Associazione di Biologia Cellulare e del Differenziamento

Stem Cells, Development and Regenerative Medicine

Organisers

Giorgio Merlo (Chair) - University of Turin

Caterina Missero (vice-Chair) - University of Naples Federico II and CEINGE

Programme & Abstracts

Salerno, 6-7 June 2014

http://SCDRM2014.azuleon.org

With the support of

Università degli Studi di Salerno

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PROGRAMME

Friday, 6 June

12:00 **Registration**

13:00 **Lunch**

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

Giorgio Merlo & Caterina Missero

Session I: Molecular mechanisms of development and differentiation

14:15-15:00 **Plenary Lecture**

Davide Gabellini (Milan)

Molecular pathogenesis of FSHD muscular dystrophy

15:00-17:00 **Brief Talks**

Chairs: D. Gabellini, A. Baldini

Maria Armentano (Turin)

Rac1 Hyper-activation, by knocking-out GTPase-activating-protein *ArhGAP15*, disrupts proper architecture and functions of cortical and hippocampal circuits

Luisa Cirillo (Naples)

Impaired epidermal differentiation leads to severe skin erosions and a systemic autoimmune disease in AEC syndrome

Gemma Flore (Naples)

Mesodermal expression of *Tbx1* is required for cortical development in mice

Filomena Gabriella Fulcoli (Naples)

Rebalancing gene haploinsufficiency in vivo by targeting chromatin

Sara Zribi (Rome)

An imbalanced deoxyribonucleoside triphosphate pool hinders DNA replication in reactivated postmitotic cells

Sharon Muggeo (Milan)

Generation of genetically-corrected induced pluripotent stem cells from osteopetrotic mice and differentiation to the hematopoietic lineage as a cell therapy approach

17:00-17:20 **Coffee break**

Session II: Tissue repair and regenerative medicine

17:20-18:40 **Brief Talks**

Chairs: H. Lickert, E. Illingworth

Shikhar Aggarwal (Turin)
Adult human renal papilla CD133+ stem/progenitor cells repair glycerol-induced kidney injury in mice

Monica Dentice (Naples)
The intracellular inactivation of thyroid hormone signaling in muscle stem cells and muscle regeneration

Daniele Conte (Turin)
Wnt5a/JNK pathway is downstream of Dlx5 and can restore normal limb morphogenesis in the mouse model of ectrodactyly Dlx5;Dlx6 double KO

Ombretta Guardiola (Naples)
Towards pharmacological modulation of Cripto in skeletal muscle regeneratioe

18:45-19:30 **Plenary Lecture**

Heiko Lickert (Neuherberg, Germany)

Beta cell development and regeneration

19:45 **Dinner**

21:00-21:45 **Poster Session**

Saturday, 7 June

Session III: Stem cell biology and cancer

8:45-9:30 **Plenary Lecture**

Gianvito Martino (Milan)

The role of neural stem cells in brain homeostasis and repair

9:30-10:45 **Brief Talks**

Chairs: G. Martino, G. Minchiotti, V. Calautti

Anna Musto (Naples)
Identification of miRNAs mediating the function of BMP4 in pluripotent stem cells

Francesco Neri (Turin)
PRC2 recruits Dnmt3L and Tet1 to maintain the promoter of bivalent genes in the hypomethylated state in embryonic stem cells

Silvia Pellegrini (Milan)
In vitro and *in vivo* characterization of human induced pluripotent stem cells (hiPSC) differentiation into insulin-producing cells

Arianna Petrizzo (Naples)
Drosophila as a model system for the study of stem cell dysfunction caused by X-linked dyskeratosis congenita

10:45-11:05 **Coffee break**

11:05-12:05 **Brief Talks**

Chairs: C. Blanpain, M. Crescenzi

Maria Prat (Novara)
Isolation and characterization of a spontaneously immortalized multipotent mesenchymal cell line derived from mouse subcutaneous adipose tissue

Mara Maldotti (Turin)
Long intergenic non-coding RNAs involved in cell cycle control

Ivan Colaluca (Milan)
Structure/function analysis of the Numb/Hdm2/p53 circuitry in the perspective of therapeutic applications in cancer

12:05-12:55 **Plenary Lecture**

Cédric Blanpain (Brussels, Belgium)

Mechanisms regulating stemness in epithelial cancers

13:00-13:10 **Closing remarks**

13:10 **Lunch**

Oral Presentations

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

Molecular pathogenesis of FSHD muscular dystrophy

D. Gabellini
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We are interested in understanding the regulation of muscle-specific gene expression in normal physiology and in disease using FSHD muscular dystrophy as a paradigm.
FSHD is the second most common muscular dystrophy in adults. It is an autosomal dominant disorder that is not due to a mutation within a protein-coding gene. Instead, FSHD patients carry deletions of 3.3 kilobase macrosatellite repeats, termed D4Z4, located at chromosome 4q35. In healthy subjects, a high D4Z4 copy number is associated to repression of 4q35 genes. In FSHD patients, D4Z4 deletion leads to chromatin remodeling causing de-repression of nearby genes. The unusual nature of the FSHD mutation and its complex effects on 4q35 chromatin make it unlikely that the root cause of the disease could be attributed to a single gene. An intriguing possibility is that FSHD could result from the cumulative effects caused by the aberrant overexpression of multiple 4q35 genes. The two main FSHD candidate genes are DUX4 and FRG1.
During my presentation, I will show how we recently discovered that FRG1 is a direct DUX4 transcriptional target pointing toward a unifying molecular pathway for FSHD. I will also summarize our results regarding to role of FRG1 in the control of muscle stem cell biology and muscle differentiation. Finally, I will illustrate how the identification of key disease pathways resulted in the development of a possible therapeutic approach for the disease.
Collectively, our results provide a long awaited molecular explanation for the muscle differentiation defects that have been frequently reported in FSHD and identified molecular pathways that can be exploited for therapeutic purposes.

Rac1 Hyper-activation, by knocking-out GTPase-activating-protein *ArhGAP15,* disrupts proper architecture and functions of cortical and hippocampal circuits

M. Armentano[1], V. Zamboni[1], E. Ciraolo[1], A. Ghigo[1], V. Carabelli[3], D. Gavello[3], N. El-Assawi[4], A. Mauro[4], L. Priano[4], M. Passafaro[2], E. Hirsch[1], G.R. Merlo[1]
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[4]Dept of Neurology, Univ. of Turin, Italy

Cytoskeletal reorganization is essential for several aspect during the life of a neuron ranging from the initial progenitor cell division, passing trough migration, neuritogenesis, axon guidance, and synaptogenesis. Proteins of the Rho-type small GTPases family (Rho, Rac and cdc42) are among the best known players directly impacting on cytoskeletal dynamics.
Rho-GTPases enzymatic activity is finely modulated by a large set of activating (GEFs) and inhibitory (GAPs) proteins, but the precise role of each of these is poorly known.
We have generated mice null for *ArhGAP15*, a Rac-specific GAP expressed in embryonic migrating interneurons and in a large fraction of both excitatory and inhibitory neurons of the adult cortex and hippocampus.
Loss of *ArhGAP15* results in hyper-activation of Rac1 in the embryonic and adult forebrain and increased retrograde actin dynamic at the growth cone, associated with defects in neuronal migration, reduced neuritogenesis and spine formation. We also observed a reduction of CR+ interneurons in cortex and hippocampus, and a strong reduction in VGAT+ inhibitory synapses on pyramidal neurons in the CA3 area. Preliminary data show a correlation between *ArhGAP15* loss and the PV network configuration in CA3 with a shift of PV intensity, from low to intermediate/high state, that is a measure of an altered synaptic plasticity. In addition, EEG spectral analysis show reduced high-frequency activity, increased lower frequencies activity, and overall reduced variability; also, micro-electrode array recordings of spontaneous activity of cultured primary neurons from *ArhGAP15* KO cortex and hippocampus show delayed onset, reduced amplitude, reduced variability and inability to synchronize.
Together, these findings are reminiscent of an Alzheimer-like spectrum phenotype and suggest that a fine modulation of Rac1 activity is essential for attaining a proper architecture and function of cortical and hippocampal circuits and plasticity.

Impaired epidermal differentiation leads to severe skin erosions and a systemic autoimmune disease in AEC syndrome

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AEC (*Ankyloblepharon- Ectodermal defects Cleft lip/palate*) syndrome is an autosomal dominant disorder, caused by missense mutations in p63 and characterized by skin erosions, ectodermal dysplasia and cleft lip and/or palate. To elucidate the molecular mechanisms associated with the pathogenesis of AEC syndrome, we generated a conditional knock-in mouse model carrying the clinically relevant L514F human mutation. $p63^{L514F/L514F}$ mice were crossed with a K14-Cre transgenic line that allows expression before birth. Mutant mice develop a progressively strong phenotype, characterized by skin erosions, skin crusting and hair loss. Owing to this severe phenotype a high percentage of mutant mice die between 8 and 15 days after birth. Alterations in cell adhesions and differentiation cause a progressive inflammation characterized by hyperplasia and hyperkeratosis, and massive infiltration of macrophages and mast cells in the dermis. Clear signs of severe inflammation are accompanied by strongly elevated levels of Thymic stromal lymphopoietin (Tslp), an IL-7 like cytokine. We find that epidermal derived Tslp is released in blood circulation, causing a B-lymphoproliferative disorder with high levels of pre- and immature B cells. Interestingly a similar autoimmune disorder has been observed in at least one AEC patient with severe skin erosions. Taken together these data indicate that in the AEC conditional mouse model, epidermal barrier is affected causing erosions, crusting closely mimicking the human condition.

Mesodermal expression of *Tbx1* is required for cortical development in mice

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Proper temporal control of neurogenesis and neural migration during embryonic development ensures the correct assembly of the mammalian cerebral cortex. Changes in the cortical distribution of projection neurons and interneurons have been associated with behavioral disorders and psychiatric diseases, including schizophrenia and autism spectrum disorders, suggesting that disrupted cortical connectivity may underlie the brain pathology. The transcription factor *TBX1* is the major gene involved in 22q11.2 deletion syndrome, a relatively common chromosomal deletion disorder that is characterized by a greatly increased risk for schizophrenia. We have previously shown that heterozygous inactivation of *Tbx1* in the mouse causes reduced prepulse inhibition, a behavioural abnormality that is associated with the human disease and with non-syndromic schizophrenia. Here we show that a *Tbx1* loss of function mutation alters mouse brain neurogenesis by promoting prematurely neuronal differentiation in the dorsal and ventral telencephalon. In addition, we found altered polarity in both radially migrating excitatory neurons and tangentially migrating interneurons, as well delayed or disrupted motility. Taken together, these phenotypes may all contribute to the altered neuronal distribution that we observe in *Tbx1* homozygous embryos at the terminal stages of corticogenesis within the neocortical layers. Since *Tbx1* mRNA expression is limited to brain vessel endothelium, the observed neural phenotypes are likely to be indirect. In fact, we show that the early mesoderm-specific inactivation of *Tbx1* can recapitulate the brain phenotype caused by global *Tbx1* inactivation, suggesting that *Tbx1* exerts a cell non-autonomous role in cortical development.

Rebalancing gene haploinsufficiency in vivo by targeting chromatin

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Tbx1 is haploinsufficient in humans and mice, and its mutation is a cause of DiGeorge syndrome, which includes congenital heart disease. The gene encodes a transcription factor of the T-box family, required in several cellular functions during development, including the regulation of the balance between proliferation and differentiation of cardiac progenitors of the second heart field. Recently, we linked at least some molecular functions of Tbx1 to interactions with a Swi-Snf-like chromatin remodeling complex and with histone methyltransferase enzymes. To explore in detail the interactions between Tbx1 and chromatin, we have carried out genome-wide ChIP-seq assays to map binding sites of Tbx1 and correlate them with gene features and chromatin profiling. The results indicated that Tbx1 occupation strongly correlates with H3K4me1 enrichment (71%) and positively regulates monomethylation of H3K4 at a significant number of regions that it occupies throughout the genome, often located at the 3' end of target genes. Thus, we hypothesized that reduced dosage of *Tbx1* may lead to target gene disregulation through insufficient H3K4me1 enrichment. We reasoned that treatment with a demethylase inhibitor may compensate for reduced dosage of Tbx1 and thus ameliorate the haploinsufficiency phenotype. Treatment of cells with Tranylcypromine (TCP), a drug approved for human use, which is a potent inhibitor of Lsd1-mediated demethylation, rescues the expression of approximately one third of all the genes dysregulated by Tbx1 suppression and it rebalances H3K4me1 levels at many Tbx1 occupied sites. In addition, TCP treatment of mouse models of the syndrome ameliorates considerably the cardiovascular phenotype. These data indicate that epigenetic drugs can be used successfully to treat gene haploinsufficiency phenotypes, including congenital heart disease and open a novel avenue to search for specific drugs to treat the adolescent and adult phenotypes associated with DGS.

An imbalanced deoxyribonucleoside triphosphate pool hinders DNA replication in reactivated postmitotic cells

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The molecular bases of the permanent withdrawal from the cell cycle that defines terminally differentiated (TD) cells are insufficiently understood. The ability to reactivate proliferation in TD cells such as neurons and cardiomyocytes would open major avenues for regenerative medicine. TD cells such as skeletal muscle myotubes (Mt) can be forced to reenter the cell cycle by several means, among which expression of the adenoviral oncogene E1A or depletion of CDK inhibitors. However, reactivated Mt cannot fully replicate DNA, leading to apoptosis or mitotic catastrophe. We regard incomplete DNA replication as the last hurdle on the way of attaining long term proliferation of TD cells.
In Mt, similar to other resting cells, the dNTP pool scarcely reaches 10% of that of proliferating myoblasts. We reasoned that the inability of reactivated Mt to restore an adequate dNTP pool might explain their failure to fully replicate nuclear DNA. To test this hypothesis, we surveyed the dNTP biosynthetic enzymes and measured dNTP concentrations in reactivated Mt. qPCR analyses showed a notable impairment of the dNTP salvage biosynthetic pathway. Strikingly, we found that reactivated Mt attempt to replicate their DNA in the presence of a severely imbalanced dNTP pool, characterized by very low levels of dGTP and dTTP and very high levels of dATP and dCTP. We assessed the dNTP pool status in different Mt-reactivating conditions and found that more extensive DNA replication correlates with better-balanced nucleotide pools.
To correct dNTP levels in reactivated Mt, we administered them with deoxythymidine and deoxyguanosine. This manipulation achieved a remarkable rebalancing of the dNTP pool; dramatic modifications of the BrdU incorporation pattern argue in favor of a significant improvement in DNA replication.
These results suggest that an insufficient/unbalanced dNTP pool is responsible, at least in part, for the incomplete DNA replication that follows cell cycle reactivation in TD Mt.

Generation of genetically-corrected induced pluripotent stem cells from osteopetrotic mice and differentiation to the hematopoietic lineage as a cell therapy approach

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Autosomal recessive osteopetrosis is a bone disease mainly caused by mutations in TCIRG1 gene that prevents osteoclasts resorbing activity and is recapitulated by *oc/oc* mice. Hematopoietic stem cell (HSC) transplantation is the unique possible treatment limited by the need for a matched donor. Therefore, generation of corrected autologous HSCs may be a novel approach to therapy and induced pluripotent stem cells (iPSc) represent an innovative source of donor cells. The aim of our project is to obtain iPSc from wt and *oc/oc* mice, to correct the mutation and to generate HSCs/progenitors able to produce functional osteoclasts. To generate iPSc we employed a lentiviral vector carrying Oct4, Sox2 and Klf4, excisable from the host genome by the Cre recombinase. Obtained iPSc with low vector copy number and normal numerical distribution of chromosomes were selected, treated with Cre and sub-cloned. Obtained cell lines showed normal karyotype, expression of stemness markers and pluripotency as assessed by teratoma formation and *in vitro* differentiation in the three embryonic germ layers. *oc/oc* derived iPSc were corrected through homologous recombination using a BAC with wt *Tcirg1* and contributed to generate chimeric mice. wt and corrected *oc/oc* iPSc were differentiated *in vitro* towards different hematopoietic lineages without additional transgene over-expression. FACS analysis revealed differentiation kinetics resembling physiologic fetal hematopoiesis, with $CD41^+$ cells gradually giving rise to $CD45^+$ cells, which comprised both mature myeloid cells and high proliferative potential colony-forming cells. Finally we differentiated myeloid cells derived from wt iPSc towards osteoclasts, the relevant cells in our model, which were functional as demonstrated by the dentine resorption assay; similar experiments with corrected *oc/oc* iPSc are ongoing. In conclusion, we will provide a proof of principle for the treatment of osteopetrosis and potentially other genetic blood disorders.

Adult human renal papilla CD133⁺ stem/progenitor cells repair glycerol-induced kidney injury in mice

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Introduction: Cell therapy is a novel and promising therapeutic approach for the treatment of renal failure. Kidney stem/progenitor cells (KSPCs) may provide a tool to modulate and foster the intrinsic capability of kidney to repair itself upon extramural insults. Adult human CD133⁺ renal cells have been identified by our group as a promising adult renal stem/progenitor located in the proximal tubules in the renal cortex and in the inner hypoxic medullary papilla region of Henle's loop and S3 limb segment. In particular, papillary CD133⁺ cells show activation of hypoxia-inducible genes and are specifically involved in Erythropoietin (EPO) and IL-15 production. However, CD133⁺ cells derived from papillary region have not yet been explored in their ability to repair kidney failure *in vivo*.
Aim: To investigate the role of adult human CD133⁺ papillary stem/progenitor cells in an *in vivo* model of kidney injury induced by glycerol injection.
Methods and materials: Kidney injury is created in immunodeficient mice using glycerol (8mg/gbw, i.m.) and adult human CD133⁺ papillary cells are injected (*i.v.*) 1 day after the primary injury. In some cases, a secondary injury (Day 7/Day 15) is also given to the mice. Mice are euthanized at different time intervals (day 15/day 30) and blood and other tissues are collected for creatinine/urea and histological/morphological analysis respectively.
Results: Adult human CD133⁺ papillary cells protect and improve the renal function in the injured mice as compared to control group, shown by lower level of creatinine and urea in serum and by morphological analysis of the tissues. CD133⁺ papillary cells also reduced development of fibrosis in the injured tissues during repair. In addition, immunohistochemical and fluorescence studies show integration of CD133⁺ papillary cells within interstitium and tubules of the injured renal tissues.
Outlook: Adult human CD133⁺ cells are attractive candidates in renal pathologies and can be used to modulate their role *in situ* using pharmacological approaches.

The intracellular inactivation of thyroid hormone signaling in muscle stem cells and muscle regeneration

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Thyroid hormone (TH) controls a number of cellular processes including cell proliferation, differentiation and survival. Precise control of thyroid hormone-dependent transcription is required by multiple cell system, including muscle stem cells, but how this is achieved is still largely unknown. Intracellular TH concentration does not simply mirror circulating TH levels, but is determined by a metabolic balance between the activating and inactivating deiodinase enzymes, D2 and D3. In functional combinations, these regulatory enzymes provide the ability to fine tune TH action at cellular level.
Skeletal muscle is a major target organ of thyroid hormone. TH has long been known to have important effects on skeletal muscle physiology by altering the proportion of slow and fast fibers, regulating the protein synthesis and catabolism and increasing muscle thermogenesis. Here, we report that in the muscle context, D3-mediated TH inactivation might play a significant role in muscle development and regeneration.
Our data demonstrate that in response to proliferative stimuli such as skeletal muscle acute injury, type 3 deiodinase (D3), the thyroid hormone-inactivating enzyme, is specifically induced in satellite cells where it reduces intracellular thyroid signaling. Satellite-specific genetic ablation of D3 impairs skeletal muscle regeneration. This impairment is due to massive satellite cell apoptosis, caused by aberrant exposure of activated satellite cells to the physiological, but spatio-temporally excessive, TH concentrations in the circulation.
In conclusion, our results indicate that the D3 enzyme is dynamically exploited in vivo to attenuate TH-signaling and simultaneously orchestrate distinct gene activation and repression programs required for the satellite cell lineage progression and survival.

Wnt5a/JNK pathway is downstream of Dlx5 and can restore normal limb morphogenesis in the mouse model of ectrodactyly Dlx5;Dlx6 double KO

D. Conte[1], G. Garaffo[1], V. Poli[1], V. Orecchia[1], R. Zeller[2], G.R. Merlo[1]

Split hand/foot malformation (SHFM), or ectrodactyly, is a developmental disorder characterized by a median cleft of the hands and/or feet and missing central finger. Mutation in the homeogenes *Dlx5* and *Dlx6* are causative of SHFM type I. These transcription factor are expressed in a transitory stratified specialization of the ectoderm, known as apical ectodermal ridge (AER), at the dorsal-ventral rim of the embryonic limb bud. The AER is a signaling center, essential for proximo-distal growth and morphogenesis of the limb, that acts on the adjacent mesodermal cells and on itself via diffusible signaling molecules. Among these, the non-canonical Wnt5a molecule regulates the orientation of movement and division of the mesenchyme cells via JNK and the activation of the Planar Cell Polarity (PCP) pathway.
Embryos null for *Dlx5;Dlx6* exhibit ectrodactyly of the posterior limbs, the central wedge of the AER is poorly stratified, fails to express FGF8 and shows reduced p63 protein level. We show that Wnt5a is a target of Dlx5 and is downregulated in *Dlx5/Dlx6*$^{-/-}$ limbs. We also show that the AER cells of mutant limbs are mis-polarized, and the expression of PCP-linked molecule RhoU GTPase is diminished. To further test the role of Wnt5a, we have set up ex vivo cultures of whole embryonic limbs. Treatment of cultured *Dlx5/Dlx6*$^{-/-}$ limbs with Wnt5a rescues AER stratification, the expression of specific ectoderm and mesoderm markers, the expression of p63 as well as the PCP-linked molecule *RhoU*. Conversely treatment of normal limbs with JNK inhibitors causes reduced *RhoU* expression and the appearance of AER defects, similar to those of the mutant limbs. These data suggest that: A) alteration of the PCP pathway is likely to be involved in ectrodactyly; B) Wnt5a can rescue a normal AER organization and function, likely resulting in restoration of normal limb morphogenesis.

Towards pharmacological modulation of Cripto in skeletal muscle regeneration

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We have recently provided new insights in TGF-b ligand-specific signaling by Cripto on satellite cells, showing that inactivation of *cripto* in adult satellite cells compromises skeletal muscle regeneration. Moreover we demonstrated that Cripto promotes myogenic committment of satellite cells and that it is mitogenic for satellite cell-derived myoblasts. These notions support the model in which Cripto possesses intrinsic activities as a transacting factor on satellite cells both in cell culture and *in vivo*. In line with this idea, we demonstrated that the viral- mediated overexpression of a soluble form of Cripto (sCripto) is able to rescue the effect of the genetic ablation of *cripto* in the adult satellite cell compartment on muscle regeneration, thus providing direct evidence that sCripto was able to fully recapitulate the function of endogenous membrane Cripto. Furthermore, sCripto overexpression improves muscle regeneration that normally occurrs in a model of acute injury, increasing myofiber CSA area. In order to investigate whether recombinant sCripto could be used as a pharmacological approach to improve skeletal muscle regeneration, we perfomed preliminary experiments by injecting sCripto intramuscularly (IM) or by using biodegradable microcarriers (gelatin hydrogel microspheres), a system that enables the protein to maintain its activity over a prolonged period of time, allowing to control the release kinetics of the protein. Our preliminary results show that myofiber CSA area significantly increased in sCripto treated mice compared to control, supporting the idea of using sCripto as biopharma either alone or in combination with other molecules to stimulate muscle regeneration mobilizing endogenous stem cells.

Beta cell development and regeneration

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[Abstract not received]

The role of neural stem cells in brain homeostasis and repair

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Regenerative processes occurring under physiological (maintenance) and pathological (reparative) conditions are a fundamental part of life, and vary greatly among different species, individuals, and tissues. Physiological regeneration occurs naturally as a consequence of normal cell erosion, or as inevitable outcome of any biological process requiring the restoration of homeostasis. Reparative regeneration occurs as a consequence of tissue damage. Although the central nervous system (CNS) has been considered for years as a 'perennial' tissue, it is now becoming clear that both physiological and reparative regeneration occur within the CNS to sustain tissue homeostasis and repair. Proliferation and differentiation of neural stem and progenitor cells (NPCs) residing within the healthy CNS, or surviving to the injuries, are considered crucial in sustaining these processes. Thus, a large number of experimental stem cell-based transplantation systems for CNS repair have recently been established. The results suggest that transplanted NPCs might promote tissue repair not only via cell replacement but also via their local contribution to changes in the diseased tissue milieu.

Identification of miRNAs mediating the function of BMP4 in pluripotent stem cells

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Embryonic Stem Cells (ESCs) are able to self-renew and to differentiate in vitro giving rise to a wide range of specialized cells upon different cues. For these characteristics, ESCs represent a powerful tool that could be used for regenerative medicine and for the study of embryonic development. However, a deeper knowledge of the mechanisms controlling self-renewal and differentiation is required before using these cells for these purposes. Two branches of TGFβ signaling pathway , i.e. BMP4 and Nodal/Activin, are key regulators of ESC fate and they act by controlling ESC differentiation at different levels. We have recently demonstrated that the fine balance between these two pathways is regulated by miR-125a at least in the first phases of ESC differentiation. This regulation goes through an efficient loop by which BMP4 controls the transcription of miR-125a that targets the BMP4 co-receptor, Dies1, and in turn this mechanism controls the proper response of ESCs to the BMP4 stimulus. We have hypothesized that this can be a general mechanism adopted by BMP4 to control ESC fate through the regulation of specific miRNAs. To this aim, we performed a miRNA expression profiling in ESCs stimulated with BMP4 and we found 20 up-regulated miRNAs. Among these miRNAs, by measuring the level of the primary transcripts and by means of ChIP-qPCR experiments, we have found a miRNA cluster (miR-23a, 24-2, 27a) highly expressed in undifferentiated ESCs, that is directly controlled by BMP4. Then, we have analyzed the function of miR-23a cluster in undifferentiated ESCs and during neuronal differentiation. We have found that the suppression of these miRNAs does not impair stemness maintenance but it increases apoptosis of a subset of cells during neuronal differentiation. These results suggest that these miRNAs can function to protect the cells from apoptosis during differentiation. Now we are searching for miRNA targets that can mediate this function in ESCs.

PRC2 recruits Dnmt3L and Tet1 to maintain the promoter of bivalent genes in the hypomethylated state in embryonic stem cells

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In mouse embryonic stem cells (ESCs), developmental gene promoters are hypomethylated, but maintained repressed by Polycomb Repressive Complex 2 (PRC2). DNA methylation increases around implantation to remain stable in fully differentiated cells. Through genome-wide analysis of DNA methylation in ESC silenced for Dnmt3L we found that Dnmt3L is a positive regulator of methylation at the gene bodies of housekeeping genes and, more surprisingly, is also a negative regulator of methylation at promoters of bivalent genes. We demonstrate that Dnmt3L is required for the differentiation of ESCs into primordial germ cells (PGCs) through the activation of the homeotic gene Rhox5. Dnmt3L interacts with the Polycomb PRC2 complex in competition with the DNA methyltransferases Dnmt3a and Dnmt3b to maintain low the level of methylation at the H3K27me3 regions.
Moreover, we found that the overlap between the repressive modification H3K27me3 and 5hmC is specific of ESCs and it is not present in other cell types. Furthermore, by ChIP-seq and protein immunoprecipitations we demonstrated that in ESCs Tet1 cooccupies with PRC2 bivalent promoters in a proximal region downstream from the TSS. We show that PRC2 interacts with Tet1 and it is required for Tet1 recruitment to the chromatin and 5hmC deposition at developmental genes.
Thus, in ESC, Dnmt3L and Tet1 counteract the activity of *de novo* DNA methyltransferases to maintain hypomethylated the promoter of bivalent developmental genes.

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In vitro and *in vivo* characterization of human induced pluripotent stem cells (hiPSC) differentiation into insulin-producing cells

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Background: New sources of insulin-secreting cells are strongly required for the cure of type 1 diabetes. Recent successes in differentiating embryonic stem cells (ESC), in combination with the discovery that it's possible to derive hiPSC from somatic cells, have raised the possibility that patient-specific β cells might be derived from patients through cell reprogramming and differentiation.
Methods: We optimized differentiation protocols already established for ESC. Read outs of the differentiation process were: Taqman for gene expression (results expressed as fold changes (FC) compared to undifferentiated hiPSC), FACS and ELISA for insulin production. HiPSC differentiated in vitro were transplanted in NOD/SCID mice and functional and an immunohistochemical analysis of grafts were performed.
Results: We obtained down-regulation of the pluripotency genes Oct4 and Nanog and up-regulation of the definitive endoderm Sox17 and Foxa2 (39,25±15,78 and 7,94±4,16 FC) and of the pancreatic endoderm genes Hnf1b, Pdx1, Ngn3 and Nkx2.2 (97,14±77,01, 596,34±368,78, 83,46±80,34 and 1,62±0,90 FC). At the end of the differentiation, the production of insulin mRNA was highly increased (1567,92±785,1 FC) and 5±2,9% cells resulted insulin-positive; terminally differentiated cells also produced C-peptide *in vitro* (1,7±0,1 ng/mL). In mice transplanted with hiPSC-derived pancreatic progenitor cells, the grafts resulted composed of a mixed population of cells containing mature pancreatic cells (Insulin$^+$, Glucagon$^+$ and Pdx1+), but also pluripotent (Sox2+ and Ki67+) and some neuronal cells (GFAP+) at 1 and 4 weeks. Mice transplanted with pancreatic progenitor cells responded to glucose secreting human c-peptide.
Conclusions: *In vitro* results show that hiPSC differentiate in insulin secreting cells. Furthermore, *in vivo* study suggests that differentiated cells engraft and survive in the recipient mice, but evidence the contamination of pluripotent cells with tumorigenic potential.

Drosophila as a model system for the study of stem cell dysfunction caused by X-linked dyskeratosis congenita

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Loss of function mutations of the human *DKC1* gene cause X-linked Dyskeratosis Congenita (X-DC), a multisystemic syndrome accompanied by telomerase defects, premature aging, increased cancer susceptibility and stem cell depletion.
The *DKC1* encoded protein, called dyskerin, belongs to a highly conserved family of pseudouridine synthases. In mammalian cells, members of this family participate to two essential cellular complexes: the H/ACA snoRNP complex, that is involved in a variety of functions, including ribosome biogenesis and pseudoridylation of cellular RNA, and the active telomerase complex, involved in the maintenance of telomere integrity. The striking evolutive conservation of snoRNP structure, coupled with a highly divergent mechanism of telomere maintenance, makes Drosophila an ideal animal model in which assessing the specific role that is played by each of these two complexes in the maintenance of the stem cell compartment.
To define this aspect, we triggered by *in vivo* RNAi the silencing of *Nop60b/mfl*, the Drosophila *DKC1* ortholog, and investigated the downstream effects on the formation of larval Adult Midgut Precursor (AMPs) cells, that represent a valuable system for the study of somatic stem cell maintenance.
We found that gene silencing totally disrupts the formation of the larval imaginal islands, the typical structures in which AMPs are organized. Moreover, gene activity proved to be specifically required within the subset of cells that express the stemness marker *escargot (esg;* member of the Snail family of transcription factors); as consequence, *esg*+ MFL-depleted cells show defective self-renewal and fail to expand in their number.
These results point out that maintenance of the stem cell compartment is a telomerase–independent function and requires the activity of the evolutively conserved eukaryotic H/ACA snoRNP complexes.

Isolation and characterization of a spontaneously immortalized multipotent mesenchymal cell line derived from mouse subcutaneous adipose tissue

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The emerging field of tissue engineering and regenerative medicine is a multidisciplinary science that is based on the combination of a reliable source of stem cells, biomaterial scaffolds, and cytokine growth factors. Adult mesenchymal stem cells are considered important cells for applications in this field, and adipose tissue has revealed to be an excellent source of them. Indeed, adipose-derived stem cells (ASCs) can be easily isolated from the stromal vascular fraction (SVF) of adipose tissue. During the isolation and propagation of murine ASCs, we observed the appearance of a spontaneously immortalized cell clone, named m17.ASC.
This clone has been propagated for more than 200 passages and stably expresses a variety of stemness markers, such as Sca-1, c-kit/ CD117, CD44, CD106, islet-1, nestin, and nucleostemin. Furthermore, these cells can be induced to differentiate toward osteogenic, chondrogenic, adipogenic, and cardiogenic phenotypes. m17.ASC clone displays a normal karyotype and stable telomeres; it neither proliferates when plated in soft agar nor gives rise to tumors when injected subcutaneously in NOD/SCID-γ null mice. The analysis of gene expression highlighted transcriptional traits of SVF cells. m17.ASCs were genetically modified by lentiviral vectors carrying green fluorescent protein (GFP) as a marker transgene and efficiently engrafted in the liver, when injected in the spleen of NOD/SCID-γ null monocrotaline-treated mice. These results suggest that this non-tumorigenic spontaneously immortalized ASC line may represent a useful tool (cell model) for studying the differentiation mechanisms involved in tissue repair as well as a model for pharmacological/toxicological studies.

Long intergenic non-coding RNAs involved in cell cycle control

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Long intergenic non-coding RNAs (lincRNAs) are polyadenylated RNAs not translated longer than 200 nt. They are expressed at low levels and cell type specific. Growing evidence suggest that lincRNAs have emerged as important regulators for diverse cellular functions. However, little is known about lincRNAs in regulation of the cell cycle. To identify novel lincRNAs in primary Mouse Embryonic Fibroblasts (MEFs) we performed a discovery through High-throughput *RNA sequencing (*RNA-seq) technology in a time course of serum induction (0, 0.5, 1, 2 hours). We found 44 lincRNAs specifically induced by serum treatment of starved MEFs. To study their biological role we performed the loss of function experiments by shRNAs in BALB/c fibroblasts. 8 lincRNAs, which showed an effective knockdown greater than 50% respect control were further characterized. We performed cellular proliferation assays and we observed a significant decrease of the proliferation of the cell growth curve, also confirmed by FACS analysis with an arrest of cells in G0/G1 phase, for two lincRNAs. The ectopic expression of both lincRNAs, resistant to silencing, were able to rescue the phenotype. To characterize the molecular mechanism by which these lincRNAs affect the cell cycle we first performed a subcellular fractionation and we found that one cell cycle-related non-coding RNA (lincRNA-CCR492) had a predominant cytoplasmic localization while the other (lincRNA-CCR1022) was nuclear. These results were confirmed by RNA-FISH. To better understand the molecular mechanism by which these lincRNAs are involved in cell cycle progression we will perform RNA-seq analysis.

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

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

Mechanisms regulating stemness in epithelial cancers

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For the vast majority of cancers, the cell at the origin of tumor initiation is still unknown. Two epithelial skin cancers are frequent in human populations: the basal cell carcinoma and the squamous cell carcinoma. We developed genetic lineage tracing approaches to identify the cells at the origin of these two types of cancer in mice, isolate these oncogene targeted cells by flow cytometry and determined the molecular changes associated with tumor initiation (Youssef NCB 2010, Youssef NCB 2012, Lapouge PNAS 2011).
Cancer stem cells (CSCs) have been described in various cancers including skin squamous cell carcinoma. Using different approaches in mice, we and others have recently shown that mouse squamous skin tumors contain cancer stem cells characterized by their greater ability to be propagated long term upon transplantation into immunodeficient mice (Lapouge EMBO J 2012) or by their ability to fuel tumor growth using lineage tracing experiments (Driessens Nature 2012).
To define the mechanisms that regulate skin cancer stem cells, we transcriptionally profiled cutaneous CSCs at different stages of tumor progression and identified genes preferentially upregulated in CSCs. Using state of the art genetic gain and loss of function in mice, we are defining how some of these genes regulate tumor stemness and malignant transition in vivo within their natural environment. I will discuss how the combination of extrinsic factors such as the vascular niche (Beck Nature 2011) and intrinsic factors, such as the expression of Sox2, a transcription factor expressed in a variety of developmental progenitors and adult stem cells, regulate tumor heterogeneity, renewal and invasive properties of CSCs during skin cancer progression (Boumhadi Nature 2014).

This work is supported by WELBIO, the FNRS, and the European Research Council (ERC).

POSTERS

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P1

Isolation and characterization of neural stem cells from dystrophic mdx mouse

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Our previous researches have been focused on the study of the alterations of dystrophin and dystrophin-associated protein complex (DAPs) in the glial cells of dystrophic mdx mice, a murine model of the Duchenne muscular dystrophy in parallel with the study of the alteration of the blood brain barrier (BBB) and of the vessels permeability. In this study, we isolated for the first time from the brain of the mdx and control mice, the neural stem cells (ANSCs) and characterized them by FACS, western blotting, real time-PCR, electron microscopy, and immunofluorescence, and confocal microscopy analysis. Moreover, we studied the expression of the DAPs aquaporin-4 (AQP4), potassium channel Kir4.1, β-dystroglican (βDG), α-syntrophin (αSyn), and short dystrophin isoform Dp71 proteins. The results showed that the mdx ANSC expressing the CD133, nestin and NOTCH receptor were reduced in the number compared to controls, they showed a retard in the cycle cell and ultrastructurally, appeared 50% size reduced with a few cytoplasmic organelles compared to control ones. Finally, the mdx ANSC expressed the Dp71 and DAPs proteins like the controls, but they were strongly reduced at post-trascriptional level, and not arranged on the ANSC plasmamembrane, as observed in the controls, after immunofluorescence confocal analysis.
Overall, these results confirm a deficiency in the Dp 71 and DAPs proteins in the mdx brain, which affect a population of glial precursors cells, namely the neural stem cells. In this context, these precocious alterations may be responsible of the altered mdx glial differentiation and BBB injuries in mdx mice.

[T. Annese is a fellowship of Dottorato di Ricerca in Trapianti di tessuti ed organi e Terapie cellulari.]

P2

A composite enhancer regulates p63 gene expression in epidermal morphogenesis and in keratinocyte differentiation by multiple mechanisms

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p63, a master regulator of keratinocyte gene expression, is the first epidermal marker to be expressed in the surface ectoderm during embryonic development, and becomes progressively confined to the basal proliferative layer of the developing epidermis. The mechanisms that mediate *p63* expression in the developing skin and in epidermal differentiation are still poorly understood. Here we report the identification of a novel genomic regulatory element (C38) that, combined with the previously identified C40 element, constitutes a keratinocyte-specific enhancer (p63LRE). Both elements are in an open and active chromatin state in keratinocytes. The genomic region encompassing the two elements drives strong and basal cell-specific expression in epidermis and this regulation is dependent upon the p63 protein as determined in a *p63*-null background. Consistently p63 protein binds uniquely to both regulatory elements in the *p63* locus. A search for other transcription factors involved in p63 regulation through the p63 LRE revealed that members of the POU domain-containing proteins Skin1a and Tst1, and the CAAT enhancer binding proteins C/EBPα and C/EBPβ repress *p63* expression directly through the p63LRE enhancer, consistently with their role in keratinocyte differentiation. In contrast the Interferon Regulatory Protein Irf6 indirectly inhibits p63LRE activity and *p63* expression at the RNA level with a mechanism that is dependent on the p63 protein itself. Collectively our data reveal that two long range regulatory elements contribute in an additive and partly redundant modular fashion to the overall p63 expression pattern in the epidermis. While p63 sustains its own transcription, a subset of transcription factors involved in commitment to differentiation, namely C/EBPα/β and Skin1a/Tst1 act as repressors of p63 transcription in differentiated keratinocytes.

P3

Quick production of spherical aggregates of human cardiac progenitor cells for scaffold-less tissue engineering by means of a novel methylcellulose hydrogel-based system

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Although slow, the self-renewing ability of adult myocardium is ackowledged since more than 10 years and it has raised great expectations in the field of cardiac regenerative medicine. However, Cardiac Progenitor Cells (CPCs), once transplanted as single cell suspension in the heart wall were found to be rather inefficient, since most of them were lost in short time. On the other side, the conventional tissue engineering approach based on the transplantation of cells loaded on scaffolds could lead to problems, such as inflammation and fibrosis. An alternative more recent approach employs scaffold-less cell aggregates, such as cell sheets or cardiospheres. We have used a quick and cheap home-made methylcellulose (MC) hydrogel well system to produce implantable spherical clusters of human cardiac progenitor cells (hCPCs) for innovative scaffold-less cardiac tissue engineering. Cell spheres could be obtained in 16-18 h and 5000 cells were judged to be optimal dose. More than 90% of the cells within them were alive and, when they were seeded in conventional culture plates, cells easily migrated and reached confluency. Moreover the cells migrated from the 3D spheres were superior to hCPC maintained in 2D conventional culture systems in their ability to migrate in a wound healing assay in response to an agonist monoclonal antibody directed against the Hepatocyte Growth Factor Receptor. The spheres maintained the expression of stemness/mesenchymal (CD90, CD44 and vimentin) and ECM (collagen I, fibronectin and laminin) markers, as well as of some early cardiac markers (connexin 43, GATA-4 and MEF2C), although at a low level. When the spheres were injected in the mouse heart wall, hCPC were able to migrate, engraft and survive into the host tissue, at least for 1 week after transplantation.

P4

The wound healing assay revisited: a transport phenomena approach based on time-lapse microscopy and image analysis

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The wound healing or scratch assay is a straightforward and economic technique widely used to investigate *in vitro* cell dynamic behavior during wound repair. In this assay, an artificial scratch is created mechanically on a confluent cell monolayer by scraping off an area of cells. In response to the injury, the cells on the edges of the newly created gap proliferate and move toward the center of the denuded area until the wound is closed. In fact, the wound closure process is driven by the migration and proliferation ability of the cells, aimed to the recovery of the cell-free area. Fibroblast-like cells cover the wound space moving as individual units, while epithelial-like cells migrate into the wound space as closely connected populations.
In our work we carried out an experimental investigation based on wound healing assays coupled with automated time-lapse video microscopy, equipped with a cell incubation system. Wound closure kinetics were obtained by measuring cell-free area reduction in time through automated image analysis algorithms. The experimental data were interpreted according to mathematical models, based on transport phenomena concepts, used to quantify both cell motility and proliferation mechanisms. For fibroblast-like cells, cell motility can be described in terms of a motility coefficient, analogous to the Fickian diffusion coefficient, while cell proliferation can be described by models of logistic growth.
This methodological approach couples a well-established, popular and low cost assay, to an innovative and advanced data analysis methodology, and can be used to measure quantitatively the contribution of cell proliferation and migration to the wound closure. The applications range from the measurement of tumor invasion, to the high throughput screening of drugs and chemicals, to identify molecules and signaling pathways which play an important role in cell migration.

P5

In vivo effects of natural compounds present in *Chelidonium majus* on stem cells using a simple animal model

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Planarians are a well-known model for regeneration and provide an excellent system to study the behavior of stem cells *in vivo*. Despite the potential attractiveness of planarians these organisms have not been used yet in large-scale chemical screenings to see the effects of compounds on different key aspects of regeneration as, for instance, stem cell proliferation and differentiation. Our work focuses on the analysis of the effects produced by the main alkaloids (chelidonine, berberine, sanguinarine, chelerytrine, protopine and coptisine) present in *Chelidonium majus* (Papaveraceae), an herb showing interesting therapeutical properties. We demonstrate that chelidonine produces a significant anti-proliferative effect on planarian stem cells, supporting the possibility that this alkaloid acts on cell cycle progression by inhibition of tubulin polymerization. Berberine treatment perturbs the regenerative pattern. Although berberine does not influence cell proliferation/apoptosis, this compound causes abnormal regeneration of the planarian visual system. Our findings, sustained by RNAi-based investigations, support the possibility that berberine effects are critically linked to anomalous extracellular matrix remodeling. Abnormal head regeneration has also been observed following sanguinarine treatment. Preliminary results provide evidence that sanguinarine induces apoptosis through a caspase-dependent mechanism, but does not influence cell proliferation. The study presented here might become a good test to determine the potentiality of planarians as a model to analyze drug effects. At the same time, such screenings and experiments could help to better understand the process of planarian regeneration itself by providing novel information about how proliferation, differentiation and/or morphogenesis and patterning are regulated during this amazing process.

P6

p53 suppression partially rescues the cardiovascular defects of Tbx1 mutants

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The 22q11.2 deletion syndrome is the most common deletion syndrome in humans. Mutation of TBX1, located within the deletion, is sufficient to recapitulate the phenotype in patients. Tbx1 has a critical role in cardiovascular and pharyngeal development. During mouse heart development, Tbx1 modulates positively cardiac progenitors proliferation and negatively their differentiation.
We aim at genetic and pharmacological strategies to rescue the mutant phenotype and we considered cell proliferation as a potential target. We tested whether reduced dosage of p53, a positive regulator of p21 that result be repressed by Tbx1, may modify the Tbx1 mutant phenotype in vivo. Results showed a near complete rescue of the typical haploinsufficient phenotype, using p53 genetic ablation, and a less dramatic rescue using a drug. p53 mutation did not rescue the more complex Tbx1-/- phenotype, instead in a Tbx1Neo2 hypomorphic mutant, p53 deletion improved the heart phenotype.
qRT-PCR results on E8.5 embryos showed that p53 deletion rescues the expression of Gbx2, a gene down-regulated in Tbx1+/- embryos and important for pharyngeal arch artery remodeling; and a ChIP assay demonstrated that both Tbx1 and p53 might regulate this gene directly.
In addition, p53 deletion had a significant impact on the proliferation of cardiac progenitors, improving the Tbx1 mutation-induced cell proliferation defects in the second heart field.

P7

Spatio-temporal distribution of RSPO1/DKK1 machinery in testis embryonic development and its role in testicular angiogenesis

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Testicular vasculogenesis is one of the key processes regulating male gonad morphogenesis. The knowledge of the molecular cues underlining this phenomenon could represent a central element in the understanding of the onset of testicular morphogenetic disorders. RSpondin-1 (RSPO1) has been clearly established as a candidate for ovary determination both in mice and humans. On the contrary, very few data are available on the expression and role of RSPO1 during testicular development. This study aims to clarify the distribution pattern of RSPO1 as well as of other partners of its machinery during the entire period of testicular morphogenesis and to indicate the role of this complex system in testicular development. To this end we first studied the distribution pattern of RSPO1, its antagonist Dickkopf-1 (DKK1), and the signaling effector β-catenin during testis development, from 11.5 to 18.5 dies post coitum (dpc). Our results clearly demonstrate that RSPO1 and DKK1 distribution pattern partially overlaps but, intriguingly, RSPO1 is always detectable in the testicular coelomic portion, the region that organizes testicular vasculature, whilst DKK1 is never detectable in this area. Moreover, performing organ culture experiments of embryonic testes, we demonstrated that DKK1 acted as an inhibitor of testis recruitment of endothelial mesonephric cells. Interestingly, this inhibition was rescued by the co-administration of RSPO1. In addition, RSPO1 alone has been able to enhance, in culture, testicular vasculogenesis. These results let us to conclude that RSPO1 machinery is involved in the control of the correct testicular vascular compartment organization.

P8

L-Proline remodels non-coding RNA gene expression profiles in Embryonic Stem Cells

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Naturally occurring amino acids are emerging as key players in the regulation of the phenotypic plasticity of stem cells. We have recently shown that a rapid increase in the extracellular availability of the non-essential amino acid L-Proline (L-Pro) forces Embryonic Stem Cells (ESCs) towards a phenotypic transition named embryonic stem cell-to-mesenchymal-like transition (esMT) that converts compact-adherent ESCs into mesenchymal-like spindle-shaped, highly motile, invasive and metastatic stem cells named L-Pro-induced cells (PiCs). Despite the relevance of such phenomenon, the molecular mechanisms underlying L-Pro control of ESC behavior/identity remain largely unknown. To get clues on the molecular mechanisms driving L-Pro-induced esMT, we searched for L-Pro-responsive mRNAs, miRNAs and lncRNAs by performing genome-wide expression analysis. In particular, we identified a number of miRNAs and Ultra Conserved Regions (UCRs) that are specifically and differentially expressed in PiCs with respect to ESCs. Our results reveal a unique time-dependent signature of non-coding RNAs during L-Pro-induced transition of ESCs.

P9

Circulating factors released during cardiac rehabilitation in patients with heart failure stimulate beneficial adaptations in human endothelial cells

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Exercise-based Cardiac Rehabilitation (CR) is effectively used as an adjuvant therapy in Heart Failure (HF). However, CR remains considerably underutilized especially in elderly and there are few data concerning its underpinning molecular mechanisms.
Aim of this study was to evaluate the effects of CR in old patients with stable post-ischemic HF from both functional and molecular point of view.
Blood samples from 50 patients undergone a 4-weeks CR program were collected before and at the end of the CR. Lipid peroxidation, 8-hydroxy-2-deoxyguanosine, Superoxide Dismutase (SOD) and catalase (Cat) activities, NAD+/NADH and NADP+/NADPH ratios and Nitric Oxide (NO) bioavailability were measured in patients' sera. Sirtuin 1 (Sirt 1) activity was quantified in patients' lymphocytes. Serum samples isolated from patients before and after CR were used to condition Human Umbilical Vein Endothelial Cells (ECs) either exposed or not to oxidative stress with H_2O_2. Activities of antioxidants, SOD and Cat; Endothelial Nitric Oxide Synthase levels and NO bioavailability were measured. Cellular senescence (by β-gal assay) was also evaluated. Inhibitors of Sirt1 and Cat activities were utilized to investigate the potential role of these enzymes in modulating endothelial cell senescence. An animal model of post-ischemic HF was used to confirm the effect of the exercise on cellular senescence.
CR improves systemic antioxidants capability in failing patients and, noteworthy, changes in serum Cat activity correlate with changes in cardiopulmonary stress test duration in HF patients. A role for Sirt 1 and Cat in the senescence regulation and as markers of CR effectiveness is suggested. CR triggers cellular adaptations that lead to enhance the oxidative stress response and prevent the senescence of human endothelial cells.

P10

Dissecting Foxe1 function in basal cell carcinoma

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Basal cell carcinoma (BCC) is the most common form of human skin cancer and arise from constitutive activation of Sonic hedgehog (Shh)/GLI signalling pathway. The Forkhead box E1 transcription factor (Foxe1) is a downstream target of Shh/GLI pathway and is expressed both in human and mouse BCCs. Comparative transcriptomic analyses in skin BCC versus normal epidermal cells indicate that Foxe1 is one of the most significantly induced genes in BCCs. The aim of this work is to dissect the role of Foxe1 in BCC through using mouse models and cell culture systems. Overexpression and depletion studies in cell culture reveal that Foxe1inhibit cell proliferation and positively regulate a subset of genes involved in extracellular matrix remodeling and cell adhesion. To study the function of Foxe1 in vivo in the context of BCC, we crossed K5-Gli2 with a conditional Foxe1 deficient mouse (K5-Gli2;K14-Cre;Foxe1$^{fl/fl}$). Mice carrying Foxe1 deletion in skin cells develop more proliferative and locally invasive tumorigenic lesions. These data suggest a role for Foxe1 in inhibiting BCC proliferation and/or invasion explaining the relative benign behaviour of these tumors.

P11

Novel pancreas organogenesis markers refine the pancreatic differentiation roadmap of Embryonic Stem cells

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The adult stem cell population within the pancreas is poorly defined despite extensive investigation, embryonic stem cells (ESCs) offer a significant theoretical advantage to generate large amounts of a desired cell type. However, ESCs direct differentiation protocols, reveal heterogeneous metastable cell states hamper to accurately recapitulate the molecular signaling underlining cellular specification. The in vivo organogenesis signaling governing pancreas organogenesis improved direct ESCs differentiation toward pancreatic cells. Much is missing from current in vitro direct differentiation from ESCs to generate functional cells with respect to normal ontogeny. In particular, there is still a gap of information about intrinsic factors that govern pancreatic progenitor cells (PPCs) specification and differentiation. The PPCs specification is temporally and spatially enriched during pancreas budding processes, and it is signed by a multipotent phase occurring mainly at E10.5, and a high proliferative state by the stage E13.5. These considerations prompted us to search for novel PPCs intrinsic factors among the genes that are specifically expressed in pancreatic bud at the developmental stage E10.5. In our work, we identified a cohort of genes (Bex1, Nepn, Pcbd1, Prdxdd1, Rnf160, Slc2a1, and Tff3) which expressions marked, temporally and spatially, in vivo pancreatic development. Noticeably, the expressions of those genes, marked a novel naïve pancreatic steady state cell population induced during the in vitro directed pancreas differentiation of ESCs.
The expression of our newly identified molecular signature represent a proper way of validating the pancreatic development program, as well as provide clues to direct ESCs pancreatic proper differentiation that refines the map of the ontogenetic transitional states. Our data could have considerable applications in regenerative medicine through the improvement of in vitro cell expansion and cell differentiation protocols from either ESC, and induced Pluripotent Stem cells.

P12

Reducing Glypican-4 in ES cells improves recovery in a rat model of Parkinson's disease by increasing the production of dopaminergic neurons and decreasing teratoma formation

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The heparan sulphate proteoglycan Glypican 4 (Gpc4) is strongly expressed in mouse ES cells where it controls the maintenance of self-renewal by modulating Wnt/β-catenin signaling activities. Here we show that mouse ES cells carrying a hypomorphic Gpc4 allele, in a single-step neuronal differentiation protocol, show increased differentiation into dopaminergic neurons expressing tyrosine hydroxylase and Nurr1. In contrast to wild type cells, these differentiating Gpc4-mutant cells expressed high levels of DOPA decarboxylase (DDC) and the dopamine transporter (DAT), two markers expressed by fully mature dopaminergic neurons. Intrastriatal transplantation of Gpc4 hypomorphic cells into a 6-OHDA rat model for Parkinson's disease improved motor behavior in the cylinder test and amphetamine induced rotations at a higher level than transplanted wild type cells. Importantly, Gpc4 hypomorphic cell grafts, in contrast to wild type cells, did not generate teratomas in the host brains, leading to strongly enhanced animal survival. Therefore, control of Gpc4 activity level represents a new potential strategy to reduce ES cell tumorigenic features while at the same
time increasing neuronal differentiation and integration.

P13

Cripto is required for embryonic stem cell to epiblast stem cell transition and regulates DLK1-Dio3 cluster expression

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Increasing evidence indicate that mouse embryonic stem cells (mESCs) fluctuate between a *naive* inner cell mass-like state and a *primed* epiblast-like state when cultured in serum. This metastable condition ensures both self-renewal and predisposes ESCs for differentiation to Epiblast stem cells (EpiSCs). The transition from naive to primed cells represents a pivotal event in cellular differentiation; the extracellular signals that control this fundamental process remain still unclear. Our findings indicate that the extracellular protein Cripto plays a crucial role in this complex scenario. We demonstrate that Cripto is metastable in mESCs and that separation of ESCs on the basis of Cripto expression levels yields to cell populations characterized by different stemness-associated features. Unexpectedly, genome-wide transcriptional profiling shows a significant downregulation of the DLK1-Dio3 cluster in Cripto KO ESCs. Interestingly, recent evidences indicate that expression of DLK1-Dio3, which is the largest microRNA cluster in the mammalian genome, correlates with pluripotency in ESCs and induced Pluripotent Stem Cells (iPS). According to the trancriptome analysis, *microRNAome* profiling shows that >50% of the microRNAs deregulated in Cripto KO ESCs belong to the DLK1-Dio3 cluster. In line with the idea that high level of Cripto correlates with "full pluripotency", a combination of loss-and-gain of function approaches show that Cripto regulates *ESC*-to-EpiSC *transition, and* is required both to induce and to maintain the EpiSC-like state. Together our preliminary data suggest a novel role for Cripto in EpiSC self-renewal, likely through modulation of the Dlk1-Dio3 cluster.

P14

Role of the histonemethylase SMYD3 in embryonic stem cells differentiation

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SMYD3 is a histone methylase that is highly expressed in the embryo, in adult skeletal muscle and in few other tissues. It is frequently upregulated in cancers and its transcriptional activity is involved in the proliferation of tumor cells. The role played by SMYD3 in embryonic development has not been studied yet.
It has been recently reported that sh SMYD3 may be involved in cardiac development and myogenesis in zebrafish and therefore we decided to investigate SMYD3 role in an in vitro model of cardiac differentiation. We employed mouse embryonic stem (ES) cells induced to differentiate toward the cardiac lineage.
To determine the role of SMYD3 in cardiac lineage differentiation, we analyzed SMYD3 expression pattern in differentiating mouse embryonic stem cells and the effect of SMYD3 depletion by ShRNA intereference on the expression of markers of cardiovascular lineages.
We observed that the expression levels of cardiac markers (TnnT2, β-MHC) were increased in embryoid bodies depleted of SMYD3 and allowed to differentiate to cardiac lineage for 14 days. The number of spontaneously beating embryoid bodies was also increased in cells with reduced levels of SMYD3. Markers of vascular and endothelial lineages, which derive from cardiovascular progenitor cells, were expressed more robustly in embryoid bodies depleted by SMYD3.
Our studies indicate that SMYD3 may play a role during EMT in embryonic stem cells differentiation and it may modulate genes involved in the early stages of embryonic stem cells differentiation.

P15

Role of exosomes in pericardial fluid-mediated cardiac regeneration

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Background: We recently demonstrated that epicardial stem cells participate to the regenerative response following myocardial infarction (MI) and factors released in the pericardial fluid (PF) may play a key role in this process. Exosomes are secreted microvescicles of endocytic origin, identified in most body fluids, which may contain proteins able to modulate cell functions. Here, we investigated whether exosomes are present in the PF and their role in epicardial stem cell-mediated cardiac repair.
Methods and Results: Exosomes were identified in PFs from non-infarcted patients (PFC) and patients with acute myocardial infarction (PFMI). A shotgun proteomics analysis of exosomes from PFC and PFMI was used to identify differentially expressed proteins in these microvescicles. We identified Clusterin in exosomes isolated from PFMI but not from PFC. Notably, Clusterin is an important –induced epithelial-to-mesenchymal transition (EMT), aβmediator of TGF key process which regulates epicardial cell activation and differentiation. Both q-RTPCR and Immunohistochemical studies showed cardiomyocytes expressing Clusterin in the infarcted region 3 days following MI. The importance of EMT in PF-induced epicardial cells was confirmed by gene expressions studies on epicardial cells from infarcted mouse hearts in the presence and in the absence of PF. Three days following MI, the expression of genes related to EMT, i.e. Snai1 and Twist1 were increased in the presence of PF. Noteworthing, EMT genes were specifically upregulated in epicardial cells expressing the stem cell antigen c-kit as demonstrated by in vitro studies. In vivo studies are ongoing to demonstrate the involvement of Clusterin in the PFMI induction of EMT and myocardial repair.
Conclusions: Exosomes are present in the PFs. Clusterin was identified only in PFMI and may account, at least in part, for epicardial EMT and myocardial regeneration following MI.

P16

Involvement of soluble forms of the urokinase receptor in hematopoietic stem cell homing

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The urokinase (uPA) receptor (uPAR) plays a key role in cell migration. uPAR is expressed on the surface of various cell types, both in full-length and cleaved forms, lacking the N-terminal DI domain (DIIDIII-uPAR). Full-length uPAR binds uPA and vitronectin (VN) and regulates integrin activity. The receptor can be shed from the cell surface, generating full-length (suPAR) and cleaved (DIIDIII-suPAR) soluble forms, both detected in human plasma and urine. suPAR can bind uPA and VN, unlike DIIDIII-suPAR. Indeed, DIIDIII-suPAR exposing the chemotactic SRSRY sequence at its N-terminus, is a ligand for fMLF chemotaxis receptors.
We previously demonstrated the involvement of uPAR soluble forms in the G-CSF-induced mobilization of human $CD34^+$ hematopoietic stem cells (HSCs). Further, we demonstrated that DIIDIII-suPAR can induce mobilization of hematopoietic stem/progenitor cells in mice. Since HSC mobilization and homing are specular processes which utilize same mediators and similar signaling pathways, we investigated whether uPAR soluble forms could be also involved in HSC homing.
We found that human bone marrow (BM) stromal cells produce suPAR and DIIDIII-suPAR. Both forms of suPAR increased the number of adherent clonogenic progenitors in long term cultures (LTC) of G-CSF mobilized HSCs. Further, we found a significant increase in DIIDIII-suPAR levels in sera from leukaemia patients, as compared to healthy donors, and its significant decrease following the pre-transplantation chemotherapy-based conditioning.
Overall, these results suggest that BM stroma produces soluble forms of uPAR. Full-length suPAR could contribute to an efficient $CD34^+$HSC engraftment to BM; viceversa, DIIDIII-suPAR, given its mobilizing activity, may be required to be down-regulated to allow an efficient homing.

P17

A comprehensive transcriptome analysis of GABA-differentiating neural stem cells aimed at dissecting molecular mechanisms underlying cell cycle exit, neuronal commitment and differentiation

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Understanding stem cell maintenance and differentiation is a fundamental step for basic research that moreover paves the way to challenging medical objectives such as regenerative medicine. The central nervous system is a particularly relevant tissue both for its limited regenerative potential and for the severe diseases associated to its altered development and maintenance.
Here we aim to understand cell cycle exit and differentiation in deep molecular terms, as a way to reveal novel pathways and regulations. Specifically, we focused on differentiation of GABAergic neurons, adopting the model of the adherent Neural Stem (NS) cells. We generated RNA-seq and miRNA profile data at four time points, from proliferating NS to the mature neuron stage.
We examined differential expression when these cells exit the cycle, after removal of FGF. While their expression of differentiation markers and of synaptic genes progressively increases over time, cell-cycle regulators are rapidly shut off. We identified three down-(15b, 25 and 27a) and five up-(149, 652, 30c, 125b-5p and 342-3p) regulated miRs; integrating these data with DE analyses we identified some cognate target:miRNA pairs engaging in negative regulations.
Next we examined alternative polyadenylation usage, a phenomenon recently implicated in proliferation and differentiation. Several (45) mRNA are shorter in differentiating NS cells and only few (5) are longer, following a known trend in other contexts. We have identified miR seed enriched in the lost 3'UTR portions, suggesting shortening as a possible way to escape specific miRNA based regulations.
Finally, comparing WT with *Dlx5;Dlx6* KO NS cells, where GABAergic differentiation is retarded, we will be able to focus only on the functionally relevant regulations.
Overall, we hope to de-convolute RNA-based networks essential for GABA differentiation, leading to a systems biology comprehension of the molecular regulations underlying interneuron genesis and maturation.

P18

Structure, expression and developmental functions of the *astacin* metalloproteinase gene family in planarians

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Planarians (Platyhelminthes) are a model system well known for regenerative potential and continuous cell turnover. These characteristics depend on neoblasts, a population of pluripotent stem cells present throughout the *mesenchyme* of adult worms. A rich *extracellular matrix* (ECM) surrounds neoblasts and instructs their behaviour, acting as a source of regulatory signals. Many members of the zinc-endopeptidase astacin family play important functions during development and regeneration, by processing precursors to mature functional ECM proteins and growth factors. In planarian, however, the roles of the astacin-like genes remain elusive. We identified eight *astacin*-related genes by in silico analysis of the *Schmidtea mediterranea* genome. Most of them encode astacins with the minimal structure that characterizes the secreted forms, and are highly conserved in the planarian *Dugesia japonica.* Astacin transcripts are expressed in secretory cells distributed in the mesenchymal tissue around the pharynx and, as confirmed by qRT-PCR, are early upregulated during regeneration. Functional ablation of these genes by RNAi shows different phenotypes in intact animals, including body lesions, as well as abnormal tissue growth in front of the pharynx. During regeneration most of the RNAi phenotypes show delay in the blastema growth, altered morphogenesis or do not regenerate at all. Additionally, behavioural defects concerning movements have been observed. Preliminary molecular modeling analysis is in progress to identify possible conformation changes and/or drug binding sites for specific molecular targets.

P19

Identification of high-fidelity Myc and Max genomic targets in mouse embryonic stem cells

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Myc is a master transcription factor that has been demonstrated to be required for embryonic stem cell (ESC) pluripotency, self-renewal, and inhibition of differentiation. Although recent works have identified several Myc-targets in ESCs, the list of Myc binding sites is largely incomplete due to the low sensitivity and specificity of the antibodies available. To systematically identify Myc binding sites in mouse ESCs, we used a stringent streptavidin-based genome-wide chromatin immunoprecipitation (ChIP-Seq) approach with biotin-tagged Myc (Bio-Myc) as well as a ChIP-Seq of the Myc binding partner Max. This analysis identified 4325 Myc binding sites, of which 2885 were newly identified. The identified sites overlap with more than 85% of the Max binding sites and are enriched for H3K4me3-positive promoters and active enhancers. Remarkably, this analysis unveils that Myc/Max regulates chromatin modifiers and transcriptional regulators involved in stem cell self-renewal linking the Myc-centered network with the Polycomb and the Core networks. These results provide insights into the contribution of Myc and Max in maintaining stem cell self-renewal and keeping these cells in an undifferentiated state.

P20

Inducible pluripotent stem (iPS) cell-derived human astrocytes as a new disease model to shed light into the molecular pathogenesis of megalencephalic leukoencephalopathy with subcortical cysts (MLC)

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a rare inherited, autosomal recessive form of childhood onset spongiform leukodystrophy characterized by macrocephaly, subcortical cysts, brain edema and myelin vacuolation. To date MLC is an incurable disease and the management of MLC patients is limited to treatment of disease symptoms. Available evidence indicates that the majority of MLC patients carry mutations in a gene, *MLC1*, that encodes a protein (MLC1) of unknown function, highly expressed in brain astrocytes. In our previous works aimed at identifying MLC1 function(s), we used human astrocytoma cells overexpressing wild type or mutated MLC1 and demonstrated that MLC1 is involved in the regulation of intracellular calcium influx and volume control in response to osmotic stress. However, the tumoral origin of these cells does not allow to readily translate the information obtained into the clinics for therapeutic purposes. Since astrocytic dysfunction is the primary cause of MLC and human astrocytes cannot be easily obtained from patients, astrocytes differentiated from induced pluripotent stem cells (iPSCs) derived from patient skin fibroblasts will represent the most appropriate cellular model to investigate astrocyte dysfunctions mediated by MLC1 pathological mutations. Therefore, iPSC clones have been generated by reprogramming of human fibroblasts from control subjects (one sample) and MLC patients (two samples). After selecting the most appropriate iPSC line, a differentiation protocol to obtain astrocyte-enriched cultures will be applied and the iPSC-derived astrocytes will be used to study further the molecular mechanisms underlying MLC disease and identify targets for potential drug candidates.

P21

Tbx1 impacts the cardiac commitment of Vegfr2/Flk1+ cells

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The understanding of the developmental logic of heart disease and the identification of new approaches to arrest or reverse its progression represents the major goal of regenerative medicine. Research on cardiac precursor cells from adult heart or in vitro differentiation of embryonic stem cells has improved our understanding of mechanisms of cardiac differentiation.
During embryonic development, *Tbx1* is expressed in cardiac precursor cells regulating their proliferation and differentiation. Here we show the generation of an ESC line carrying a tetracyclin-regulatable *Tbx1* cDNA. Using this cellular system, we demonstrated that Tbx1 overexpression reduces the number of Vegfr2/Flk1+ cells while it increases the number Nkx2.5+ cells in a differentiating ES cell population. Moreover the induction of Tbx1 in the isolated Flk1+ cells reduces the expression of Flk1 while induces the expression of the Baf60c cardiac differentiation marker. Furthermore, clonal analysis of individual Flk1+ cells, fated to become endothelial cells, has shown that Tbx1 overexpression induces the expression of *Nkx2.5* and *SM-actin* genes, which are cardiac and smooth muscle markers, respectively.
We performed developmental expression analysis of the Flk1 gene and we found a previously undescribed domain in a region bordering the second heart field, near the inflow region. Surprisingly, in Tbx1 null mice this domain of expression is extended into the posterior second heart field, a region populated by cardiac precursor cells. Together, these data indicate that Tbx1 suppresses (directly or indirectly) the expression of *Flk1* and suggest that Tbx1 may modulate the fate of Flk1+ precursors by activating cardiac precursor markers.

P22

3T3L1 cell differentiation was affected by prostate cancer cell conditioned medium

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Prostate cancer (PCa) is the most common diagnosed cancer in men. There is extensive evidence suggesting the role of environmental factors, such as obesity, in PCa progression. Ongoing research is now dissecting the molecular links underlying the association between obesity and PCa aggressiveness. Recently, the importance of cancer-associated adipocytes, adjacent to/or infiltrating into tumors, has been stressed. The interaction between cancer cells and adjacent adipocytes is bidirectional and likely is modulated by the local environment, which includes fibroblasts, macrophages, lymphocytes and endothelial cells. Aim of this study is to evaluate whether PCa cell may influence 3T3L1 cell (mouse embryonic fibroblast) differentiation. 3T3-L1 cell have been differentiated, according to American type culture collection (ATCC) protocol, with or without conditioned medium (CM) of PC3, androgen-independent PCa cell line. On day 8, we extracted total RNAs and we evaluated, by RT- PCR, whether the presence of PC3 cell CM affect the expression of peroxisome proliferator-activated receptor γ (PPAR γ), the master regulator gene of adipogenesis, and of adipocyte fatty acid-binding protein 2 (aP2). The lipid contents were quantified by Oil Red O staining. Interestingly, PC3 CM significantly reduced 3T3L1 lipid accumulation up to 40% and PPAR γ and aP2 mRNA levels by 1.4 and 1.7 fold over control, respectively. Our results showed that PC3 CM is able to reduce the differentiation of 3T3-L1 adipocytes, suggesting that PCa cell promote a more "fibroblastic" phenotype in adipose tissue, enriching it of cells with supportive role on the tumor cell growth.

P23

A planarian *kuzbanian-like* gene is a key regulator during regeneration of the nervous system

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Kuzbanian is a crucial member of the ADAM family of metalloproteinases. Kuzbanian-mediated cleavage of Notch represents in fact one of the fundamental steps leading to activation of the Notch pathway during early neurogenesis. This enzyme also plays important roles in later stages of neural development, modulating axon guidance and extension. Planarians are a model system well known for regenerative potential. These characteristics depend on a population of adult stem cells that guarantees replacement of all differentiated cell types, including nerve cells. Here we report the functional characterization of a planarian *Kuzbanian-like* gene (*Smed-kuzbanian3*). *Smed-kuzbanian3* is expressed in the mesenchyme and in the central nervous system (CNS) and is overexpressed during regeneration of this structure. Functional ablation of *Smed-kuzbanian3* by RNAi causes abnormal CNS regeneration. Anomalous eye morphogenesis, including atypical axonal projections of the optic chiasm, is observed. The architecture of the regenerating cephalic ganglia also appears unusual, with an aberrant number and distribution of nerve cells. We speculate that Smed-Kuzbanian3, similarly to that observed in other organisms, plays different roles during CNS regeneration. To test whether *Smed-kuzbanian3* plays a conserved role during neurogenesis we plan to use *Xenopus laevis* embryos as a model system. Gain of function experiments will allow us to demonstrate the involvement of *Smed-kuzbanian3* in modulating early Notch-mediated lateral inhibition mechanisms and the guidance of axonal extensions at later developmental stages.

P24

HOXB7 and lung cancer: biological relevance of a homeobox gene in cancer progression

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HOXB7 is a homeobox transcription factors known to play essential roles in embryo development and maintenance of tissue homeostasis and found overexpressed in a variety of cancers. Recently, we identified HOXB7 in a prognostic gene signature for early stages lung adenocarcinoma.
To identify a role for HOXB7 in lung cancer progression, we analyzed the biological effect of its expression modulation in lung cancer cells. When we forced HOXB7 expression in lung cancer cell lines we observed an increase in cell migration, proliferation and the induction of epithelial to mesenchymal transition (EMT), as reported in literature. On the contrary, HOXB7 knockdown in a high expressing lung adenocarcinoma cell line (A549) displayed anti-proliferative and pro-apoptotic effects. To get more insights about the role of HOXB7 in the regulation of cancer relevant pathways, we performed gene-set enrichment analyses (GSEA) on gene expression datasets of about 1000 lung adenocarcinoma (profiled by RNA-seq and affymetrix). More than 3000 sets of genes, representing virtually all known cancer molecular mechanisms, were analyzed. Among the top scoring gene-sets positively correlating with high level of HOXB7, we identified several gene-sets involved in tumor progression, metastasis, EMT, and strikingly in stem cell homeostasis and IPS. This points toward a possible role for HOXB7 in cancer stem cell biology, further contributing to the enhanced aggressiveness of HOXB7 positive lung tumors. A more detailed analysis of stem cell and EMT biomarkers revealed the overexpression of a gene known to be involved in cancer progression, EMT, reprograming and stemness. By luciferase and ChIP assays, we finally demonstrated this gene to be directly targeted by HOXB7. Moreover, A549 lung cancer cell viability resulted to be dependent on the expression of both HOXB7 and this newly discovered target. We are now exploring the relevance of this two-gene interaction and its role in lung cancer progression.

P25

Metformin reduces self-renewal and tumorigenicity of breast cancer stem cells by inhibiting the Notch signaling pathway

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Several reports showed that the anti-diabetic drug metformin inhibits cancer cells growth in a wide range of solid tumors through PI3K/AKT/mTOR pathway deregulation. Recent studies also reported that metformin selectively kills cancer stem cells (CSCs) in pancreatic, hepatoma and breast cancer cell lines. Although the inhibition of the inflammatory response has been proposed as a mechanism for CSCs growth arrest, the molecular events involved in such mechanism remain still unclear. The Notch signaling pathway has been shown to be upregulated in several solid tumors, including breast cancer. Despite its simple molecular design, as it contains a very small signalling core, the Notch pathway plays a critical role in maintaining the balance between cell proliferation, differentiation, stemness and apoptosis. In this study, we examined the effects of metformin on Notch signaling pathway in breast adenocarcinoma cell line (MCF-7) and in breast CSCs through generation of mammospheres from MCF-7 cells. After 24 hours exposure, metformin significantly decreased MCF-7 cell growth and wound-healing capacity. We found that metformin induced Notch pathway down-regulation and that this effect is proteasome-independent. Metformin also decreased sphere-forming capacity of MCF-7 cells, induced mammospheres growth arrest and altered mammosphere shape, impairing self-renewal and proliferation of CSCs. Moreover, metformin treatment induced down-regulation of Notch target gene expression in mammospheres, suggesting a correlation between Notch signaling pathway and the anti-proliferative effect of metformin. These data demonstrate that the down-regulation of the Notch signaling pathway may represents a new molecular mechanism by which the anti-diabetic drug metformin exerts anti-tumoral effects and open new insights for targeted therapy of breast cancer.

P26

Identification and functional characterization of novel lincRNAs in mES differentiation

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Most of the mammalian genome encodes for many thousands of large non-coding transcripts including a class of large intergenic ncRNAs (lincRNAs). LincRNAs are emerging as key regulators of the chromatine state and other cellular processes. They form structures able to interact with protein or DNA and mediate the recruitment and the assembly of protein-protein or protein-DNA complex or may act like modular scaffolds for multi-protein histone modification complexes. Recent evidence characterizes lincRNAs in different models of terminal differentiation stages. Here we identify novel Differentiation Associated LincRNAs (DAL), involved in early stage of differentiation in embryonic bodies (EBs), a three-dimensional structure differentiation model. We have identified 150,000 transcripts using the TopHat spliced-mapper. After that initial filtering we've obtained 50 novel lincRNAs that do not overlap with any know annotated transcript and have an optimal intron/exon profile. After than we subdivided our novel DAL on the bases of their expression level in the different lineages: neuro-ectoderm and meso-endoderm. In order to obtain information about the functional role of these lincRNAs we perform loss of function experiments by RNA interference approaches. We identify a neuroectoderm-associated DAL389 in mouse able to induce a downregulation of neuroectodermal marker genes olig2 and nestin. DAL389 is localized on chromosome 14 between two specific genes involved in neuroectodermal differentiation stages. Moreover DAL389 is conserved and it is always localized between two genes as previously identified.Together our data provided evidence for a long non-coding RNA with a role in neuroectoderm differentiation, and provide new information on the regulation of the early stages of ES differentiation.

P27

Abnormalities in keratinocyte proliferation and apoptosis in mice with a conditional deletion of rictor in the epidermis

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mTOR (mechanistic target of rapamycin) is a serine-threonine protein kinase that regulates diverse cellular processes such as growth, metabolism, proliferation, survival and differentiation and mTOR signaling abnormalities are a hallmark of many types of cancer. mTOR nucleates two distinct protein complexes characterized by their differential sensitivity to rapamycin, namely mTORC1 and mTORC2. Rictor is an adaptor protein that is essential for mTORC2 activity. mTORC2 phosphorylates both Akt and PKC isoforms at regulatory residues, thereby modulating their signaling activity.
Since rictor-knockout mice die before the development of stratified epithelia, to explore the biological functions of rictor/mTORC2 in the epidermis we generated mice with a conditional deletion of rictor in this tissue. Rictor-deficient keratinocytes displayed abrogation of Akt phosphorylation at Ser473 and subtle alterations in Akt isoforms expression and signaling activity. Although these animals did not display an obvious epidermal phenotype, cell proliferation was impaired in rictor-deficient keratinocytes, both *in vivo* and *in vitro*. Epidermal hyperplasia induced by the tumor promoter TPA was reduced in rictor-deficient epidermis, suggesting that rictor/mTORC2 may favor epidermal tumor progression. However, we surprisingly found that rictor-deficient keratinocytes are protected from cell senescence and have an intrinsic tendency to immortalize spontaneously. In addition, rictor-deficient keratinocytes display increased resistance to apoptosis induced by several stimuli.
Thus, our findings suggest that rictor/mTORC2 inactivation on one hand impairs epidermal cell proliferation, and at the same time inhibits fail-safe mechanisms of tumor suppression such as keratinocyte death and senescence. These results indicate that caution should be taken in the design of anti-cancer therapeutic strategies based on mTORC2 inhibition.

P28

Conditional/inducible deletion of Dlx5 to analyze the role of Parvalbumin+ interneurons in the adult mouse brain

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Dlx5 is a pan-interneuron marker, expressed in most developing and adult interneurons (INs). Although INs represent only 20% of cortical neurons, they are essential components of the balance between excitation and inhibition and are increasingly implicated in cognitive deficits and age-related mental decline.
The molecular regulations underlying the organization and electrical functions of INs in the adult cortex are still poorly known. Using a *Cre-lox* strategy, we are able to conditionally delete *Dlx5* only in Dlx5+ mature INs of the adult brain. The model consists in *Dlx5*-flox mice crossed with *Dlx5-Cre-ERT2* knock-in. Tamoxifen (Tam) treatment efficiently depletes *Dlx5* in INs, both in embryos and adults. Tam-treated animals showed a specific reduction in parvalbumin (PV) expressing INs, but not other INs, in cortex and hippocampus, confirmed by a reduction of PV mRNA; this phenotype is not due to increased apoptosis.
PV+ INs are fast-spiking neurons that control the flow of excitation within the cortex. We recorded the EEG spectra of Tam-treated mice, under sedation, and detected a reduced power of high frequency oscillations with a shift of the activity peaks towards lower frequencies, and the appearance of polyspikes elements, a feature of sub-clinical seizure-like events. Thus, in the absence of *Dlx5*, the mouse cortex is more excitable and shows reduced high frequency oscillations.
PV+ INs are involved in higher cognitive functions. We plan to address their role in memory consolidation and emotional association by deleting *Dlx5* in selected adult brain regions, i.e. cortex, hippocampus and amygdala. To do this, we stereotaxically inject Tam in these areas and induce local PV impairment. Preliminary data show efficient *Cre*-mediated recombination, proving the feasibility of this approach. We will adopt a Pavlovian fear-conditioning protocol of associative learning and memory, and dissect the contribution of PV+ INs in each brain region.