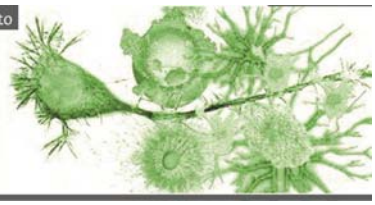
Associazione di Biologia Cellulare e del Differenziamento

ABCD



SAPIENZA
UNIVERSITY OF ROME

ABCD MEETING

Stem cells, Development and Regenerative Medicine

Parma, 9-10 aprile 2010

Programme & Abstracts

Venerdì, 9 aprile

Registrazione fino alle 12:30

12:30 **Lunch**

13:45 **Apertura meeting**

14:00-17:30 **Stem cells biology**
Chair: Lorenzo Silengo

14:00 **Keynote lecture**
Paolo Bianco *introduced by Antonio Musarò*
Beyond mesenchymal stem cells

14:30 **Michela Palmisano**
Modulating the teratogenic potential of the mouse Embryonic Stem Cells

14:45 **Ilaria Bronzini**
Cryopreservation of equine peripheral blood-derived mesenchymal stem cells (ePB-MSC) for therapeutic application

15:00 **Letizia De Chiara**
Generation of functional hepatocytes from mouse germline cell-derived pluripotent stem cells *in vitro*

15:15 **Giovanni Luca**
Xenograft of microencapsulated pre-pubertal porcine sertoli cells reverse type 1 diabetes in nod mice by inducing regeneration of new pancreatic islet beta-cells

15:30 **Maria Chiara Quitadamo**
Engraftment capacity of hES- derived alveolar epithelial type II pneumocytes into silica and bleomycin lung-damaged mice

15:45 **Maria A. Mariggio'**
Stem cells from amniotic fluid: a promising source for cellular therapy?

16:00 **Viviana Meraviglia**
Human mesenchymal cells from first trimester chorionic villi: determination of in vitro plasticity and differentiation potential

16:15 **Rosalia Pellitteri**
Olfactory Ensheathing Cells: an unusual glial cell population

16:30 **Matteo Vecellio**
Human cardiac stromal cells reprogramming by defined epigenetic modulators

16:45 **Valentina Di Felice**
Cardiac progenitor cells, scaffolds for in vivo and in vitro myocardial tissue engineering

17:00 **Stefano Cavalli**
Tracking the homing, engraftment and differentiation of stem cells for myocardial regeneration

17:15 **Antonietta Gentile**
Cultured human epicardium-derived cells fuse with high efficiency with skeletal myotubes and differentiate towards the skeletal muscle phenotype *in vitro* and *in vivo*

17:30-17:45 **Coffee break**

17:45-19:45 **Development and differentiation 1**
Chair: Ada Maria Tata

17:45 **Giulia Pacini**
A simple and reliable method to generate and study differentiating serotonergic neurons in vitro from murine ES cells

18:00 **Raoul Manenti**
Role of the alternative transcripts and targets of the Ci-POU IV gene in the development of the Peripheral Nervous System of the tunicate *Ciona intestinalis*

18:15 **Marina Boido**
Graft of embryonic neural precursors and adult mesenchymal stem cells in an experimental model of spinal cord hemisection

18:30 **Cristina Giacinti**
Cdk9-55 a new player in muscle differentiation program

18:45 **Salvatore Campo**
Identification and putative role of Versican isoforms during *Xenopus* development

19:00 **Pompeo Macioce**
The interaction with HMG20a/b proteins suggests a potential role for β-dystrobrevin in neuronal differentiation

19:15 **Pasquale Caramanica**
Regulation of adult NSC properties by RE1-Silencing Transcription factor

19:30 **Alessandro Dell'Anna**
Differentiation and organization of the nervous system of two Hydrozoan crawling planulae

20:30 **Social dinner**

Sabato, 10 aprile

8:30-10:30 **Development and differentiation 2**
Chair: Massimo Santoro

8:30 **Keynote lecture**
Massimo Santoro *introduced by Mario Pestarino*
The zebrafish model system: from development to stem cell biology

9:00 **Antonello Mallamaci**
Inhibition of gliogenesis and promotion of neuronogenesis by patterned overexpression of *Emx2* and *Foxg1*

9:15 **Giorgio Merlo**
Wnt5a regulated GABAergic differentiation of neural progenitor cells, *in vitro* and *in vivo*

9:30 **Ada Maria Tata**
Different muscarinic receptor subtypes modulate oligodendrocyte progenitor survival, proliferation and differentiation

9:45 **Elisa De Luca**
Characterization of zebrafish sra and nemo mutants: a way to discover new key-genes in cardiovascular development

10:00 **Elena Parmigiani**
Expression and trafficking of GPR17 in immortalized oligodendrocyte precursor cells

10:15 **Nicla Romano**
In-vivo and in *ex-vivo* heart regeneration in zebrafish (*Danio rerio*)

10:30 **Coffee break**

10:45-13:45 **Tissue regeneration and regenerative medicine**
Chair: Alessandro Vercelli

10:45 **Roberta Tasso**
The recruitment of host progenitor cells after mesenchymal stem cells (MSC) implantation plays a key role in the development of the tissue-engineered bone

11:00 **Massimo Riccio**
Osteogenic differentiation occurring in 2D and 3D cultures of human dental pulp stem cells

11:15 **Sandra Zecchi-Orlandini**
Dermal matrix scaffold engineered with adult mesenchymal stem cells and platelet rich plasma as a potential tool for tissue repair and regeneration

11:30 **Simone Merlin**
Bone marrow transplantation cures hemophilia A

11:45 **Raffaella Spina**
Zinc finger protein 521: a novel regulator of the normal and malignant immature cell compartment in the haematopoietic and neural system

12:00 **Rosa Curci**
Association of collagen VI alpha1 chain to monocytes-derived macrophages podosomes

12:15 **Guglielmo Sorci**
RAGE is re-expressed in skeletal muscle satellite cells after muscle injury and deletion of RAGE results in delayed muscle regeneration

12:30 **Paola Aulino**
Analysis of regenerative potential and homeostasis in cachectic muscles: the role of stem cells

12:45 **Elisabetta Meacci**
Effects of sphingosine 1-phosphate on skeletal muscle repair/regeneration after eccentric contraction-induced damage

13:00 **Paola Lorenzon**
A novel potential trophic effect of neural agrin on the regenerative potential of aged human skeletal myoblasts

13:15 **Grazia Esposito**
Exogenous high mobility group box 1 protein regulates myocardial remodelling through miR206 modulation and induces myocardial regeneration in chronically failing hearts

13:30 **Andrea Zamperone**
Human cardiac progenitor/stem cells for regeneration of heart muscle tissue in an innovative "scaffold-less" strategy: generation, characterization and in vivo implantation of cell-sheets: preliminary results

13:45 **Concluding remarks**
Mario Pestarino, Antonio Musarò

14:00 **Lunch**

ABSTRACTS

Analysis of regenerative potential and homeostasis in cachectic muscles: the role of stem cells

Paola Aulino, Dario Coletti, Emanuele Berardi, Veronica Cardillo, Mario Molinaro, Sergio Adamo
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Cachexia, a muscle wasting syndrome associated to many chronic diseases, is a negative prognostic factor, interferes with therapy and worsens quality of life. Cachexia is characterized by severe skeletal muscle atrophy, muscle frailty and weakness, accounted for by increased catabolism of sarcomeric proteins. With the aim to investigate the mechanisms underlying cachexia, we investigated a murine model of cancer-cachexia consisting in mice bearing the C26 colon carcinoma. We noted that cachectic muscle fibers display sarcolemmal damage, as shown by EBD incorporation. We also observed that cachectic muscles are enriched in several stem cell populations, including satellite cells, as an attempt to cope with the damage. However, we observed that the skeletal muscles of tumor-bearing mice display loss of regenerative potential. This phenomenon is not due to lack of satellite cell activation and, indeed, satellite cells show a higher rate of proliferation upon cachexia. Following focal injury, inflammation is exacerbated in tumor-bearing mice, suggesting that elevated levels of cytokines hamper the regenerative potential by inhibiting satellite fusion into nascent myofibers. However, in the presence of elevated levels of cytokines, stem cell participation to myofiber formation can be rescued by gene delivery, pharmacological or hormonal approaches. In conclusion, cachexia is characterized by an unbalance between increased muscle damage and decreased muscle regeneration, which ultimately could account for muscle wasting. The inflammatory cytokines characteristic of cachexia promote the proliferation and inhibit the differentiation of stem cells with myogenic potential. Antagonizing cytokine effects on myogenic cells could be a therapeutic approach to ameliorate muscle homeostasis.

Graft of embryonic neural precursors and adult mesenchymal stem cells in an experimental model of spinal cord hemisection

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Spinal cord injury (SCI) can determinate neurological deficits below the injury site, producing a functional damage to local neurons and axons fibres, and many secondary events (glial activation, inflammation, oxidative stress, glial scar formation, cell death).
Serotonergic raphespinal projections promote functional recovery after SCI, but spontaneous regeneration of most severed axons is limited by the glial cyst and scar that form at the lesion site.
In the present study we have examined whether stem cell transplantation could offer a promising approach for inducing regeneration through the damaged area, comparing the effects of transplantation of embryonic neural precursors (NPs) and adult mesenchymal stem cells (MSCs).
Spinal cord hemisection was performed at the L2 neuromer in adult mice. Two weeks post-injury, we transplanted NPs or MSCs into the cord, caudal to the hemisection site (L3 neuromer). Injured mice without a graft served as controls. In order to value the functional recovery, mice underwent a battery of motor tasks (Basso Mouse Scale, posture, grip test, foot-fault test and hindlimb flexion).
Twenty-eight days after transplantation, animals were sacrificed and analyzed for survival of grafted cells, for effects of engraftment on the local cellular environment and for the sprouting of serotonergic axons.
Both types of stem cells survived several weeks and were integrated into the injured host spinal cord; moreover NPs were able to express neuronal markers (5-HT, MAP-2 and NeuN), but were negative for the astrocyte marker GFAP. All transplanted animals displayed an increased number of 5-HT-positive fibres caudal to the hemisection, compared to untreated mice. Finally stem cell transplantation close to the lesion site significantly improved functional recovery in animals with SCI.
These results point to a therapeutic potential for such cell grafting: both cell types probably could deliver trophic and immunomodulatory factors in proximity of lesion site, inducing axonal regeneration.
Supported by Girotondo Onlus and AIM Foundations and by Regione Piemonte grants to AV.

Cryopreservation of equine peripheral blood-derived mesenchymal stem cells (ePB-MSC) for therapeutic application

Ilaria Bronzini[1], Martinello Tiziana[1], Maccatrozzo Lisa[1], Iacopetti Ilaria[2], Mascarello Francesco[1], Sampaolesi Maurilio[3], Patruno Marco[1]
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In equine medicine bone marrow is still considered the best source of adult mesenchymal stem cells (MSC) although also peripheral blood is regarded as a new potential tissue because of its lower invasivity and easier accessibility.
The aim of this project was to isolate and fully characterize MSC from the equine peripheral blood (ePB-MSC). Moreover, given that the percentage of circulating stem cells in mammalian peripheral blood is very low and their successful isolation in equine is of 33% we studied cryopreservation effects for obtaining quickly functional stem cells of horse. Equine peripheral blood-derived mesenchymal stem cells were isolated and analyzed immediately and after frozen in liquid nitrogen for one year. Both fresh and cryopreserved cells showed the typical morphology, growth curve and population doubling time referred in literature to stem cells population. FACS analysis had showed significant positivity for mesenchymal markers as CD90, CD44, CD117 and CD13 but not for hematopoietic markers as CD34 and CD45. Fresh and cryopreserved ePB-MSC have demonstrated a strong alkaline posphatase reactivity and telomerase activity. Therefore, another aim of this work was to verify the proliferative capacity and the ability to differentiate into different cell types of ePB-MSC without losing stem features after long term cryopreservation. In fact, both fresh and frozen cells were able to differentiate in non-hematopoietic lineages, such as osteogenic, myogenic and adipogenic, proving a great differentiative potential.
Our results demonstrated that cryopreservation of ePB-MSC provides a convenient tool for in vitro applications because they possess the same stem characteristics of fresh isolated cells. The possibility of maintaining the stem cell features of ePB-MSC after a long term storage will have important implications for an autologous cellular-based therapy in veterinary medicine.
In collaboration with the Department of Veterinary Clinical Sciences autologous cryopreserved ePB-MSC were used in a particularly severe OCD lesion in the tarsal joint of a foal. The follow up is encouraging although other clinical trials are required.

Identification and putative role of Versican isoforms during *Xenopus* development

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Versican is a main proteoglycan (PG) of the extracellular matrix of the early embryo and our recent findings in *Xenopus* suggest that it is expressed as a maternal gene. A number of our previous and other investigators' studies in the amphibian (*Mexican axolotl*) and chick embryos have indicated a clear importance of this PG in the control of neural crest (NC) cell migration, although the modes through which the PG affects this movement and the significance of its effect are currently controversial. As the ablation of the Versican gene in mouse causes early embryonic death, the precise role of this PG during development remains largely obscure. Thus, to better resolve the role of Versican in NC cell migration we have cloned the *Xenopus* Versican gene, defined its spatio-temporal distribution and undertaken a gene loss-of-function approach. We find that XVersican is duplicated in two differentially expressed and alternative spliced genes generating a total 8 isoforms of the PG (versus 4 found in higher vertebrates). These include the 2 parental V0 forms, 3 that are homologous to the chick/mouse/human V1 isoform, 2 that resemble the V2 isoform and one corresponding to the amphibian V3 orthologue. Expression analysis during development revealed an early expression of the V0, V1 and V3 variants, highlighting a discordance with the expression pattern seen in avian and mammals where the V3 variant appears at late stages of development. These transcriptional data have further been validated by in situ hybridization and immunoblotting using previously described and proprietary antibodies. A preliminary set of experiments suggest that unilateral morpholino knockdown of XVersican causes an altered migration of NC-derived pigment cell precursors. We are currently performing DNA microarray experiments to establish whether loss of XVersican causes specific or more global gene expression changes secondarily affecting cell movement. Similarly, rescuing experiments entailing co-injection of XVersican mRNA are currently pursued to establish the specificity of the inhibitory effects of XVersican on NC cell migration. Collectively, these experiments are expected to resolve the role of XVersican during NC cell migration and final homing, as well as corroborate the previous hypothesis that Versican acts as a haptotactic motility factor during NC development.

Regulation of adult NSC properties by RE1-Silencing Transcription factor

Pasquale Caramanica, Camilla Toselli, Gabriella Augusti-Tocco, Stefano Biagioni, Emanuele Cacci

A compelling factor involved in neuronal differentiation is the RE1 silencing transcription factor (REST). REST is a multifunction transcription factor that represses or silences many genes in both neural and non-neural cells fundamental in brain function, including neuronal receptors and synaptic vesicle proteins, adhesion molecules, and signaling and channel proteins. Furthermore, REST plays a key role during embryogenesis, neural stem cells (NSC) differentiation and in several neural and non-neural diseases. Our group has demonstrated that the neurotransmitter acetylcholine activates a neuronal differentiaton program through the modulation of REST/NRSF expression (Salani, Anelli et al., 2008). Nevertheless, the function of REST in regulating its target genes remains largely unclear and seems to depend upon the developmental stage and cell type.
We are currently investigating the contribution of REST in various biological functions of NSCs derived from mouse adult subventricular zone (aSVZ). In order to investigate the role of REST on self-renewal, cell proliferation, differentiation and neural cell death of adult NSCs, we have been using an adenovirus carrying the dominant negative form of REST which is known to antagonize the effects of REST (Wood et al., 2003).
We have established appropriate conditions to infect the clonal adult NSC line aNSC1 with high efficiency. Under these conditions around 80% of the cells were infected. Interestingly we found that several genes, including β-3 tubulin, Synapsin I, Snap25, Celsr3, Unc3a, are modulated by DN:REST overexpression. Studies aimed to reveal the functional consequences of DN:REST overexpression in aNSC1 are in progress.
In collaboration with N.J. Buckley laboratory (King's College, London), by using chromatin immunoprecipitation (ChIP) we will also investigate if specific genes, possibly involved in the modulation of adult NSCs differentiation and computationally identified as REST targets, directly bind REST.
Understanding the transcriptional mechanisms that regulate NSC properties is in fact crucial to unlock their potential.

Tracking the homing, engraftment and differentiation of stem cells for myocardial regeneration

Stefano Cavalli [2-4], Caterina Frati [2-4], Alessandra Giuliani [6], Gallia Graiani [2-4], Denise Madeddu [2-4], Lucia Prezioso [1], Alessandra Rossini [5], Francesco Fagnoni [6], Roberto Sala [3], Eugenio Quaini [7], Costanza Lagrasta [2-4], Franco Rustichelli [6], Federico Quaini [1-4]

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Clinical and experimental observations have shown an unexpected developmental plasticity of adult stem cells (SC) and the safety of cell therapy, documenting positive effects on cardiac functional recovery. However, cell fate in the host myocardium and its contribution in reparative processes remains to be determined, in order to face the unresolved issue on whether the beneficial effect of SC on cardiac repair is primarily due to their direct differentiating ability or to regenerative processes and angiogenesis of the host tissue. Using different cell tracking methods, we tested the in vitro and in vivo properties of several cell types including rat Cardiac Progenitor Cells (CPCs), human cardiac derived (CMSC), bone marrow derived (BMMSC) and placenta derived (PMSC) Mesenchymal Cells. The in vitro growth characteristics and biologic properties were assessed on cells labelled by different fluorochromes or by genetic manipulation. After intramyocardial injection in the infarcted rat heart, in vivo cell fate was analysed by tracking methodologies, including GFP-lentivirus cell transfection, Quantum dots (QDts), CM-DiI and FISH. Further evidence of in vivo cell behaviour, was tested by 3D images obtained with Micro Computed Tomography (CT). Injection of all cell types showed positive effects on tissue repair, but data revealed significant changes in homing, engraftment and differentiation. QDts and GFP methodologies, showed important differences among cells in terms of sensing the damage and migration to the infarcted myocardium. When GFP labelling was examined to determine SCs differentiation, the ability of the injected cells to generate new cardiomyocytes and vessels was also of different magnitude. In our preliminary experiments, using CT we have imaged and characterized 3D distribution of CPCs and CMSCs inside infarcted rat heart at early time points. The 3D images obtained so far constituted a very innovative progress, as compared to the usual 2D histological images, which do not provide the correct position of CPCs within the heart. Thus, suitable methodologies can be employed to dissect fundamental regenerative properties of SCs for the reconstitution of the damaged heart.

Association of collagen VI alpha1 chain to monocytes-derived macrophages podosomes

Rosa Curci[1], Camilla Pellegrini[1], Alessandra Zamparelli[1], Patrizia Sabatelli[1], Francesca Gualandi[2], Elena Martoni[2], Luciano Merlini[2], Stefano Squarzoni[1], Alessandra Ferlini[2] Nadir Mario Maraldi[1]
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Resident macrophages, as well macrophages originated from the systemic circulation, play a crucial role related to tissue remodeling and regeneration. With respect to the extracellular matrix, macrophages have been considered to be destructive, however, recent data suggest that alternatively activated macrophages contribute to tissue stabilization by secreting matrix components. In particular, monocytes-derived macrophages produce and secrete collagen VI, however relationships to macrophages function have never been elucidated.
Although ubiquitous, collagen VI has a critical role in maintaining skeletal muscle integrity; in fact mutations in COL6A genes cause a group of inherited muscular dystrophies. Collagen VI is also implicated in tissue remodelling and wound healing, because variation in collagen VI expression have been reported in many pathological conditions.
To give insights into collagen VI function in the matrix produced by macrophages, we performed a biochemical, immunofluorescence and electron microscopy study on monocytes-derived macrophage cultures obtained from healthy subjects. We found that collagen VI was expressed at the macrophage cell membrane, as assessed by electron microscopy analysis of replicas obtained from in vivo labeled rotary shadowed samples. Interestingly, by using alpha1(VI) single chain antibody, we found a specific labelling of podosomal structures. Lysis-squirting technique, used to expose ventral membranes, revealed a specific labelling of alpha1(VI) chain at the protoplasmic face of the ventral cell membrane associated to podosome rosettes, visualized as aggregate of conical particles. Collagen VI analysis in macrophages of two collagen VI deficient patients carrying mutations in COL6A2 and COL6A3 genes showed a reduced expression at the cell surface and double labelling with anti-talin antibodies revealed a reduced podosome formation in both collagen VI deficient patients. Podosomes are dynamic structures present in highly motile cells, however their function is not only related to cell motility but also to cell adhesion and matrix degradation, with implications in tissue remodelling. The association of collagen VI alpha1 chain in macrophage podosomes and the reduced podosome formation in collagen VI deficient patients may unveil novel collagen VI pathogenetic mechanisms.
This work has been supported by Telethon grant n° GUP08006.

Characterization of zebrafish sra and nemo mutants: a way to discover new key-genes in cardiovascular development

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To follow the physiological growth of developing organs and to respond to tissue damage the cardiovascular system is continuously and dynamically remodeled during life. The identification of molecular pathways involved in cardiac and blood vessel development, differentiation, and maturation is crucial to study the biogenesis of cardiovascular system in normal and pathological conditions. The transparency and easy genetic manipulation of zebrafish embryos render this system a powerful tool for analysis of these cardiovascular processes. Here, we focus on the analysis of two zebrafish mutant lines, both derived from a large-scale forward genetic screening (Suk-Won Jin et al., Dev. Biol. 2007). These mutants, named stradivari (sra) and nemo (nem), are characterized by vascular regression, heart defects and impaired circulation. sra mutants show a collapsed heart due to a progressive regression of the endocardium starting at 24 hpf. This results in the absence of blood flow in heart chambers, although myocardial contractility is mantained. This abnormal heart morphology is due to apoptosis of endocardial cells, as demonstrated by TUNEL and active-caspase3 assays, while myocardial cells are not affected. Interestingly, Nem mutants are characterized by blood regurgitation in heart chambers starting at 48hpf. The shape and contractility of heart chambers, however, appears normal until 72hpf. A progressive blood retention in the heart leads to massive dilatation of both atrial and ventricular chambers. Confocal time lapse data show an arhythmic heart contraction in early phases of development. A non physiological contraction is evident in Nem where the atrio-ventricular valve will form as well in the outflow tract. Positional cloning will allow the identification of the genes mutated in stradivari and nemo, thus discovering new players involved in the cardiovascular development.

Differentiation and organization of the nervous system of two Hydrozoan crawling planulae

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In their life cycle Hydrozoan have a diploblastic planula larva possessing a simple network of neural cells, forming a diffused nervous system.
The aim of this work is to investigate morphological and functional aspects of the crawling planulae of *Clava multicornis* and *Eudendrium racemosum* (Hydrozoa).
In contrast to most swimming planulae, these larvae display a smooth gliding movement on the substrate, characterized by alternate bending of the anterior pole.
Ultrastructural and immunocytochemical analysis revealed a remarkable complexity of the nervous system of these planulae. At least three different types of neural cells have been identified: ganglionic cells; ciliated sensory cells and club-shaped sensory cells. Ganglionic cells form a nerve plexus in the anterior portion of the planulae.
Immunostaing with an anti-β-tubulin antibody showed numerous neural cells concentrated in the anterior portion of the body of the planula sending their processes toward the posterior pole. Two distinct population of cells, positive to GLWamide and RFamide, are localized in the ectoderm of the anterior pole.
In both species, the differentiation and the organization of the nervous system are affected by treatment with retinoic acid (RA), a molecule known for its role in patterning anterior-posterior axis of chordates. After exposure of the embryos to RA, the nerve plexus resulted completely disorganized and some RFamide positive cells were shifted in posterior position.
Given the common behavioral constraints encountered by animals with benthic locomotory activity, we can argue the organization of the nervous system of "crawling" cnidarian planula might be comparable to that of simple bilaterians with similar behavior.

Cardiac progenitor cells, scaffolds for in vivo and in vitro myocardial tissue engineering

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The main goal in the last few years in cardiac research has been to isolate cardiac potential progenitor cells from adult myocardium and to demonstrate their differentiation potential. Until now, who isolated cardiac resident cells have differentiated them on a layer of neonatal cardiomyocytes or onto a flat scaffold, obtaining the expression of structural proteins (myosin or troponin) in what it can be considered a bi-dimensional culture. Other groups have injected these cells in vivo inside the heart without showing each passage of differentiation.
Drawing from this background, we demonstrated that c-Kit positive cardiac progenitor cells are able to organize themselves into a tissue-like cell mass. In this 3D mass, they can produce a high concentration of natural extracellular matrix, can create vessels, a capsule and, with the help of an OPLA scaffold, many cells can create an organized elementary myocardium. Starting from this commercially available scaffold, which induced a good differentiation into cardiomyocytes, we are collaborating with a group of engineers to create the best scaffold for myocardial tissue engineering. This three-dimensional culture may be used in the future as a biodegradable patch for the surgical repair of the heart wall and as an experimental model to study the differentiation pathways of cardiac progenitor cells.

Exogenous high mobility group box 1 protein regulates myocardial remodelling through miR206 modulation and induces myocardial regeneration in chronically failing hearts

Grazia Esposito[1], Federica Limana[2], Daniela Arcangelo[1], Chiara Bertolami[1], Anna Di Carlo[1], Antonella Mangoni[1], Giulio Pompilio[2], Jan Kajstura [3], Piero Anversa[3], Antonia Germani[4], Maurizio C. Capogrossi[1]
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Background: The local delivery of HMGB1 in mouse hearts acutely after infarction, induces a regenerative response by activating resident cardiac stem cells (CSCs). We examined whether HMGB1 injection in failing hearts could generate a similar scenario improving left ventricular function and partially preventing myocardial remodeling. Moreover we analyzed mechanisms involved in HMGB1-mediated effects.
Methods and Results: Three weeks after coronary artery ligation, C57BL/6 mice that developed heart failure, received 200 ng of purified HMGB1 or denatured HMGB1 in the peri-infarcted region. Three days after treatment, enhanced CSC number of was observed in the infracted area of HMGB1-treated hearts. Echocardiographic and hemodynamic parameters at 2 and 4 weeks demonstrated a significant recovery of cardiac performance in HMGB1-treated mice. Ejection Fraction was 6% and 9% higher in HMGB1-treated than control mice at 2 and 4 weeks after injection, respectively. HMGB1 enhanced left ventricular (LV) developed pressure (LVDP; 73.2 ± 8.3 vs 64.1 ± 7) and lowered LV end diastolic pressure (LVEDP; 16.8 ± 3.2 vs 19.6 ± 3.2). Further, HMGB1 significantly attenuated LV remodeling as indicated by the reduced LV volume and increased infarcted wall thickness ($p<0.05$). Importantly, HMGB1 affected infarct scar formation: the infarcted region of HMGB1-injected hearts displayed reduced collagen deposition and enhanced MMP9 production. In this area vessel density increased (14.5 ± 6.5 vs 7 ± 3 mm/mm3; $p<0.05$) and foci of regenerating cardiomyocytes were detected. Recent studies have identified critical roles for microRNAs (miRNAs) in a variety of biological process, including regeneration. To investigate mechanisms involved in HMGB1-mediated effects, microRNAs (miRNAs) expression was analyzed in untreated and HMGB1-treated failing hearts. Results showed that miR206 was upergulated in the infarcted area of HMGB1-treated hearts. TargetScan software and Real Time PCR identified collagen and Tissue Inhibitor of Metalloproteases (TIMPS) as potential miR206 targets. Notably, HMGB1 enhanced miR206 expression in cardiac fibroblasts and miR206 overexpression inhibited collagen production as well as TIMPs expression.
Conclusions: HMGB1 administration in failing hearts enhances LV function and tissue regeneration. miR206-mediated reduction of collagen and TIMP activity may promote cardiac c-kit cell invasion and differentiation into the scar tissue, thereby inducing regeneration and attenuating myocardial remodeling.

Generation of Functional Hepatocytes from Mouse Germline Cell-derived Pluripotent Stem Cells *in vitro*

Sharmila Fagoonee[1], Robin M. Hobbs[2], Letizia De Chiara[1], Daniela Cantarella[3], Rosario M. Piro[1], Emanuela Tolosano[1], Enzo Medico[3], Paolo Provero[1], Pier Paolo Pandolfi[1,2], Lorenzo Silengo[1], Fiorella Altruda[1]
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Germline cell-derived pluripotent stem cells (GPSCs) are similar to embryonic stem (ES) cells in that they can proliferate intensively and differentiate into a variety of cell types. Previous studies have revealed some inherent differences in gene expression between undifferentiated mouse ES cells and GPSCs. Our aims were to generate functional hepatocytes from mouse GPSCs in vitro and to investigate whether the differences in gene expression may impact on the hepatocyte differentiation capacity of the GPSCs compared to ES cells. Mouse GPSCs and ES cells were induced to differentiate into hepatocytes through embryoid body formation, with very high efficiency. These hepatocytes were characterised at cellular, molecular and functional levels. The GPSC-derived hepatocytes expressed hepatic markers and were metabolically active as shown by albumin and haptoglobin secretion, urea synthesis, glycogen storage and indocyanine green uptake. We also performed an unprecedented DNA microarray analysis comparing different stages of hepatocyte differentiation. Gene expression profiling demonstrated a strong similarity between GPSC and ES cells at different stages of induced hepatic differentiation. Moreover, Pearson correlation analysis of the microarray datasets suggested that, at late hepatic differentiation stages, the in vitro-derived cells were closer to fetal mouse primary hepatocytes than to those obtained from neonates. We have shown for the first time that adult GPSCs can be induced to differentiate into functional hepatocytes in vitro. These GPSC-derived hepatocytes offer great potential for cell replacement therapy for a wide variety of liver diseases.

Cultured human epicardium-derived cells fuse with high efficiency with skeletal myotubes and differentiate towards the skeletal muscle phenotype *in vitro* and *in vivo*

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Several recent studies have underscored a role for the epicardium as a source of multipotent cells which in the adult retain much of their embryonic plasticity. Here, we investigate the myogenic potential of adult human epicardium-derived cells (EPDCs) and analyze their ability to undergo myogenesis when cultured alone or with differentiated primary myogenic cultures. Results are compared to those ones obtained with mesenchymal stromal cells (MSCs), a cell type whose ability to differentiate towards the skeletal muscle phenotype has been extensively demonstrated in vitro and in vivo, and with endothelial cells, a cell type previously reported to be refractory to myogenic reprogramming. We demonstrate that EPDCs spontaneously fuse with pre-existing myotubes with an efficiency that is particularly enhanced compared to the other cell type tested. We additionally show that, although with a very low efficiency, endothelial cell may also contribute to myotube formation. In all cased analyzed, once entered the myotube, non-muscle nuclei are reprogrammed to express muscle-specific genes. Conversely, myogenic differentiation of EPDCs, MSCs and endothelial cells prior to their contact with pre-existing myotubes was not detected. The fusion propensity of non myogenic cells in vitro parallel their ability to reconstitute dystrophin expression in mdx mice. Finally, fusion of human non-muscle cell to mouse myobtubes occurs through an IL-4 independent mechanism.

Cdk9-55 a new player in muscle differentiation program

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Adult skeletal muscle contains a specialized population of myogenic quiescent stem cells, termed satellite cells, which contribute to repair myofibers after injury. During muscle regeneration, satellite cells exit their normal quiescent state, proliferate, activating MyoD and Myf-5 expression, and finally differentiate and fuse to reconstitute the injured muscle architecture. We have previously reported that cdk9 is required for myogenesis in vitro by activating MyoD-dependent transcription. In myoblasts induced to differentiate, MyoD recruits cdk9 on the chromatin of muscle-specific regulatory regions. This event correlates with chromatin-modifying enzyme recruitment and phosphorylation of cdk9-specific target residues at the carboxyl-terminal domain of RNA polymerase II. Here we report that a second cdk9 isoform, termed cdk9-55, plays a fundamental role in muscle regeneration and differentiation. This alternative form is specifically induced in injured myofibers and its activity is strictly required for the completion of muscle regeneration process. We also show that cdk9-55 is definitely involved in muscle differentiation program also during embryonic myogenesis.

A novel potential trophic effect of neural agrin on the regenerative potential of aged human skeletal myoblasts

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Neural agrin has been first described as a trophic factor released by the motor neuron due to its ability to promote the formation of the postsynaptic apparatus. Nowadays, emerging experimental evidence demonstrates that the effect of neural agrin is not spatially limited to the endplate region. For example, neural agrin causes remodelling of the skeletal muscle cell, i.e formation of microprocesses and reorganisation of cytoskeletal elements. We have also demonstrated that the neural agrin improves the maturation of the excitation-contraction apparatus and membrane electrical properties in myotubes originated by human satellite cells *in vitro* (Bandi *et al*, *Am J Physiol Cell Physiol*, 2008, 294: C66-73; Jurdana *et al*, *Cell Mol Neurobiol*, 2009, 29: 123-131).
The aim of the present work was to explore the possible trophic role of neural agrin on the regenerative potential of aged human myogenic cells. To do this, human myoblasts, derived from satellite cell isolated from aged donors (58- and 66-year-old), were cultured in the presence of 1 nM neural agrin. Our results demonstrated that neural agrin could enhance the proliferative potential of human myoblasts. When cultivated in the presence of neural agrin, the proliferative capacity of aged myoblasts improved: they underwent a higher number of cell divisions before reaching the replicative senescence. Interestingly, the treatment with neural agrin did not alter the myogenic potential of aged human myoblasts: the percentage of desmin positive cells remained constant up to the terminally nondividing state. Agrin-treated myoblasts exhibited a fusion index as well as a number of nuclei per myotube similar to untreated human cells. In addition, preliminary experiments showed that myotubes generated by treated aged myoblasts exhibited a functional skeletal type excitation-contraction coupling mechanism.
Our results suggest that neural agrin could enhance the regenerative potential of aged human satellite cells. Further experiments are required to explore its role, if any, in the impaired plasticity and regeneration of aged skeletal muscle tissue.

Xenograft of microencapsulated pre-pubertal porcine sertoli cells reverse type 1 diabetes in nod mice by inducing regeneration of new pancreatic islet beta-cells

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Introduction and Aims. Sertoli cells (SC) provide an immuno-protective environment for transplantation of pancreatic islets for treatment of insulin-dependent diabetes (T1DM). Aim of this work was to verify if intraperitoneal transplantation of SC, enveloped in barium alginate-based microcapsules (BaMCs) would reverse spontaneous Type 1 Diabetes (T1DM) in NOD mice with generation of newly formed islets, associated with functional β-cells.
Methods. BaMCs were prepared, according to our method, by a mono air-jet device system, and thereafter examined as far as: (a) SC morphology by light microscopy; (b) SC viability, by fluorescence microscopy after staining with ethidium bromide and fluorescein diacetate (EB+FDA); (c) SC in vitro function (α-aromatase activity and IGF-I secretion); (d) reversal of T1DM in spontaneous diabetes NOD mice, were concerned.
Results. BaMCs exhibited excellent features in terms of size, morphology, sphericity and coalescence. SC viability was very high (over 90%). Efficient α-aromatase activity and IGF-I secretion were observed on the examined SC preparation. Graft of BaMCs induced significant reversal (88%) of spontaneous T1DM diabetes in the NODs'. The treated mice showed dramatic increase of regulatory T-limphocytes (about 14,5% of the total CD4+T cell count) as compared with the diabetic NOD's (about 5.8% of the total CD4+ T cells) treated with empty capsules only. Histological examination of the pancreas retrieved from the SC transplanted animals showed total absence of insulitis in the NOD's. In contrast, the pancreata of mice associated with successful SC therapy, reappearance of islets occurred, in absence of invasive insulitis and with mild or no peri-insulitis, the latter fading over time. Immunofluorescence examination of the pancreas of SC-treated animals revealed that the increased Ngn3 expression reinstalled ability of the islets to produce insulin, glucagon, and somatostatin. The occurrence of glucagon and somatostatin double-stained cells reflected the process of embryonic stem cell development, thereby suggesting the occurrence of authentic β-cell regeneration.
Conclusions. SC may be enveloped in BaMCs with no long-term loss of their functional and morphological identity either in vitro or in vivo. Xenograft of SC-containing BaMCs induced significant reversal (88%) of spontaneous T1DM diabetes in diabetic NOD mice, based on SC-related both, powerful immunomodulatory effects and cell regeneration properties.

The interaction with HMG20a/b proteins suggests a potential role for β-dystrobrevin in neuronal differentiation

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α And β dystrobrevins are cytoplasmic components of the Dystrophin-associated Protein Complex (DPC), which are thought to play a role as scaffold proteins in signal transduction and intracellular transport. In the search of new insights into the functions of β-dystrobrevin, the isoform restricted to non-muscle tissues, we performed a two-hybrid screen of a mouse cDNA library to look for interacting proteins. Among the positive clones, one encodes iBRAF/HMG20a, an HMG (High-Mobility Group)-domain protein that activates REST (RE-1 Silencing Transcription factor)-responsive genes, playing a key role in the initiation of neuronal differentiation. We characterized the β-dystrobrevin-iBRAF interaction by in vitro and in vivo association assays, localized the binding region of one protein to the other, and assessed the kinetics of the interaction as one of high affinity. We also found that β-dystrobrevin directly binds to BRAF35/HMG20b, a close homologue of iBRAF and a member of a co-repressor complex required for the repression of neural specific genes in neuronal progenitors. In vitro assays indicated that β-dystrobrevin binds to RE-1 and represses the promoter activity of synapsin I, a REST-responsive gene which is a marker for neuronal differentiation. Altogether, our data demonstrate a direct interaction of β-dystrobrevin with the HMG20 proteins iBRAF and BRAF35, and suggest that β-dystrobrevin may be involved in regulating chromatin dynamics, possibly playing a role in neuronal differentiation.

Inhibition of gliogenesis and promotion of neuronogenesis by patterned overexpression of *Emx2* and *Foxg1*

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Neural stem cells (NSCs) give rise to all cell types forming the cortex, neurons, astrocytes and oligodendrocytes. The transition from the former to the latter ones takes place via lineage-restricted progenitors and is mastered by large sets of genes, among which some implicated in CNS pattern formation. Aim of this study was to disentangle the kinetic and histogenetic roles exerted by two of these genes, *Emx2* and *Foxg1*, in cortico-cerebral precursors.
For this purpose, we set up a new integrated in vitro assay design. Embryonic cortical progenitors were trasduced with lentiviral vectors driving overexpression of *Emx2* and *Foxg1* in NSCs and neuronal progenitors (NPs). Cells belonging to different neuronogenic and gliogenic compartments were labeled by spectrally distinguishable fluoroproteins, driven by cell-type-specific promoters, as well as by cell-type-specific antibodies. They were subsequently scored, via multiplex cytofluorometry and immuno-cytofluorescence. Finally, these assays were complemented by immunofluoro-cytometric profiling of neural cultures constitutively loss-of-function for each of these two genes.
A detailed picture of *Emx2* and *Foxg1* activities in cortico-cerebral histogenesis resulted from this study. Unexpectedly, we found that both genes may inhibit gliogenesis and promote neuronogenesis, through distinct mechanisms, and *Foxg1* also dramatically stimulates neurite outgrowth. Remarkably, such activities, alone or combined, may be exploited to ameliorate the neuronal output obtainable from neural cultures, for purposes of cell-based brain repair.

Role of the alternative transcripts and targets of the Ci-POU IV gene in the development of the Peripheral Nervous System of the tunicate *Ciona intestinalis*

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Some members of the POU gene family regulate the neural patterning and differentiation in both vertebrate and invertebrate embryos. The tunicate *Ciona intestinalis* has only three genes encoding for POU transcription factors and the gene *Ci-POU-IV* is specifically expressed in all the peripheral nervous system (PNS) territories and in some cells of the central nervous system during development. Within chordates, tunicates represent the sister group of vertebrates and their larvae have a typical chordate body plan. Notably larval nervous system is formed by few cells whose organization mirrors that of vertebrates. The aim of this work was to study the role played in neural differentiation by the two alternative transcripts of *Ci-POU-IV*, we discovered, and to identify the targets of this gene.
We designed Morpholino oligos to perform gene knock-down experiments for the different isoforms, a short and a long one. Preliminary results from these experiments revealed that the expression of the serotonin rate-limiting synthesis enzyme, tryptophane hydroxylase (TPH), and glutamate transporter (vGlut) could be regulated by the long transcript. To verify if the alternative transcripts are expressed in different neuron populations, we synthesized a probe selective for the short isoform. The in situ hybridizations, compared to the whole expression profile of *Ci-POU-IV*, showed a lack of expression of the "short" form in the sensory epidermal neurons of the trunk. Furthermore, we look for the *Ci-POU-IV* targets by a bioinformatic approach. The possible consensus sequences were obtained by bibliographic research of those known for the POU IV family in both invertebrates and vertebrates. We used these sequences to build a matrix that was employed to perform a bioinformatic research in the whole C. intestinalis genome with a software elaborated by the Lemaire team of the IBDML of Marseille. We identified 19 possible targets of *Ci-POU-IV* and we preliminary selected 8 regions corresponding to 6 genes, including TPH. The activity of the selected regions is being evaluated. Results from this work allows us to hypothesize that the two isoforms play different roles during nervous system differentiation and will help us to delineate the signal cascade of *Ci-POU-IV* during PNS differentiation.

Stem cells from amniotic fluid: a promising source for cellular therapy?

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Amniotic fluid (AF) contains human stem cells able to differentiate into multiple lineages. Unlike the embryonic ones, AF-derived mesenchymal stem cells (AF-MSCs) appeared not to be tumorigenic after transplantation in mice (De Coppi et al, Nat Biotechnol 2007;25:100) and this makes them good candidates for therapeutic purposes. There are a lot of findings on the differentiation potential of AF-MSCs, but they appear fragmentary and, up today, there are no standardized and reproducible protocols.
Aim of this study is to verify whether AF-MSCs were really able to transdifferentiate in neurons. To this purpose we performed immunophenotypical characterization, analyses of the main stemness markers expression, and functional electrophysiological measurements to evaluate the static electric membrane potential and the presence of Na+ and K+ currents typical of neuronal cells.
AF cell samples were obtained from amniocentesis (16th week pregnancy) and collected at the "Salesi University Medical Center" of Ancona following informed consent. After washes with PBS, cells were cultured in Mesenchymal Stem Cell Medium (MSCGM, Lonza Ltd, Switzerland) for at least 3 weeks. Then, the AF-MSCs were incubated with Neurobasal medium plus B27 (Gibco, Italy) up to 7-10 days, with or without different trophic factors (guanosine, GTP, adenosine, ATP, retinoic acid (RA) or BDNF), known to induce a neuron-like phenotype.
FACS and PCR analyses revealed that AF-derived cells showed a mesenchymal phenotype and Oct4 and Nanog expression after 3 weeks culture in MSCGM. These cells also showed intracellular calcium variations similar to that observed in other mesenchymal models. Among the tested trophic factors, only RA and BDNF induced neuronal-like morphological changes but they failed to significantly modify the functional cellular signalling.
These results reveal that AF-MSCs could be a potential neurogenic model, but the tested experimental approaches failed to induce the fully differentiated functional phenotype in vitro. This requires the definition of a standardized optimal differentiation protocol.

Effects of sphingosine 1-phosphate on skeletal muscle repair/regeneration after eccentric contraction-induced damage

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Skeletal muscle regeneration is severely compromised in case of extended damage. The current challenge is to find factors capable of limiting muscle degeneration and/or activating the inherent regenerative program. Recent studies from our groups and others have shown that the bioactive lipid, sphingosine 1-phosphate (S1P), promotes myoblast differentiation and exerts a trophic action on denervated skeletal muscle fibres. In this study, we examined the effects of the sphingolipid on eccentric contraction (EC)-injured mouse EDL muscle fibers and the resident satellite cells. EC-injured fibers showed plasma membrane depolarization and reduced membrane resistance together with morphological and biochemical signs of muscle damage and cell death. Treatment with exogenous S1P attenuated the EC-induced tissue damage, protected skeletal muscle cells from apoptosis and affected extracellullar matrix remodelling through the up-regulation of MMP-9 expression. Moreover, S1P greatly potentiated satellite cell activation and enhanced their attitude to fuse into multinucleated myotubes once isolated from the single fibers. Notably, the activity of sphingosine kinase 1 (SphK1), but not SphK2, and the levels of endogenous S1P were significantly higher in the injured fibres and satellite cells, stressing the involvement of SphK1/S1P axis in skeletal muscle protection and repair. Together, these findings are in favour for a role of the pro-survival factor in skeletal muscle healing and regeneration and offer new clues for the identification of novel therapeutic approaches to manage skeletal muscle damage and disease.

Human mesenchymal cells from first trimester chorionic villi: determination of *in vitro* plasticity and differentiation potential

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Introduction: Mesenchymal stromal cells (MSCs) have recently raised interest in regenerative medicine due to their capacity to differentiate along distinct lineages. MSCs were originally isolated from bone marrow (BMSCs), but similar populations were found in other adult organs and in fetal tissues. Aim of this study was to isolate and characterize MSCs derived from placental first trimester chorionic villi (CSVCs).
Methods and results: A protocol was developed to optimize isolation and expansion of CSVCs; each preparation has been expanded in different culture media: (i) a culture medium made of IMDM, with 20%FBS and bFGF (10ng/ml); (ii) the dedicated AmniomaxII, a cell culture medium specifically tailored to grow chorionic villi culture in prenatal diagnosis. In both cases CSVCs were fibroblast-like shaped and expressed the mesenchymal membrane markers CD105, CD73, CD90. As expected, CD34, CD45, HLA-DR were negative. The pluripotency genes Nanog, Oct-4 and Sox-2 were also detectable at a level similar to BMSCs used as control. Further CSVCs were able to differentiate in vitro in: a) adipocytes (determined by Oil Red-O staining); b) osteocytes (determined by Von Kossa staining); c) endothelial cells (according to the presence of VWF and to the ability to form capillary-like structures on Cultrex); d) skeletal myocytes (based on the ability to form elongated bi- and poly-nucleated myotubes and on the expression of myocyte-specific markers such as MYH-3 and CHRNA1). The genomic stability of CSVCs was demonstrated by karyotype analysis before and after treatment to differentiate. Notably, cells expanded in AmniomaxII exhibited a faster growth kinetic and responded more effectively to in vitro differentiation compared with those isolated in IMDM, 20%FBS, bFGF, indicating that the culture conditions is crucial for in vitro cell characterization.
Conclusion: CSVCs represent an excellent candidate for future cell therapy application as they possess high proliferative potential, wide differentiation ability and stable karyotype in vitro.

Bone marrow transplantation cures hemophilia A

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Identification of cells capable of synthesizing and releasing FVIII will be critical for therapeutic development in hemophilia A. Recent studies indicated that endothelial cells, particularly liver sinusoidal endothelial cells, are a major source of FVIII, although the origin of endothelial cells is incompletely defined and FVIII could potentially be expressed in additional cell types. To determine whether donor bone marrow-derived cells could produce FVIII, we studied hemophilia A mice subjected to bone marrow (BM) transplantation. We analyzed FVIII production as well as correction of hemophilia by several assays. BM was replaced extensively after transplantation of 2x106, as well as 10x106 of total BM cells. Remarkably, In 86 hemophilia A mice subjected to BM transplantation, we found lesser therapeutic correction in recipients of 2x106 BM cells compared with 10x106 BM cells (55% versus 100%, respectively). FVIII activity was measured by chromogenix or fluorogenic assays at least 2 months after BM transplantation. Plasma FVIII activity was >5% to 22% of normal levels in mice showing correction of hemophilia and this remained stable for12 month at least. Moreover, hemophilia A mice with correction of plasma FVIII activity survived tail clip-induced bleeding. J02, MCT or CCl4 treatments did not improve therapeutic correction in hemophilia A mice and, despite hepatic and endothelial injury to recruit transplanted cells, donor bone marrow-derived hepatocytes or endothelial cells were extremely rare, and did not account for therapeutic benefits. By contrast, FVIII was produced in mononuclear cells and mesenchymal stromal cells derived from donor bone marrow, which expressed FVIII both mRNA and protein. Finally, to establish the applicability of these findings in humans, we studied FVIII mRNA in total human BM and BM cell fractions, i.e., CD133+ progenitor cells, CD33+ myeloid cells, CD105+ mesenchymal and stromal cells. FVIII mRNA was expressed in myeloid and stromal cell fractions of BM. Moreover, injection of healthy Kupffer cells (liver macrophage/mononuclear cells), which predominantly originate from BM, or of healthy BM-derived mesenchymal stromal cells protected hemophilia A mice from bleeding challenge. In immunostaining MSC were positive for vimentin and for FVIII. Therefore, we concluded that bone marrow transplantation cured hemophilia A through reconstitution of donor mononuclear cells and mesenchymal stromal cells. These insights in cellular origins of FVIII offer new mechanisms for understanding pathophysiological alterations in FVIII synthesis and production and for developing further therapies in hemophilia A and provides exciting opportunities for addressing the potential of human BM-derived cells.

Wnt5a regulated GABAergic differentiation of neural progenitor cells, *in vitro* and *in vivo*

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We are investigating the biological and molecular functions of Wnt signalling on the proliferation and differentiation of neural progenitor (NP) cells. Wnt ligands use several transduction pathways: the best known one (canonical) depends on stabilization/nuclear translocation of beta-catenin, and generally promotes self-renewal and proliferation of stem cells. Conversely, beta-catenin independent pathways, collectively known as non-canonical, either use JNK phosphorylation or Ca-dependent activation of PKC. In general, activation of non-canonical Wnt pathways promote neuronal maturation/differentiation.
Our approach combines the examination of basal forebrain development, in particular in Dlx-mutant strains of mice, with the use of cultured neural progenitors. In the basal forebrain, NP cells occupy a specific niche at the wall of the lateral ventricle, and are fate-committed towards the GABA+ interneuron lineage. As NP cells exit the cell cycle, they migrate either to the olfactory bulb (OB), a process that continues well into adulthood, or to the cortex. In the mouse brain, the transcription factors Dlx2 and Dlx5 are essential for migration and differentiation of GABA progenitors. We provide evidence that Dlx2 and Dlx5 regulate transcription of Wnt5a, and in turn Wnt5a ligand promotes GABA+ differentiation, both in vitro and in vivo, and rescues the Dlx5 defect. This observation links the activity of Dlx transcription factors with a diffusible pro-differentiation signal.
The molecular/intracellular pathway by which Wnt5a modulates differentiation of NP cells is poorly known. To examine this, we derive and examine NP cells from the embryonic brain, using a recently described protocol for adherent expansion of NP cells and their GABA+ differentiation. In these cells we observe that canonical Wnt3a specifically activates beta-catenin, while non-canonical Wnt5a promotes phosphorylation of JNK. Furthermore, JNK phosphorylation is reduced in the forebrain of Dlx5 null mice. Wnt5a interacts with the tyrosine-kinase receptor Ryk, and Ryk activation/cleavage promotes the differentiation of cortical NP. Our preliminary data show that application of a Ryk neutralizing antibody on basal NP alters their differentiation properties. Defining the pathway that links Wnt5-Ryk interaction with JNK during NP cell differentiation may open the way to activate/inhibit key steps of this regulation and thereby control GABA+ differentiation of NP cells.

Modulating the teratogenic potential of the mouse Embryonic Stem Cells

Michela Palmisano, Raffaella Molteni, Barbara Clissi, Ruggero Pardi

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

Expression and trafficking of GPR17 in immortalized oligodendrocyte precursor cells

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The recent deorphanised G-protein coupled purinoreceptor GPR17 has been described as a new player in the remyelination process. This dualistic receptor is activated by both uracil nucleotides and cysteinyl-leukotrienes, signalling molecules that are abundantly released by damaged cells. GPR17 is up-regulated in in vivo injury models characterized by neuronal damage and demyelination, and it is thought that it may be involved in their differentiation. To set the basis for the possible exploitment of GPR17 for stimulation of adult oligodendrocyte precursor cells (OPCs) differentiation, we characterised the expression of GPR17 in an immortalized OPC line (Oli-neu) with the aim of establishing an in vitro model useful for investigating GPR17 trafficking and signalling. Biochemical and immunofluorescence analysis of Oil-neu differentiation in neuronal conditioned medium and simultaneous investigations of the expression of GPR17 and myelin proteins, showed that GPR17 is almost absent in undifferentiated Oli-neu cells. The receptor is detected after 24 hours' incubation in conditioned medium, but not with other differentiating agents; its expression plateaus after 48-72 hours, and decreases after 4-5 days in culture. On the contrary, the myelin marker MAG is expressed after 48 hours in culture, its expression increases over time and remains high after 4-5 days. When GPR17 was knocked down by means of RNA interference, fewer Oli-neu cells were capable of differentiating. Having established that GPR17 is expressed in Oli-neu cells, we investigated its subcellular distribution and found that the receptor is mainly located at the plasma membrane. It can also be internalized and partially colocalizes with markers of the endocytic and lysosomal compartments. Furthermore, the metabolic labelling of Oli-neu showed that a substantial amount of GPR17 is rapidly degraded by lysosomes and/or proteasomes. Taken together, these data indicate that Oli-neu cells can express significant amounts of GPR17 under specific culture conditions and can down-regulate receptor expression during differentiation. This cell line is therefore a useful tool for investigating the trafficking and signalling of GPP17, a new potential therapeutic target in demyelinating diseases.

A simple and reliable method to generate and study differentiating serotonergic neurons *in vitro* from murine ES cells

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Serotonergic neurons are localized in the *raphe nuclei* of the brainstem where serotonin (5-HT) is synthesized through the activity of the rate-limiting enzyme *Tryptophan hydroxylase 2*, *Tph2*, selectively expressed in serotonergic neurons. In the Central Nervous System (CNS) serotonergic neurons provide a widespread axonal network to the whole CNS playing an important role in the control of several behavioral and physiological functions and impairment to the normal 5-HT neurotransmission has been causally linked to several neuropsychiatric disorders such as depression, anxiety, schizophrenia and autism.
Embryonic Stem (ES) cells represent an excellent model to study the differentiation of specific cell types, as well as for stem cell-based therapies designed to treat neurological disorders. In the last years, efficient protocols have been set up to obtain serotonergic neurons from differentiating mouse ES cells *in vitro* based on the use of specific growth factors (FGF2, Shh, FGF8, FGF4). However protocols using onerous reagents, such as growth factors, have limited applicability in fields where 5-HT neurons are expected to be obtained on large scale, such as for example high-throughput screening. An affordable culture condition to obtain serotonergic neuron from differentiating ES cells, would then allow several application in this fields, from high throughput screening to the study several processes of serotonergic neuron development and functions, such as differentiation, migration and axonal outgrowth.
We tested several culture conditions using *E14Tg2A* murine ES cells and an affordable treatment was identified to reproducibly and efficiently differentiate ES cells toward serotonergic neurons as assessed by the expression of serotonergic markers. On the whole, our differentiation strategy provides a simple and reliable method to generate an in vitro model to study the development and the biology of serotonergic neurons.

Olfactory Ensheathing Cells: an unusual glial cell population

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Olfactory mucosa is considered a potential source of neural stem cells. When olfactory receptor neurons (ORNs) die, new neurons are produced by division of basal cells of the deepest layer of epithelium. The ability of ORNs to regenerate throughout life in the adult olfactory system is thought to be due in part to particular glial cells of the olfactory nerve, termed Olfactory Ensheathing Cells (OECs). OECs, described by Golgi and Blanes at the end of 19th century, show exceptional plasticity and are able to ensheathe unmyelinated olfactory axons and exhibit antigenic and morphological characteristics both of astrocytes and of Schwann Cells (SCs). As a matter of fact they express an astrocyte-specific marker (GFAP) and low-affinity p75 nerve growth factor receptor (p75 NGFr), S100, as well as adhesion molecules such as laminin and N-CAM like SCs. In addition, OECs express, 04, vimentin, neuropeptide Y, calponin and are positive to nestin in embryonic and postnatal stages, a stem cells marker. Immunocytochemical studies reveal that OECs are able to produce different growth and survival factors, such as NGF, bFGF, BDNF, GDNF, CNTF, NT4, NT5. In vitro, OECs promote axonal growth, probably by secretion of neurotrophic growth factors that support axonal elongation and extension. In vivo studies have shown that OECs can form myelin promoting remyelination of damaged axons, expressing P0, a myelin constituent. These findings have stimulated many researches to transplant OECs into transected spinal cord demonstrating their ability to promote regeneration and functional recovery. In our opinion, OECs probably have this capability as source of growth factors and adhesion molecules. Moreover, when transplanted, they stimulate extensive sprouting and axonal regeneration of multiple axons. As OECs appear to exert a neuroprotective effect for functional restoration and for neural plasticity in neurodegenerative disorders, they might be considered a suitable approach to functional recovery. These properties might render them potential clinical agents and of trophic support to CNS injury, showing some advantages over SC thanks to their different capacity to intermingle with astrocytes after implantation in lesion sites.

Engraftment capacity of hES- derived alveolar epithelial type II pneumocytes into silica and bleomycin lung-damaged mice

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Pulmonary fibrosis encompasses a broad range of diseases with limited treatment options. These progressive diseases result from abnormal wound healing processes of the lung, leading to the accumulation of fibroblasts and myofibroblasts and a progressive deposition of extracellular matrix proteins and connective tissue.
The purpose of this study is to evaluate the engraftment capacity of airway epithelium generated from human embryonic stem cells (hES; HUES3) in silica and bleomycin-damaged mouse model.
HUES-3 cells were in vitro treated with SAGM medium for 3-5-8 days. Immunohistochemical analyses were performed for assessing the expression of pulmonary specific markers such as surfactant protein B, C (SP-B; C), and also of ZO-1 (gap junction) and CFTR, allowing their correct localization within SAGM-treated cells.
Moreover differentiated cells, grown on permeable supports showed also a transepithelial resistance >150±?/cm2, specific for transepithelial chloride ion transport.
Molecular analysis conducted by Real Time RT-PCR showed the expression of differentiation markers of epithelial lung such as CXCR4; TTF1; SP-C and SP-B.
Successively in vivo experiments were performed to validate the engraftment capacity of SAGM-committed cells. Firstly 100 nude mouse were intranasal/intratracheal injected with silica [50mg/mL] and bleomycin [5U/Kg] for 15 days. Histochemical data evidenced the damage in silica and bleomycin-mouse lung specifically in type II pneumocytes by both ematossilin-eosin and Masson analyses. Results reveal a typically fibrosis area of inflammation, epithelial hyperplasia, interstitial edema and deposition of silica-quartz particles. Moreover Real Time RT-PCR was performed on mouse lung to assess the level of expression of inflammatory cytokines such as IL-6; TNF-?; Mip-2; Col-1-?1 and CXCL12.
About 3x106 SAGM-committed cells (3 days of treatment) were inoculated in damaged mice.
One month after, animals were sacrificed for evaluating the presence of human SAGM cells within mouse lung. Real-time PCR (Quantifiler kit), immunohistochemical for Human Nuclear antigen and FISH analyses demonstrated the presence of human DNA into silica and bleomycin lung.
Human SP-C and Cytocheratin expression are in progress to characterize the engrafted human cells
Cytokines expression, detected by Real Time RT-PCR, resulted strongly decreased, thus demonstrating a clear reduction of inflammation status in lung of inoculated mice.
Our results demonstrate the usefulness of hES for repairing damage in compromised lung as happens in Pulmonary fibrosis by a cell therapy approach.

Osteogenic differentiation occurring in 2D and 3D cultures of human dental pulp stem cells

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The aim of this study was to characterize the in vitro osteogenic differentiation of dental pulp stem cells (DPSCs) in 2D and 3D cultures. DPSCs were separated from dental pulp by enzymatic digestion and then isolated by magnetical cell sorting using antibodies against c-Kit, CD34 and STRO-1 surface antigens. After sorting, in a first phase, cells were differentiated toward osteogenic lineage on 2D surface of culture flask by using an osteogenic medium. Differentiated cells express specific bone proteins like Runx-2, Osx, OPN and OCN with a sequential expression analogous to those occurring during osteoblast differentiation and produce extracellular calcium deposits.In a second phase DPSCs were cultured in MatrigelTM and Collagen 3D scaffolds in order to differentiate cells in a 3D space that mimes the physiological environment. Cells cultured on these scaffolds show an improved osteogenic differentiation and produce a mineralized extracellular matrix. In MatrigelTM we observed cells differentiated with osteoblast/osteocyte characteristics and calcified nodules containing cells connected by gap junction constituting a 3D intercellular network. On the other hand DPSCs differentiated in collagen sponge actively secrete human type I collagen micro-fibrils and form calcified fibres assembling in trabecular-like structures. These neo-formed DPSCs-scaffold devices may be used in regenerative surgical applications in order to resolve pathologies and traumas characterized by critical size bone defects.

In-vivo and in *ex-vivo* heart regeneration in zebrafish (*Danio rerio*)

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Zebrafish has the remarkable ability to regenerate the heart, by a process referred to as epimorphic regeneration, the regrowth of amputated structures from an anatomical complex stump. Networks of transcription factors regulate heart development, maintenance, and regeneration in a dose-dependent manner, but the effects of translational regulation on the titration of these pathways are largely unknown. Here, with In Situ Hybridization (ISH) experiments, it was tested in regenerating hearts the presence and the
timing expression of genes involved in FGF regulation pathway. In-vivo experiments showed that the response to the injury started already at 3 hours post amputation (hpa), just around the surgered site, by the reactivation of developmental genes such as *dusp6* (mkp3), *erm*, *pea3*, *raldh2*, and *sef*. Between 6 and 12 hpa all the amputated hearts evidenced the expression of other genes such as *etv5* and *sprouty4*, showing the reactivation of development genes. With the aim to optimize the media to reproduce the regeneration process, it was also tested the ability of zebrafish heart to survive in ex-vivo cultures after the amputation of ventricular apex. Injured hearts cultured with BCI, FGF-2, Thrombin, and with a cocktail of FGFs were able to maintain expression of *raldh2* between 7 and 14 days post amputation (dpa), whereas PDGF and Basement Membrane Extract (BME) supplemented with FGFs cocktail allowed the *raldh2* expression until 30 dpa. Regenerating hearts in ex-vivo conditions were able to survive and make contractions, and surprisingly showed different degree of cell replication in
dependence to the culture media. The highest level of duplication, detected by BrdU incorporation, was observed at 14 dpa in hearts cultured with addiction of BCI. However operated hearts in-vivo showed a complete regeneration after 30 dpa whereas ex-vivo cultured the hearts displayed only a partial regeneration. Moreover, in *ex-vivo*, a big diminution of the clot site was observed in hearts cultured with BCI, suggesting that this compound could
interact with FGF pathway, stimulating and supporting the heart regeneration process.

RAGE is re-expressed in skeletal muscle satellite cells after muscle injury and deletion of RAGE results in delayed muscle regeneration

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RAGE (receptor for advanced glycation end products) is a multiligand receptor of the immunoglobulin superfamily playing an important role in innate immunity and in endothelial cell activation and vascular smooth muscle proliferation in atherosclerosis and inflammation (*J Clin Invest 108:949-55, 2001; J Mol Med 83:876-86, 2005*). RAGE is expressed in several cell types during development, repressed at completion of development and re-expressed in the course of certain pathological conditions (*J Clin Invest 108:949-55, 2001; J Mol Med 83:876-86, 2005*). The expression of RAGE in several cell types during development suggests that RAGE might not be regarded simply as a transducer of inflammatory cues. RAGE is expressed in skeletal muscle fibers during prenatal and postnatal development being repressed thereafter (*Mol Cell Biol 24:4880-94, 2004*). Also, RAGE is expressed in proliferating and differentiating myoblasts, and once activated by its ligand, HMGB1, it transduces a promyogenic, pro-apoptotic and anti-proliferative signal in myoblasts and rhabdomyosarcoma cell lines via activation of a Rac1/Cdc42/MKK6/p38 MAPK pathway (*Mol Cell Biol 24:4880-94, 2004; J Biol Chem 281:8242-53, 2006; Am J Pathol 171:947-61, 2007*). We show here that following damage, RAGE becomes expressed in skeletal muscle satellite (i.e., $Pax7^{+}$) cells (SCs) and in regenerating myofibers (likely as a result of expansion of activated SCs and fusion of RAGE/myogenin-expressing SCs, in the latter case), becoming repressed at completion of regeneration, and that deletion of RAGE results in an elevated SC basal number, a strong infiltration of undamaged tissue with activated SCs at early and late regeneration phases, and delayed muscle regeneration. Also, primary $RAGE^{-/-}$ myoblasts exhibit high Pax7 levels, enhanced proliferation, migration and invasiveness, and defective differentiation compared with wild-type myoblasts, and transfection of $RAGE^{-/-}$ myoblasts with full-length RAGE, but not a RAGE mutant lacking the cytoplasmic and transducing domain rescues their myogenic potential. HMGB1/RAGE represses Pax7 expression via a p38 MAPK/myogenin axis in myoblasts with myogenin binding to four (in growth medium) and six (in differentiation medium) recognition sites in the Pax7 gene promoter and stimulating proteosomal degradation of Pax7. Collectively, our results suggest that HMGB1/RAGE might physiologically contribute to muscle regeneration.

Zinc finger protein 521: a novel regulator of the normal and malignant immature cell compartment in the haematopoietic and neural system

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The transcription co-factor ZNF521 was identified and cloned in our laboratory because of its strikingly differential expression between early haematopoietic progenitors and mature leukocytes. ZNF521 is a 1311-AA-long protein with 30 zinc fingers and an N-terminal NuRD-binding motif shared with transcriptional co-repressors such as FOG-1 and 2 and the SALL proteins. ZNF521 is abundant in haematopoietic stem cells, but its expression rapidly declines to undetectable levels during their differentiation. Similarly to the cognate protein, ZNF423, implicated in the maintenance of the immature state in olfactory progenitors and in cerebellar development, ZNF521 inhibts the activity of EBF1, a transcription factor of crucial importance in B-cell development and neuronal differentiation.

In the haematopoietic system, RNAi-mediated silencing of ZNF521 in stem cells results in their depletion and, in appropriate culture conditions, in a strong enhancement of B-lymphoid differentiation, suggesting that one of the mechanisms by which ZNF521 may contribute to the maintenance of the immature haematopoietic compartment is by counteracting the EBF-driven commitment to the B-cell lineage. High levels of ZNF521 mRNA are detected in most AMLs, particularly in leukaemia-initiating cells compared to stem cells-depleted fractions. Strong expression is consistently observed in AMLs with MLL rearrangements. Silencing of ZNF521 in these cells impairs their growth and clonogenicity, suggesting a role in regulating the homeostasis of the leukaemia-initiating cell compartment.

ZNF521 is strongly expressed in brain and is one of the limited number of genes whose expression is shared between haematopoietic and neural stem cells. Extremely high expression of Zfp521 is observed in the granule layer of the developing cerebellum, that hosts the cerebellar granule neuron precursors, regarded in turn as the cells-of-origin of medulloblastoma (MB). This is mirrored by strong expression of ZNF521 in a subset of MBs with high tendency to metastasize. Overexpression of ZNF521 in the human MB cells, DAOY, induces the expansion of a sub-population with stem-like features capable of generating tumours in xenotranspants, in a process that depends on the integrity of the NuRD-binding motif.

Thus, ZNF521 appears to play a regulatory role in normal and malignant, aematopoietic and neural stem cells. The identification of its molecular partners and downstream targets will shed further insight into its mechanism of action and help define its role in the control of stem cell homeostasis.

The recruitment of host progenitor cells after mesenchymal stem cells (MSC) implantation plays a key role in the development of the tissue-engineered bone

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Following implantation of porous ceramic cubes seeded with mouse Mesenchymal Stem Cells (MSC) into immunocompetent syngenic mice, we demonstrated bone tissue formation by cells of host origin within the implanted scaffold after 6-8 weeks. Seeded MSC appeared pivotal at the early stages of tissue development. No bone formation was observed when the porous ceramic cubes were implanted without the addition of MSC.
Green Fluorescent Protein-positive (GFP+) MSC/scaffold constructs were implanted in syngenic, GFP- recipients. Implants were harvested after 3, 7, and 11 days, and collagenase-digested to generate single-cell suspensions. Recovered cells were sorted, based on GFP expression, in order to distinguish GFP+ implanted MSC and GFP- recruited cells.
We identified two subgroups of cells, distinct for the expression of mainly two cell surface antigens, CD14 and CD45. CD14+CD45+ double-positive cells (DP) presented characteristics similar to the not specific cells recruited into implanted empty scaffolds. On the contrary, the number of CD14-CD45- double-negative cells (DN) progressively increased from 3 to 11 day implantation time. Day 7-DN cells were enriched in CD31+ endothelial cells, while day 11-DN cells were enriched in CD146+ cells and possessed osteogenic properties. This phenomenon indicated that the nature of the seeded and implanted cells influences not only the implant vascularization, but also the mobilization of host cells with an osteogenic potential, thus confirming that a tight link between host endothelial cells and host osteoprogenitor cells recruitment exists.
A deeper knowledge of the nature of stem/progenitor cells locally present or recruited in the bone regenerative niche, and a better comprehension of their cross talk and interactions are mandatory to elucidate cellular and molecular control mechanisms behind the bone formation/regeneration process and the possible translation of this knowledge to the clinical application.

Different muscarinic receptor subtypes modulate oligodendrocyte progenitor survival, proliferation and differentiation

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It is known that neurotransmitters (e.g. ACh) can modulate neurogenesis controlling neural stem and progenitor cell proliferation, as well as neuron and glial cell survival. ACh receptors have been found in several glial cell types suggesting an active role for ACh in the maturation and physiology of the glial cells. Recently we have demonstrated that rat Schwann cells express different muscarinic receptor subtypes, whose activation negatively modulates Schwann cell proliferation, promoting their progression to myelinating phenotype. This suggest that as for Schwann cells, ACh could also influence OL proliferation and/or differentiation. In the present work, using purified cultures of oligodendrocyte progenitors, we have firstly characterized the expression of muscarinic receptor subtypes at mRNA and protein levels in progenitor and mature oligodendrocytes. Oligodendrocyte progenitors (OPC) express mainly M3, M4 and M1 subtypes and low levels of M2 and M5 while mature oligodendrocytes express low level of all muscarinic subtypes. We then demonstrated that the exposure to muscarine, enhanced OPC proliferation. The muscarinic antagonists 4-DAMP, pirenzepine and tropicamide, counteracted the muscarine-induced proliferation indicating that respectively M3, M1 and M4 subtypes are involved in the control of OPC proliferation. The M2 receptors is not involved in this process, but their activation by agonist arecaidine impaired oligodendrocyte progenitor survival, inducing concentration-dependent cell death. Muscarinic receptor activation increased the expression of PDGFR-α and inhibited the expression of MBP and ErbB 3/ErbB4 receptors. These findings suggest that ACh, possibly cooperating with other molecules (e.g. growth factors), may influence the development of oligodendrocytes promoting the maintenance of immature proliferating progenitor pool.

Human cardiac stromal cells reprogramming by defined epigenetic modulators

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INTRODUCTION: We recently isolated a cardiac population of stromal cells (CMSC) that exhibited tissue-specific properties, revealing higher competence for differentiation toward the myocardial and vascular lineages than their syngeneic bone marrow counterparts. Despite their specific plasticity, CMSC did not spontaneously exhibit cardiac stem cell markers, including c-Kit and MDR-1. Since serum and epigenetic drugs, e.g. nitric oxide (NO), Retinoic Acid (RA), and phenyl butyrate (PB), can modify cell fate and induce functional reprogramming, the aim of the present work was to design an epigenetically-based strategy to enrich the CMSC population in putative cardiovascular precursors.
METHODS AND RESULTS: After a round of expansion in their regular medium, in the presence of 20% fetal bovine serum (FBS), CMSC were exposed to culture medium supplemented with 5% FBS for 3, 7 and 14 days either in the presence or in the absence of a defined "epigenetic cocktail" (EpiC) containing 5μM ATRA, 5μM PB and 200 μM Deta/NO. Different parameters were evaluated to assess the biological effects of the EpiC treatment. EpiC significantly inhibited cell proliferation, without increasing cell apoptosis, and induced the expression of pluripotency/stemness associated genes. Specifically c-Kit, VEGF-R2 and MDR-1 were up-regulated either at mRNA or protein level compared to cells cultured in complete medium or in low serum alone. Further, MDR-1 was functionally active as indicated by the rhodamine extrusion assay. In all conditions tested, the expression of Nanog, OCT4, Sox2 and Klf4 was unchanged.
CONCLUSIONS: Adult CMSC can be pharmacologically reprogrammed to express markers specific of a more immature cell population belonging to the cardio-vascular precursor family.

Human cardiac progenitor/stem cells for regeneration of heart muscle tissue in an innovative "scaffold-less" strategy: generation, characterization and in vivo implantation of cell-sheets: preliminary results

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The recently identified cardiac progenitor cells (CPC) within the myocardium are involved in cardiac homeostasis and may provide the cellular component for the ex vivo regeneration of cardiac proto-tissues to replace the damaged myocardium. To circumvent many problems related to the use of biomimetic scaffolds in regenerative medicine (suitable scaffold geometry and stiffness, possible inflammation and fibrosis, ..), an alternative "scaffold-less" tissue engineering strategy, named "cell sheet engineering", can be applied, which uses temperature-responsive polymer (e.g. poly-N-isopropylacrylamide = PNIPAAm). These sheets can be layered to build cell-dense thick ex-vivo proto-tissues to be implanted without scaffold.
CPC were isolated from human atrial heart biopsies after enzymatic digestion, migration from heart fragments and immunomagnetic selection for c-kit expression. Most selected hCPCs expressed stemness and mesenchymal markers (Sca-1-like, CD105, CD90), very few were positive for CD34, CD31, FLK-1, a few were positive for CX43. They were negative for Abcg2 and cardiac specific markers (GATA-4, Nkx 2.5, caveolin 3, MHC, ?-sarcomeric actin). hCPCs were multipotent, since they differentiated into adipocytes, osteblasts and chondrocytes. When cultured with neonatal mouse-cardiomyocytes, hCPCs could be committed to cardiomyocytic phenotype (expression of the cardiac markers GATA-4, CX43 and cardiac actinin). When seeded on PNIPAAm, hCPCs generated cell-sheets with functional cell to cell interactions (CX43 at cell membrane), retaining the expression of the stemness markers Sca-1-like, c-kit and CD105. Moreover they up-regulated genes relative to components of extracellular matrix, angiogenesis and cardiac progenitor early commitment (Islet-1 and GATA-4, myocardin), while down-regulating genes related to cell cycle progression and inhibition of differentiation, as assessed by a microarray analysis (938 genes).
Finally, in preliminary experiments, hCPC cell-sheets (labeled with vybrant staining) were implanted in immunoincompetent mice with experimentally induced infarction. Three days after implantation mice were killed and it was found that cell-sheets adhered to the heart and hCPCs started to migrate inside the heart wall.
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Dermal matrix scaffold engineered with adult mesenchymal stem cells and platelet rich plasma as a potential tool for tissue repair and regeneration

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The purpose of this study was to investigate the efficacy of Integra®, an artificial dermal matrix used as a dermal template for skin regeneration, to form a multifunctional scaffold with human bone marrow-derived mesenchymal stem cells (hMSCs) and platelet-rich plasma (PRP) for tissue engineering and regenerative technology. First, we showed that PRP, used as a supplement for growth medium represented an optimal substitute for animal serum as well as a source of multiple growth factors, was able to satisfactorily support cell viability, induce significant cell proliferation and influence the gene expression involved in stemness maintenance in hMSCs, such as Oct4 and Sox2. Moreover, Integra® appeared to be a suitable substrate for HMSCS colonization, as judged by two-photon microscopy combined with fluorescence lifetime imaging (FLIM) and confocal analysis. In the combination strategy, the cells were then seeded on Integra® + PRP for 24 and 48 hrs. Notably, in these conditions, the seeded cells exhibited a greater attitude to colonize the scaffold and showed improved cell adhesion and spreading, as compared with those cultured on Integra® alone, indicating that the bioengineered scaffold provided an appropriate environment for cellular growth and differentiation. In conclusion, the results of the present study, even though preliminary, provide inputs into the design of novel biomaterials for stem cell-based regeneration strategies to repair and restore skin functions.