SIBBM Seminar
“Frontiers in Molecular Biology”
Trieste, 26-28 May 2011

Programme & Abstracts

Sponsors


BIO-RAD


BMR
Genomics


charles river


DBA


Eurocione
serving science through innovation


PerkinElmer
For the Better


Promega Italia


QIAGEN


Roche


SIGMA-ALDRICH


TEMA
RICERCA

Thursday, 26 May

14:00-14:30 Welcome

Session I » Stem cell, reprogramming and regeneration – *Chair: Mauro Giacca*

14:30-15:10 **ABCD Lecture:** *Michele de Luca (Modena)* Human epithelial stem cells and regenerative medicine

15:10-15:35 **EMBO YIP Lecture:** *Davide Corona (Palermo)* Chromatin Binding, Nucleosome Spacing and ncRNA-mediated Regulation of the Remodeling ATPase ISWIing ATPase ISWI

15:35-16:00 *Serena Zacchigna (Trieste)* Novel therapies for old hearts

16:00-16:15 *Silvia Parisi (Naples)* Dies1: a new regulator of ESC pluripotency

16:15-16:30 *Marcello Tortorici (Pavia)* Catalysis and combinatorial assembly of histone demethylase LSD1 complexes

16:30-18:00 Coffee break and Poster Session I

18:00-18:25 *Pier Lorenzo Puri (Rome & La Jolla, CA, USA)* Epigenetic control of gene expression during muscle regeneration

18:25-18:40 *Livia Modica (Milan)* The role of Prep1 in regulating hematopoietic stem cell maintenance

18:40-18:55 *Roberta Benetti (Trieste)* b1 at the interface of cancer and stem cell function: miR-335 as a bridge

19:00 Cocktail

Friday, 27 May

Session II » Signalling pathways: how to govern differentiation proliferation and metabolism – *Chair: Valerio Orlando*

9:00-9:40 **Keynote Lecture:** *Stefano Piccolo (Padua)* TGF-beta signaling in development and cancer

9:40-10:05 *Lawrence Banks (Trieste)* The role of the Human Papillomavirus E6 oncoprotein in malignant progression

10:05-10:30 *Emilio Hirsch (Turin)* Integration of PI3K scaffold and catalytic functions in health and disease

10:30-10:45 *Mariaceleste Aragona (Padua)* Role of YAP/TAZ in mechanotransduction

10:45-11:10 Coffee break

11:10-11:35 *Guidalberto Manfioletti (Trieste)* Function of the HMGA molecular network in cancer cells

11:35-11:50 *Carolina Prezioso (Rome)* Signal dependent dynamics of two different prc2 polycomb complexes control skeletal muscle differentiation

11:50-12:05 *Marco Sandri (Padua)* Signaling pathways that control ubiquitin-proteasome and autophagy-lysosome systems in skeletal muscles

12:05-12:20 *Sara Calabretta (Rome)* Activation of MNK2/eIF4E pathway by the splicing factor SF2/ASF supports pancreatic cancer cell proliferation and survival to genotoxic stress

12:20-12:35 *Silvia Di Agostino (Rome)* Mutant p53 and Plk2 proteins are components of an oncogenic autoregulatory feedback loop

12:35-14:30 Lunch break

Session III » Postgenomic approaches to cell and molecular biology – *Chair: Stefano Piccolo (Padua)*

14:30-15:10 **Keynote Lecture:** *Gianni Cesareni (Rome)* Mapping the human phosphatome on growth pathways

15:10-15:35 *Stefano Gustincich (Trieste)* Long non-coding antisense RNA controls UchL1 translation through the 5' overlapping region and an embedded SINEB2 repeat

15:35-16:00 *Diego Di Bernardo (Naples)* Systems Biology approaches to elucidate gene function and drug mode of action

16:00-16:15 *Giulio Di Minin (Trieste)* A genome-scale protein interaction profile of Drosophila p53 uncovers additional nodes of the human p53 network

16:15-16:30 *Livia Caizzi (Turin)* Ligand independent-binding of Estrogen Receptor alpha revealed by genome-wide analysis in breast cancer cells

16:30-16:45 *Anna Sofia Eulalio (Trieste)* High-throughput functional screening identifies miRNAs controlling cardiomyocyte proliferation

16:45-17:40 Coffee break and Poster Session II

Session IV » Effective communication and publishing – *Chair: Giannino Del Sal*

17:40-18:20 *Bernd Pulverer (Chief Editor, The EMBO Journal)* The future of scientific publishing

18:20-19:10 *Eric May (Consultant and Trainer, Germany)* Communicating Science – understanding the public and the news media

19:10-20:10 General SIBBM Assembly

20:30 Social dinner

Saturday, 28 May

Session V » Cancer stem cells and epigenetics – *Chair: Giulia Piaggio*

9:30-10:10 **Keynote Lecture:** *Pier Giuseppe Pelicci (Milan)* Cancer stem cells

10:10-10:25 *Marco Napoli (Trieste)* A Pin1 / mutant p53 axis promotes aggressiveness in breast cancer

10:25-10:40 *Sara Sessa (Aviano)* Twist1 interacts with and promotes p53 degradation

10:40-11:00 Coffee break

11:00-11:25 *Salvatore Pece (Milan)* Cancer stem cells and breast carcinogenesis: a new outlook on the molecular,biological and clinical heterogeneity of breast cancers

11:25-11:50 *Salvatore Oliviero (Siena)* Myc-dependent epigenetic modifications that contribute to stemness

12:00 Chiara D'Onofrio "Giovani" Award (prize to be awarded to the best Selected Talk) and Final remarks

Oral Presentations

(in alphabetical order of presenting author)

Human epithelial stem cells and regenerative medicine

M. De Luca
Centre for Regenerative Medicine "Stefano Ferrari", Univ. of Modena and Reggio Emilia, Modena, Italy

Adult stem cells are cells with a high capacity for self-renewal that can produce terminally differentiated progeny. Stem cells generate an intermediate population of committed progenitors, often referred to as transit amplifying (TA) cells, that terminally differentiate after a limited number of cell divisions. Human keratinocyte stem cells are clonogenic and are known as holoclones. Human corneal stem cells are segregated in the limbus while limbal-derived TA cells form the corneal epithelium. Self-renewal, proliferation and differentiation of limbal stem cells are regulated by the ΔNp63 (α, β and γ), C/EBPδ and Bmi1 transcription factors. Cultivated limbal stem cells generate sheets of corneal epithelium suitable for clinical application. We report long-term (up to 10 years) clinical results obtained in an homogeneous group of 112 patients presenting with corneal opacification and visual loss due to chemical burn-dependent limbal stem cell deficiency. The corneal epithelium and the visual acuity of these patients have been restored by grafts of autologous cultured limbal keratinocytes. In post hoc analyses, success was associated with the percentage of p63-bright holoclone-forming stem cells in culture. Graft failure was also associated with the type of initial ocular damage and postoperative complications. Mutations in genes encoding the basement membrane component laminin 5 (LAM5) cause junctional epidermolysis bullosa (JEB), a devastating and often fatal skin adhesion disorder. Epidermal stem cells transduced with a retroviral vector expressing the β3 cDNA can generate genetically corrected cultured epidermal grafts able to permanently restore the skin of patients affected by LAM5-β3-deficient JEB. The implication of these results for the gene therapy of different genetic skin diseases will be discussed.

Chromatin Binding, Nucleosome Spacing and ncRNA-mediated Regulation of the Remodeling ATPase ISWIing ATPase ISWI

D.F.V. Corona
Dulbecco Telethon Institute c/o STEMBIO, Università di Palermo

Chromatin modifications, occurring without changes in the DNA sequence, set different chromatin functional states and constitute the epigenetic marks of our genome. Despite the wealth of data concerning the mechanisms of action of chromatin remodeling factors and histone modifying enzymes, relatively little is known about how their activities are coordinated and inherited to regulate chromatin structure, gene expression and other nuclear functions. Therefore, we got interested in dissecting the functional network of regulation existing between ATP-dependent remodelers and chromatin factors.
ISWI is an evolutionarily conserved nucleosome sliding factor playing essential roles in transcription, DNA replication, and chromosome organization. Using the fruit fly as a model system and a combination of genome wide and bioinformatic approaches we found that ISWI binds genes near their promoters affecting nucleosome spacing at their transcription start site. Our work shows that higher eukaryote transcription and chromosome organization is regulated genome-wide by the activity of the chromatin remodeling factor ISWI.
Further, Using an in vivo assay to identify factors regulating ISWI activity, we recovered a genetic interaction between ISWI and hsrω. The hsrω gene encodes a non-coding RNA that is essential for the assembly and organization of hnRNP-containing nucleoplasmic omega speckles. Our findings highlight a novel role for chromatin remodelers in organization of nucleoplasmic compartments, providing the first example of interaction between an ATP-dependent chromatin remodeler and a large ncRNA.

Novel therapies for old hearts

S. Zacchigna
ICGEB, Trieste

Although there has been substantial progress over recent decades in the development of pharmacological and surgical therapies for heart failure, on average these strategies increase lifespan by a few months or years at most. There are no existing drugs to 'cure' heart failure. The understanding of the molecular mechanisms leading to cardiac dysfunction, together with the recognition that the adult heart possesses a stem cell compartment, is now raising the unique opportunity to rebuild dead myocardium after infarction, to repopulate the hypertrophic, decompensated heart with functioning myocytes and new vessels and, perhaps, to reverse ventricular dilation and wall thinning. Novel therapeutic approaches, based on both gene and cell delivery, will be presented and discussed.

Dies1: a new regulator of ESC pluripotency

S. Parisi[1,2], M. Battista[1,2], A. Musto[1,3], A. Navarra[1,3], M. Stante[1], G. Minopoli[1,3] and T. Russo[1,3]
[1]CEINGE Biotecnologie Avanzate, Napoli
[2]SEMM, European School of molecular Medicine, Napoli
[3]Dipartimento di Biochimica e Biotecnologie Mediche, Università degli Studi di Napoli "Federico II", Napoli, Italy

During the past decade Embryonic Stem cells (ESCs) became particularly attractive for translational medicine for their ability to give raise to specialized and functionally active cell types. However, before using ESCs for therapeutic purposes, such as transplantation and tissue regeneration, it will be essential to improve our ability to modulate and control ESC differentiation into lineage-specific derivatives. In this context, our aim is to identify and characterize molecules able to control ESC differentiation. To achieve this goal we have developed an in vitro system based on the RNA interference technology that allowed us to identify many factors strictly required for proper neuronal differentiation. Among these candidates we have selected a gene encoding for a membrane protein that we have named Dies1 for more detailed study. Dies1 suppression blocks ESC differentiation by maintaining the cells in an undifferentiated state. We have demonstrated that this protein is required to allow neuronal differentiation by participating to the signaling pathway of BMP4 that plays a crucial role in the balance between ESC stemness and differentiation. Indeed, Dies1 suppression induces an impairment of BMP4 signalling and on the other hand it induces an up-regulation of Nodal/Activin targets. Thus, Dies1 seems to be responsible for the balance between BMP4 and Nodal pathways in the decision of ESC fate. We have demonstrated a direct interaction between BMP4 receptor complex and Dies1 by means of FRET microscopy and biochemical approaches. Finally, we are investigating the regulation of Dies1 in ESCs by microRNAs.

Catalysis and combinatorial assembly of histone demethylase LSD1 complexes

M. Tortorici, S. Pilotto, D. Bonivento, G. Ciossani, A. Karytinos, A. Mattevi
Dept of Genetics and Microbiology, Univ. of Pavia, Italy

In the last years, we have studied a nuclear protein complex formed by the association of histone deacetylase 1, a co-repressor protein CoREST, and lysine-specific histone demethylase LSD1 that specifically acts on Lys4 of histone H3[1]. Our structural studies of the CoREST/LSD1 complex highlighted a uniquely specific binding-site for the histone H3 N-terminal tail and these insights have been critical for our efforts towards structure-based development of demethylase inhibitors. These newly designed inhibitors were evaluated with a cellular model of acute promyelocytic leukemia and marked effects on cell differentiation and an unprecedented synergistic activity with anti-leukemia drugs were observed [2].
It has been recently discovered that the transcription factor Snail1 binds to LSD1/CoREST and that the three proteins are over-expressed in cancer cell lines and breast tumors. Snail1 controls the epithelial-mesenchymal transition, which is essential for numerous developmental processes (including metastasis). Structure determination of the ternary complex LSD1/CoREST/Snail1 peptide has revealed that the N-terminal residues of Snail1 bind in the active site cleft of LSD1 effectively mimicking the histone H3 tail. Therefore, Snail1 is a potential endogenous inhibitor of LSD1. [3].
A challenge for future studies will be to extend these structural investigations to visualize nucleosome binding by LSD1-containing protein complexes through biophysical methods and crystallography.

[1] Forneris F et al LSD1: oxidative chemistry for multifaceted functions in chromatin regulation. Trends Biochem Sci, 2008, 33, 181-189.
[2] Binda C et al. Biochemical, Structural, and Biological Evaluation of Tranylcypromine Derivatives as Inhibitors of Histone Demethylases LSD1 and LSD2. J Am Chem Soc 2010, 285:36849-36856.
[3] Baron R et al Molecular Mimicry and Ligand Recognition in Binding and Catalysis by the Histone Demethylase LSD1-CoREST Complex. Structure, 2011, 19, 212-220

Epigenetic control of gene expression during muscle regeneration

P.L. Puri
Dulbecco Telethon Institute (DTI), IRCCS Fondazione Santa Lucia, Rome, Italy and Sanford-Burnham Medical Research Institute, La Jolla, US

Muscle regeneration relies on the coordinated activity of a multitude of distinct cell types that compose the regeneration environment and are exposed to different external cues. Therefore, an important task is to decipher the mechanism by which regeneration cues influence the activity of different cell types in physiological and pathological conditions. Our studies have revealed the intracellular signaling cascades that convert external cues into the epigenetic information, which controls gene expression at the chromatin level in distinct populations of muscle-derived stem cells. In muscle satellite cells, we have elucidated the molecular and biochemical basis by which differentiation-activated p38 kinases direct the genome distribution and activity of two key epigenetic regulators – the Polycomb and SWI/SNF chromatin remodeling complexes. Furthermore, we have identified a HDAC-regulated network that controls the expression of specific miRNAs, which target structural components (namely the Brg1/Brm-associated factors – BAFs) of the SWI/SNF chromatin remodeling complex to form sub-complexes containing mutually exclusive BAF60 variants that direct the myogenic or the fibro-adipogenic program in a novel population of muscle-derived pluripotent cells. This unanticipated regulatory axis is an important "epigenetic disease modifier", since it provides the restriction point for dystrophic muscle decision to either undergo compensatory regeneration or adipose infiltration and fibrosis - two deleterious events in the progression of muscular dystrophies.

These results illustrate a new link between HDAC, chromatin remodelers and the fate decision of muscle-derived pluripotent cells, and identify key targets for pharmacological interventions in the treatment of muscular dystrophies.

The role of Prep1 in regulating hematopoietic stem cell maintenance

L. Modica, G. Iotti, F. Blasi
IFOM, Istituto FIRC di Oncologia Molecolare, Milano

Prep1, a homeodomain transcription factor, belongs to the TALE family and plays an essential role during early development. A hypomorphic mutation of the Prep1 gene ($Prep1^{i/i}$) causes embryonic lethality between E17.5 and P0 with a pleiotropic embryonic phenotype that includes defects in all hematopoietic lineages. Deficiency in hematopoietic stem cells (HSCs) might be responsible for the downstream hematopoietic phenotype observed.
We observed that Long-Term Hematopoietic Stem Cells (LT-HSCs), identified as $Lin^{-}Sca1^{+}ckit^{+}CD150^{+}CD48^{-}CD41^{-}$ in E14.5 fetal livers (FLs), are strongly reduced in Prep1i/i FLs compared to wild type (wt), and show strongly impaired ability to form colonies in LTC-IC assays *ex vivo*. *In vivo* limiting dilution experiments have shown a reduced frequency of LT-HSCs in $Prep1^{i/i}$ FLs compared to wt. Furthermore, in long-term competitive transplantation assays, $Prep1^{i/i}$ HSCs are considerably less efficient than wt cells in establishing HSC and progenitor compartments in lethally irradiated repicients. Importantly, the $Prep^{i/i}$ stem cell pool shows defects in self-renewal ability.
Interestingly FL $Prep1^{i/i}$ LT-HSCs also showed a strongly reduced G0 pool suggesting a role of Prep1 in the maintenance of the sel-renewing quiescent HSC pool. Moreover, we could observe the activation of proliferative pathways in $Prep1^{i/i}$ LT-HSCs as demonstrated by the increased phosphorilation of Stat1 and by the overexpression of Sca1. This feature might force HSCs towards the active proliferating state.
All these data suggest a crucial role of Prep1 in regulating critical features of HSCs.

Rb1 at the interface of cancer and stem cell function: miR-335 as a bridge

M. Scarola[1], S. Schoeftner[2], C. Schneider[1], R. Benetti[1]
[1]LNCIB, Area Science Park, Cancer Epigenetic Group, Trieste
[2]Present position: IEO - Istituto Nazionale Tumori Regina Elena, Roma

Loss of function mutations of Retinoblastoma family (Rb) proteins drive tumorigenesis by overcoming barriers to cellular proliferation. Consequently, factors modulating Rb function are of great clinical import.
We show that miR-335 is differentially expressed in human cancer cells and that it tightly regulates the expression of Rb1 (pRb/p105) by specifically targeting a conserved sequence motive in its 3'UTR. We found that by altering Rb1 (pRb/p105) levels, miR-335 activates the p53 tumor suppressor pathway to limit cell proliferation and neoplastic cell transformation. DNA damage elicited an increase in miR-335 expression in a p53-dependent manner. miR-335 and p53 cooperated therefore in a positive feedback loop to drive cell cycle arrest. Together, these results indicate that miR-335 helps control proliferation by balancing the activities of the Rb and p53 tumor suppressor pathways.
Further developments on the role of miR-335 will be anticipated using mouse embryonic stem cells (mESC), which are an ideal and mutation free model system to study retinoblastoma function in the context of proliferation control, self renewal and differentiation.

TGF-beta signaling in development and cancer

S. Piccolo
Dept Medical Biotechnologies (DIMBM), Univ. of Padua, Padua, Italy

How does a cell read TGF-beta signaling? I will present an overview of the TGF-beta signaling cascade, starting from a description of its basic components and of the apparent simplicity of its cascade. Then I will present examples of how the pathway is regulated in quantitative and qualitative ways to order to achieve specificity of effects and precise dosage of signaling intensity and duration. Crucial for these controls is the crosstalk with other signaling pathways. As biological paradigms, I will refer to embryonic development, where TGF-beta family members control early patterning of pluripotent cells, and tumor progression, during which TGF-beta responsiveness changes from tumor suppressive to pro-metastatic.

The role of the Human Papillomavirus E6 oncoprotein in malignant progression

L. Banks
International Centre for Genetic Engineering and Biotechnology ICGEB, Trieste, Italy

Human Papillomaviruses are the causative agents of cervical cancer, which is the second major cause of cancer-related death in women worldwide. A striking feature of these tumours is the continued expression of the two viral oncoproteins, E6 and E7, many years after the initial immortalising events. Indeed, ablation of their expression causes cessation of tumour growth. Thus both viral oncoproteins represent excellent targets for therapeutic intervention in HPV-induced malignancy.Only a small number of HPV types are associated with cancer development, and these are collectively referred to as high-risk types, with HPV-16 and HPV-18 being the most prevalent. Numerous studies have been performed to investigate the mechanism by which E6 contributes to malignant progression, and interactions with p53 and a subset of PDZ domain-containing cellular proteins appear critical. We are interested in the association between E6 and two such PDZ domain-containing substrates, Discs Large and Scribble, which are potential tumour suppressors and are involved in the regulation of cell polarity. Data will be presented that defines how E6 interacts with these substrates and how different HPV types might perturb their respective functions. Furthermore we will provide evidence that targeting Discs Large enhances the resistance of HPV infected cells to undergo anoikis, whist targeting Scribble enhances the malignant potential of HPV transformed cells.

Integration of PI3K scaffold and catalytic functions in health and disease

E. Hirsch
Molecular Biotechnology center, University of Torino, Torino, Italy

Phosphoinositide 3-kinases (PI3K) are key players in receptor-mediated signal transduction and play a role in a large variety of biological processes. PI3K consist of heterodimers of a 110 kD catalytic (p110) as well as a regulatory/adapter subunit and are required for the production of a membrane bound phosphorylated lipid (PIP3) that acts as a secondary messenger molecule. Class I p110s (p110α, β, γ and δ) share significant homology but studies using genetically engineered mice show that they all play non-redundant roles. While these reports recently provided support for PI3K as promising drug targets they also unexpectedly revealed that these proteins not only work as kinases but also as scaffolds for protein-protein interactions. For example, we showed that p110β catalytic activity is required for male fertility and for Erbb2 driven mammary gland cancer development. On the other, hand loss of p110β blocks endocytosis and results in reduced fibroblast proliferation and embryonic lethality. Similarly, p110γ plays a crucial role in the mounting of inflammatory reactions as well as in leukemia but it is also part of a complex that, independently of its kinase function, controls cardiac contractility. Indeed, we found that p110γ is a scaffold protein for a complex that mediates a reciprocal control between cAMP and PIP3 signaling. These data not only support the view of PI3K as drug targets for ATP binding site competitors but also for more sophisticated protein-protein interaction inhibitors.

Role of YAP/TAZ in mechanotransduction

S. Dupont[1], L. Morsut[1], M. Aragona[1], E. Enzo[1], S. Giulitti[2], M. Cordenonsi[1], F. Zanconato[1], M. Forcato[3], S. Bicciato[3], N. Elvassore[2], S. Piccolo[1]
[1]Dept of Histology, Microbiology and Medical Biotechnologies, Univ. of Padua School of Medicine, Padua, Italy
[2]Dept of Chemical Engineering (DIPIC), Univ. of Padua, Padua, Italy
[3]Center for Genome Research, Dept of Biomedical Sciences, Univ. of Modena and Reggio Emilia, Modena, Italy

Cells perceive their microenvironment not only through soluble signals but also in term of physical and mechanical cues, such as extracellular matrix (ECM) rigidity or confined adhesiveness. By mechanotransduction systems, cells translate these stimuli into biochemical signals controlling multiple aspects of cell behavior, including growth, differentiation and cancer progression; but how mechanosensing is ultimately linked to activity of nuclear transcription factors remains poorly understood. Here we report the identification of the transcriptional coactivators YAP/TAZ as nuclear relays of mechanical signals exerted by ECM rigidity and cell-shape. This regulation requires Rho activity and tension of the acto-myosin cytoskeleton but is independent from the Hippo cascade. Crucially, YAP/TAZ are functionally required for differentiation of mesenchymal stem cells induced by ECM rigidity and for survival of endothelial cells regulated by cell geometry; conversely, forced expression of activated YAP dominates over physical constraints in dictating cell behavior. These findings identify YAP/TAZ as sensors and mediators of mechanical cues instructed by the cellular microenvironment.

Function of the HMGA molecular network in cancer cells

R. Sgarra, S. Pegoraro, E. Maurizio, G. Ros, L. Arnoldo, I. Pellarin, G. Manfioletti
Dept Life Sciences, Univ. of Trieste

The High Mobility Group A (HMGA) proteins constitute a family of nuclear factors that play important roles in the execution of multiple biological processes in eukaryotic cells. These architectural factors, by interacting with AT-rich DNA sequences and nuclear factors, are able to assemble or modulate DNA/nucleoprotein macromolecular complexes thus participating in a variety of biological processes such as embryogenesis, differentiation, and neoplastic transformation. HMGA overexpression is a constant feature of human malignant neoplasms and in the last years the causal role of HMGA proteins in neoplastic transformation has been firmly established using both cellular and animal models.
With the advent of proteomic tools for the identification of protein-protein interactions, the number of HMGA molecular partners has increased rapidly. This has led to the extension of our knowledge of the functional involvement of HMGA from the transcriptional regulation field to RNA processing, DNA repair, and chromatin remodelling and dynamics.
We addressed the relevance of the molecular network orchestrated by HMGA in cancer cells by silencing the expression of HMGA1 in the highly aggressive and metastatic triple-negative MDA-MB-231 breast cancer cell line. HMGA1-depleted cells undergo a dramatic change in the neoplastic phenotype: they grow in 2D as a regular monolayer, they assume acini-like structures in 3D culture, and moreover they show an inhibition of migration and invasion. Molecular profiling of gene expression show a consistent alteration in pathways related to tumor progression. Altogether these results demonstrate that HMGA1 coordinates a chromatin network that is crucial in maintaining the metastatic potential in MDA-MB-231 cancer cells.

Signal dependent dynamics of two different prc2 polycomb complexes control skeletal muscle differentiation

Z. Jasencakova[1,9,*], C. Prezioso[1,*], L. Stojic[1,7,*], A. Stutzer[2], B. Bodega[1,3], D. Pasini[4,8], R. Klingberg[5], R. Margueron[6], D. Schwarzer[5], K. Helin[4], W. Fischle[2], V. Orlando[1]
[1]DTI, IRCCS Fondazione Santa Lucia, Rome, Italy
[2]Max Planck Institute for Biophysical Chemistry, Gottingen, Germany
[3]Dept of Biology and Genetics for Medical Sciences, Univ. of Milan, Milan, Italy
[4] BRIC, Univ. of Copenhagen, Copenhagen N, Denmark
[5] FMP, Dept of Chemical Biology , Berlin, Germany
[6] Curie Institute,Paris, France
[7] Present address: Dept of Oncology, Cancer Research UK Cambridge Research Institute, Cambridge, UK
[8] Present address: Dept of Experimental Oncology, IFOM IEO Campus, Milan, Italy
[9] Present address: BRIC, Univ. of Copenhagen, Copenhagen N, Denmark
*equal contribution

PcG proteins, the transcriptional repressors that prevent changes in cell identity by chromatin modifications, form two major complexes, PRC1 and PRC2. Although much is known about the PcG regulated processes, little is known about their dynamics and the relative signalling dependent pathway during skeletal muscle cell differentiation. We used three different systems, the C2C12 mouse cell line, the human myoblasts and the satellite cells as skeletal muscle differentiation model to gain insight into the role of different PRC2 components during this process and into the signalling pathways that regulates PRC2 dynamics at muscle loci. We report that two different PRC2 complexes are present during skeletal muscle differentiation: the classical PRC2 complex, PRC2 EZH2, that is predominat in myoblasts, and the novel PRC2 EZH1, containing EZH1 but devoid of EZH2, specific for post-mitotic myotubes. Interestingly, these two PRC2 complexes are differentially associated with muscle regulatory regions. In particular, we show that PRC2 EZH1 complex seems to replace PRC2 EZH2 complex at Myog promoter during the switch from myoblasts to myotubes and this dynamics regulates its correct transcriptional activation timing, via recruitment of MyoD and RNA Polymerase II. In respect with their opposite chromatin association, we verified that a MSK1 dependent signaling, controlling H3S28ph, is specifically involved in PRC2 EZH2 displacement from Myog and mCK regulatory regions, allowing muscle differentiation. As expected, PRC2 EZH1 chromatin binding is not affected by MSK1 H3S28ph. Thus, our data add another important layer of epigenetic regulation of skeletal muscle differentiation, introducing a novel function of PRC2 Ezh1 complex in post mitotic cells.

Signaling pathways that control ubiquitin-proteasome and autophagy-lysosome systems in skeletal muscles

M. Sandri[1,2,3]
[1]Dept Biomedical Science, Univ. of Padova, Italy
[2]Venetian Institute of Molecular Medicine, Padova, Italy
[3]Dulbecco Telethon Institute, Padova; Italy

Muscle mass represents 40-50% of human body and, in mammals, is one of the most important sites for the control of metabolism. Moreover, during catabolic conditions, muscle proteins are mobilized to sustain gluconeogenesis in liver and to provide alternative energy substrates for organs. However excessive protein degradation in skeletal muscles is detrimental for the economy of body and it can lead to death. Ubiquitin-proteasome and autophagy-lysosome systems are the major proteolytic pathways of the cell and are coordinately activated in atrophying muscles. However the role and the regulation of autophagy pathway in skeletal muscle is still largely unknown. We will focus on regulation of ubiquitin-proteasome system and autophagy pathway and discuss their beneficial or detrimental role for the maintenance of muscle mass.

Activation of MNK2/eIF4E pathway by the splicing factor SF2/ASF supports pancreatic cancer cell proliferation and survival to genotoxic stress

S. Calabretta[1,2]*, L. Adesso[1,2]*, F. Barbagallo[2], R. Geremia[2], G. Capurso[1], G. Delle Fave[1], C. Sette[2]
[1]Medical surgical department of tecnobiomedical clinical sciences and translational medicine, Univ. of Rome La Sapienza, Rome, Italy
[2] Dept of Public Health and Cell Biology, Univ. of Rome Tor Vergata, Rome, Italy
*The autors contributes equally

Alternative splicing modulates the expression of oncogenic protein variants. Splicing factors regulating this process can themselves act as oncogenes, sustaining cell transformation and tumor progression. To understand if splicing factors are involved in resistance to chemotherapy, we chronically exposed pancreatic cancer cells with gemcitabine. Among the splicing factors tested, expression of SF2/ASF was reproducibly increased. Importantly, SF2/ASF overexpression is sufficient to transform fibroblast by controlling the alternative splicing of many genes, including the MNK2 kinase. The MNK family comprise two genes, MNK1 and 2, that are alternatively spliced to yield two variants, a and b. The main MNK substrate is the translation factor eIF4E, whose phosphorylation is strongly increased in cancer. We found that gemcitabine induced SF2/ASF-dependent splicing of the MNK2b variant, which displays the highest basal activity toward eIF4E and is independent of upstream MAPK signalling. Importantly, eIF4E phosphorylation is stimulated in response to chemotherapeutic treatment of pancreatic cancer cells, in concomitance with upregulation of the MNK2b variant. Depletion of the endogenous MNK2 protein abolished eIF4E phosphorylation and decreased cell proliferation and survival to gemcitabine. The same effects were obtained by preventing eIF4E phosphorylation with pharmacological inhibition of MNK activity. Immunohistochemistry of patient specimens indicated that eIF4E phosphorylation represents an independent prognostic factor of pancreatic cancer, which predicts early disease onset and worse prognosis. Thus, treatment of pancreatic cancer cells with gemcitabine triggers a positive feedback through SF2/ASF-mediated splicing of MNK2b and phosphorylation of eIF4E, which protects cancer cells from drug-induced genotoxic stress. These results suggest that inhibition of MNK activity might represent a novel therapeutic strategy for this non curable disease.

Mutant p53 and Plk2 proteins are components of an oncogenic autoregulatory feedback loop

S. Di Agostino[1], F. Fausti[2], T. Shay[3], F. Biagioni[1], G. Fontemaggi[1], E. Domany[3], M.B. Yaffe[4], S. Strano[2], G. Blandino[1]
[1]Translational Oncogenomics Unit, Regina Elena Cancer Institute, Rome Italy
[2]Molecular Chemoprevention Group, Scientific Direction, Regina Elena Cancer Institute, Rome Italy
[3]Department of Physics of Complex Systems, Weizmann Institute of Science, Rehovot, Israel
[4]David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA

Aberrant activation of kinases has emerged to be a key event along with tumor progression, maintenance of tumor phenotype and response to anticancer treatments. This study documents the existence of an oncogenic auto-regulatory feedback loop that includes the Polo-like kinase-2 (Snk/Plk2) and mutant p53 proteins. Plk2 protein binds to and phosphorylates mutant p53, thereby potentiating its oncogenic activities. Phosphorylated mutant p53 binds more efficiently to p300 consequently strengthening its own transcriptional activity. Plk2 gene is regulated at a transcriptional level by both wt- and mutant p53 proteins. This leads to growth suppression or enhanced cell proliferation and chemo-resistance, respectively. In turn, the siRNA-mediated knock down of either mutant p53 or Plk2 proteins significantly curtails the growth properties of tumor cells and their chemo-resistance to anticancer treatments. Therefore, this paper identifies a novel tumor network including Plk2 and mutant p53 proteins whose triggering in response to DNA damage might disclose important implications for the treatment of human cancers.

Mapping the human phosphatome on growth pathways

F. Sacco[1], PF. Gherardini[1] , A. Ragnini-Wilson[1,2], S. Paoluzi[1], M. Helmer-Citterich[1], L. Castagnoli[1], G. Cesareni[1]
[1]Dept of Biology, University of Rome "Tor Vergata", Italy
[2]High-throughput Microscopy facility; Dept of Translational and Cellular Pharmacology, Consorzio Mario Negri Sud, SM. Imbaro, Italy

The ability to address, on a large scale, the functional consequences of knocking down the expression of any gene of interest has considerably sped up gene annotation in complex eukaryotic systems. Typically, the consequences of interfering, by siRNA, large collections of genes, up to entire genomes, on any convenient phenotypic readout can be investigated by established approaches. Thus, genes may be associated to a function of interest if the alteration of their gene products perturbs the phenotypic readout. The mapping procedure, however, is low resolution because, given the intricacy of the gene interaction web in the cell, two genes affecting the same readout may map to different, distant signaling pathways.
We have set out to develop an approach that could map, at a higher detail, gene products onto complex pathways. We focused on the task of mapping the 300 human phosphatase gene products onto the growth pathways that respond to cytokine, growth factors and nutrients. To this end we have used a high content phenotypic screening based on siRNA and automated fluorescence microscopy and we have monitored the cell state after knocking down each of the phosphatase genes. Cell state is a "complex" phenotype defined by a combination of five readouts monitoring the activation of five key "sentinel" proteins chosen for their centrality in the pathways and for the robustness of the activation assay.
By modeling the available information on the growth pathways that we are testing we can predict the effects of perturbing each node of interest on the cell state defined by the activation/inactivation pattern of the sentinel proteins.
Finally, by matching the experimentally determined cell states with the one predicted by the pathway model we can infer the pathway nodes that are likely to be affected by the phosphatase knock down.

Long non-coding antisense RNA controls UchL1 translation through the 5' overlapping region and an embedded SINEB2 repeat

C. Carrieri[1], L. Cimatti[1], A. Beugnet[2], C. Santoro[3], S. Zucchelli[1,4], P. Carninci[5], S. Biffo[3,6], E. Stupka[7], S. Gustincich[1,8]
[1]Sector of Neurobiology, International School for Advanced Studies (SISSA), Trieste, Italy
[2]Laboratory of Molecular Histology and Cell Growth, DIBIT, San Raffaele Scientific Institute, Milano, Italy
[3]Dept of Medical Sciences, Univ. of Eastern Piedmont, Novara, Italy
[4]Istituto Italiano di Tecnologia IIT, SISSA Unit
[5]Omics Science Center, RIKEN Yokohama Institute, 1-7-22 Suehiro-chô, Tsurumi-ku, Yokohama, Kanagawa, Japan
[6]Dept of Environmental and Life Sciences, Univ. of Eastern Piedmont, Alessandria, Italy
[7]UCL Cancer Institute, Paul O' Gorman Building, University College London, London, UK
[8]The Giovanni Armenise-Harvard Foundation Laboratory, Trieste, Italy

Pervasive transcription of the mammalian genome involves long non coding RNA (lncRNA) and repetitive sequences, such as SINEs (short interspersed nuclear element). A large percentage of these transcripts are nuclear-enriched with unknown function. Antisense lncRNAs may participate in sense/antisense pairs (S/AS) overlapping with a protein coding gene on the opposite strand and regulate epigenetic silencing, transcription and mRNA stability.
Here we identify AS Uchl1 as a nuclear-enriched lncRNA AS to Uchl1, a gene involved in neurodegenerative diseases. AS Uchl1 is expressed in dopaminergic neurons of the mesencephalon, the site of degeneration in Parkinson's disease. Its transcription is under control of Nurr1, a major transcription factor involved in differentiation and maintenance of dopaminergic neurons. AS Uchl1 RNA is down-regulated in neurochemical models of PD in vitro and *in vivo*.
AS Uchl1 increases UchL1 protein synthesis at post-transcriptional level, identifying a new functional class of lncRNAs. AS Uchl1 activity depends on the presence of a 5' overlapping sequence and an embedded inverted SINEB2 element. In addition, mTORc1 inhibition by rapamycin causes an increase in UchL1 protein that is associated to shuttling of AS Uchl1 RNA from the nucleus to the cytoplasm and recruitment of the overlapping sense protein-encoding mRNA to active polysomes for translation.
Thus, AS Uchl1 is the first identified lncRNA able to stimulate translation of specific mRNAs, in conditions in which CAP-dependent translation is reduced.

Systems Biology approaches to elucidate gene function and drug mode of action

D. di Bernardo
Telethon Institute of Genetics and Medicine & University of Naples "Federico II", Naples

One of the main aim of Systems Biology is inferring, or ‘reverse-engineering’, gene networks. This process can be defined as that of identifying gene interactions from experimental data through computational analysis.We will show how we used these techniques to identify the function of disease genes and to identify "master regulators" of gene signatures. In addition, we will also present published results on a new method to elucidate the mode of action of drugs using gene expression profiles following treatments with more then 1300 compounds.

A genome-scale protein interaction profile of Drosophila p53 uncovers additional nodes of the human p53 network

G. Di Minin [1,2,*], A. Lunardi[1,2,*], P. Provero[3], M. Dal Ferro[1,2], M. Carotti[1,2], G. Del Sal[1,2], L. Collavin[1,2]
[1]Laboratorio Nazionale Consorzio Interuniversitario per le Biotecnologie (LNCIB), Area Science Park, Trieste, Italy
[2]Dipartimento di Scienze della Vita, Univ. degli Studi di Trieste, Trieste, Italy
[3]Molecular Biotechnology Center and Dipartimento di Genetica, Biologia e Biochimica, Università degli Studi di Torino, Torino, Italy
[*]GDM and AL contributed equally to this work

The genome of the fruitfly Drosophila melanogaster contains a single p53-like protein, phylogenetically related to the ancestor of the mammalian p53 family of tumor suppressors.We reasoned that a comprehensive map of the protein interaction profile of Drosophila p53 (Dmp53) might help identify conserved interactions of the entire p53 family in man. Using a genome-scale in vitro expression cloning approach, we identified 91 previously unreported Dmp53 interactors, considerably expanding the current Drosophila p53 interactome. Looking for evolutionary conservation of these interactions, we tested 41 mammalian orthologs and found that 37 bound to one or more p53-family members when overexpressed in human cells. An RNAi-based functional assay for modulation of the p53 pathway returned five positive hits, validating the biological relevance of these interactions. One p53 interactor is GTPBP4, a nucleolar protein involved in 60S ribosome biogenesis. We demonstrate that GTPBP4 knockdown induces p53 accumulation and activation in the absence of nucleolar disruption. In breast tumors with wild-type p53, increased expression of GTPBP4 correlates with reduced patient survival, emphasizing a potential relevance of this regulatory axis in cancer.

Ligand independent-binding of Estrogen Receptor alpha revealed by genome-wide analysis in breast cancer cells

L. Caizzi[1,2], S. Cutrupi[1,4], A. Testori[1,3], D. Corà[1,3], F. Cordero[1,6], O. Friard[1,4], C. Ballare[8], R. Porporato[3], G. Giurato[7], A. Weisz[7], E. Medico[1,3], M. Caselle[1,5], L. Di Croce[8,9], M. De Bortoli[1,3]
[1]Center for Molecular Systems Biology, Univ. of Turin, Italy; [2]Bioindustry Park Silvano Fumero, Colleretto Giacosa, Italy; [3] Dept Oncological Sciences, SP142, Candiolo, Italy; [4]Dept of Human and Animal Biology, Turin, University of Turin, Italy; [5] Dept of Theoretical Physics, Turin, Univ. of Turin, Italy; [6] Dept of Computer Science, Turin, Univ. of Turin, Italy; [7]Dept of General Pathology, Second Univ. of Naples, Italy; [8]Center for Genomic Regulation, Barcelona, Spain; [9]ICREA and Center for Genomic Regulation, Barcelona, Spain

The growth and differentiation of epithelial mammary cells, as well as mammary tumor development, is critically dependent on the action of estrogenic hormones mediated by Estrogen Receptors. Recently, it has been shown that ligand independent activity of Estrogen Receptor alpha (ERα) is essential to maintain basal expression of epithelial genes in breast cancer cells (Cardamone et al., 2009). In this work, we set out to examine the binding of ERα to chromatin and possible epigenetic and transcriptional effects in human breast cancer cells. First, available ERα ChIP-seq datasets from experiments of MCF7 and T47D cells cultured in estrogen-deprived conditions (Cicatiello et al., 2010; JS Carroll, unpublished) were explored and the data obtained from these experiments crossed. Individual ERα ChIP-qPCR analysis was used to validate a number of peaks in MCF7 cells transfected with control or ERα siRNA. This approach confirmed a number of bona-fide ERα binding sites, both in promoters or in intragenic locations, in absence of ligand. Next, using the same experimental setting, i.e. MCF7 cells transfected with control and ERα siRNA, ERα ChIP Seq was performed. 10,778 ER-binding peaks (p-value 0.005) were found. Constitutive ERα peaks were present in intronic and intergenic regions (45.90% and 43.93%, respectively) as well as in gene promoters and exonic regions (4.62% and 2.51%, respectively). The search for transcription factor binding sites showed significant enrichment for EREs motifs (identified in 47% of ER-binding peaks), as well as a number of other putative binding motifs (AP1, AP2, RXR, CTCF). Furthermore, we have performed microarray experiments in the same conditions, obtaining a list of genes that could be regulated by ERα knockdown, suggesting that ERα may indeed regulate gene expression in a ligand independent manner. Analysis of histone PTMs in and around constitutive binding sites is under way.

High-throughput functional screening identifies miRNAs controlling cardiomyocyte proliferation

A. Eulalio, M. Mano, M. Giacca
Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

In mammals, enlargement of the heart during embryonic development is primarily dependent on the increase in cardiomyocyte number, but shortly after birth cardiac myocytes stop proliferating and further growth of the myocardium occurs through hypertrophic enlargement of existing myocardial cells. As a consequence of the limited proliferation capacity of adult cardiomyocytes, the ability of the mammalian heart to regenerate itself following injury is very restricted. The exact molecular mechanisms controlling proliferation and differentiation of cardiac myocytes remain largely unknown.
A novel, exciting possibility is that miRNAs, evolutionarily conserved small noncoding RNAs that regulate gene expression at the post-transcriptional level, control cardiomyocyte proliferation. Several miRNAs have been implicated in different aspects of heart function, though only two of them have so far clearly been shown to take part in the control of cardiomyocyte proliferation, miR-1 and miR-133. Taking into consideration the vast number of miRNAs and the impact that the few examined so far have in heart development and disease, it is clear that further functions of miRNAs in cardiac function are awaiting discovery.
We performed a high-content, fluorescence microscopy-based high-throughput screening in rat neonatal cardiomyocytes using a library of miRNA mimics using different read-outs for cell proliferation (EdU incorporation, Ki-67 expression, phospho-histone H3 and AuroraB-kinase positivity). We identified several miRNAs able to significantly increase cardiomyocyte proliferation up to 4-fold, as well as miRNAs able to completely block proliferation. Ongoing work aims at characterizing the mechanisms underlying the function of the identified miRNAs with the ultimate goal of exploiting the ensuing knowledge to promote cardiomyocyte proliferation in vivo by modulation of miRNA levels, which may be the basis for the development of novel therapeutic approaches against heart failure.

The future of scientific publishing

B. Pulverer
Chief Editor, The EMBO Journal

Publishing in selective journals is an increasingly important component of research assessment. It is therefore essential that reputable journals optimize peer review and editorial processes, and that they set strong publication policies. Access to research information has changed dramatically with advanced search functionality and on account of the exponentially growing, increasingly specialized literature. Still, online technology has not bee exploited to its full potential in Scientific publishing. I will outline recent enhancements at EMBO to fortify the publishing processes and EMBO's vision of the 'paper of the future'.

Communicating Science – understanding the public and the news media

Eric May
Media Consultant

Participants will get practical skills and techniques to understand the sources of commonly held perceptions and stereotypes about science; express complex topics clearly for non-scientific audiences; identify compelling story angles that attract different types of audiences; identify key priorities for communicating science effectively to the news media and the general public.

A Pin1 / mutant p53 axis promotes aggressiveness in breast cancer

M. Napoli[1,2], J.E. Girardini[1,2,#], A. Rustighi[1,2], S. Piazza[1], C. Marotta[1,2], V. Capaci[1,2], E. Scanziani[3], A.Thompson[4,5], A. Rosato[6], T. Crook[4], A. R. Means[7], G. Lozano[8], G. Del Sal[1,2]
[1]Laboratorio Nazionale CIB (LNCIB), Area Science Park, Trieste, Italy
[2]Dip. Scienze della Vita, Università degli Studi di Trieste, Italy
[3]Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Università di Milano, Italy
[4]Dundee Cancer Centre, University of Dundee, UK
[5]Department of Surgical Oncology, MD Anderson Cancer Center, Houston, Texas, USA
[6]Dip. di Scienze Oncologiche e Chirurgiche, Università di Padova, Italy
[7]Dept of Pharmacology and Cancer Biology, Duke University, Durham, USA
[8]Dept of Cancer Genetics, M.D. Anderson Cancer Center, Houston, Texas, USA
[#]current address: Instituto de Biologia Molecular y Celular de Rosario (IBR-CONICET), Argentina

TP53 missense mutations dramatically influence tumor progression, however their mechanism of action is still poorly understood. Here we demonstrate the fundamental role of the prolyl isomerase Pin1 in mutant p53 oncogenic functions. Pin1 enhances tumorigenesis in a Li-Fraumeni mouse model and cooperates with mutant p53 in Ras-dependent transformation. In breast cancer cells, Pin1 promotes mutant p53 dependent inhibition of the anti-metastatic factor p63 and induction of a mutant p53 transcriptional program to increase aggressiveness. Furthermore, we identified a transcriptional signature associated with poor prognosis in breast cancer and, in a cohortof patients, Pin1 overexpression influenced the prognostic value of p53 mutation. These results define a Pin1/mutant p53 axis that conveys oncogenic signals to promote aggressiveness in human cancers.

Twist1 interacts with and promotes p53 degradation

S. Sessa, F. Pivetta, S. Piccinin, R. Maestro
Unit of Experimental Oncology 1, CRO Aviano National Cancer Institute, Aviano (PN)

Twist1, a bHLH transcriptional factor, plays a critical role in mesodermal development. Recently, a role for Twist1 as an oncogene has been proposed. In fact, Twist1 has been shown to inhibit the p53 pathway and to promote EMT (epithelial-mesenchymal transition). Moreover, Twist1 has been shown to play a role in stemness.
In particular, we have demonstrated that Twist1 antagonizes oncogene-induced apoptosis and senescence by interfering with the p53 pathway, at least in part by repressing ARF expression. However, Twist1 is capable of antagonizing the p53 response also in ARF null settings. Indeed, we collected evidence that Twist1 interacts with the C-terminal regulatory region of p53 through the Twist box.
Here we provide evidence that Twist1 promotes MDM2-mediated degradation of p53 independently of ARF. Intriguingly, we found that Twist1 is capable of binding also MDM2, disclosing the possibility that Twist1 may favour p53 degradation by bridging the interaction between p53 and its ubiquitin ligase.

Cancer stem cells and breast carcinogenesis: a new outlook on the molecular,biological and clinical heterogeneity of breast cancers

S. Pece
Molecular Medicine for Care Program, Istituto Europeo di Oncologia, Milan, Italy
Milan Univ. Medical School, Milan, Italy

The emerging view of the stem cell origin of cancer holds that a handful of cells, functionally identifiable as cancer stem cells, sit at the heart of tumorigenesis representing the true responsible for the onset and development of tumors. Several lines of evidence also converge on the idea that cancer stem cells are also responsible for therapy failure and disease recurrence. A corollary of this concept, of paramount importance for its clinical implications, is that cancer stem cells also hold the key for the definitive cure of cancer. Related to breast cancer, we have recently highlighted the crucial molecular traits of normal and cancer stem cells, thus paving the way for the development of clinical strategies to improve the management of breast cancer patients. For instance, we have provided knowledge that the intrinsic content of cancer stem cells in breast tumors is proportionally higher in biologically aggressive and poor prognosis breast tumors compared to well-differentiated and good prognosis ones. Relevant to patient stratification, we found that the cancer stem cell content of tumors can be used to distinguish breast cancer patients according to their pathological, molecular and clinical features, based on the use of suitable biomarkers for the identification of cancer stem cells in routine histopathological procedures. We will discuss how these findings, once subjected to extensive clinical validation, are susceptible to refine the currently available algorithms for diagnosis, prognosis and prediction of treatment responsiveness through the introduction of novel cancer stem cell biomarkers, and therefore to guide more accurately tailored clinical choices in the clinical management of the breast cancer neoplastic disease.

Myc-dependent epigenetic modifications that contribute to stemness

F. Neri, A. Zippo, A. Krepelova, A. Cherubini, M. Rocchigiani, S. Oliviero
Dip Biotecnologie, Siena, Italy

Mouse embryonic stem cells (ESC) pluripotency and self renewing capabilities rely on independent regulatory networks which include the stem cell factors Oct3/4, Sox2 and Nanog, the Polycomb complexes, micro RNAs and Myc. Myc over-expression in adult cells induces cell proliferation, metabolism, and block of differentiation. In cooperation with other oncogenes like mutated Ras Myc induces cell transformation while it cooperates with Oct3/4, Sox2, and Klf4 to reprogram adult differentiated cells into induced pluripotent stem cells (iPS), acting at an early stage during the process of reprogramming. Thus, Myc acts both as an inducer of cell cycle and metabolism but also a repressor of genes involved in differentiation. We studied in more detail this latter aspect of Myc regulation as this mechanism is relevant both in cell reprogramming and in transformation. We observed that Myc is a direct regulator of the Polycomb repressive complex 2 (PRC2) both in fibroblasts and in ES cells. Silencing of Myc proteins in ES cells leads to the reduction of expression of all the components of the PRC2 complex, resulting in a global reduction of H3K27me3 and a specific reduction of the H3K27me3 mark at "bivalent" genes. We suggest that Myc-dependent epigenetic modification at developmentally regulated genes are required for ES cells pluripotency ability and contribute to revert the differentiated phenotype in adult cells. Implications in transformation will be discussed.

Poster Abstracts

P1

Insights into Ca^{2+} oscillations generated by neurons in organotypic spinal slices

I. Abate, A. Fabbro, L. Ballerini
Life Science Dept, Univ. of Trieste, Trieste, Italy

In the spinal cord, intracellular Ca2+ dynamics are thought to be crucial signals contributing to the maturation of motor networks into functional circuits. In fact, Ca2+ signals might detail the emergence of specific neuronal phenotypes or guide the formation of cellular connectivity. Organotypic slice cultures developed from the embryonic mouse spinal cord recapitulate, during in vitro growth, several molecular and physiological events of spinal networks formation usually described in vivo. Using this model system we characterized distinct forms of Ca2+ activities, such as waves, bursts and oscillations. Here we focused on a repetitive Ca2+ signal generated by small clusters of ventral spinal neurons: the so-called Ca2+ oscillations, displaying a specific duration and period.
We used organotypic embryonic spinal slices at the second week of in vitro development to study the possibility to modulate the appearance of Ca2+ oscillations by transiently interfering with the intracellular Ca2+ handling. In particular, by means of Ca2+ imaging techniques, we monitored the effect of an acute or chronic treatment with 30 μM BAPTA-AM, an intracellular Ca2+ chelator, on Ca2+ oscillations generated by small groups of ventral neurons. We observed that acute BAPTA-AM application completely suppressed Ca2+ oscillations. In contrast, slices chronically incubated (24 h; 2 h washout) with BAPTA-AM showed an increased number of oscillating neurons when compared with untreated slices. Notably, we observed a significant difference in the duration of Ca2+ oscillations without any particular differences in the period, when comparing treated and untreated slices. In a second det of experiments, patch clamp recordings (whole cell voltage clamp) from control and chronic-BAPTA treated slices of visually identified ventral interneurons, suggested that the changes in Ca2+ oscillations dynamics were accompanied with changes in the expression of voltage-dependent K+ currents. In fact, our preliminary results showed an increased in the amplitude of total voltage activated K+ currents in BAPTA treated neurons.

P2

Emodin prevents intrahepatic fat accumulation, inflammation and redox status imbalance during diet-induced hepatosteatosis in rats

A. Alisi[1], A. Pastore[2], L. Da Sacco[3], N. Panera[1], S. Petrini[4], G. Bruscalupi[5], A. Masotti[3], F. Piemonte[6], V. Nobili[1]
[1]Liver Unit, [2]Laboratory of Biochemistry, [3]Microarray Unit, [4]Microscopy Unit and [6]Neuromuscular and Neurodegenerative Diseases Unit, of "Bambino Gesù" Children's Hospital and Research Institute, Rome, Italy
[5]Dept of Biology and Biotechnology "C. Darwin", "La Sapienza" Univ., Rome, Italy

High-fat and/or high-carbohydrate diets may predispose to several metabolic disturbances including fatty infiltration in the liver alone (hepatosteatosis) or associated with necro-inflammation and fibrosis (steatohepatitis). Lifestyle intervention, including correction of dietetic regimen coupled with exercise, is the mainstay of therapeutic intervention. However, in the last years, several studies have emphasized the hepatoprotective effect of some natural agents.
In this study, we investigated the potential therapeutic effects of the treatment with Emodin, an anthraquinone derivative with anti-oxidant and anti-cancer abilities, in rats that develop diet-induced hepatosteatosis and steatohepatitis.
Sprague-Dawley rats fed, for 15 weeks, with a standard diet (SD), a high-fat diet (HFD), or a high-fat/high-fructose diet (HFD/HF). After 5 weeks, Emodin was added to the drinking water of a part of SD, HFD and HFD/HF rats. The experiment was termed after additional 10 weeks.
Emodin treatment caused a weight gain (20-30%) in all rats, even though it prevented the intra-hepatic accumulation of liver triglycerides in HFD and HFD/HF rats. Furthermore, Emodin exerted anti-inflammatory activity by inhibiting the HFD- and HFD/HF-induced increase of circulating levels of lypolysaccharides (LPS) and tumor necrosis factor (TNF)-alpha, and LITAF (LPS-induced TNF-alpha) mRNA and protein expression levels. Emodin also affected the levels of the HFD- and HFD/HF-induced increase of glutathionylated/phosphorylated proteins, such as LITAF and phosphatase and tensin homolog (PTEN).
In conclusion, we demonstrate that a natural agents, such as Emodin is able to prevent liver from hepatosteatosis, preserving liver from pro-inflammatory and pro-oxidant damage caused from high-fat and high-fructose diet. These findings are promising, regarding the possibility to use Emodin to treat hepatosteatosis avoiding its progression to steatohepatitis. However, further studies are needed.

P3

Acetylation of critical lysine residues of APE1 controls its subnuclear trafficking during genotoxic damage

G. Antoniali[1], L. Lirussi[1], C. Vascotto[1], M. Poletto[1], C. D'Ambrosio[2], L. Cesaratto[1], M. Romanello[1], A. Scaloni[2], K. Bhakat[3], G. Tell[1]
[1]Dept Medical and Biological Sciences, Univ., Udine, Italy
[2]Proteomics and Mass Spectrometry Laboratory, ISPAAM, National Research Council, Naples, Italy
[3]Dept Biochemistry and Molecular Biology, Texas Medical Branch Univ., Galveston, Texas, US

The apurinic endonuclease 1/redox factor-1 (APE1) is a mammalian multifunctional protein involved in the BER pathway of DNA lesions caused by oxidation and alkylation and in transcriptional regulation of gene expression, having a crucial role in redox signalling. Recently, a new unexpected APE1 function in RNA metabolism, modulated by NPM-1 within nucleoli, has been discovered. Recent studies identified APE1 as an excellent target for sensitizing tumor cells to chemotherapy. These evidences reflect APE1 pleiotropic role in mammalian cells and underline the importance of understanding the molecular mechanisms responsible for fine-tuning its different biological functions. Our previous data have demonstrated that APE1 N-terminal acetylation on lysines residues (Lys27-35) influences its DNA repair activity and its binding to NPM1. Here, we studied APE1 acetylation role and its dynamics after genotoxic damage. We showed that: (i) MMS-induced acetylation of Lys27-35 controls APE1 binding to NPM-1 and its subnuclear relocalization from nucleoli to nuclei; (ii) cell lines expressing an APE1 mutant mimicking constitutive acetylation show a complete APE1 nucleolar exclusion which confers an increased genotoxic stress resistance but an impairment in cell proliferation; (iii) SIRT-1 controls the acetylation status of Lys27-35 and thus possibly regulates APE1 subnuclear shuttling from nucleus to nucleoli; iv) there is a crosstalk between acetylation status of K27-35 and K6/K7. Altogether, these results suggest an emerging role for the nucleolus in modulating APE1 DNA repair function in BER and highlight the importance of post-translational modifications of APE1 N-terminal domain in vivo, in better coordinating and fine-tuning protein BER activity and function on RNA metabolism. Our data clearly show that nucleoli not only act as a storage site for APE1 but also that nucleolar APE1 may control cell proliferation possibly through its RNA cleansing function in ribosome biogenesis.

P4

Silencing of ZEB1 and SNAI2 promotes Mesenchymal-to-Epithelial transition and downregulation of stem properties

S. Borgna, R. Maestro, M. Santarosa
Experimental Oncology, CRO IRCCS, Aviano, Italy

In breast cancer, EMT (Epithelial-to-Mesenchymal transition) and stemness are strictly connected phenomena. A breast cancer cell population, isolated by $CD44^{+}/CD24^{-/low}$ cell surface profile, has been demonstrated to be endowed with cancer stem cell features such as self-renewal, tumorigenicity and multi-lineage differentiation capacity. Further, this population expresses several EMT-inducing transcription factors including ZEB1 and SNAI2. ZEB1 and SNAI2 can bind directly to the E-cadherin promoter repressing its transcription; functional loss of E-cadherin by transcriptional regulation has been considered an important hallmark of occurred EMT.
To investigate the role of SLUG and ZEB1 in the possible control of both EMT and stemness, we knockdown these transcription factors by RNA interference in basal breast cancer cellular models, known to resemble cancer stem cell like features. The hypothesis is that by silencing these factors, we could observe both the MET (Mesenchymal-to-Epithelial transition) and the downregulation of stem properties, through E-cadherin and CD24 re-expression, respectively.
ZEB1 downregulation performed in MDAMB-231 and MDA-157 cells results mainly in the expression of E-cadherin as well as other cell adhesion molecules (by qRT-PCR and/or Western Blot) and a small CD24 positive population (by FACS analysis). Re-expression of E-cadherin is detectable also in SNAI2 silenced cells in HS578T cellular model, while other markers are being evaluated.
Thus, the reversion of both EMT and stemness induced by silencing key EMT-driving genes might be the basis of a promising model directed to identify novel common molecules involved in stemness and EMT.

P5

Isolation of glioma stem cells with tumor supporting characteristics

A. Pucer[1], E. Bourkoula[1], D. Musiello[1], A. Calio[1], B. Toffoletto[1], T. Ius[2], M. Vindigni[2], V. Pecile[3], A. P. Beltrami[1], D. Cesselli[1], M. Skrap[2], C.A. Beltrami[1]
[1]Centro Interdipartimentale di Medicina Rigenerativa dell'Università degli Studi di Udine, Italy
[2]Azienda Universitario-ospedaliera Santa Maria della Misericordia di Udine, Dipartimento di neurochirurgia, Udine, Italy
[3]S.C. Laboratorio di Genetica Medica, IRCCS Burlo Garofolo, Trieste, Italy

Recently several groups have described the existence of cancer stem cells in human brain tumors of different phenotypes. The work done in our laboratory was focused on gliomas, the most common form of primary central nervous system tumors. The most aggressive form of gliomas (glioblastoma, WHO grade IV) have a median survival of 10-12 months, while the low grade tumors (WHO grade I and II), despite having a better median prognosis, may relapse and give rise to high grade tumors. Up to date, the ability to predict their clinical behavior is inadequate. We have developed a new method to efficiently isolate multipotent stem cells with aberrant growth properties from low- (n=41) and high- (n=64) grade human gliomas. These cells are characterized by mesenchymal cell surface immunophenotype, expression of pluripotent state-specific transcription factors Oct-4 and NANOG, clonogenicity and multipotency, being able to differentiate into all three neural lineages. Moreover, these cells show anchorage-independent growth and the ability to modify significantly clonogenicity and migratory properties of two glioma cell lines, U87MG and A172. However, at a genetic level, these cells do not present the glioma mutations that characterize the respective tumor tissue, as observed by a whole genome SNP analysis (n=4). Importantly, in vitro properties of cell lines derived from high-grade gliomas (e.g. population doubling time, CD49a and CD10 expression) significantly correlate with patient survival, suggesting that isolated cell lines could represent an in vitro model mirroring the in vivo behavior of the original tumor. The aim of the future research will be to further correlate the biological properties of cell lines derived from low-grade gliomas with the characteristics of tumor in vivo in order to determine whether this optimized system can be used to identify new prognostic and/or predictive factors.

P6

The RNA recognition motif protein RBM11 is a novel tissue-specific splicing regulator

S.Pedrotti[1,2], C. Compagnucci[1,2], R. Busà[1,2], C. Sette[1,2]
[1]Dept of Public Health and Cell Biology, Section of Anatomy, Univ. of Rome "Tor Vergata", Rome, Italy
[2]Laboratory of Neuroembryology, Fondazione Santa Lucia IRCCS, Rome, Italy

Mammalian tissues display a remarkable complexity of splicing patterns. Nevertheless, only few examples of tissue-specific splicing factors are known. We have characterized a novel tissue-specific splicing factor named RBM11, which contains an RNA Recognition Motif (RRM) at the amino terminus and a region lacking known homology at the carboxyl terminus. RBM11 is selectively expressed in brain, cerebellum and testis, in a developmentally regulated manner that correlates with differentiation events occurring in neurons and germ cells. Changes in RBM11 levels mirror the expression of two alternatively spliced isoforms. High RBM11 levels correlate with splicing of a criptic intron in the 3'UTR. Moreover, Luciferase reporter assays showed that the splicing of this intron in the 3'-UTR regulates the stability and translation of the encoded mRNA. Deletion analysis of the RBM11 ORF indicated that the RRM is required for RNA binding, whereas the carboxyl terminal region permits nuclear localization and homodimerization. RBM11 is diffusely localized in the nucleoplasm, with specific enrichment in SFRS2-containing splicing speckles. Transcription inhibition/release experiments indicated a dynamic movement of RBM11 between nucleoplasm and speckles, suggesting that its localization is affected by the transcriptional status of the cell. Splicing assays revealed a role for RBM11 in the modulation of alternative splicing. In particular, RBM11 affected the choice of alternative 5' splice sites in BCL-X by binding to specific sequences in exon 2. Thus, our findings identify RBM11 as a novel tissue specific splicing factor with potential implication in the regulation of alternative splicing during differentiation of neurons and germ cells.

P7

A novel population of Neuropilin-1-expressing Mononuclear Cells (NEMs) contributes to tumor vessel stabilization and normalization

A. Carrer[1], S. Zacchigna[1], S. Moimas S[1], G. Ruozi[1], M. Mano[1], L. Zentilin[1], F. Maione[2], F. Bussolino[2], E. Giraudo[2], M. Giacca[1]
[1]Molecular Medicine Laboratory, International Center for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy
[2]Dept of Oncological Sciences and Division of Vascular Biology, Institute for Cancer Research and Treatment (IRCC), Univ. of Torino School of Medicine, Candiolo, Italy

The exaggerated production of angiogenic factors in the tumor mass results in the formation of an aberrant vascularization, characterized by excessive endothelial sprouting and lack of mural cell coverage. This leaky vasculature paradoxically favors tumor growth by rendering the tumor microenvironment constantly acidic and hypoxic, two conditions that induce the secretion of a variety of pro-tumorigenic growth factors. In this respect, it has been demonstrated that Semaphorin3A (Sema3A) promotes vascular normalization in RIP-Tag mice, thus inhibiting tumor growth. As we recently showed that Sema3A promotes vessel maturation through the recruitment of a myeloid population of Neuropilin-1 Expressing Mononuclear cells (NEMs), we wondered whether NEMs might be causally involved in the anti-tumor activity of Sema3A.
To address this issue, we exploited AAV-mediated gene transfer to overexpress Sema3A at the site of tumor inoculation in immunocompetent mice. We found that Sema3A reduced tumor growth up to 70%, recruited tumor-infiltrating NEMs and promoted a more mature vascular phenotype. To definitively prove that NEMs induce tumor vessel normalization, we purified these cells from either Sema3A-expressing muscles or the bone marrow and subsequently injected them into growing tumors. Notably, NEM administration significantly reduced tumor growth rate, eventually resulting in a normalized tumor vasculature, as determined by the relative increase in a-SMA+ and NG2+ mural cell coverage, the formation of a thicker vessel wall and the acquisition of a more regular lumen shape. Consistent with an improvement of the vascular network, NEM injection also relieved tumor-associated hypoxia, as shown by pimonidazole staining and by the reduced activation of HIF-1α.
Molecular profiling of NEMs supports the concept that these cells may contribute to vessel maturation through the paracrine secretion of PDGFb, TGF-beta and several chemokines, ensuing in the activation and proliferation of tissue-resident mural cells.

P8

Characterization of brain and spinal cord neural stem cells (NSCs) derived from E13.5 SMAΔ7 mouse model

S. Vincenti[1], A. Malgieri[2], A. Luchetti[2], M.C. Buè[1], F. Sangiuolo[2], M.G. Farace[1], S.A. Ciafrè[1]
[1]Dept of Experimental Medicine and Biochemical Sciences, Tor Vergata Univ., Rome, Italy
[2]Dept of Biopathology and Diagnostic Imaging, Tor Vergata Univ., Rome, Italy

Spinal muscular atrophy (SMA) is a motor neuron disease representing one of the most common genetic diseases leading to death in childhood. It is an autosomal recessive disorder caused by depletion of survival motor neuron1 (SMN1) protein and characterized by degeneration of α-motor neurons in the spinal cord. Even if SMN1 depletion is common to all cell types in the body, SMA affects specifically motor neurons. The molecular mechanisms explaining these cell-specific effects are still far from being understood. For our study, we employed the SMNΔ7 SMA mouse, an extensively used model accurately mimicking the human disease.
We isolated neurosphere-forming Neural Stem Cells (NSCs) from brains and spinal cords of 13.5 dpc SMNΔ7 SMA mice (wt, hz and SMA), in order to study the SMN1 role in neurodevelopment and in neuromaintenance.
We confirmed the phenotype-genotype correlation by the analysis of SMN mRNA and protein for all three cell types, and we provided results showing how brain and spinal cord NSCs of all genotypes express the typical neural progenitor markers such as Nestin, Olig2, Sox2 and Pax6. Moreover, spinal-cord NSCs express the hindbrain/spinal cord homeobox genes Hoxb4 and Hoxb9 whereas brain NSCs are positive for Otx2, Foxg1, Six3 and Tlx.
By propidium iodide staining and flow cytometry we analysed the cell cycle and the proliferation rates of wt, SMA and hz cells, showing differences in the distribution of SMA cells in the S phase of the cycle, compared to the other genotypes.
qPCR analysis show that SMA NSCs display a pathological modification of the expression of the spliceosome components, confirming a defect in snRNP biogenesis.
We also profiled, by microarray analysis, the miRNome expression of SMA vs wt NSCs, providing preliminary data about a specific modulation of a number of microRNAs in spinal cord SMA NSCs.
In summary, our results may open new perspectives into the comprehension of the motor-neuron-specific defects in SMA.

P9

Exploring Class IIa Histone Deacetylases functions in breast cancer

A. Clocchiatti[1], E. di Giorgio[1], C. Brancolini[1]
[1]Medical and Biological Sciences Dept, Univ. of Udine, Udine, Italy

Histone Deacetylases (HDACs) are considered promising drug targets for cancer treatment. While Class I HDACs involvement in tumor biology is well established, another group of enzymes, Class IIa HDACs, is emerging as a new player in the control of this disease. Among the Class IIa members, HDAC4 is significantly mutated in human breast cancer. Hence, we investigated its function using different breast cancer cell lines that mimic breast tumor heterogeneity. We observed that expression of HDAC4 enzyme is higher in ER- cell lines, with the exception of the HCC1937 cells, where this protein was found mutated. To evaluate HDAC4 activity we investigated the mRNAs levels of a set of MEF2- target genes in MCF7 and MDA-MB-231 cells over-expressing or down-regulating HDAC4. Interestingly, only the silencing of multiple Class IIa HDACs (HDAC4, 5 and 9) promotes MEF2 dependent transcription in ER+ MCF7 cells but not in ER- MDA-MB-231 cells. KLF2 was the only MEF2-target gene up-regulated after Class IIa silencing. Curiously the expression of another MEF2 target, FRK was repressed under the same conditions. Although the siRNA experiments indicate a differential repressive competence of Class IIa, binding between HDAC4 and MEF2 and HDAC4/MEF2 protein complexes > 660 kDa were similarly observed, in MCF7 and MDA-MB-231. Since in these two cell lines there is an inverse relationship between HDAC4 and HDAC3 protein levels we speculated a possible alteration in the repressive activity of the HDAC4/HDAC3 complex in MDA-MB-231. Indeed HDAC activity measured after HDAC4 co-immunoprecipitation is reduced in ER- cells. Finally, the knock down of these Class IIa HDACs reduces proliferation and sensitize to etoposide induced apoptosis only in MCF7 cells. These results suggest an impairment of Class IIa HDACs function in ER- cancer cells while they could represent a possible therapeutic target for ER+ breast tumours.

P10

Functional induction of the cystine-glutamate exchanger system X_c^- activity in SH-SY5Y cells by unconjugated bilirubin

C.D. Coda-Zabetta[1], P.J. Giraudi[1], C. Bellarosa[1], C.Tiribelli[1,2]
[1]Centro Studi Fegato, AREA Science Park, Basovizza, Trieste, Italy
[2]Dept ADEM, Univ. degli Studi di Trieste, Trieste, Italy

A marked mRNA upregulation of the Na^+ -independent cystine:glutamate exchanger System X_c^- (SLC7A11 and SLC3A2 genes) has been observed in SH-SY5Y neuroblastoma cells after 24h of unconjugated bilirubin treatment by our group. In the present study we demonstrate that also the activity of this transporter is highly increased after a 24h treatment. This is accompanied by the lack of significant changes in the mRNA expression and functionality of the other two cystine transporter (X_{AG}^- and GGT) described in neurons. The total glutathione content was 2 folds higher than controls, supporting the hypothesis that the internalized cystine is used in gluthathione synthesis by the neuronal cells. Of notice the observation that after the bilirubin treatment, the cells are less sensitive to an oxidative stress induced by H_2O_2. The present data suggest that bilirubin can modulate the GSH levels in neuroblastoma cells throught System Xc- induction and this modulation elicit an higher resistance to oxidative stress.

P11

Ser46 phosphorylation and Pin1 mediated isomerization of p53 are key events in p53-dependent apoptosis induced by mutant Huntingtin

A.Grison[1,3], F. Mantovani[1,2], A. Comel[1,2], F. Persichetti[3], G. Del Sal[1,2]
[1]Laboratorio Nazionale CIB, Area Science Park, Padriciano,Trieste, Italy
[2]Dept of Life Sciences, Univ. of Trieste, Trieste, Italy
[3]Sector of Neurobiology, International School for Advanced Studies (SISSA), Trieste, Italy

Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG repeat expansion in the gene coding for Huntingtin protein. Several mechanisms have been proposed by which mutant Huntingtin (mutHtt) may trigger striatal neurodegeneration, including mitochondrial dysfunction, oxidative stress and apoptosis. mutHtt has been reported to induce DNA damage and to activate a stress response, and a critical role of p53 in mediating toxic effects of mutHtt has been established. Therefore it is of primary interest to dissect the molecular pathways that induce p53 apoptotic functions downstream of mutHtt. The phosphorylation dependent prolyl isomerase Pin1 has been shown to be a key regulator of several signaling pathways and among them the p53 pathway. We observed that in protein extracts of HD patients' brains, as well as in neuronal cells challenged by transient overexpression of mutHtt, p53 is phosphorylated on the Pin1 target site Ser46. As a consequence, expression of mutHtt promoted the interaction of p53 with Pin1, both in vitro and in the brains of homozygous HdhQ111 KI mice. We demonstrated that, upon expression of mutHtt, the apoptotic kinase HIPK2 mediates p53 activation; p53, thus phosphorylated on Ser46, interacts with Pin1 that assists its dissociation from the apoptosis inhibitor iASPP thereby leading to apoptotic gene expression. Importantly, pharmacologic inhibition of Pin1 was effective in reducing mutHtt-induced cell death. Furthermore knockdown of HIPK2, as well as inhibition of the upstream kinase ATM, can prevent mutHtt-dependent apoptosis of neuronal cells. These results provide rationale for use of small molecule inhibitors of Pin1 and of stress-responsive protein kinases as a potential therapeutic strategy for treatment of HD.

P12

Stemness in complex epithelial/mesenchymal organ: the liver specific derivatives common origin

A. Conigliaro[1], F. Garibaldi[1], B. Sacchetti[2], M. Riminucci[2], P. Bianco[2], L. Amicone[1], M. Tripodi[1]
[1]Dept Cellular Biotechnologies and Hematology, Univ. Sapienza of Rome, Italy
[2]Dept Experimental Medicine,Univ. Sapienza of Rome, Italy

Our work focuses on liver stem cell plasticity: we gathered evidences for a common origin of liver specific epithelial/mesenchymal derivatives.
We previously characterized a number of stable stem cell lines named RLSCs (from Resident Liver Stem Cells) that spontaneously differentiate into hepatocyte RLSCdH (from RLSC derived Hepatocytes) (*Conigliaro et al.CDD 2008 Jan*). Notably RLSCs were proved also to recapitulate the hepatocitic post-differenziative patterning defined "zonation"; their spontaneous differentiation, in fact, generates periportal hepatocytes that may be induced to switch into perivenular hepatocytes by means of the convergence of Wnt signalling on the HNF4a-driven transcription (*Colletti et al. Gastroenterology 2009 Aug*).
Recently we gathered data on: 1) the RLSC capacity to give rise to both epithelial and mesenchymal liver specific derivatives; 2) the niche factors influencing their plasticity; 3) the molecular mechanisms allowing the mesenchymal/epithelial diffentiations.
In summary we found that:
1) in orthotopic transplants RLSCs give rise to both parenchymal (hepatocyte and colangiocyte) and stromal cells (Hepatic Stellate Cells, HSC). The morphology and anatomical localization of exogenous cells match the expected one: while polarized HNF4-positive cells are arranged in hepatic cords within the lobuli architecture, mesenchymal GFAP-positive cells were found scattered throughout the organ and under the vessel (Disse space). This evidences are of particular relevance considering HSCs involvement in liver homeostasis and fibrogenesis;
2) in heterotopic co-transplants of RLSCs and HUVEC, the endothelium provides a pivotal instruction allowing RLSCs to execute four different destinies: self renewal, hepatocitic, cholangiocytic and HSC differentiation;
3) the RLSCs plasticity is based on their metastable heterogeneity characterized by co-expression of mesenchymal and ephitelial markers

P13

Shaping substrates at the nanoscale for single cell characterization

S. Corvaglia[1], L. Ianeselli[2], D. Cesselli[4], A. P. Beltrami[4], G. Scoles[234], D. Scaini[1], L. Casalis[3]
[1]Univ. of Trieste, Trieste, Italy
[2]SISSA, Scuola Internazionale Superiore di Studi Avanzati, Trieste, Italy
[3]ELETTRA, Sincrotrone Trieste, Basovizza, Trieste, Italy
[4]Centro Interdipartimentale Medicina Rigenerativa, University of Udine, Udine, Italy

Cell shapes induced by cell–substratum interactions are linked with proliferation, differentiation or apoptosis of cells. Microscale technologies are emerging as powerful tools that allow for an unprecedented ability to control the cellular microenvironment in culture and miniaturize assays for high-throughput applications. We present a versatile root for promoting cell adhesion and viability on differently functionalized bio-compatible materials controlling at the nanoscale the morphology and the stiffness of this cell substrates. A suite of techniques, known collectively as 'soft lithography', has been developed over the last years to fabricate microscale systems compatible with cells, proteins, and other soft materials with features that are exactly the size of a cell (10-100 μm). Using this technology, we aim at designing a device that offers the possibility for a single/ few cells study that is important for the parallel characterization of rare cells (e.g. adult stem cells). Such a device would allow us to resolve the heterogeneity in the cell populations under study with the sensitivity required for analytical processes to detect rare events.

P14

Neural precursors are present in adult spinal cord meninges and migrate to the parenchyma upon spinal cord injury

I. Decimo[1], F. Bifari[2], F.J. Rodriguez[3], S. Dolci[1],V. Lavarini[2], S. Pretto[1], S. Vasquez[3] , G. Malpeli[4], M. Sciancalepore[5], A. Montalbano[5], M. Krampera [2], G. Fumagalli[1]
[1]Dept of Public Health and Community Medicine, Section of Pharmacology, Univ. of Verona, Italy
[2]Dept of Medicine, Stem Cell Research Laboratory, Section of Hematology, Univ. of Verona, Italy
[3]Group of Molecular Neurology, Hospital Nacional de Parapléjicos, Toledo, Spain
[4]Dept of Pathology, Section of Pathological Anatomy, Univ. of Verona, Italy
[5]Dept of Life Sciences and B.R.A.I.N., Centre for Neuroscience, Univ. of Trieste, Italy

We have previously described stem/progenitor cells with neural differentiation potential in postnatal rat brain located in the meninges (arachnoid and pia mater) covering the parietal cortex. Based on this finding we hypothesize that adult spinal cord meninges may be a niche for a neural stem cell-like population.
In this study we describe a new population of cells resident in the adult rat spinal cord meninges that express the neural stem/precursor markers nestin and doublecortin. A neural stem cell population was extracted from dissociated meningeal tissue, cultured in vitro as neurospheres up to several month and subsequently differentiated into functional neurons and mature oligodendrocytes.
We also provide evidences of participation of meninges-derived neural precursors to the parenchymal reaction to injury. Indeed we observed that the proliferation rate and number of neural stem/precursor cells in meninges increased in vivo following spinal cord injury. By in vivo labeling with GFP lentiviral transduction, we followed the migration of the meninges-derived neural precursors and found that they penetrate the parenchyma and contribute to the glial scar formation. Our data indicate for the first time that spinal cord meninges are potential niches harboring injury-responsive neural stem/precursor cells. For the superficial location of the meninges and the presence of neural precursors in adults, meninges may be considered an important alternative source of neural stem/progenitor cells for regenerative medicine in autologous setting.

P15

Kidins220 directly interacts with PDZRN3 via a PDZ-binding motif

S. Marracci, E. Landi, B. D'orsi, M. Barilari, A. Iervolino, M. Andreazzoli, L. Dente
Dept Biology, Pisa Univ., Pisa, Italy

The neural protein Kidins220 (kinase D–interacting substrate of 220 kD) is also named ARMS (Ankyrin Repeat-rich Membrane Spanning) for the presence of ankyrin repeats and transmembrane domains, together with other binding modules and recognition motifs. The presence of multiple domains underlines the potential of such dynamic multifunctional protein in contacting specific and different targets. We have identified the protein PDZRN3 (PDZ domain-containing Ring Finger protein) as a binding partner for Kidins220 by phage display screen. The interaction between the two proteins has been confirmed by glutathione S-transferase-pull-down and coimmunoprecipitation experiments. The minimal region that is determinant for the binding to the first PDZ domain of PDZRN3 protein was identified by using a deletion mutant, missing the last three aminoacids at the carboxyl-terminus of Kidins220/ARMS.
The two proteins are evolutionary conserved, sharing high homology among zebrafish, amphibians and mammalians. We are performing the analysis of the spatial and temporal expression of the two genes in X. laevis embryos, using whole-mount in situ hybridization and quantitative real-time RT-PCR analysis. Preliminary results show the two genes co-expressed during X. laevis development, suggesting a potential involvement in common functions.

P16

Epigenetic regulation of Notch1 signaling during cardiomyocyte differentiation

G. Felician, C. Collesi, M. Lusic, M. Giacca
Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

The Notch signaling pathway plays a key role during heart development and finely tunes the balance between proliferation and differentiation of stem and progenitor cells in several different settings, including heart tissue.
In a model of primary cultures of rat neonatal cardiomyocytes (CMs), we previously demonstrated that the proliferative potential of immature myocytes relies on Notch1 activity; however, in vivo, the forced activation of this pathway through an Adeno-Associated Virus coding for either activated Notch1 or a soluble form of its ligand Jagged1 was not sufficient to trigger CM proliferation. We therefore assessed the possibility that epigenetic modifications occurring at the Notch1 promoter and at the promoters of its target genes might impede reactivation of the Notch pathway after terminal cardiomyocyte differentiation.
We found that Notch1 and its target genes (Hes1, Hey1, Hey2, CyclinD1, CyclinD3) are highly expressed in proliferating neonatal CMs; interestingly, chromatin at these promoters was enriched for H3K4 trimethylation, an active chromatin mark. The loss of the proliferative potential of CMs paralleled to a decrease in H4K4 trimethylation at these promoters, confirming that the transcriptional state of Notch1 changes during the differentiation process. Interestingly, the repressive mark H3K27 trymethylation, linked to silenced chromatin, increased along the differentiation process, further confirming the existence of a repressive environment at the selected genes in differentiated CMs. These findings were strengthened by an increased promoter occupancy of Ezh2 (subunit of the Polycomb Group protein2 responsible for H3K27 trimethylation) in terminally differentiated CMs, implying a role of Polycomb in the epigenetic silencing of Notch target genes in these cells.
These results provide the first evidence of an epigenetic mechanism of regulation of Notch1 signaling pathway in cardiac cell proliferation.

P17

AAV-IGF1 Gene transfer improves muscle performance: a mouse model of gene doping

A. Macedo[1#], M. Moriggi[2#], M. Vasso[2,4], S. De Palma[2], M. Sturnega[1], G. Friso[3], C. Gelfi[2,4], M. Giacca[1], S. Zacchigna[1]
[1]Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy
[2]Dipartimento di Scienze e Tecnologie Biomediche, Università degli Studi di Milano, Italy
[3]Istituto di Biofisica - CNR Area della Ricerca di Pisa, Italy4 Istituto di Bioimmagini e Fisiologia Molecolare, CNR Milano, Italy
[#] these authors contributed equally to this work

The recent progresses in gene therapy have hinted at the potential misuse of gene transfer in sports to achieve better athletic performance, while escaping from traditional doping detection methods. Suitable animal models of gene doping are therefore required in order to develop proper screening and detection strategies. Here we describe a mouse model of gene doping based on the AAV-mediated delivery of the IGF-1 cDNA to multiple muscles. This treatment determined marked muscle hypertrophy, neovascularization and fast-to-slow fiber type transition, similar to endurance exercise. In functional terms, treated mice showed impressive endurance gain, as determined by an exhaustive swimming test. The proteomic profile of the transduced muscles at 15 and 30 days after gene delivery revealed induction of key proteins controlling energy metabolism. At the earlier time point, enzymes controlling glycogen mobilization and anaerobic glycolysis were found induced, while they were later replaced by proteins required for aerobic metabolism, including enzymes related to the Krebs cycle and oxidative phosphorylation. These modifications were paralleled by the induction of several structural and contractile proteins, in agreement with the observed histological and functional changes. Collectively, these results support the use of AAV-IGF1 as a doping agent, give important insights into the biological response of the muscle to IGF1 in vivo and provide a reliable animal model for the development of gene doping-detection strategies.

P18

Molecular cloning and expression analyses of the RBM20 gene that is associated to familial dilated cardiomyopathy

A. Filippello, P. Lorenzi, C. Morandi, M.G. Romanelli
Dept of Life and Reproduction Sciences, Section of Biology and Genetics, Univ. of Verona, Italy

Recent studies have indicated that mutations in RBM20, a gene encoding a novel ribonucleic acid – binding protein, cause dilated cardiomyopathy (DCM). The molecular bases of the effects of the RBM20 mutations are unknown. In the present study we have analyzed the expression of RBM20 in mouse and human tissues and undertaken a molecular characterization of the protein in terms of functional domains and intracellular distribution. The gene, mapped to chromosome 10q25.2, encodes a protein of 1227 amino acids, which contains an RS domain, an RNA recognition motif (RRM) and a Zinc-finger double-stranded RNA-binding. The functional domains of the protein are highly conserved in the mouse RBM20 homologue. We have analyzed the expression of RBM20 and of two additional ribonucleoproteins, RBM15 and RBM27, by RT-PCR from tissues and cell lines. We found that RBM20 transcripts showed tissue specificity and were more represented in the cardiac and muscle tissues compared to brain, lung and liver tissues. Actually we are producing GFP-RBM20 fusion proteins in order to map the functional domains of the protein that contribute to subcellular distribution and RNA interactions. Over the past decade, genetic heterogeneity in DCM has been demonstrated, and many of the causative genes have been identified. These genes encode mutant proteins of the cytoskeleton, sarcolemma, and sarcomere. RBM20 is the first RNA binding protein associated to DCM. Structural and functional characterization of RBM20 may contribute to understand the molecular pathogenesis of familiar DCM.

P19

CD34⁺ stem cells as a *in vitro* model to assess toxicity of antimalarial drugs on human developmental erythropoiesis during pregnancy

S. Finaurini[1,3], L. Ronzoni[2] , A. Colancecco[2], M.D. Cappellini[2], D. Taramelli[1]
[1]Dip. di Sanità Pubblica, Microbiologia-Virologia, Univ. di Milano, Milano, Italy
[2]Fondazione Policlinico Mangiagalli, Regina Elena, IRCCS, Milano, Italy
[3]Laboratorio Nazionale TASC, CNR-IOM, Area Science Park Basovizza, Trieste, Italy

Malaria is a parasitic disease caused by protozoan parasites of the genus Plasmodium and it has a worldwide distribution with an incidence of more 500 million of new cases every year. During pregnancy, malaria causes serious clinical effects on the mother and the foetus. Unfortunately, resistance to the preventive treatment is increasing and more information about the safety of new drugs to cure malaria during gestation is urgently needed. Artemisinin derivatives represent the first line treatment: however, the World Health Organization (WHO) recommends to avoid them during the first trimester of pregnancy, since recent studies showed a marked depletion of animal embryonic red blood cells. As long as no human clinical studies can confirm these evidences, safety information about the potential toxicity in pregnant women is urgently required.
CD34⁺ stem cell committed towards erythrocytes are a useful *in vitro* model reproducing all the stages of human erythropoiesis. In order to predict embryo-toxicity on the developmental erythropoiesis, this model allowed to investigate the effect of dihydroartemisinin (DHA), the active metabolite of artemisinin derivatives and to characterize the target erythroid stage. Plasma concentrations of DHA were added on stem cells, early erythroid progenitors, pro-erythroblasts, on basophilic erythroblasts or polychromatic erythroblasts.
Results showed that DHA specifically affects pro- and basophilic erythroblasts in a dose- and time-dependent manner. No effects were observed on other stages. In addition, the CD34⁺ erythroid cell model showed that DHA significantly targets foetal and not adult haemoglobin synthesis, suggesting that the primitive erythropoiesis, occurring in the yolk sac during the the first trimester of human pregnancy, may be affected by artemisinin treatment. Therefore our data support current WHO recommendations for use of artemisinin derivatives during pregnancy.

European Antimal Project 18834 is acknowledged

P20

Tbr2 controls retinoic acid responsiveness during corticogenesis

A. Sessa[1], C. Icoresi Mazzeo[1], S. Liang[2], C. Mao[2], V. Broccoli[1]
[1]San Raffaele Scientific Institute, Milan, Italy
[2]Univ. of Texas, M.D. Anderson Cancer Center, Houston TX, USA

The cerebral cortex, the most complex structure in the mammalian brain, grows drastically compared to the other districts of the brain. We focused on particular neural transit amplifying precursors within the cortex, called Intermediate (basal) Progenitors Cells (IPCs). IPCs, originated from Radial Glial Cells in the embryonic Ventricular Zone (VZ), form the Sub-Ventricular Zone (SVZ) and symmetrically divide one or few times giving rise to postmitotic neurons. In this work we investigated the molecular network controlled by Tbr2 transcription factor in the formation of IPCs; intersecting both the data sets resulting from both microarray analysis, of Tbr2 mutant mice, and Chromatin Immuno-Precipitation, followed by sequencing, we have identified an interesting target of this TF: the Zinc Finger Protein 423 (Zfp423) a co-receptor for Retinoic Acid (RA), a strong neural-differentiative cue. Firstly we confirmed the direct repression on Zfp423 transcription exerted by Tbr2, and secondly, that this downregulation affects the whole cellular response to RA, in vitro.
NSCs over-expressing Tbr2 are infact less prone to RA mediated differentiation.
Using in utero electroporation approach, we then assessed that Zfp423 is able to promote premature differentiation in the developing cortex.
Finally, based on our "in-vivo RA reporter" assay, we suggest a model in which, during corticogenesis, RA responsiveness is modulated in an oscillatory manner: from low in proliferative RGCs, to high in differentiative RGCs to becoming low again in IPCs. We postulated that Tbr2, with its repressive action on RA signaling through Zfp423, could have a role in the formation of IPCs in the SVZ delaying RA-promoted differentiation.

P21

The role of different isoforms of VEGF-A in human tumour angiogenesis, development, and prognosis

M. Kazemi[1], A. Carrer[1], S. Zacchigna[1], P. Prelazzi[2], N. De Manzini[2], M. Giacca[1]
[1]Molecular Medicine Laboratory, ICGEB, Trieste, Italy
[2]Dept of Surgery and Anesthesiology, Faculty of Medicine, University of Trieste, Trieste, Italy

Vascular Endothelial Growth Factor A as a master angiogenesis regulator is crucially involved in cancer progression. However, the role and relative abundance of various VEGF splicing isoforms in tumour angiogenesis are poorly investigated. Here we determined the relative abundance of different isoforms of VEGF-A, specifically of those composed of 165 and 121 amino acids, in a set of tumour samples harvested from colorectal cancer patients and from paired normal mucosa. No obvious tendency in the absolute levels of these splicing isoforms could be appreciated in the neoplastic tissues compared to corresponding mucosa. However, in low grading tumours, the VEGF-$A_{165/121}$ ratio was significantly increased, suggesting a role of VEGF-A_{165} in hampering tumour progression. Accordingly, a small subset of patients who strongly upregulated VEGF-A_{165} in the malignant tissue (*$VEGF_{165}^{hi}$ patients*), showed reduced lymph node infiltration and better prognosis.
In mouse models we have already characterized VEGF-A_{165}, but not VEGF-A_{121}, as a cytokine fully able to sustain vessel maturation during angiogenesis. Hence, we wondered whether the upregulation of VEGF-A_{165} might inhibit human tumour progression through the normalization of tumour vascular network, an event leading to the suppression of several pro-tumourigenic stimuli, most notably hypoxia. Accordingly, immunofluorescence staining showed that $VEGF_{165}^{hi}$ patients develop a more mature tumour vasculature, as revealed by increased α-SMA coverage of tumour vessels.
Altogether these data support the idea that VEGF-A_{165}, contrary to its shorter splicing isoform, VEGF-A_{121}, is able to induce full vessel maturation also in human pathology.
Accordingly, relative VEGF-A_{165} expression was found to be strongly enhanced by hypoxia, as assessed both *in vitro* using cell culture systems and *in vivo* by analyzing the hypoxic core of tumour mass. Again, this is consistent with a superior ability of VEGF-A_{165} in promoting vascular function.

P22

Defining the role of YAP1 in 11q22-amplified cancer cell lines

E. Lorenzetto[1], M. Boeri[2], P. Gasparini[2], S. Rossi[3], E. Piccinin[1], F. Facchinetti[2], A.Grizzo[1], G. Sozzi[2], M. Massimino[4], R. Maestro[1], P. Modena[1]
[1]Dept of Experimental Oncology 1, C.R.O. Aviano National Cancer Institute, Aviano, Italy
[2]Dept of Experimental Oncology and [4]Dept of Pediatric Oncology, Fondazione Istituto Nazionale Tumori, Milano, Italy
[3]Dept of Pathology, Treviso Regional Hospital, Treviso, Italy

The transcriptional coactivator YAP1 (Yes-associated protein1) is a critical effector of the human Salvador-Warts-Hippo pathway. Despite the fact that YAP1 exogenous overexpression has been reported to exhibit oncogenic properties in in vitro and in vivo models, additional studies link YAP1 overexpression to tumor-suppressive functions in distinct in vitro models and in breast cancer. Furthermore, genomic amplification events comprising YAP1 gene have been detected in multiple tumor types but, due to the extension of the amplicons and the presence of a number of candidate genes located within, no direct evidence of the oncogenic role of endogenous YAP1 has been reported so far.
Therefore, in order to reach this goal, we identified a panel of cancer cell lines, representative of different tumor types, harboring genomic amplification and overexpression of YAP1 and we studied the effects of downregulation of endogenous YAP1 by RNA interference strategies on transcriptional profiling, cell proliferation, colony forming capacity, anchorage-independent growth, response to pharmacologic treatments and in vivo tumorigenic potential. Overall, our results demonstrate that in the YAP1-amplified cancer cell lines under study the silencing of endogenous YAP1 is associated with a significant decrease of multiple transformed traits, supporting the hypothesis that YAP1 is a direct oncogenic target of the observed 11q22 amplicons. We also investigated the frequency of YAP1 amplification in a panel of human tumor samples from cancer types corresponding to the cancer cell lines used in our study and we show that the amplification event is actually present in 3-23% of the cases, depending on the tumor type, suggesting that YAP1 amplification comprises a relatively rare but general strategy adopted by cancer cells to inactivate the tumor suppressive function of Hippo pathway.

P23

Increased AAV transduction in postmitotic tissues correlates with the downregulation of DNA-damage response proteins

J. Lovric, M. Mano, L. Zentilin, S. Zacchigna, M. Giacca
International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

Despite the increasing utilization of vectors based on the Adeno-Associated Virus (AAV), several aspects of their biology still remain obscure. In particular, limited information is available on molecular determinants of AAV tissue permissivity. The tissues that show high-level permissivity to AAV transduction are those containing postmitotic cells. Previously, we demonstrated that the host cell proteins involved in double-stranded DNA break response (DSB), namely MRN complex, negatively regulate AAV genome processing and restrict AAV permissivity in replicating cells. In this study, we aimed to elucidate the molecular mechanisms underlying the high AAV permissivity of postmitotic cells and to clarify whether it could be mediated by the diminished expression of proteins involved in DSB response in terminally differentiated cells. In tissue extracts from adult mice, we observed decreased expression levels of MRN complex proteins in postmitotic tissues (heart, skeletal muscle) in comparison with tissues with high proliferative activity known to be poorly permissive of AAV transduction (spleen, bone marrow). As cardiomyocytes soon after birth withdraw from the cell cycle, we analyzed MRN expression in mouse heart from the early postnatal days until the adult age. We observed an abrupt decrease in MRN expression at day 14, which, most interestingly, correlated with increased efficiency of cardiac transduction upon systemic delivery of AAV. In primary cultures of neonatal cardiomyocytes, as well as in a cell model of myoblast differentiation, we also demonstrated that AAV transduction increased with the cell differentiation status. Consistently, we observed a gradual decrease in the expression of the MRN complex along the differentiation process. Taken together, these results indicate that the downregulation of the DNA damage response machinery in postmitotic cells results in the stabilization of rAAV genomes leading to increased functional transduction.

P24

PML nuclear bodies determine formation of a repressive environment restricting HIV-1 gene expression in primary human lymphocytes

M. Lusic[1,2], B. Marini[1,3], M. Giacca[1,3]
[1]ICGEB, Molecular Medicine laboratory, Padriciano,Trieste
[2]San Raffaele Scientific Institute, Milano
[3]Scuola Normale Superiore, Pisa

Nuclear bodies (NBs) are important components of nuclear architecture, involved in processes such as DNA replication and RNA Pol II-mediated transcription. In particular Promyelocytic Leukemia (PML) NBs are involved in the epigenetic control of gene expression (1, 2). The PML protein is a member of tripartite motif (TRIM) protein family, which has a well documented role in antiviral defense, although its function in HIV-1 infection remains elusive. We have demonstrated that CDK9 and cyclinT1, members of P-TEFb complex and main regulators of HIV-1 transcription, localize inside PML NBs (3, 4); we have thus hypothesized that the viral genome actually resides in the proximity of PML NBs.

By 3D-immuno DNA-FISH, we visualized latent HIV-1 proviruses in a close proximity to PML NBs, whereas transcriptional activation induced by TNF-α or phorbol esters (TPA) led to the displacement of PML NBs. This displacement of PML from the transcribing HIV LTR was also confirmed by ChIP, showing PML occupancy at the HIV LTR in resting conditions and its dynamic release after induction. PML degradation and NB disruption by a shPML-containing lentiviral vector or by arsenic trioxide led to a strong increase in viral transcription concomitant with the changes in the chromatin at the viral genome, as revealed by ChIP for specific histone marks. We found that PML anchors HIV-1 to facultative heterochromatin and serves as a bridging factor for the specific histone methyltransferase G9a.

Taken together, our results indicate that PML restricts viral gene expression by forming a particular repressive nuclear neighbourhood for HIV-1 silencing. These findings can have importance for the development of new therapeutical approaches aimed at the eradication of HIV-1 infection.

[1] R. Zhao et al., Curr Opin Genet Dev 19 (2009), 172-9
[2] S. Zhong et al., Nat Cell Biol 2 (2000), 85-90
[3] Marcello et al., EMBO J 22 (2003), 2156-66
[4] A. Sabo et al., Mol Cell Biol 28 (2008), 2201-12

P25

A miRNA-based system for selecting and maintaining the pluripotent state in human induced Pluripotent Stem Cells

B. Di Stefano[1,3*#], S. M. Maffioletti[1*], B. Gentner [2*], F. Ungaro[1], G. Schira[2], L. Naldini[2¶] and V. Broccoli[1¶]
[1]Stem Cells and Neurogenesis Unit, Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy
[2]TIGET, San Raffaele Scientific Institute, Milan, Italy
[3]Policlinico San Matteo, Pavia, Italy
[#]Present adress: Hematopoietic Stem Cell Biology and Differentiation Group Dept. of Differentiation and Cancer, Centre for Genomic Regulation (CRG), Barcelona, Spain
*These authors contributed equally to this work
¶Co-senior authors

iPS cell technology has provided researchers with a unique tool to derive disease-specific stem cells for the study and possible treatment of degenerative disorders with autologous cells. The low efficiency and heterogeneous nature of reprogramming is a major impediment to the generation of personalized iPSC lines. Here, we report the generation of a lentiviral system based on a microRNA-regulated transgene that enables for the efficient selection of mouse and human pluripotent cells. This system relies on the differential expression pattern of the mature form of microRNA let7a in pluripotent versus committed or differentiated cells. We generated microRNA responsive GFP and Neo reporters for the specific labeling and active selection of the pluripotent cells in any culture condition. We used this system to establish Rett syndrome and Parkinson's disease hiPSCs. The presented selection procedure represents a straightforward and powerful tool for facilitating the derivation of patient-specific iPSCs.

P26

High-throughput, genome-wide siRNA screening unravels cellular factors critical for transduction of gene therapy vectors based on the Adeno-Associated Virus (AAV)

M. Mano, R. Ippodrino, J. Lovric, L. Zentilin, M. Giacca
Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

Over the last few years, viral vectors based on Adeno-Associated Virus (AAV) have gained increasing popularity due to several favorable characteristics, including the excellent safety profile, lack of inflammatory response, prolonged transgene expression and high efficiency of transduction of post-mitotic tissues such as muscle, heart, brain and retina. Nonetheless, it has become evident that significant improvements need to be achieved before attaining broader clinical application. Given the simplicity of AAV vector particles, the determinants of permissivity to vector transduction appear to reside mostly on the host cell.
To systematically identify the host cell factors involved in the internalization, intracellular trafficking, processing of AAV genome and AAV gene expression, we have performed a high-throughput screening using a genome-wide siRNA library (18175 gene targets).
Differences in AAV transduction in HeLa cells were assessed using a recombinant AAV vector expressing the firefly Luciferase reporter gene. Analysis of the results obtained from this primary screening identified 1528 genes affecting transduction by AAV vectors by more than 4-fold (184 genes by more than 8-fold); of these genes, 993 are inhibitors of AAV transduction, whereas 535 are required for efficient transduction by AAV vectors. The effect of these genes on AAV transduction was further confirmed in secondary screenings based on high-throughput fluorescence microscopy. Gene ontology analysis of the inhibitor genes revealed a clear overrepresentation of genes related to DNA recombination and repair and cell cycle control, whereas in the subset of genes required for infection an overrepresentation of genes involved in endocytosis, intracellular trafficking and transcription was observed. Work in progress aims at investigating the mechanisms of action of the identified genes and exploiting this information to develop RNAi or pharmacological strategies to improve AAV transduction in vivo.

P27

The impact of nuclear topology on HIV-1 integration and replication

B. Marini[1,3], H. Ali[1], M. Giacca[1,3], M. Lusic[1,2]
[1]ICGEB, Molecular Medicine, Trieste, Italy
[2]San Raffaele Scientific Institute, Milano, Italy
[3]Scuola Normale Superiore, Pisa, Italy

Recent technologies have highlighted that the nucleus is a heterogenous organelle, consisting of distinct chromatin domains and several proteinaceous subcompartment; chromosomes occupy non random positions within the nuclear space, and nuclear topology indeed plays a role in gene regulation. On the other hand, the HIV-1 provirus exploits cellular mechanisms to control its own gene expression and needs to integrate into the host genome in order to complete its replication. Integrating HIV-1 shows preferences for certain genomic regions (hot spots of integration), indicating the importance of chromatin in target-site selection. Since nuclear topology contributes to chromatin structure, we explored the possibility that nuclear topology itself may determine the choice of HIV-1 integration sites.
To verify whether the HIV-1 provirus preferentially integrates in some nuclear compartments to the detriments of others, we selected a number of highly targeted genomic regions (hot spots of integration), and probed them in 3D-immuno-DNA-FISH. The association of these hot spots with the nuclear membrane was determined in primary CD4+ T-cells: certain hot spots indeed localized at the nuclear periphery, whereas more central localization was observed for cold spots, control regions rarely targeted by HIV-1 integration.
Consistently, FISH analysis of infected human primary CD4+ T-cells showed that HIV-1 almost exclusively resides at the periphery of the nucleus in both productive and latent infection. Nuclear pore staining indicated that the integrated HIV-1 colocalizes in the tight proximity with the nuclear pore compartment. We are currently performing ChIP with different nuclear pore proteins to verify the direct association of HIV-1 with the nuclear pore. Interestingly, nuclear pore proteins have been shown to facilitate DNA looping and contribute the activation of inducible genes in yeast. Since tge HIV-1 provirus forms a transcription-dependent gene loop structure, we will verify by 3C-ChIP, whether HIV-1 gene loop may also be regulated by nuclear pore components.

P28

Development of glycinergic transmission in G93A mouse model of ALS: an in vitro study in organotypic slices

M. Medelin*, V. Rancic*, G. Cellot, A. Villari, L. Ballerini
Life Science Dept, Univ. of Trieste, Italy
*these authors equally contributed to this work

In order to provide a dynamic in vitro model of the amyiotrophic lateral sclerosis (ALS), we developed organotypic slice cultures from wild type (WT) and SOD1 mutated (G93A) embryonic spinal cords. We characterize the appearance and development of glycinergic IPSCs via patch clamp (whole cell and voltage clamp configuration) recordings from visually identified ventral interneurons. We pharmacologically isolate glycinergic IPSCs in the presence of CNQX (10 μM), and SR95531 (10 μM). Glycinergic events, which disappear in strychnine 1 μM, display progressively faster kinetics (from 15 ms to 8 ms τ values, 1 and 3 weeks in WT) along with growth in vitro in both culture groups. Notably, τ values became significantly different between the two groups at 3 weeks (8 ms and 5 ms, WT and G93A respectively). We decided therefore to investigate whether such differences may be related to a diverse modulation in G93A of GlyRs subunit expression during development in vitro. Here, we further investigate the τ values of miniature glycinergic IPSCs in the presence of 1 μM TTX and we used outside-out patches to characterize GlyRs subtypes in the somatic membrane of spinal interneurons at different ages in vitro. We also estimate the conductance of synaptic receptors, determined by direct resolution of channel events in the presence of low concentrations of strychnine (200 nM) to assess whether these values are similar to those detected in outside-out patches. We detected differences in channel conductances during development that most likely correspond to the physiological progressive maturation of GlyRs leading to the switch from α2 to α1 subunits.

P29

Identification of a novel human bone marrow-derived population with anti-tumor activity

S. Moimas[1], A. Carrer[1], M. Kazemi[1], S. Zacchigna[1], M. Mano[1], A. Balani[2], N. De Manzini[2], M. Giacca[1]
[1]Molecular Medicine Laboratory, ICGEB, Trieste, Italy
[2]Dept of Surgery and Anesthesiology, Faculty of Medicine, Univ. of Trieste, Italy

We have recently described a population of mouse bone marrow-derived Neuropilin-1 (Nrp-1)-Expressing Mononuclear cells (NEMs; CD11b+, Nrp1+, Gr-1-, CD11c+), which are recruited to the sites of ongoing angiogenesis by Nrp-1 ligands, such as Semaphorin3A (Sema3A). NEMs were reported to sustain vascular maturation and stabilization through a paracrine activity. Moreover, studies performed in tumor animal models showed that NEMs exert also anti-tumor activity, likely contributing to tumor vessel normalization.To assess whether NEMs might be significant for human pathology, we analyzed tissue samples from colorectal carcinoma-bearing patients. Notably, patients who expressed high levels of Sema3A in the neoplastic tissue showed significantly reduced lymph node infiltration, absence of metastasis and longer survival, strongly supporting a tumor-protective effect of Sema3A. We found infiltrating CD11b+/Nrp-1+ cells (putatively NEMs) in the primary tumor mass of those patients. Most importantly, analysis of tumor vessels revealed that Sema3A-overexpressing tumors indeed had a more mature vessel phenotype, as revealed by α-SMA immunostaining. These data again associate the presence of NEMs with vessel stabilization, consistent with our previous findings in the mouse models.
The presence of CD11b+/Nrp-1+ cells was confirmed in both human bone marrow and peripheral blood, again supporting the existence of human NEMs.
Exhaustive flow-cytometry characterization of both mouse and human CD11b+/Nrp-1+ cells was performed in order to identify NEMs as a unique cell population. Interestingly, NEMs scored positive for CD14, confirming their myelo-monocyte specification, and negative for Gr-1 (mouse) or CD16 (human), thus confirming the relative equivalence of the latter two markers for identification. The anti-tumor activity of human NEMs will be screened in vivo exploiting adoptive human cancer animals models.

P30

Functional interactions between ubiquitin conjugating E2 enzymes and TRIM proteins

L.M. Napolitano[1], E.G. Jaffray[2], R.T. Hay[2], G. Meroni[1]
[1]Cluster in Biomedicine (CBM), Trieste, Italy
[2]College of Life Science, University of Dundee, Scotland, UK

The TRIM or RBCC family of proteins is characterized by the presence of the tripartite motif module, composed of a RING domain, one or two B-box domains and a Coiled-coil region. TRIM proteins are involved in many cellular processes and represent the largest subfamily of RING-containing putative ubiquitin E3 ligases, the components of the ubiquitylation cascade responsible for the specific transfer of the ubiquitin peptide to the target. Even if it is clear that ubiquitin conjugating enzymes (UBE2s or E2s) and E3 ligases work together to generate different forms of substrate modification including mono-, multi- and poly-ubiquitin chains of up to seven different linkage types, much has still to be uncovered about E2/E3 specific interactions and how this is achieved and regulated. TRIM role as E3 ubiquitin ligases has been in several cases established, however, little is known about their specific and required connections with the ubiquitin conjugating enzymes.
We performed a thorough screening of interactions between the TRIM and UBE2 families. We observed that the majority of the TRIM proteins tested interact with UBE2 enzymes and we also found a general preference of the TRIM proteins for the D and E classes. Two important exceptions were observed: TRIM9-UBE2G2 and TRIM32-UBE2V1/2 specific interactions. We confirmed representative interactions and also demonstrated that the TRIM E3 activity is only manifest with the UBE2 they interact with. For the most specific interactions we could also observe subcellular co-localisation of the TRIM involved and its cognate UBE2 enzyme suggesting that the specific selection of TRIM-UBE2 pairs has physiological relevance. As many efforts in recent years have been addressing the key role of UBE2 enzymes in governing the type and processivity of the assembled ubiquitin chains, our findings represent the basis for future studies on the specific reactions catalyzed by the TRIM E3 ligases in determining the fate of their targets.

P31

Autophagy deregulation and intra-hepatic lipid accumulation during fatty liver development

N. Panera[1], R. Raso[1], M. Pezzullo[2], S. Petrini[3], G. Bruscalupi[4], A. Alisi[1], V. Nobili[1]
[1]Liver Unit, [2]Core Facilities, and [3]Microscopy Unit, of "Bambino Gesù" Children's Hospital and Research Institute, Rome, Italy
[4]Dept of Biology and Biotechnology "C. Darwin", "La Sapienza" University, Rome, Italy

Nonalcoholic fatty liver disease (NAFLD) is actually considered one of most common liver diseases worldwide. As multifactorial disease, NAFLD development depend on the regulation of several molecular mechanisms. Defects in the autophagy, a lysosomal process involved in the intracellular organelles and macromolecules degradation, have been recently described as potential hallmarks of intra-hepatic fat accumulation and its consequences.
Here, we studied "if and why" alterations of the autophagic process can be found in NAFLD. To this aim we used liver samples from an animal model of diet-induced NAFLD and children affected by this disease.
Rats were made NAFLD by 14-week treatment with different diets: standard diet (SD), diet enriched in fats (HFD), diet enriched in fructose (HFr), and diet with high-fat and high-fructose (HFD-HFr). Our results showed a reduction in the levels of LC3 (microtubule-associated protein 1 light chain 3) proteins, an increase of the levels of p62 (polyubiquitin-binding protein), and an accumulation of ubiquitinated proteins in livers from HFD, HFr and HFD-HFr rats compared to SD. Similar results were obtained by the immunohistochemical analysis of liver tissues from NAFLD children, that in addition highlighted a significant correlation between LC3 decrease and elevated grades of steatosis and NASH. Moreover, interestingly, we demonstrated that PTEN/Akt/mTOR signal transduction pathway, closely related to the control of autophagic process, is altered in liver extracts from rats fed fructose-enriched diets.
In conclusion, our findings demonstrate the presence of a deregulation of autophagic process during NAFLD development and suggest a potential regulatory role of PTEN/Akt/mTOR signaling in this effect. Although further analysis are required, our data emphasize the relevance of a corrected autophagy to maintain lipid homeostasis in liver, thus providing novel potential targets to design safe and efficient therapeutic approaches to NAFLD.

P32

Establishment of Doxorubicin-Resistant Hepatic cell lines

N. Rosso*, D. Pascut*, C. Tiribelli
Fondazione Italiana Fegato, ONLUS, AREA Science Park Basovizza, Trieste, Italy
*equal contribution

Background: Hepatocellular carcinoma (HCC) is a relatively chemo-resistant tumor and is highly refractory to chemotherapy. There is no convincing evidence so far that systemic chemotherapy improves overall survival in advanced HCC patients. Doxorubicin (DOX), is a widely used drug for the treatment of HCC but its efficacy is restricted by multidrug resistance.
Aim: Establish DOX-resistant different hepatic cell lines, IHH (immortalized human hepatocytes) and JHH6 (undifferentiated HCC) by survival selection in increasing concentrations of DOX. During the selection process, we assessed drug sensitivity ($LC_{50)}$, intracellular drug accumulation, cell proliferation and expression of the main multidrug resistance proteins (MDR1, MRP1 and BCRP).
Results: During selection cells undergo significant morphological changes (presence of multinucleated giant cells; marked cytoplasmic vacuolization). Normal morphology is restored once the selection-dose is reached Selected JHH6 and IHH present a 1.6 to 2-fold increase DOX LC_{50} respectively vs. control cells (CC). Resistance is accompanied by higher MRP1 and MDR1 mRNA expression (JHH6:2.5 and 1.4-fold; IHH:2 and 3-folds, respectively), while the expression of BCRP is decreased in both cell lines. By exposing cells to 2.5 μM DOX for 24h, selected cells present a reduced intracellular drug accumulation (IHH:13% JHH6:20% vs. their respective CC). Interestingly, when treating CC with the same DOX selection doses for 24h G2/M a cell cycle arrest occurs which was absent in resistant cells.
Conclusions: Altogether this data reinforces the role of MRP1 and MDR1, but not BCRP, as one of the responsible players to DOX-resistance in HCC. Moreover this model represents a valid tool for studying the underlying mechanisms involved in DOX resistance, which have not been fully elucidated yet.

P33

Exploring telomerase silencing effects in HCC

D. Pascut, N. Rosso, C. Tiribelli
Fondazione Italiana Fegato, ONLUS, AREA Science Park Basovizza, Trieste, Italy

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death worldwide. Orthotopic Liver transplantation (OLT) or liver resection represents the only curative treatments for HCC. However, most patients cannot undergo surgical treatments and chemotherapy represent the alternative. Unfortunately the establishment of the multidrug resistance leads to new therapeutically approaches such as gene therapy. Targeting essential and specific cancer related genes represent the new challenge in anticancer molecular techniques. By screening of a cohort of 21 HCC patients, telomerase (hTERT) resulted the best candidate for the gene silencing therapy since its mRNA expression decreased significantly from tumoral to distal tissue, and was not expressed in normal tissue. This gene is involved in telomere elongation (preventing senescence) and cell stability/survival by p53 pathway interactions. An undifferentiated HCC cell line (*JHH6*) was used for hTERT *in-vitro* silencing experiments. The cells were treated for 72h with 25, 50, 100nM of siRNA. The silencing resulted in a reduction in hTERT mRNA expression up to 63% *vs.* control with no inflammatory response. The transfection was coupled with morphological changes: undifferentiated cells (fibroblast-like) became slightly more differentiated (hepatocyte-like). The albumin expression (an hepatic hallmark) was increased in silenced cells. Silencing induced a 44% ($P<0.01$) decrease in cell viability compared to control cells, this percentage is significantly higher ($P<0.05$) than the results obtained by exposure to 25nM for 72h doxorubicin (commonly used antineoplastic agent). Furthermore, hTERT silencing induced a G1 arrest in the cell cycle. This work evidences the pivotal role of hTERT not only in maintaining telomere length but also in cell survival and proliferation.

P34

Molecular imaging of NF-Y transcriptional activity maps proliferation sites in live animals

F. Goeman[1,5,6], I. Manni[1,5], S. Artuso[1,5], B. Ramachandran[2], G. Toietta[3], G. Rando[2], C. Cencioni[3], S. Germoni[4], S. Straino[3], M. Capogrossi[3], S. Bacchetti[1], A. Maggi[2], A. Sacchi[1], P. Ciana[2], G. Piaggio[1]
[1]Dept Experimental Oncology, Regina Elena Cancer Institute, Rome, Italy
[2]Dept Pharmacological Sciences, Milan Univ. , Milan, Italy
[3]Lab. Patologia Vascolare, Istituto Dermopatico dell'Immacolata, Rome, Italy
[4]SAFU, Regina Elena Cancer Institute, Rome, Italy
[5]equal contribution
[6]present address: Translational Oncogenomics Unit, Regina Elena Cancer Institute, Rome, Italy

In vivo imaging involving the use of genetically engineered animals is an innovative powerful tool for the noninvasive assessment of the molecular and cellular events that are often targets of therapy. We have generated a transgenic reporter mouse, MITO-Luc, which represents an unprecedented and powerful experimental model for monitoring cell proliferation in living animals. Based on the knowledge that activity of the NF-Y transcription factor is restricted to proliferating cells, we engineered transgenic mice expressing the luciferase gene under control of a NF-Y-dependent promoter thus enabling bioluminescence imaging (BLI) of NF-Y activity in every body district of a living organism under true physiological conditions. In this animal model we were able to visualize areas of cell proliferation, both physiological and during regeneration in response to injury. Using this tool, we highlight for the first time an unknown function of NF-Y activity in liver regeneration. The MITO-Luc reporter mice should facilitate investigations on the involvement of genes in cell proliferation as well as provide a useful model for studying aberrant proliferation in disease pathogenesis. It should be also useful in the development of new anti/pro-proliferative drugs and the assessment of their efficacy and side effects on non target tissues.

P35

The N-terminal domain of human APE1 modulates its nucleic acids binding activity

M. Poletto[1], C. Vascotto[1], G. Antoniali[1], D. Marasco[2,3], L. Vitagliano[3], G. Tell[1]
[1]Dept of Medical and Biological Sciences, Univ. of Udine, Udine, Italy
[2]Dept of Biological Sciences, Univ. of Naples "Federico II", [3]Institute of Biostructures and Bioimaging, National Research Council, Naples, Italy

Human apurinic/apyrimidinic endonuclease 1 (hAPE1) is an essential and multifaceted protein, involved in base excision DNA repair (BER) and in regulation of gene expression, acting both as a redox co-activator for several transcription factors and as a transctiptional repressor on negative calcium response elements (nCaRE)-containing promoters. Moreover, recent findings from our laboratory suggest a novel role for hAPE1 in RNA metabolism, which is modulated through its interaction with nucleophosmin (NPM1). Here, we characterized the molecular requirements essential for the interaction of APE1 with NPM1 and nucleic acids. Data show that: (i) hAPE1-nucleic acids binding activity depends on the secondary structure adopted by the oligonucleotide chain; (ii) the N-terminal tail of hAPE1, which emerges as an independent domain in fine-tuning the different activities of hAPE1, is able, per se, to bind nucleic acids in a structure-dependent manner. However, this region is not sufficient for the stable binding of to NPM1. Finally, through a "domain swapping" approach, we demonstrate that the N-terminal tail of hAPE1 might represent a phylogenetic gain of function for the protein, in terms of nucleic acids and protein-protein interaction.

P36

Intrinsic disorder and induced order in the transcriptional repressor HES1

M. Coglievina, M. Popovic, C. Guarnaccia, A. Pintar, S. Pongor
Protein Structure and Bioinformatics Group, International Centre for Genetic Engineering and Biotechnology (ICGEB), AREA Science Park, Padriciano, Trieste, Italy

HES1 is a transcriptional repressor of the basic helix-loop-helix (bHLH) family and one of the main downstream effectors in Notch signaling. Its domain architecture is composed of a bHLH region necessary for DNA binding, an Orange domain of yet unclear function, and a C-terminal region required for the recruitment of corepressors. As a general rule, HES1 regulates tissue morphogenesis by maintaining precursor cells in an undifferentiated state. Recently, it has been discovered that undifferentiated cancer cells may use HES1 to escape irreversible differentiation, senescence, or apoptosis, taking advantage of the same mechanisms used by normal cells to remain quiescent [1]. Despite the key function of HES1 in cell differentiation, the underlying molecular mechanisms are not known due to a severe lack of biochemical and structural data.
We addressed the biophysical and structural characterization of HES1, showing that different degrees of structural order are present in the different regions of HES1, and proposed that order/disorder transitions in the different domains are associated not only with binding to DNA, but also with protein homo- and hetero-dimerization [2]. We are currently determining, through NMR or X-ray crystallography, the 3D structure of the different HES1 domains. Structural data will provide the grounds not only to understand the interaction between HES1 and its target DNA sequences, but also to design peptides targeted at the disruption of specific homo- and heterodimer interfaces, thus acting as protein-DNA and protein-protein interaction inhibitors.

Key references:
[1] L. Sang, J.M. Roberts, H.A. Coller. Hijacking HES1: how tumors co-opt the anti- differentiation strategies of quiescent cells. Trends Mol.
Med. (2010) 16:17-26.
[2] M. Coglievina, C. Guarnaccia, A. Pintar, S. Pongor. Different degrees of structural order in distinct regions of the transcriptional repressor HES-1. Biochim. Biophys. Acta (2010) 1804:2153-61.

P37

DNA-protein dynamics at the LaminB2 replication origin

L. Puzzi[1,2], G. Abdurashidova[2], M. Giacca[2], †Arturo Falaschi[1,2,3]
[1]Laboratorio di Biologia Molecolare, Scuola Normale Superiore, Pisa, Italy
[2]International Centre for Genetic Engineering and Biotechnology, Trieste, Italy
[3]Istituto di Fisiologia Clinica-CNR, Pisa, Italy

Efficient duplication of the eukaryotic genome requires a harmonic sequence of cascade checkpoints and licensing events most of which are well conserved from yeasts to humans. The cis-acting elements necessary for the activity of DNA replication origins and the epigenetic factors, like chromatin environment, are still poorly understood. Recent data highlighted that oncoproteins are relevant actors involved in the initiation of DNA replication and pointed out the importance of DNA topology for origin site selection and firing.
In this work we investigate the topological transition occurring at the human LaminB2 replication origin by using a high resolution DNA analysis. We found that two oncoproteins belonging to the AP-1 complex, c-Fos and c-Jun, interact with this origin during late G1 phase of the cell cycle. We demonstrated that this binding involves two DNA regions which, during the transition from middle to late G1, interact each other confirming that, in a high order structure, chromatin topology is a fundamental requisite for the activation of the pre-RC complex.

P38

Expression of multidrug resistance protein 3 in liver of β-thalassemic mouse model

M. Qaisiya, C. Bellarosa, C. Tiribelli
Centro Studi Fegato , Univ. of Trieste, Trieste, Italy

Introduction: The liver serves a critical role in the elimination of xenobiotics compounds. Hepatocytes have a fundamental system of efflux proteins that protect cells from toxic insults. Defect of functional transporter in hepatocytes were associated with many diseases and alteration in the expression of specific transporter was associated with disease-complicated symptoms as a result of metabolic abnormalities. Hepatic Mrp3 expressions are induced *in vivo* in response to non-hemolytic hyperbilirubinemia. The function of this up-regulation is a compensatory mechanism in which substrates are exported from hepatocytes back to bloodstream for renal excretion.
Aim: To analyze the hepatic Mrps expression profile in vivo during hemolytic hyperbilirubinemia.
Methods: β-thalassemic mouse was used as an animal model of chronic hemolysis. Another hemolytic animal model were used, group of WT mouse were treated with Phenylhydrazine (PHZ-WT) to induce acute hemolysis.
Results: Total serum bilirubin was 4 fold and 2 fold higher in β-thalassemic and PHZ-WT mouse compared to WT mouse. Hepatic Mrp3 mRNA was normal, while unexpectedly, Mrp3 protein was 75% down-regulated in β-thalassemic mouse. To confirm further this down-regulation during hemolysis we used PHZ-WT mouse. Mrp3 mRNA was significantly induced in PHZ-WT compared to WT mouse, while again, Mrp3 protein was 60% down-regulated. To determine whether other members of Mrps family may be modulated to compensate for this down-regulation, we analyzed the closely related transporters, Mrp1, 2 and 6 mRNA and protein expression were normal.
Discussion: For the first time we observed a clear down-regulation for hepatic Mrp3 protein during hemolysis and this down-regulation neither transcriptionally occurred nor related to bilirubin. We suggest that a similar decrease occurs in patients of β-thalassemia. This decrease is probably disrupt the biological substrates of Mrp3 and thus may affects liver function under this condition.

P39

An electrophysiological and morphological characterization of dendritic spines in potentiated glutamatergic neurons

R. Rauti[1], L. Ballerini[1], D. Scaini[1,2]
[1]Life Science Department, University of Trieste, Trieste, Italy
[2]ELETTRA, Sincrotrone Trieste S.C.p.A., Trieste, Italy

Dendritic spines are tiny protrusions that stud the surface of neurons and form the postsynaptic component of most of the excitatory synaptic connections in the brain. It has long been suggested that increasing the size and/or number of dendritic spines would enhance the strength of connections between neurons. This process is thought to underlie cellular mechanisms of learning and memory such as LTP in the hippocampus [1]. As with synaptic plasticity, changes in spine morphology are bidirectional, with stimuli that induce LTP causing spine growth[2] and stimuli that induce long-term depression (LTD) causing spine shrinkage [3]. Changes in spine structures have long been proposed to contribute to synaptic plasticity.
One difficulty in evaluating the relationship between spine and synaptic plasticity is that the mechanisms underlying NMDAR-dependent changes in spine morphology are poorly understood. To define whether these two processes are related or distinct, we simultaneously propose to integrate electrophysiology measurements, AFM technology and optical microscopy to develop new generation tools to understand neuronal plasticity at the nanoscale. In particular, we monitored EPSCs and dendritic spines morphology, combining patch-clamp recording and AFM technology in cultures of dissociated rat hippocampal neurons. To induce synaptic changes, repetitive stimulation (60 pulses at 1Hz) was applied to the presinaptic neuron. Measurements of the amplitude of EPSCs revealed a persistent increase in synaptic efficacy after the repetitive stimulation.
References
[1]Harris KM, Kater SB(1994).Annu Rev Neurosci. 17:341-71
[2]Matsuzaki M,Honkura N,Ellis-Davies GC,Kasai H(2004) Nature 429:761–766
[3]Okamoto K,Nagai T,Miyawaki A,Hayashi Y(2004). Nat Neurosci 7:1104 –1112.

P40

Cell cycle alterations in hyperbilirubinemic Gunn rat cerebella

M.C. Robert, S. Gazzin, C. Tiribelli
Fondazione Italiana Fegato- Area Science Park Basovizza and Dept of ACADEM Univ. of Trieste, Trieste, Italy

Neonatal hyperbilirubinemia in jj Gunn rat results from a deficiency of the hepatic bilirubin conjugating enzyme UDP-glucuronosyl transferase 1A1, homologous to human patients with Crigler Najjar type I Syndrome and analogous to the reduced enzyme activity seen in neonates during the first days of life. The high levels of unconjugated bilirubin (UCB) in plasma can lead to the accumulation of bilirubin in the brain. The hyperbilirubinemic jj Gunn rat develop a marked cerebellar hypoplasia, with the greatest damage occurring in brain areas that mature postnatally. Recently, an effect of UCB on cell cycle progression has been described with cell cycle arrest in the late G1 phase.
The aim of this study was to investigate if cerebellar hypoplasia in hyperbilirubinemic jj Gunn rat could be due to a cell cycle arrest.
Cerebellum from hyperbilirubinemic (jj) and normal (JJ) Gunn rat at 9 days after birth was dissected and divided in two parts. The first was used to evaluated the mRNA relative expression of Cyclin D1, A and Cdk2 genes using Real Time q-PCR (n=11); and the other one to determinate its protein levels by quantitative Western Blot (n=9). At the mRNA level, we observed a slight reduction in Cyclin D1 expression in jj rats (JJ 1.00 ± 0.18 vs. jj 0.80 ± 0.30), whereas the mRNA expression of Cyclin A and Cdk2 was unchanged. However when its protein relative expression were studied a significantly reduction in jj animals was observed (Cyclin D1: 0.813 ± 0.09 vs. 0.69 ± 0.12 $p < 0.05$, Cyclin A: 2.24 ± 0.37 vs. 1.83 ± 0.29 $p < 0.01$ and Cdk2: 1.11 ± 0.11 vs. 0.84 ± 0.07 $p < 0.001$, JJ vs. jj respectively).
Based on our in vivo results we hypothesizes that bilirubin could arrest the cell cycle through reduction of the protein level of Cyclin D1, Cyclin A and, more importantly, Cdk2 in cerebellum of developing hyperbilirubinemic Gunn rat. The exact point of the cell cycle at which bilirubin is acting needs to be further elucidated.

P41

An *in vivo*, AAV-based, functional selection screening for the identification of novel genes promoting cardiomyocyte protection or sustaining cardiac function

G. Ruozi[1], S. Zacchigna[1], M. Dal Ferro[2], A. Macedo[1], F. Bortolotti[1], L. Zentilin[1], M. Giacca[1]
[1]Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy
[2]Azienda Ospedaliera-Universitaria "Ospedali Riuniti di Trieste", Ospedale di Cattinara, Trieste, Italy

The need to develop novel biotherapeutics for myocardial ischemia and heart failure is increasingly pressing. In our effort to identify novel genes regulating cardiomyocyte proliferation and survival that might be exploited therapeutically, we devised an innovative, in vivo functional selection strategy. According to this strategy, a cDNA library corresponding to the mouse secretome is directly selected in vivo under ischemic conditions upon subsequent rounds of delivery with vectors derived from the Adeno-Associated Virus (AAV). These vectors are particularly suitable for in vivo screening, as they can be produced at high titers, efficiently transduce the heart and drive persistent transgene expression.To support the feasibility of the strategy, we initially tested a pool of 30 AAV vectors expressing different factors. After intracardiac injection of this pool, animals were subjected to myocardial infarction, and, after 15 days, persisting vector DNA was recovered, re-cloned into AAV and used for subsequent cycles of selection. From this initial set of experiments, we were able to observe a marked enrichment for surviving cardiomyocytes transduced with AAV9-Ghrelin, a hormone involved in endocrine metabolism. The cardioprotective potential of Ghrelin was further characterized by morphological and functional assays. In vitro transduction with AAV9-Ghrelin increased cardiomyocytes survival after treatment with pro-apoptotic drugs, while in vivo it preserved cardiac function and reduced infarct size upon myocardial infarction, as assessed by echocardiography and histological analysis. This effect was paralleled by a decreased number of apoptotic cells at early time points after infarction, and by the reduced expression of markers of heart failure, such as miR-21 and MMP-2.
In conclusion, these results support the feasibility of our innovative in vivo selection approach and identify Ghrelin as a novel, powerful molecule, able to provide benefit after cardiac damage.

P42

Melatonin triggers p53Ser phosphorylation and prevents DNA damage accumulation

R. Santoro[1], M. Marani[1], G. Blandino[2], P. Muti[3], S. Strano[1]
[1]Molecular Chemoprevention Group, Molecular Medicine Area, Italian National Cancer Institute "Regina Elena", Rome, Italy
[2]Translational Oncogenomic Unit-ROC, Molecular Medicine Area, Italian National Cancer Institute "Regina Elena", Rome, Italy
[3]Scientific Director Office, Italian National Cancer Institute "Regina Elena", Rome, Italy

Several epidemiological studies have shown that high levels of melatonin, an indolic hormone secreted mainly by the pineal gland, reduces the risk of developing cancer. This appears to suggest that melatonin triggers the activation of tumor suppressor pathways that lead to the prevention of malignant transformation. We illustrate for the first time that melatonin induces phosphorylation of p53 at Ser-15 causing cell proliferation inhibition and prevention of DNA damage accumulation of both normal and transformed cells. This activity requires p53 and PML expression and efficient phosphorylation of p53 at Ser-15 residue. Melatonin-induced p53 phosphorylation at Ser-15 residue does not require ATM activity, while it is severely impaired upon chemical inhibition of p38 MAPK activity. Largely, these findings imply that the activation of the p53 tumor suppressor pathway is a critical mediator of melatonin and its anticancer effects, thus providing molecular insights into growing observational evidence for the role of melatonin in cancer prevention activity.

P43

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

G. Sorrentino[1], M. Mioni[1], F. Mantovani[1,2], P. Pinton[3], G. Del Sal[1,2]
[1]Laboratorio Nazionale Consorzio Interuniversitario Biotecnologie (LNCIB), Area Science Park, Padriciano, Trieste
[2]Dipartimento di Scienze della Vita, Università di Trieste, Trieste
[3]Dept of Experimental and Diagnostic Medicine, Univ. of Ferrara, Ferrara

The major tumor suppressive activity of p53 is the induction of apoptosis in response to stress, relying on both regulation of transcription and on direct roles at the mitochondria. In the nucleus p53 is able to induce the expression of key proapoptotic genes in response to genotoxic stress. A key regulator of this pathway is the prolyl-isomerase Pin1, which is able to transduce phosphorylation of p53 into conformational changes in order to trigger its proapoptotic transcriptional activity. At the mitochondria p53 induces outer membrane permeabilization. We hypothesize that Pin1 might regulate also the mitochondrial apoptotic activity of p53, given the fact that the prolyl-isomerase has been previously shown to regulate other apoptotic proteins acting at the mitochondria, such as BIMEL, Bcl2 and p66-Shc. Here we show that Pin1 is essential to p53-mediated transcription-independent apoptosis. Indeed Pin1 is necessary for efficient localization of p53 to mitochondria by promoting its HDM2-mediated monoubiquitination. Furthermore we identified phosphorilation of p53 on Pin1 binding site Ser46 by HIPK2 as a post-translational modification essential for the proapoptotic function of Pin1. Chemoterapeutic drugs and small molecule RITA were able to activate HIPK2 and thus to promote p53-mediated transcriptional independent apoptosis in a Pin1 mediated fashion.

P44

The role of stem cells in human primary liver cancers: identification and expression

C.H.C. Sukowati, B. Anfuso, L.S. Crocè, C. Tiribelli
Fondazione Italiana Fegato, AREA Science Park, Trieste, Italy

Fast growing evidences of cancer cells hierarchy and interaction have been recognized in both circulating and solid cancers. However, their identification and expression *in vivo* in human primary liver cancers (PLCs) are still limited. In this study, we had identified a cells population from PLCs hepatocellular carcinoma and cholangiocarcinoma with stem cells (SCs) characteristic, also supported by *in vitro* model. Furthermore, we had studied also the expression of common SCs markers in several groups of liver pathologies. The isolated cells from paired neoplastic and non-neoplastic tissues were maintained in selective medium and characterized by FACS and RT-PCR. The isolated cells co-expressed CD90 and CD44, and further analysis showed the expression of mesenchymal markers and pluripotency factors, might also indicate a cancer-promoting phenotype. This population had low expression of cytokeratin but not committed hepatocytes as they did not or weakly express albumin. When they were plated at low density, they had ability to clone and form colonies. After induction into insulin producing cells, they showed up-regulations of markers of pancreatic cells, indicating potency to trans-differentiate from mesencymal to endodermal lineage. One line had the capacity to differentiate into adipogenic lineage as confirmed by fat droplets accumulation in the cytoplasm and high expression of PPARG. From mRNA distribution data of clinical samples, we found that the distribution of SCs markers in liver tissues were highly variable. Interestingly, it showed a tendency, especially for marker CD90 and EpCAM, that the expressions of these genes were higher in the diseased than in normal tissues and in the lesion than distal tissues, may represent the tumorigenesis process from normal to malignant tissues. The data in this study has supported the presence of SCs in liver cancer and might be related with cancer SCs theory.

P45

Inside the mechanism of apoptotic resistance to type I IFNs in glioblastoma cells

A. Sgorbissa[1], A. Tomasella[1], H. Potu[1], I. Manini[1], C. Brancolini[1,2]
[1]Dipartimento di Scienze Mediche e Biologiche, Università degli Studi di Udine, Udine, ITALY
[2]MATI Center of Excellence, Università degli Studi di Udine, Udine, ITALY

Glioblastoma is among of the most aggressive human cancer characterized by a strong resistance to apoptosis and by the frequent deletion of type I IFNs genes. These features prompted us to investigate the contribution of the IFN autocrine signalling to INF-α induced apoptosis, in glioblastoma cells. For this purpose we took advantage of U87MG cells, which hold a deletion of type I IFN genes and of T98G cells which conserve the spontaneous IFN signalling. IFN-α treatment showed a limited up-regulation of TRAIL in U87MG, whereas other interferon stimulated genes (ISGs) were efficiently up-regulated. Analysis of TRAIL promoter sequence and activity revealed no differences between the two cell lines. Drugs reversing DNA methylation up-regulated TRAIL transcription, but without overt differences between the two cell lines. Moreover, we have demonstrated that TRAIL mRNA stability is influenced by IFN-α, but again without differences between U87MG and T98G cells. By silencing IFNAR1 we provide evidences that the spontaneous IFN signalling loop was required to sustain elevated levels of TRAIL expression, possibly through the regulation of IRF-1. Oncomine analysis confirmed that the pattern of IRF1 mRNA expression mirrors TRAIL mRNA levels in several cell lines and glioblastoma tumors. USP18 is an important negative regulator of IFN-α induced apoptosis, but its down-regulation overcame apoptotic resistance only in T98G cells. USP18 down-regulation was not able to maintain high levels of TRAIL expression in U87MG cells, as instead we observed in T98G cells. We have also discovered alterations in other elements of the extrinsic apoptotic pathway in U87MG, such as in Bid and c-FLIP, that can reinforce apoptotic resistance. Finally, only the induction of ER-stress in combination with silencing of USP18 efficiently restored apoptosis in U87MG. During this apoptotic response an important role was played by the BH3-only protein Noxa.

P46

Morphofunctional impact of carbon nanotubes scaffolds on spinal explants during *in vitro* re-growth

A. Villari[1*], A. Fabbro[1*], J. Laishram[1], D. Scaini[1], F.M. Toma[2], A. Turco[2], M. Prato[2], L. Ballerini[1]
[1]Life Science Dept, Univ. of Trieste, Trieste, Italy
[2]Dept of Pharmaceutical Sciences, Univ. of Trieste, Italy
*these authors equally contributed to this work

Tissue engineering has increasingly involved nanotechnology for the development of super-molecular architectures to sustain and promote neural regeneration following injury. Due to the potential role of nanomaterials in directing sub-cellular interactions for neuronal structures, we investigated the impact of carbon nanotubes (CNT), that are roughly in the same size scale as of small axons and dendrites, in the assembly of neuronal circuits in culture. We investigated the impact of a CNT-covered growth support on the morphology and the neuronal activity of developing organotypic spinal explants, where only part of the tissue is directly interacting with the CNT meshwork. We compared organotypic cultures grown on glass coverslips (control cultures) and organotypic cultures grown on CNT-layered coverslips (CNT cultures). We measured, by means of confocal and atom force microscopy, the growth of neuronal processes exiting the explants. We detected an increased density of fibres in CNT cultures when compared to control ones. Such fibres displayed differences in morphology, were longer and more flattened, when interacting with the CNT layer instead of control glass. By optical and scanning electron microscopy we identified and quantified a 70% increase in the number of growth cones in CNT cultures.
We further revealed by patch clamp recordings of ventral horn interneurons in CNT cultured slices, a significantly increase in poli-synaptic responses to dorsal root ganglia stimulations, when compared to control ones. When recording spontaneous activity, the mean amplitude of spontaneous postsynaptic currents (PSC) recorded from CNT cultures was also significantly increased, when compared to aged-matched controls, while PSC occurrence was not significantly affected. These results show that CNT scaffolds are able to instruct spinal slice explant in vitro potentiating growth and activity.

P47

Role of the prolyl isomerase pin1 in the oncogenic functions of the notch1 intracellular domain

A. Rustighi[1], L. Tiberi[1,2], A. Zannini[1], G. Sorrentino[1], G. Del Sal[1]
[1]National Laboratory CIB, Trieste, Italy. and Dept of B.B.C.M. Univ. of Trieste, Trieste, Italy
[2]present address: Laboratory of Neurophysiology, Institute for Interdisciplinary Research (IRIBHM), Free Univ. of Brussels, Belgium

Recently, a crucial role in regulating signaling pathways has emerged for the prolyl-isomerase Pin1. This enzyme binds to and catalyzes cis/trans isomerization of prolines on phosphorylated S-T/P motifs in many proteins, thereby transducing phosphorylation signaling in conformational changes that profoundly affect their functions. We have recently shown that Pin1 activity is crucial in controlling DNA damage checkpoint pathways, by regulating the activities of p53 tumor suppressor and p73. Pin1 is also an important modulator of cell proliferation and other cellular events, and is involved in many diseases, such as cancer. More recently we have demonstrated that Pin1 interacts also with Notch1 receptor, affecting its cleavage by γ-secretase, and leading to an increase of the active intracellular domain. Moreover, we found that Notch1 directly induces transcription of Pin1, generating a positive loop. Several findings suggest that the stability of N1ICD could be an important regulator of intracellular signalling thresholds and that abrogation of the Notch1 degradation machinery could predispose cells for transformation. An important E3 ubiquitin-ligase that targets nuclear N1ICD and another member of the Notch family, N4ICD, for proteasomal degradation is Fbxw7. This ligase has been shown to cause suppression of the Notch signal, acting as a potent tumour suppressor. Owing to the strong correlation between high nuclear accumulation of N1ICD and Pin1 over-expression, that we observed in a breast cancer tissue array, we investigated the role of Pin1 in the Fbxw7 dependent downregulation of both, N1ICD and N4ICD. We have found that Pin1 contributes to sustained high levels of N1ICD and N4ICD by interfering with proteasomal degradation mediated by the E3 ubiquitin ligase Fbw7. Our results furthermore indicate that Pin1 and N1ICD cooperate in N1ICD dependent transcription and transforming activities in breast cancer cells.

P48

Post-translational modification by acetylation regulates VEGFR2 activity

A. Zecchin, L. Pattarini, M.I. Gutierrez, M. Giacca
Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB), Padriciano, Trieste, Italy

The tyrosine kinase receptor Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) is a key regulator in developmental angiogenesis, by triggering proliferation, migration and survival of endothelial cells. Activity of the receptor follows interaction with its cognate ligands, primarily the members of the VEGF family, and involves phosphorylation of various tyrosine residues in the intracytoplasmic portion of the receptor. By combining biochemical and proteomics studies, here we provide the first evidence that VEGFR2 activity is essentially regulated by acetylation; the enzyme responsible for this modification is p300/CBP, a well-characterized acetyltransferase. In both endothelial and non-endothelial cells, membrane-associated VEGFR2 was found to be acetylated in at least four lysine residues that form a dense cluster in the kinase insert domain, and in a single lysine located in the activation loop. We found that VEGFR2 acetylation essentially regulates receptor stability, since a mutant in which the five lysines identified as acetylated were substituted with arginines, was significantly less stable than the wild type protein. Furthermore, we observed that over-expression of p300 dramatically increased the levels of receptor tyrosine autophosphorylation upon ligand binding. VEGFR2 mutants that were not acetylated, showed impaired levels of both phosphorylation and tyrosine kinase activity, also leading to reduced ability in intracellular signal transduction. Finally, and most important, receptor hyperacetylation was found to counteract the process of receptor desensitization following VEGF stimulation, maintaining the levels of VEGFR2 expressed on the cell membrane high over time and still allowing receptor phosphorylation and intracellular signalling upon prolonged ligand stimulation.Taken toghether these findings indicate that post-translational modification by acetylation is a critical mechanism that directly affects VEGFR2 receptor biology and function.

support services:

Azuleon snc

azuleon.com

meetings@azuleon.com