechanisms and the extent of its involvement in cancer, highlighting new potential targets for therapeutic intervention.

P3. Tumour Necrosis Factor alpha Impair Oligodendrocytes differentiation by modulating mitochondrial physiology

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Tumour Necrosis Factor alpha (TNF alpha) is an inflammatory cytokine able to regulate both cell death or survival depending on cellular contest. This dual ability appears fundamental in the pathogenesis of Multiple Sclerosis (MS) where oligodendrocytes death and loss in remyelinization activity appear engaged by TNF alpha, resulting in the neuronal degeneration and apoptosis that produce symptoms appearance.
Mechanisms of oligodendrocytes death are poorly characterized from the side of the mitochondrial physiology and calcium homeostasis, largely known as key regulator of different cell death mechanism. Moreover an increasing collection of evidence reveals mitochondria as actors in the pathogenesis of MS.
We evaluated the effects of TNF alpha and IFNgamma on intracellular Ca2+ homeostasis in oligodendrocytes precursor revealing that only TNF alpha is able to cause a reduction in mitochondrial calcium uptake. Therefore no effects in cytosolic calcium were observed after TNF alpha exposure and other glial cell types such as astrocytes were not affected, concluding that TNF alpha is able to exert a selective effect on oligodendrocytes mitochondria.
Moreover a reduction of mitochondrial membrane potential (MMP) was observed, but anyone variation occur in mitochondrial morphology following TNF treatment.
Interestingly mitochondrial alterations induced by this cytokine were not associated with caspase3 activation or reduction in cell number, but with a reduction in ability to differentiate in adult oligodendrocytes.
Conversely the chronic exposure with low FCCP concentrations reproduces MMP reduction and the impairment in differentiation exerted by TNF.
Taken together, these results suggest that TNFalpha impair oligodendrocytes differentiation by altering mitochondrial functions.

P4. Development of highly sensitive and rapid detection assays for the identification of minority mutated alleles in myeloproliferative neoplasms

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Myeloproliferative neoplasms (MPN) include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Somatic mutations of the thrombopoietin receptor MPL were described in a minority of ET or PMF patients. In this work, we aimed at assessing MPL mutation status in MPN patients as well as to identify possible new mutations. We isolated DNA from circulating granulocytes and T lymphocytes and developed a new high-resolution melt (HRM) assay for exon 10 MPL mutation scanning. All the HRM results were compared with those obtained by direct sequencing. HRM identified mutations in granulocytes at very low levels, such as 1% W515A and 5% W515K mutant alleles. HRM analysis and direct sequencing detected the following MPL mutations in circulating granulocytes but not in T lymphocytes, indicating that these were acquired somatic variants: W515L, W515K, W515A, W515R, S505C, and V501A – these latter two being novel substitutions. HRM detected mutant alleles in few patients that sequencing scored as wild-type. These data confirm the high sensitivity of HRM in detecting very low proportions of minority alleles which need to be further characterized by sequencing. In the search of an ultra sensitive genotyping approach we thus developed conditions for ultra deep sequencing with the 454 GS-FLX Titanium system. We applied this technology to investigate three patients who had been previously found to carry two different MPL mutations (V501A+W515L, V501A+W515R, S505C+W515L) in circulating granulocytes. This strategy allowed for the first time to characterize the presence of two mutations located on the same allele in MPN patients. Our findings demonstrate that in the molecular analysis of pathologies characterized by the presence of minority mutant alleles the diagnostic strategy cannot rely on a unique approach. Ultra deep sequencing is very promising and provides an unprecedented opportunity to characterize these

P5. ErbB2 modulation by Trastuzumab treatment in human breast cancer cell line

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ErbB2 is a receptor tyrosine kinase member of the EGFR family (ErbB1-4) and is the favorite heterodimerization partner of the other ErbBs. ErbB2 over expression has been identified in approximately 25-30% of primary breast cancers and correlates with poor prognosis and cancer relapse. For these reasons, ErbB2 represents an attractive target for immunotherapy. Trastuzumab (marketed as Herceptin) is a humanized monoclonal IgG1 against ErbB2 currently used in advanced breast cancers therapy, that exerts anti-proliferative effects on ErbB2-positive breast tumor cell lines. Despite its therapeutic success, resistance to Trastuzumab has been frequently observed. Therefore, the study of the molecular mechanisms involved in Trastuzumab action represents an important aim of our research work.

Data previously obtained in our laboratory suggested that, at early stages of treatment (2 to 120 minutes), Trastuzumab promotes the formation of active ErbB2-EGFr heterodimers in SKBR-3 human breast cancer cell line. Biochemical analysis performed on SKBR-3 cells showed the activation of MAPK pathway as outcome of Trastuzumab administration. In particular, we observed ERK 1/2-phosphorylation and Akt dephosphorylation. Surprisingly, we found that Akt dephosphorylation was strongly dependent on ERK1/2 phosphorylation.

In addition to these data, we did not detect ubiquitylation of ErbB2 at early time of treatment and we also observe ErbB2 internalization and recycling.

As consequence of our results, we aimed to investigate whether signalling and endocytic/recycling events are dependent upon the formation of ErbB2-EGFR heterodimers. To this aim, I spent the first four months of my PhD program to set up RNAi interference of EGFR in SKBR-3 cells. SKBR-3 cells transiently silenced for EGFr will be analyzed by western blotting and confocal immunofluorescence.

P6. A new FLVCR isoform able to support erythropoiesis

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Feline Leukemia Virus subgroup C Receptor (FLVCR) was originally identified and cloned as a cell-surface protein receptor for feline leukemia virus subgroup C causing pure red blood cell aplasia in cats. Recent studies have demonstrated that FLVCR is a heme exporter essential for erythroid differentiation: Flvcr-null mice die in utero due to the failure of fetal erythropoiesis.
We have recently identified a novel FLVCR isoform (FLVCRb) coding for a putative 7 transmembrane domain-containing protein ubiquitously expressed and with a different sub-cellular localization than the canonical isoform (FLVCRa); while FLVCRa is localized at the cell membrane, FLVCRb is expressed in intracellular compartment, suggesting different roles of the two proteins in intracellular heme trafficking.
To gain insights into the specific roles of the two isoforms, we have generated Flvcr mutant mice different from those previously reported by specifically deleting FLVCRa while FLVCRb is still expressed (FLVCRa-null mice). When Flvcr-a+/- mice were intercrossed, no Flvcr-a-/- newborns were obtained and the analysis of the embryos from timed Flvcr-a+/- intercrosses showed that the Flvcr-a-/- genotype was lethal between E14.5-E16.5. Flvcr-a-null embryos showed multifocal and extended hemorrhages, visible in the limbs, head and throughout the body wall, as well as subcutaneous edema. Alcian blue-alizarin red staining demonstrated skeletal abnormalities in limbs and head similar to that observed in Diamond-Blakfan anemia patients. Interestingly, flow cytometric analyses of E14.5 fetal liver cells double-stained for Ter119 and CD71 show normal erythropoiesis in Flvcr-a-null embryos, opposite to the previously reported Flvcr-null mice.
Taken together, these data demonstrated that FLVCRb is sufficient to support fetal erythropoiesis, but not to prevent endothelial ruptures responsible for hemorrhages, thus suggesting that FLVCRa is needed for detoxifying heme excess at these sites.

P7. Molecular basis of Charcot-Marie-Tooth type 2B disease. CMT2B-associated Rab7 mutants inhibit neurite outgrowth

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Charcot-Marie-Tooth diseases (CMT) are the most common group of inherited peripheral neuropathies.
Four missense mutations in Rab7, a small GTPase that controls late endocytic trafficking, cause the CMT type 2B disease, an axonal peripheral disorder characterized by distal muscle weakness and atrophy, sensory loss, and normal nerve conduction velocities. These mutations (Leu129Phe, Lys157Asn, Ans161Thr and Val162Met) target highly conserved amino acids that alter interaction with the nucleotides. Indeed, we demonstrated that these Rab7 mutant proteins have higher K_{off} for nucleotides compared to the wt protein (especially for GDP), and, as a consequence, lower GTPase activity. In addition, these mutant proteins are predominantly GTP-bound in the cells, and are able to rescue Rab7 function when expressed in Rab7-silenced cells.
However, these biotechemical data do not explain how mutations in Rab7, a ubiquitous protein, affect only peripheral neurons.
Interestingly, we previously demonstrated that a Rab7 dominant negative mutant (T22N) stimulates neurite outgrowth in PC12 cells, while a constitutive active mutant (Q67L) inhibits it. Therefore, we decided to test if CMT2B mutants affected also neurites outgrowth.
Interestingly, expression of CMT2B-causing Rab7 mutant proteins in PC12 and in Neuro2A cells caused a strong inhibition of the outgrowth of neurites longer than 50 mm. In addition, inhibitedexpression of neuronal differentiation markers, Gap43 in PC12 and NeuN in Neuro 2A, was observed. These data, that have to be confirmed in neuronal cells, indicate that the Rab7 mutant proteins impair neurite outgrowth and neuronal differentiation.
The onset of CMT2B is during the third decade of life and, therefore, development is not impaired in CMT2B patients. This suggests that the Rab7 mutant proteins selectively affect regeneration of neurons at later stages. Strategies to control and lower Rab7 activity in neurons could be a targeted therapy for CMT2B.

P8. Role of Necdin in mesoangioblasts dependent skeletal muscle differentiation and regeneration

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Cell therapy holds great potential for the treatment of muscular dystrophies. Mesoangioblasts (MABs) are vessel-associated stem cells recently identified as ideal candidates in this regard. Indeed, it has been shown that, when delivered through the arterial circulation, they are able to significantly improve the dystrophic phenotype of different animal models.
In order to dissect the molecular pathways that regulate the growth and the differentiation of these cells we focused our study on the role of Necdin (Ndn), a MAGE protein expressed in both developing and adult skeletal muscle. Our group provided the first evidence that Ndn plays a pivotal role in muscle tissue, being required for proper myoblasts differentiation, for the first phase of muscle fibers growth and for efficient repair upon muscle injury. We also show thatNdn acts at different levels promoting myoblasts differentiation and survival.
These data encouraged us to verify if such role of Ndn can be exploited to enhance the therapeutic potential of mesoangioblasts.
We have isolated and characterized adult muscle-derived MABs from wt mice and transgenic mice for Ndn, both gain and loss of function. We have also generated a line of MABs constitutively over-expressing Ndn, by a lentiviral infection (NdnMABs).
Over-expression of Ndn in vitro increases the differentiation ability of MABs and inhibits cell death induced by several toxic stimuli. In order to study the contribution of these cells to muscle regeneration in vivo we have performed intra-muscular and intra-arterial injections of NdnMABs and control MABs in a murine dystrophic model, the alpha-sarcoglycan (a-SG) null mice. Preliminary data show that muscles injected with NdnMABs have a greater restoration of a-SG expression.
Understanding more in detail the molecular mechanism of the effect of Ndn on MABs is the next crucial step towards the optimization of cell therapy with MABs or other stem cells.

P9. Unravelling the molecular mechanisms promoting cardiac specification and differentiation in Embryonic Stem Cells

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In vitro differentiation of Embryonic Stem cells (ESCs) represents a powerful model system to identify key regulators of cardiac specification in mammals for which the molecular control is largely unknown. The EGF-CFC Cripto is a key regulator of cardiac myogenesis in ESCs.
We have identified the Angiotesin Type- I Like Receptor (AGTRL-1/APJ/*msr1*) and its ligand apelin as previously unrecognized downstream targets of Cripto/Smad2 signaling both *in vivo* and in ESCs. Gain of function experiments show that APJ suppresses neuronal differentiation, which spontaneously occurs in Cripto$^{-/-}$ ESCs, and restore the cardiac program, activating the expression of genes pivotal for cardiac specification and terminal differentiation in cripto mutant ESCs. Furthermore, loss of function experiments revealed, for the first time, a central role for APJ/apelin signaling in the gene regulatory cascade promoting ESC cardiac specification and differentiation.
Remarkably, we show that apelin promotes cardiomyogenesis via activation of pERK/p70S6 through coupling to a Go/Gi protein. Together our data point for a previously undescribed functional link between Cripto/Smad2 and APJ/apelin in the signaling pathways that govern mesoderm patterning and cardiac specification in mammals.
Finally, to get insight into the role of Apj/apelin signaling in the control of ESC differentiation, we have generated ESC lines,which allow overexpression of APJ in a time-specific manner, by using the tetracycline (Tet)-regulated transactivator system.The biological effect of Apj inducible overexpression in ESCs is currently under investigation.

P10. The mitofusin of *Drosophila melanogaster:* genetic and functional analysis to understand pathogenesis of Charcot Marie Tooth type IIa

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Mitofusin-2 (MFN2) is a mitochondrial dynamin-related protein mutated in Charcot-Marie-Tooth type IIa (CMTIIa). MFN2 localizes on the outer mitochondrial membrane like mitofusin-1 (MFN1), but enriched in mitochondria associated membranes (MAMs) where it tethers mitochondria and ER in addition to its function in coordinating mitochondrial fusion together with other mitochondrial proteins. However MFN2 has roles not shared by MFN1. All vertebrates possess two mitofusins, whereas only one mitofusin is present in almost all invertebrates reigns. There is not much knowledge about the appearance of 2 mitofusins during evolution from invertebrates to vertebrates. *C.elegans*and *S.cerevisae* possess only one mitofusin which is called Fzo. *Drosophila* possesses two Mfn homologues: Fzo has a restricted spermatid expression pattern, while *Marf* is ubiquitous.We decided to focus our analysis on Marf, which displays 47% of identity with both Mfns.

We tried to understand in which extension Marf can functionally complement mammalian mitofusins, and if it is more functionally related to Mfn1 or to Mfn2. The expression of Marf induces mitochondrial elongation in *Mfn1, Mfn2-/-* and both *Mfns-/-* MEFs, rescueing the fragmented phenotype caused by the abscence of either or both mitofusins, indicating that it can substitute for both Mfns.

In Drosophila ubiquitous, muscle and nervous system specific depletion of Marf result in lethality. We tried to rescue Marf depletion by neuronally expressing MFN2 or MFN1 in flies. Simultaneous expression of MFN2 and *Marf*-RNAi transgenes results in partial survival to adulthood, thus rescuing the lethality due to Marf depletion. In contrast, individuals simultaneously expressing MFN1 and *Marf*-RNAi in the nervous system die as pupae, thus displaying a phenotype indistinguishable from that of *Marf*-RNAi alone. In conclusion Marf expression rescues mitochondrial morphology in *Mfn1 -/-* and *Mfn2-/-* MEFs.

On the other hand *in vivo* experiments show that MFN2, but not MFN1 rescues the lethal phenotype of Marf knock-down in *Drosophila melanogaster*, thus we can speculate that Marf and MFN2 are functionally more closely related and Mfn1 may have diverged later during mammalian evolution.

P11. An antisense oligodeoxyribonucleotide targeting *uPAR* mRNA down-regulates uPAR expression in retina endothelial cells and inhibits neoangiogenesis both *in vitro* and, if applied to corneal, in an animal model of productive retinopathy

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Aims. For two decades, the main focus of the laboratory in which I have started my doctorate project was to investigate on cancer gene function by modulating their expression with antisense strategy. We have previously demonstrated that an antisense oligodeoxyribonucleotide (aODN) targeting the urokinase plasminogen activator receptor (u-PAR)efficiently inhibited uPAR expression and thereby reduced tumor invasiveness and metastasis. The role of uPA/uPAR in facilitating the development of new vessels in the retina as well as an increased level of urokinase in proliferative retinopathies, including diabetes retinopathy and age related macular degeneration, have been also reported. We tested whether the systemically delivered of a chemically modified anti-uPAR aODN could inhibit uPAR expression in retinal vessels and reduce neovascularization using mice with oxygen induced retinopathy (OIR) as experimental models.
Results. *In vitro experiments*. When applied at 10 μM concentration combined with cationic lipida (e.g. Lipofectamin), the anti-uPAR aODN reduced capillary morphogenesis by more than 40% and matrigel membrane infiltration by more than 50% in a human retinal endothelial cell line (hREC). *In vivo experiments*. The anti-uPAR aODN was tested in a mouse model of oxygen-induced retinopathy of prematurity (ROI): from day 7 to day 12 after birth mice were incubated in hyperoxia an then transferred to normoxia until day 17, to simulate an hypoxic condition occurring in ROI. When applied intraperitoneally in mice, the anti-uPAR aODN reached retinal compartments and lowered oxygen induced neovessel profiles, reducing significantly the avascular area (-50%, P<0,001) and the retinopathy score (-25%, P<0,001).
Conclusions. Systemically delivered aODN directed against the uPAR is a potential novel treatment for ocular neovascularization related disorders.
Aknowledgements. Grants from Ente Cassa di Risparmio di Firenze and Agenzia Spaziale Italiana.

P12. Effect of morgana haploinsufficiency in mice

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Mutations in morgana result in centrosome amplification and lethality in both Drosophila and mouse. In mouse cells, morgana forms a complex with Hsp90 and ROCK I and II, and directly binds ROCK II. Morgana downregulation promotes the interaction between ROCK II and nucleophosmin, leading to an increased ROCK II kinase activity, which results in centrosome amplification.
morgana+/- primary cells display an increased susceptibility to neoplastic transformation and *morgana +/-* mice are more prone to tumour development after treatment with the chemical carcinogen DMBA.
In addition, tumor tissue array histochemical analysis revealed that morgana is underexpressed in a large fraction of breast and lung human cancers. Thus, morgana appears to prevent both centrosome amplification and tumorigenesis (Ferretti, 2010).
Now we are interested in the analysis of morgana heterozygous mice to determine their spontaneous susceptibility to cancer compared to wild type controls. Preliminary results revealed that about 50% of *morgana+/-* mice became ill and die mostly between 14 and 16 months of age. These sick mice are frankly anemic and show the presence of immature forms in the peripheral blood. These *morgana+/-* mice present an increase in bone marrow cellularity and the 30% of dead heterozygous mice present symptoms resembling myeloproliferative disease–like myeloid leukemia (MPD-like ML).
In addition, the morgana heterozygous cohort developed more frequently lung tumours.
These observations suggest us that morgana heterozygosity may promote spontaneous oncogenic transformation in vivo.

P13. Role of hemopexin and hypoxia in duodenal heme absorption

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Although heme represents the most bioavailable iron source in the diet, the mechanism of heme absorption remains poorly understood. Recently, the identification of intestinal importers and exporters for heme highlighted the possibility that heme may be taken up from intestinal lumen and transferred directly to the plasma, as for inorganic iron.
This work investigates the role of hemopexin, a plasma high affinity-carrier for heme, in physiologic heme absorption. Moreover, as a low oxygen condition is known to induce inorganic iron absorption, the role of hypoxia as a putative regulatory stimulus of heme absorption is analyzed.
Despite of a normal ability to take up heme from intestinal lumen, analyses on hemopexin-null mice revealed a defect on heme export from the duodenum to the plasma. Reduced heme export is associated with increased duodenal expression and activity of heme oxygenase, which in turn results in iron loading. Conversely, in hemopexin-null livers and skeletal muscles, hemopexin deficiency is associated to enhanced expression of 5-aminolevulinic synthase.
These findings suggest the existence of a direct heme transfer pathway from the duodenum to the liver, controlled by the plasma factor hemopexin.
In order to identify further stimuli controlling this pathway, we performed in vitro experiments on human epithelial colorectal adenocarcinoma Caco-2 cells. Cell exposure to hypoxia for 24 hours showed, as expected, an increase in the mRNA level of the main inorganic iron transporters. Interestingly, higher mRNA levels were also observed for the heme importer heme carrier protein 1 (PCTF/HCP1), the heme exporter ATP-binding cassette, subfamily G, member 2 (ABCG2) and for the putative intestinal heme exporter Feline Leukemia Virus, subgroup C, Receptor (FLVCR).
Further studies will be aimed at elucidating in detail the hypoxia-mediated mechanism of heme absorption and at defining the role of FLVCR in this process.

P14. New therapeutic strategies in the treatment of non small cell lung cancer: irreversible tyrosine kinase inhibitors

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The tyrosine kinase Epidermal Growth Factor Receptor (EGFR) is an established new target for the treatment of epithelial tumors, including non-small cell lung cancer (NSCLC).
Small molecules inhibitors, such as erlotinib and gefitinib, have proven to be a useful addition to standard therapy in advanced NSCLC. However, tumor cells often acquire resistance to these reversible EGFR inhibitors. In this study our laboratory has investigated new therapeutic approaches to circumvent acquired gefitinib resistance. The design and synthesis of new tyrosine kinase inhibitors is a rational approach to treat acquired resistance to gefitinib. In approximately half of NSCLC cases that showed an initial response to reversible EGFR tyrosine kinase inhibitors and subsequently progressed, resistance was associated with the emergence of a secondary mutation within the EGFR kinase domain: substitution of threonine 790 with methionine. A second generation of 4-anilinoquinazolines or 4-anilino-3-cyanoquinolines, carrying a Michael acceptor group at the 6-position that irreversibly alkylates a cysteine residue (Cys797) close to the ATP binding site of EGFR, has been shown to overcome resistance. Several of these inhibitors, such as CI-1033, HKI-272, BIBW-2992, PF-299804 and EKI-785 are currently undergoing clinical testing in patients that initially responded to gefitinib and subsequently relapsed. These compounds contain a driver portion, which assures target recognition, and a warhead at the 6-position, that binds covalently to Cys797 within the kinase domain of EGFR. We performed a systematic exploration of the role for the warhead group, introducing different cysteine-trapping fragments at position 6 of a 4-anilinoquinazoline or 4-anilino-3-cyanoquinoline scaffold. We found that different reactive groups were able to irreversibly inhibit EGFR at significant lower concentrations than gefitinib. Moreover, we are now extending the chemical diversity of new irreversible EGFR inhibitors.

P15. Transcription-wide molecular regulation during development of the olfactory axonal pathway

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During embryonic development, the coordinated morphogenesis and differentiation of the olfactory placode (OPL) and the forebrain leads to the formation of the olfactory sensory system. Starting from early stage (E9.5), immature olfactory receptor neurons (ORN) elongate their axons to reach and contact the anterior forebrain. Axonal elongation is accompanied by migration of the $GnRH^+$ neurons, a process specifically impaired in the Kallmann's syndrome. The adult olfactory system undergoes a physiological turnover of the ORNs and their connections, a regenerating process that is likely to recapitulate (some of) the molecular events occurring during embryonic development.

Axon elongation is mediated by signals and interactions between the epithelium and the adjacent mesenchyme (i.e. morphogens, guidance molecules, ECM), but recent evidences identify specific transcription factors (i.e. *Dlx5, Fez, Emx2*) as master regulators of this complex process.

Our aim is to investigate the molecular network underlying this developmental process, and identify a set of genes essential for axon connectivity. We focus on theanalysis of mice in which homeogene *Dlx5* has been inactivated. *Dlx5*isexpressed in early ORNs and is essential for axon connectivity. We have obtained expression profiles of normal OPL at three time-points of development, as well as profiles that compare wild-type and *Dlx5*-/- OPL. Combining these data with other databases and prediction tools (PWM, genome conservation, co-regulations), we can now raise novel hypotheses on transcription regulation and signals coordinating epithelial-mesenchymal interactions, neuroepithelial development and axon elongation. We are testing 20 genes that survived these analyses, candidate targets of *Dlx5* and relevant for axon guidance.

Our great hope is to next transfer the knowledge that we gather from the embryonic model onto the adult system, as this may have more general implication for the biology of nerve regeneration and repair.

P16. Functional role of ArhGAP15, a new RacGAP

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Rho GTPases activation is regulated by cycling between inactive GDP-bound and active GTP-bound states. Guanine nucleotide exchange factors (GEFs) promote the activation of GTPases by stimulating the exchange of GDP to GTP, while GTPase-activating proteins (GAPs) accelerate hydrolysis of GTP, returning the GTPase to an inactive form. The fact that about 0.5% of all predicted human genes encode putative GAPs suggests that these proteins have widespread and important roles in GTPase regulation (Bernards A. and Settleman J., 2004). Although, the role of Rac isoforms in different cell types has been well established, how RacGAPs regulate leukocytes functions is still obscure. ArhGAP15 is a RacGAP highly expressed in macrophages (Costa et al., 2007) constituted by a pleckstrin homology (PH) domain at C-terminus required for its localization on plasma membrane and a RhoGAP domain at N-terminus (Seoh et al., 2003). To explore the role of ArhGAP15 in vivo, we generated ArhGAP15-deficient mice. Here we report that ArhGAP15 functions as a RacGAPs in vivo, regulating multiple aspects of innate immunity. ArhGAP15-null macrophages displayed an altered morphology with no effect on migration. On the contrary, ArhGAP15-null neutrophils showed increased directional migration correlating with higher Rac activity which triggers enhanced ROS production. These distinctive features protect ArhGAP15-deficient mice upon CLP (cecal ligation and puncture) treatment. Taken together, these data provides evidence for the involvement of ArhGAP15 in cytoskeleton remodelling, polarization, migration and ROS production in leukocytes.

P17. Regulation of plasma Retinol-Binding Protein (RBP4) secretion by vitamin A

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Retinol (vitamin A alcohol) deficiency causes the inhibition of RBP4 secretion from hepatocyte and its accumulation in the endoplasmic reticulum (ER); retinol repletion in the cell medium promptly results in resumption of RBP4 secretion. We have characterized immortalized murine hepatocytes (*3A cells*), that represents an innovative tool for *in vitro*studies on liver function and a useful model to investigate the regulation of RBP4 synthesis and secretion. Similarly to what happens *in vivo*, in this cell line RBP4 gene transcription is not affected by retinol availability, but ligand is essential for a regular secretion of the protein from the cell. Using 3A cells, we are studying: *a)*the mechanism by which unknown ER factors keep apoRBP in the ER during vitamin A deficiency and *b)*factors that limit intracellular apoRBP accumulation during vitamin A deficiency. *3A*cells express another secretory protein, the transthyretin (TTR), involved in thyroid hormone transport, which interacts with RBP4 before secretion from the hepatocyte and circulates as RBP/TTR complex delaying RBP4 kidney filtration.Little is known about the intracellular complex formation mechanism; it takes place before secretion and it could be helped by other proteins, maybe in relation to retinol availability in the cell.

P18. Alterations of endocytic proteins in tumor: Epn3 as a case in point

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Epsin3 (Epn3) belongs to the Epsin family of endocytic proteins. Unlike other Epsin members which are ubiquitously expressed, Epn3 expression has been reported to be restricted to migrating keratinocytes and down-regulated following cell differentiation, suggesting that its expression may be spatially and temporally regulated. Furthermore, Epn3 has been found specifically up-regulated in pathological conditions, including human cancer.

In our lab, we have analyzed the expression of Epn3 in human breast cancers by tissue microarray analysis and we have shown that it is, indeed, overexpressed in approximately 30% of the breast tumors. Importantly, Epn3 expression significantly correlates with aggressive clinical parameters, such as poorly differentiated and highly proliferating tumors.Moreover, western blot and Q-PCR experiments showed that several breast tumor cell lines overexpress Epn3 in comparison to normal counterparts.

Based on this, we propose now to study the function of Epn3 and its involvement in human tumors, employing both *in vitro* and *in vivo* approaches. To characterize *in vitro* the role of Epn3 we initially set-up stable knock down (KD) of Epn3 in BT474 cells overexpressing Epn3, and we performed a series of functional studies, including classical tumorigenic assays. Our preliminary data suggest that ablation of Epn3 impairs cell growth in 3D, as assessed by soft-agar assays. Interestingly, inhibition of the Notch pathway in the same setting phenocopies Epn3 ablation, suggesting that the tumorigenic potential of Epn3 might be linked to aberrant Notch signaling. We are currently investigating this at the transcriptional level, following the expression of Notch target genes upon Epn3 KD.

Furthermore, to substantiate the role of Epn3 in cancer, generation of transgenic mouse model expressing Epn3 under the control of a mammary-specific promoter is ongoing. **Results will be presented.**

P19. Role of PTEN in the self-renewal of human limbal-corneal stem cells

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PTEN (phosphatase and tensin homologue deleted on chromosome ten) is a tumor suppressor gene implicated in a wide variety of human cancers. It negatively regulates intracellular levels of phosphatidylinositol-3, 4, 5-triphosphate (PIP3) in cells, and functions as the main negative regulator of the PI3K/AKT signaling pathway. Recently, it has been demonstrated that PTEN expression and activity is critical for stem cell maintenance and/or differentiation in a variety of tissues, but the precise role of this signaling protein is highly cell-type specific and context-dependent. Our goal is to define the role of PTEN in the maintenance and differentiation of human limbal-corneal stem cells. Such cells are essential for the maintenance and repair of the corneal epithelium in vivo, and after expansion ex vivo, they can be used in clinical applications aimed to the repair of the corneal epithelium. Therefore, it is of paramount importance to define the signaling pathways implicated in stem cell maintenance for therapeutic purposes.

Our experimental strategy is based on lentivirus-mediated PTEN knockdown in limbal keratinocyte cultures enriched for stem cells followed by molecular and cellular analysis, to determine the effects of a loss of PTEN functions on stem cell self-renewal and differentiation.

Our preliminary data show that different levels of PTEN silencing impact differently on limbal cell self-renewal ability, and that specific levels of PTEN knockdown may favor stem cell expansion ex vivo. Furthermore, our data suggest that PTEN downregulation influences the proliferative potential of adult limbal stem cells, but not of cells at later stages of commitment to differentiation. Thus, the pharmacological manipulation of the PI3K/PTEN signaling module may lead to an improvement of cell-therapy protocols aimed to the repair of damaged corneal tissues.

P20. Endocytosis is constitutively altered in celiac disease (CD)

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Celiac Disease CD is frequent disease (1:100) and an interesting model of disease induced by food. It consists in an immunogenic reaction to wheat gliadin that happens in a specific genetic background. We have previously investigated the interaction between gliadin, and epithelial intestinal cellsfinding that gliadin and the so-called gliadin toxic peptide (P31-43) induce EGF dependent proliferation and actin rearrangements, on several cell lines and intestinal crypts enterocytes of CD patients delaying the activated receptor decay by interfering with the endocyticpathway.To understand whether any alteration of this pathway could represent a predisposing condition to gliadin effects we have investigated the endocytic pathway in mucosa from CD patients, both in the active phase of the disease and on gluten free diet (GFD), and in potential CD respect to controls. Potential CD are relatives of CD patients at gluten containing diet, with predisposing HLA, positive for auto-antibodies in the serum, but without intestinal alterations. The analysis of the endocytic compartment in CD mucosa showed that more EEA1 positive vesicles are present in CD enterocytes respect to controls. Enterocytes from potential CD have many EEA1 positive vesicles comparable to the active CD. Indicating that alteration of the endocytic compartment could be one of the first signs of damage in the CD mucosa preceding intestinal atrophy and a possible marker of the disease. We also show that the endocytic compartment in CD biopsies is malfunctioning as peptide P31-43 is delayed in EEA1 positive vesicles in biopsies from CD patients, but not in controls. In conclusion we show that the endocytic pathway, the same pathway gliadin-peptides can interfere with, is constitutively altered in CD mucosa, this explaining the specificity of gliadin-peptides effects in CD. These data also imply that the interaction between common alimentary proteins and epithelial intestinal cells needs to be farther studied and clarified.

P21. Role(s) of mast cells and their mediators in human thyroid carcinomas

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Increased numbers of mast cells are found in many tumors and the number of tumor infiltrating mast cells correlates with increased intratumoral microvessel density, tumor growth and tumor invasion. We evaluated the density of tryptase-positive mast cells in 96 papillary thyroid carcinomas (PTCs) compared to normal thyroid tissues from healthy individuals. Mast cell density was increased in 95% of PTCs with respect to normal thyroid tissue. Mast cell infiltrate degree correlated with extrathyroidal extension (p=0.0005) of PTCs. Conditioned media (CM) from different thyroid cancer cell lines induced mast cell chemoattraction *in vitro.* This effect was mediated by VEGFA since it was abrogated by VEGFA-neutralizing antibodies. Thyroid cancer cell CM also promoted histamine release and cytokine synthesis in human mast cells. Thus, thyroid cancer cell derived soluble mediators can induce chemoattraction and activation of mast cells. CM from two human mast cell lines (LAD2 and HMC-1), and from primary human lung mast cells (HLMC) induced thyroid cancer cell invasion, survival and DNA synthesis *in vitro*. The effect of mast cell CM on PTC cell proliferation was mainly mediated by three mast cell-derived mediators: histamine, and chemokines CXCL1/GROαand CXCL10/IP10. Thus, mast cell derived soluble mediators can induce invasive ability, proliferation and resistsnce to apoptosis of thyroid cancer cells. Human mast cells, injected in the tail vein of athymic mice, were recruited to xenografts of human thyroid cancer cells (8505-C) and potentiated their proliferation.Accordingly, local co-injection of human mast cells and 8505-C cells accelerated the growth of thyroid carcinoma xenograft in athymic mice. This effect was mediated by increased proliferation and vascularization and was reverted by sodium cromoglycate (Cromolyn), a specfic mast cell inhibitor. These data suggest that the blockade of mast cells recruitment at tumor site or the inhibition of their ability to release pro-angiogenic, pro-proliferative and pro-invasive mediators can be exploited as a potential antitumoral therapy.

P22. Study of Ryk functions in the development of olfactory bulb GABAergic interneurons

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We are investigating the biological and molecular functions of Wnt signalling on the proliferation and differentiation of neural progenitor (NP) cells. Wnt ligands utilize several transduction pathways: the best characterized (canonical) depends on stabilization/nuclear translocation of b-catenin, while the others (non-canonical) do not. It is commonly accepted that the canonical pathway promotes self-renewal and proliferation of NP, while activation of non-canonical pathway(s) promotes neuronal maturation/differentiation.
Our general approach combines studies on the developing basal forebrain with studies on cultured neural progenitors (NS cells). In the basal forebrain, progenitor cells occupy a specific niche at the wall of the lateral ventricle, and are fate-committed towards the GABA+ interneuronal lineage. As NP cells exit the cell cycle, they migrate either to the olfactory bulb (OB), a process that continues well into adulthood, or to the cortex.
The molecular/intracellular pathway by which Wnt5a modulates differentiation of NS cells is poorly known. To examine this we derive and expand neural progenitors from the embryonic brain, using a recently described protocol for adherent cultures NP and GABA differentiation. In these cells we observe that (canonical) Wnt3a specifically activates β-catenin, while (non canonical) Wnt5a promotes phosphorylation of JNK. We are now focussing on the tyrosine-kinase receptor Ryk, as Wnt5a interacts with Ryk, and Ryk activation/cleavage promotes the differentiation of cortical NP. Our preliminary data show that application of a Ryk neutralizing antibody on basal NP profoundly alters their differentiation properties. Defining the Wnt pathways in NP cells may open the way to be able to activate/inhibit key steps of this regulation and thereby control GABA+ differentiation of NP.

P23. Role of scaffold molecules KSRs in cell migration and angiogenesis

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Abnormal angiogenesis is involved in many pathological processes, including tumor growth and metastasis. Endothelial cell migration is an essential component of angiogenesis.This process is controlled by several receptors which activate intracellular signaling pathways leading to cytoskeleton reorganization, gene transcription and a network of cross-talks determining the final behavior of the cell.The signal transduction pathways involved in angiogenesis are only fragmentarily known. Recent data have shown that the MAPKs have been implicated in cell migration and angiogenesis. Many growth factors have been reported to stimulate cell migration, through activation of receptor tyrosin kinase involving Ras/ERK1/2 signalling pathway. However the precise molecular mechanism by which ERK1/2 regulates angiogenesis and cell migration is still poorly defined.

As for most of the MAPKs, ERK1/2 pathway is regulated by scaffold molecules, which help to assemble MAPK pathway components into a localized signaling complex. One of the most interesting scaffold molecule is Kinase Suppressor of Ras (KSR), whose function is required in the ERK1/2 pathway. There are two *ksr* genes in mammals, KSR1 and KSR2. Although KSR1 regulates cell proliferation, adipogenenesis and oncogenesis, the role of KSRs molecules in tumor angiogenesis is still unknown.Using endothelial cells isolated from KSR1 and KSR2 deficient mice we found that the protein scaffold KSRs are required for endothelial cell migration and cytoskeleton remodelling, thus contributing to the *in vitro* and *in vivo* angiogenic phenotype. These results provide new insights about the mechanisms which regulate angiogenesis and identify KSRs as new targets of angiogenic and cancer therapies.

P24. Physical interaction and co-localization of HIPK2 and H2B at the midbody

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The Ser/Thr kinase HIPK2 is a multi-functional protein acting as co-regulator of a large number of transcription factors and co-factors involved in DNA damage response and development. In response to genomic damage, HIPK2 regulates cell cycle arrest and apoptosis in p53 dependent and independent manners and HIPK2 inactivation induces resistance to apoptosis, suggesting that HIPK2 may act as oncosuppressor. In the process of identifying new HIPK2 interactors by mass spectrometry, we found that HIPK2 binds histone H2B. We confirmed this physical interaction by in vitropull-down and in vivoco-immunoprecipitation. Furthermore, we found that HIPK2 phosphorylates H2B at Serine14 (S14)in vitro. To determine whether and in what conditions HIPK2 and H2B phosphorylated at S14 (pS14-H2B) co-localize, we performed immunofluorescence experiments in different conditions. Surprisingly, we observed that HIPK2 co-localizes with pS14-H2B at the midbody during cytokinesis, rather than at the expected DNA damage-induced pS14-H2B foci. The midbody is the organelle-like structure formed at the cleavage furrow between the daughter cells during cell division. Although midbody functions are still largely unclear, its formation is an important step for a faithful cell division and its defects can lead to cytokinesis failures. The localization of both exogenous and endogenous HIPK2 and pS14-H2B at midbody was assessed with different antibodies. HIPK2 and H2B (both total and phosphorylated forms) co-localize with a-tubulin and the kinase Aurora, respectively structural and functional components of the midbody. This colocalization was further confirmed by Western blotting upon midbody isolation and extraction.Eventually, we showed that HIPK2 and pS14-H2B co-localization at midbody is independent from the presence of chromosome bridges, suggesting a direct role in cytokinesis.

P25. HGF/SF and MET transgenic lines and tumorigenesis

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HGF/SF and its receptor MET play essential roles in embryonic development as well as in regulating cell growth, motility, invasion and angiogenesis. MET and HGF/SF are known as oncogenes: inappropriate expression of MET and/or HGF/SF is associated with multiple human solid tumors and negative clinical prognosis. The basis for the overexpression of HGF/SF in tumour stroma and MET in tumour cells is unclear. Many studies established that abnormal expression of HGF/SF or of mutated MET leads to tumorigenesis, however, they failed to explain the commonest genetic and epigenetic events leading to HGF/SF and MET overexpression and the role of such events in human cancer.
We propose to tackle this problem by constructing new transgenic lines in which tagged *HGF/SF* and *MET* genes expression is driven by their own promoters. Two vector were produced in which, downstream of its own promoter, the cDNA of HGF/SF and MET were fused to a tag sequence enabling the endogenous from transgenic protein discrimination. The two constructs, devoid of plasmid sequences, were injected in fertilized mouse oocytes and were implanted in pseudo-pregnant females. Two out of 60 mice born after microinjection of HGF/SF-F7 and 4 out of 25 mice born after microinjection of HA-MET were trangenic. We are now performing expression analysis of the two transgenes on F1 progeny from each founder and histological studies.
The aim of this study is to determine whether the over-expression of HGF/SF alone in the stroma induces over-expression of MET in epithelia and other lineages and viceversa; if the overexpression of HGF/SF or MET in their tissues is sufficient to cause carcinogenicity or both are necessary; and whether, if it is the case, there is a difference in the type of tumours resulting from abnormal expression of either genes. Moreover the studies may set the foundations for understanding the mechanisms for the genetic abnormalities responsible for the role of HGF/SF and MET in human cancer.

P26. Methylglyoxal impairs insulin signaling and endothelial function *in vitro* and *in vivo*

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It has now become evident that insulin exerts a direct action on vascular cells, thereby conditioning the outcome and progression of vascular complication associated with diabetes. However, the mechanisms through which insulin signaling is impaired in the vascular endothelium remain still unclear. Chronic hyperglycaemia per se promotes insulin resistance and plays a pivotal role in the outcome and progression of diabetes-associated vascular complications. Hyperglycaemia may act through different mechanisms, including generation of methylglyoxal (MG) and advanced glycation end products (AGEs). In this work we evaluated the role of MG and AGEs in the generation of insulin-resistance in vascular cells and isolated mouse aortae. Both MG and AGEs induced a dose- and time-dependent decrease of insulin-stimulated IRSs phosphorylation, Akt activation and GSK3 phosphorylation in both NIH3T3 fibroblasts and bovine aortic endothelial cells (BAEC). This was paralleled by a 60% decrease of insulin-induced NO production in BAEC. Intraperitoneally administration of MG (50mg/kg) to C57 mice for 7 weeks caused insulin resistance (ITT AUC: C57MG 10163±1979 vs C57 7787±1174 mg/dl/120', p=0.01) and reduced serum NO by 2.5-fold compared to untreated mice. Lysates of aortae from MG-treated mice revealed a reduction of insulin-induced Akt activation. These results suggest that both AGEs and MG impair insulin signaling and insulin effect on endothelial NO production both in vitro and in vivo. Understanding the molecular mechanisms by which hyperglycaemia compromises insulin action in vascular cells may allow to develop new strategies to preserve endothelial function in diabetic subjects.

P27. Membrane association of Rac and E-Cadherin in FRT thyroid epithelial cells

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We are interested in determining the molecular mechanism by which Rac affects the expression of the polarized phenotype in FRT thyroid epithelial cells. To this aim an inducible, constitutively-active form of Rac, ER-Rac(QL), and the inducible, dominant-negative ER-Rac(N17) were stably expressed in FRT cells. By immunofluorescence analysis and cell fractionation we determined that upon tamoxifen treatment of FRT clones expressing ER-Rac(QL), the protein moves from the cytosol to the plasma membrane. The same is true for the dominant-negative ER-Rac(N17) after tamoxifen treatment. The bulk of endogenous Rac is also localized on the plasma membrane of wild-type FRT cells. Treatment with the specific Rac inhibitor NSC23766, removes endogenous Rac from the plasma membrane. Strikingly, E-cadherin is correspondingly removed from the membrane, likely by endocytosis. Chelation of calcium in the culture medium, in a Ca^{++} switch assay, also causes internalization of E-cadherin from the plasma membrane and the partial removal of Rac. Intracellular E-cadherin and Rac do not colocalize. The coordinate regulation of the association of both proteins to the plasma membrane is under investigation.

P28. Modulating the teratogenic potential of the mouse embryonic stem cells (ESCs)

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ESCs have the potential to differentiate into all cell types, but the immunological rejection and the teratoma formation stand as obstacles in the path of ESC-based therapy. Our proposal aims at investigating and developing ESCs as a therapeutic tool for treatment of diseases by ESC genetic manipulation, in order to preserve their potential to differentiate into specific cell types, while escaping a development into teratoma. We established a mouse ESC culture protocol enabling mESCs to grow in absence of both FBS and feeder cells, allowing easier manipulation and usage of mESCs. The mESCs cultured in suspension were validated for the maintenance of stemness and pluripotency, and to confirm their differentiation potential we performed an *in vivo* teratoma formation assay. To inhibit teratoma formation we modulated the self-renewal and/or differentiation patterns, by modulating ESC-specific miRNAs that control gene expression patterns associated with pluripotency. Indeed, recent papers demonstrate a central role of selected miRNAs in the ESC cell cycle, suggesting that they promote indirectly the G1/S transition. In particular, during differentiation, the expression level of the miR-290 family is downregulated, and the G1 phase, regulated by p21 and p27, is elongated. *p21*, in turn, is a direct target of miR-294, belonging to the miR-290 family. On this basis, we demonstrated, *in vitro*, that the anti-miR-294 blocks the downregulation of p21, and the indirect upregulation of c-Myc, leading to a reduction of proliferation, without interfering with the ESC differentiation. To analyze *in vivo* the effect of the miR-294 loss-of-function phenotype, we are switching to a vector-based approach, by generating a "sponge vector" using a lentiviral backbone. Then, we will perform the teratoma-formation assay, by subcutaneous injection of engineered ESC in NOD/SCID mice, to analyze the teratogenic potential of downreguleted-miR-294 ESC.

P29. Dissecting the role of Cbl in EGFR endocytosis

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Epidermal Growth Factor Receptor (EGFR) can be internalized through two different entry routes, clathrin-mediated endocytosis (CME) and non-clathrin endocytosis (NCE). EGFR ubiquitination, triggered by the E3 ligase c-Cbl, is required for this latter pathway, while it is dispensable for CME.

A dual role for c-Cbl has been shown: it acts as a major E3 ligase in the ubiquitination of different plasma membrane receptors, targeting them to lysosomal degradation, and it functions also as an adaptor, by recruiting several proteins involved in the early phase of CME. In order to understand the molecular details of Cbl activity, we plan to systematically knock-down (KD) c-Cbl and its related proteins Cbl-b and Cbl-c in HeLa cells. This will be instrumental to i) characterize their effects on EGFR ubiquitination (if they act at different steps of the endocytic cascade or through different type of ubiquitin signals), ii) dissect their involvement in CME vs NCE and iii) split their roles as adaptors and E3 ligases through reconstitution of KD cells with different sets of mutants.

We initially set-up both transient and stable KD of Cbl proteins in HeLa cells. Our preliminary data suggest that Cbl-b has a minor impact both on EGFR ubiquitination at early time points and on receptor internalization, compared to c-Cbl, suggesting distinct roles of the two proteins in EGFR endocytosis. We are currently investigating the impact of the KDs on late endocytic steps (endosomal sorting and EGFR degradation). We are also setting-up *in vitro* ubiquitination assays with purified proteins to unmask differences between the two proteins in their E3 ligase activity.

Finally, *in vivo* reconstitution of internalization/ubiquitination phenotype with different Cbl mutants (affecting ligase vs adaptor function) is on going. Results will be presented.

P30. The protein kinase C and mitochondria axis as a key regulator of autophagy

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The protein kinase C (PKC) family of serine/threonine kinases regulates several cellular functions including cell death, proliferation and tumorigenesis. They form a heterogenous group, differing for activatory mechanisms, substrate specificity and cellular distribution. Autophagy (cellular self-eating) – a lysosome-mediated catabolic process of eukaryotic cells to digest their own constituents – is a major route for the bulk degradation of aberrant cytosolic macromolecules and organelles. Autophagy principally serves an adaptive role to protect organisms against diverse pathologies, including cancer, neurodegeneration, and aging. Several reports demonstrate that PKC family could have a dual role in apoptosis, i.e. activation of specific PKC isoforms may protect or induce cell death. On the contrary, only few data are available regarding the involvement of PKCs in autophagy. In the present work, we report a direct involvement of the members of the PKCs on the regulation of the autophagic process. In particular, PKC-ζ (Atypical PKC) and PKC-δ (Novel PKC) raise autophagy whereas PKC-β (Classical PKC) has opposite effects. One of the key regulators of autophagy is the mammalian target of rapamycin (mTOR) kinase, which is the major inhibitory signal that shuts off autophagy in the presence of growth factors and nutrients. Mitochondrial homeostasis plays a major role in the determination of cell fate, and the involvement of the energy-sensing mTOR pathway in mitochondrial signaling has been shown in several studies. To verify if the PKCs mediated regulation of autophagy involves the mitochondrial compartment, we are characterizing mitochondrial physiology by measuring mitochondrial membrane potential, ATP content and reactive oxygen species production in cells in which the PKC activity is specifically modulated. These results may reveal a primary role of the PKC in the regulation of the autophagic process through the modulation of the mitochondrial functions.

P31. Do predicted microRNAs control gene networks involved in breast cancer progression?

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MicroRNAs (miRNAs) are single strand non-coding RNAs that simultaneously modulate expression of multiple genes at the post-transcriptional level by binding to the 3' UTRs of target mRNAs, leading to RNA degradation or repression of protein synthesis. Our goal is to understand how multiple miRNAs regulate a set of genes involved in breast cancer.

We first used a Wilcoxon test analysis to find differentially expressed protein-coding genes in 4 public breast cancer datasets, dividing patients into 2 classes based on disease-free survival at 5 years. We then identified those miRNAs whose targets were significantly enriched among differentially expressed genes, using an exact Fisher test and target predictions from Targetscan, Miranda, Diana-microT and Mirbase. We obtained a group of 6 miRNAs (miR-21, miR-223, miR-19b, miR-203, miR-200c and miR-340) predicted to be up-regulated in poor versus good prognosis tumors by 3 or more algorithms in at least 2 differential gene lists. Finally, all 465 down-regulated genes, predicted to be targeted by at least 4 out of 6 miRNAs, were used to perform an Ingenuity Pathway Analysis to find crucial network hubs between them.

We decided to focus first on the mechanism of modulation of two transcription factors, such as STAT5a and JUN, assuming that they play a central role in the interplay of transcriptional and post-transcriptional regulation in breast cancer. To do so, we plan to investigate how the 6 predicted miRNAs regulate STAT5 and JUN expression in several breast cancer cell-lines, following overexpression and downregulation of the miRNAs, one at a time or in various combinations. Later on we plan to investigate other hub genes present in the networks.

P32. P130Cas/estrogen receptor cross-talk during mammary gland development: *in vivo* and *ex-vivo* studies

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p130Cas is an adaptor protein which upon growth factor receptor and integrin activation functions as a regulator of cell motility and invasion. The generation of p130Cas transgenic mice in the mammary gland established an important regulatory function of p130Cas during mammaryepithelial development and remodeling*in vivo*. It is known that ovarian hormones control normal breast development. In particular, oestrogen is a major regulator of early postnatal mammary gland development and exerts its effects through estrogen receptor alpha. Several studies suggest that loss of normal regulatory mechanisms that control expression levels of ERa in normal breast epithelium may confer an increased risk for the development of breast cancer. On these regard, our laboratory has previously shown that upon oestrogen treatment of T47d human breast carcinoma cells, p130cas associates with ER-alpha, Src and PI 3-kinase to form a macromolecular complex, supporting the central role of p130Cas in the acquirement of resistance to hormone therapy in breast cancer.
The aim of the current study is to define a possible p130Cas/ER-a interaction during mammary gland development by using a p130Cas transgenic mouse model. Estrogen treatment of ovarectomized p130cas Tg mice causes an aberrant mammary gland phenotype, characterized by impaired ductal elongation and mammary fat pad invasion. In addition, altered mammary duct epithelial architecture is observed. Altogether these results suggest that p130Cas in the presence of oestrogen leads to an abnormal mammary gland development in vivo. The mechanism associated with this p130Cas-associated phenotype is currently under investigation.

P33. P140Cap regulates dendritic spines formation

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Dendritic Spines are specialized protrusion emerging from neuronal dendrites with a pivotal role in memory and learning. Most of the excitatory inputs in the brain hippocampus converge on dendritic spines. p140Cap is an adaptor protein enriched in epithelial tissues and brain, particularly in hippocampal neurons dendritic spines (Jaworski J. et al., Neuron 2009); moreover p140Cap is able to directly bind and inhibit Src kinase. Here we show that Post Natal Day 21 (P21) mice lacking p140Cap (*p140Cap*$^{-/-}$) display a defect in the number of dendritic spines of the CA1 region hippocampal neurons compared to littermate *p140Cap*$^{+/+}$ mice. Interestingly *ex vivo* cultured hippocampal neurons from 15.5 days embryos (E15.5) *p140Cap*$^{-/-}$ compared to littermate *p140Cap*$^{+/-}$ mirror this morphology defects by displaying a mis-localized F-Actin staining outside from dendritic spine, inside to the dendritic shaft. Biochemical crude synaptosomes fractioning shows increased Src kinase phosphorylation and activity in *p140Cap*$^{-/-}$ compared to *p140Cap*$^{+/+}$ mice. Small GTPases Rac1 and RhoA and their signals to dynamic cytoskeleton had been demonstrated to be crucial for proper dendritic spine maturation and morphology. Notably, pull-down experiments from crude synaptosome fractions of *p140Cap*$^{-/-}$ show an increase in Rac1 activity and a reduction in activated RhoA compared to *p140Cap*$^{+/+}$, suggesting a deregulation of cytoskeleton actin dynamic signalling. Our results indicate that p140Cap acts as a key component of the machinery of dendritic spines formation and morphology through its ability to regulate actin dynamics into neuron dendritic spines *via* Src kinase, Rac1 and RhoA activation.

P34. Importin beta regulates mitotic spindle organization and function in human cells

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The GTPase Ran regulates nuclear transport in interphase and has clear roles in mitosis. The mechanism underlying these functions is based on RanGTP ability to bind importin beta, the major vector of nuclear transport, thereby releasing importin beta cargoes in a free, active form. We are investigating how the system operates in human cells, after nuclear envelope breakdown, when nuclear transport ceases. We have found that both Ran and importin beta co-localise at mitotic spindle poles and microtubules (MTs). Inducing an imbalance in the Ran network yields abnormal spindles. To get more insight into these processes we have analysed human cells overexpressing full-length or deletion importin beta mutant during mitosis. We find that importin beta induces spindle structural abnormalities through the region that is responsible for the formation of classical import complexes in interphase (i.e., interacting with NLS-factors). Unexpectedly, we also find that a mutant lacking this region, but retaining the binding sites for nucleoporins (NUPs), alters MT dynamics, causing mitotic delay and unstable chromosome alignment and triggering the mitotic checkpoint response. These results identify importin beta as a novel global regulator of mitosis; they indicate that importin beta regulates multiple aspects of mitosis through distinct domains and suggest that NUPs represent a novel class of importin beta targets regulating chromosome segregation.

P35. EGFR as a molecular target for FDC-sarcoma therapy

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Follicular dendritic cell sarcoma (FDCs) represents a spindle cell malignancy that can occur at nodal and extra-nodal sites. Chemotherapy and radiotherapy are only partially effective and today there's no specific therapy for this kind of sarcoma. For this reason, great attention has been done for the identification of new molecular markers expressed by these cells that could represent potential therapeutic targets for FDC sarcoma therapy. By using immunohistochemical approach we observed that, in contrast to normal FDC cells, FDC sarcoma cells express high level of the epidermal growth factor receptor (EGFR), a tyrosine kinase receptor involved in the regulation of many cellular functions, such as proliferation, differentiation, apoptosis but also angiogenesis and metastasis.
It is known that EGFR is highly expressed by many kind of tumors and the use of anti-EGFR monoclonal-antibodies (mAbs), such as Cetuximab, has been demonstrated to be particular effective against carcinomas progression.
The aim of our project is to investigate whether EGFR could represent a target for immunotherapy against FDC sarcoma.
Our results demonstrated that EGF induced the activation of ERK1/2 and PI3K and is required for FDC sarcoma proliferation. *In vitro* pretreatment with anti-EGFR mAbs demonstrated that EGFR blockade by Cetuximab affected FDC sarcoma proliferation and inhibited EGFR downstream signaling pathways.
In addition, since several human tumors contain mutations at the level of genes encoding the components of EGFR pathway, we tested whether genetic abnormalities in the MAPK (e.g. Ras, Raf) and PI3K pathways, were present in the FDC sarcoma cells. DNA sequence analysis from primary FDC sarcomas revealed no mutations in *BRAF*, *KRAS*, *EGFR* and *PTEN*, indicating that genetic alterations in the EGFR pathways are not responsible of FDC sarcoma transformation.
All together, our results indicate that EGFR could be used as a target for FDC sarcoma immunotherapy.

P36. Functional role of nuclear factors-I in hematopoietic ontogenesis

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Nuclear Factor I (NFI) transcriptional factors constitute a family of four members, NFI-A, B, C and X, that are known for their positive and negative transcriptional regulatory roles in a cell type and promoter specific context. NFI genes are differentially expressed during mouse development and cellular differentiation, which suggests that these proteins could play important roles in gene expression during development. We previously identified NFI-A as a relevant target of the myeloid regulator microRNA-223. Then we found that NFI-A levels play a key role in directing hematopoietic progenitors to the erythroid or granulocytic lineage. This prompted us to examine whether the expression of NFI-A and/or other NFIs factors could regulate primitive or definitive hematopoiesisin mouse ontogeny. To this end we initially studied the expression pattern of NFIs factors in different tissues and stages of embryo development. Our preliminary results indicate that NFI-A presents the most interesting expression pattern among NFIs factors, being express in hematopoietic tissues earlier and at the highest level during embryo development. In particular in the yolk sac, where primitive hematopoiesis first and definitive hematopoiesis later take place, NFI-A shows a time dependent upregulation. We were able to establish a culture system allowing the examination of primitive erythroblasts from early yolk sac and found that NFI-A is highly expressed; implicating it in having a possible role in primitive hematopoiesis. Gross examination of NFI-A-/- spleens shows that they are smaller in size compared to their littermate controls and that the spleens are less red in color showing hypocellularity in the erythroid red pulp component. These results suggest that NFI-A could play a key role in hematopoietic ontogeny andhighlight NFI-A as a novel and potent transcriptional regulator of hematopoiesis.

P37. Ectopically implanted poly-lactic acid scaffolds as an animal model to study cardiac stem cells *in vivo* differentiation

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In the last decade cardiac regeneration has become a very interesting topic that has captured the attention of many scientists. Cardiac stem cells are self-renewing, multipotent and clonogenic cells that are able to differentiate into the three main cardiac lineages: myocytes, smooth muscle cells (SMC), and endothelial cells (EC). Furthermore, cardiac stem cells express specific surface markers such as c-Kit, a stem cell transmembrane receptor, the hematopoietic stem cell marker, Sca-1, and the Multi-Drug-Resistance gene 1 (MDR-1).
Our research aimed to investigate cardiac stem cell biology, including their differentiation, through the isolation and characterization of a pool of progenitor cells, c-kit positive, from adult rat myocardium and we demonstrated their ability to organize themselves into a tissue-like mass. In bi and three-dimensional culture, they are able to create natural extracellular matrix, vessels and elementary myocardium. For this reason we initially used OPLA scaffolds for 3D culture, which induced a good differentiation into cardiomyocites, and then a set of poly lactic acid scaffolds produced for us from BIOtech Laboratories. In vivo experiments performed inoculating scaffolds in the dorsal subcutaneous region of athymic nude-*Foxn1nu* mice demonstrated that these cells are not cancerogenic, that there is a typical foreign body reaction with body giant cells and macrophages, that they slowly differentiate compared to the in vitro counterpart, and that they create many vessels.
The employment of this animal model in basic cardiac research may contribute to a major understanding of which one is the best biodegradable that may offer a good 3D support to cell differentiation.

P38. Role of the prolyl-isomerase Pin1 in regulating the transcription-independent apoptotic activity of p53

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The major tumor suppressive activity of p53 is the induction of apoptosis in response to stress, relying on both regulation of transcription and on direct roles at the mitochondria. In the nucleus p53 is able to induce the expression of key proapoptotic genes in response to genotoxic stress. A key regulator of this pathway is the prolyl-isomerase Pin1, which is able to transduce phosphorylation of p53 into conformational changes in order to trigger dissociation of p53 from the E3-ubiquitin ligase MDM2 and the inhibitor iASPP with a consequent increase of proapoptotic transcriptional activity of p53. At the mitochondria p53 induces outer membrane permeabilization. We hypothesize that Pin1 might regulate also the mitochondrial apoptotic activity of p53, given the fact that the prolyl-isomerase has been previously shown to regulate other apoptotic proteins acting at the mitochondria, such as BIMEL, Bcl2 and p66 Shc. Here we show that Pin1 is necessary to p53-mediated transcription-independent apoptosis. Furthermore, we demonstrate that Pin1 is necessary for efficient localization of p53 to mitochondria and that it modulates the ability of p53 to interact with BclXL.

P39. Thyroid-like function in the ascidian *Ciona intestinalis*

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The role of thyroid hormones (THs) in several metabolic and developmental events has been well established. THs are necessary for the accomplishment of functions as cell growth, proliferation and differentiation, and are really important in the economy of the whole organism. Moreover both in some vertebrates and invertebrates it has been shown that thyroid hormones are involved in the dramatic phenomenon of metamorphosis. The ascidian *Ciona intestinalis*, a marine invertebrate belonging to the phylum *Chordata*, subphylum *Tunicata*, considered a sister group of vertebrate, in its adult form presents an organ, the endostyle, which has been proposed to represent the precursor of the thyroid gland of the vertebrates since it is able to concentrate radioiodine, from sea water, and to produce monoiodotyrosine, diiodotyrosine and thyroxine. Here we show, by immunostaining using a rabbit anti-T4 antibody, that L-Thyroxine is localized in the regions 7-8-9 of the endostyle of *Ciona intestinalis*, just the regions involved in iodine uptake. We investigated also the role of THs in *Ciona intestinalis* metamorphosis by rearing hatched larvae A) in artificial sea water with very low iodine content, B) in sea water added with L-Thyroxine, C) in sea water added with the goitrogen Thiourea. The data collected so far point to a role of iodine-thyroid hormone function in *Ciona intestinalis* metamorphosis too. Furthermore a proteomic approach is currently used to identify the protein(s) used as scaffold for thyroid hormones synthesis, since no homolog of thyroglobulin has been detected in *Ciona intestinalis* genome. About this issue a detailed bioinformatic analysis is in progress and a deeper biochemical analysis, via HPLC and MS has started to check the actual presence of THs in the proteins, extracted and purified from the endostyle. This study could enlighten on the origin of the thyroid gland especially with regard to thyroid hormones synthesis and their in vivo mechanism of action.

P40*. The complex between the β_1-integrin and hERG1 potassium channels as a new molecular target in antineoplastic therapy

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Background: hERG1 channels are voltage dependent K+ channels often aberrantly expressed in primary human cancers. hERG1 channels exert pleiotropic effects in cancer cells, in turn regulating cell proliferation, cell motility and invasiveness or stimulating the process of neo-angiogenesis. hERG1 can induce such diverse effects in cancer cells since it triggers and modulates intracellular signalling cascades. This role depends on the formation, on the plasma membrane of tumor cells, of macromolecular complexes with the β1 subunit of integrin receptors. Therefore, the β1-integrin/hERG1 complex may represent a novel molecular target in antineoplastic therapy, and its molecular characterization can represent a very useful task in designing novel antineoplastic therapies.

Materials and Methods: We have characterized the β1-integrin/hERG1 channel complex by both immunoprecipitation experiments and Fluorescence Resonance Energy Transfer (FRET) microscopy using fluorochrome tagged proteins (YFP-integrin and CFP-hERG1). Several mutants of either the target proteins were also produced and used.

Resuls: The experiments we have performed have clearly indicated that hERG1 channels and β1-integrin directly interact to form a plasma membrane complex in living HEK 293 cells, characterized by an intramolecular distance lower than 4 nm. Intracellular epitopes of both the β1-integrin and the hERG1 channel are apparently involved in mediating complex formation. This result, besides providing a useful confirmation of the biochemical characterization of this complex, represents an important step to design and produce molecular tools, such as bifunctional antibodies, capable of targeting, and possibly, unlocking the complex.

Conclusions: This strategy could represent a novel targeted approach for antineoplastic therapy.

*Questo Poster verrà presentato nella sessione dispari

Design e layout

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