ABCD Meeting
Riunione Nazionale Dottorandi

Gubbio, 18-20 giugno 2009

Programme & Abstracts

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ABCD Meeting
"Riunione Nazionale Dottorandi"
Gubbio, 18-20 giugno 2009
Organizzatori: Pier Paolo Di Fiore e Paolo Pinton

Programma

Giovedì, 18 giugno

13:00-14:15	Pranzo

14:15-15:00 Registrazione e affissione posters

Chair: Antonio Musaro'

15:00-15:30 *Elena Palma (Padova)* Role of mitochondria in the pathogenesis of muscular dystrophies
15:30-16:00 *Michela Aucello (Roma)* Muscle control of motor neuron degeneration and survival in neuromuscular diseases
16:00-16:30 *Elisa Onesto (Milano)* Effect of SOD1 expression in cell and animal model of familial amyotrophic lateral sclerosis

16:30-17:00 Coffee break

17:00-19:00 Sessione posters (numeri pari)

Chair: Gianni Del Sal

19:00-19:30 *Saverio Marchi (Ferrara)* Akt activity in Endoplasmic Reticulum and mitochondria: a Ca^{2+}-dependent pathway controlling cell fate
19:30-20:00 *Paola Falletta (Genova)* Melanosome biogenesis: role of OA1 signaling pathway

20:30 Cena

Venerdì, 19 giugno

08:00-09:00 Colazione

Chair: Mario Pestarino

09:00-09:30 *Brigitte Bisaro (Torino)* p130Cas adaptor as a crucial regulator of breast cancer tumorigenesis
09:30-10:00 *Sandra Parenti (Modena)* La mesalazina inibisce la via di segnalazione proliferativa di β-catenina agendo attraverso l'induzione di μ-protocaderina in cellule di cancro colo-rettale
10:00-10:30 *Andrea Bisso (Trieste)* Peptide Aptamers targeting mutant p53 induce apoptosis in tumor cells

10:30-11:00 Coffee break

11:00-13:00 Sessione posters (numeri dispari)

13:00-14:30 Pranzo

Chair: Carlo Tacchetti

14:30-15:00 *Gilda Nappo (Milano)* Endocitosi clatrina-indipendente dell'EGFR
15:00-15:30 *Eugenio Fornasiero (Milano)* Membrane trafficking in neuronal development
15:30-16:00 *Noemi Morello (Torino)* "Impaired motor coordination and cortical hypomyelination in Hemopexin-null mice

16:00-17:00 ABCD Lecture

Pier Giuseppe Pelicci (Milano)
Regulation of self renewal in cancer stem cells

17:00-17:30 Coffee break

Chair: Paolo Bernardi

17:30-18:00 *Ting Yu (Camerino)* The expression study of the heat shock protein 70 cytoplasmic subgroup in *Tetrahymena thermophila*

18:00-18:30 *Carolina L Crespo (Milano)* The Par/aPKC complex controls the vectorial migration of medaka macrophages *in vivo*

Chair: Guido Tarone

18:30-19:00 *Emanuele Berardi (Roma)* Physical activity counteracts muscle wasting and increases life span in tumor-bearing mice

19:00-19:30 *Roberta Sartori (Padova)* Smad2 and 3 transcription factors control muscle mass in adulthood

19:30-20:00 *Marco Paoli (Padova)* Study of the mechanism of intoxication of snake presynaptic PLA2 neurotoxins

20:30 Cena

Sabato, 20 giugno

08:00-09:00 Colazione

Chair: Ruggero Pardi

09:00-09:30 *Linda M Starnes (Roma)* Role of Nuclear Factor I-A in hematopoietic lineage commitment and differentiation

09:30-10:00 *Ugo Ala (Torino)* Tissue-specific human-mouse conserved co-expression networks for prediction of mammalian genes functional properties

10:00-10:30 *Matteo J. Marzi (Milano)* I microRNA regolati da E1A accoppiano il differenziamento all'uscita dal ciclo cellulare

10:30-11:00 Coffee break

11:00 Proclamazione miglior poster e miglior tesi di dottorato

Presentazioni Orali

(in ordine cronologico)

Role of mitochondria in the pathogenesis of muscular dystrophies

Elena Palma[1], Tania Tiepolo[2], Alessia Angelin[1], Patrizia Sabatelli[3], Nadir M. Maraldi[3], Emy Basso[1], Michael A. Forte[4], Luciano Merlini[5], Luca Nicolosi[1], Francesca Finetti[6], Paola Braghetta[2], Grégoire Vuagniaux[7], Jean-Maurice Dumont[7], Cosima T. Baldari[6], Paolo Bonaldo[2], Paolo Bernardi[1]
Departments of [1]Biomedical Sciences and [2]Histology, Microbioloy and Biomedical Biotechnologies, University of Padova, Italy; [3]IGM CNR c/o Istituto Ortopedico Rizzoli, Bologna, Italy; [4]Vollum Institute, Oregon Health and Science University, Portland, USA; [5]Section of Medical Genetics, University of Ferrara, and Laboratory of Cell Biology, Istituto Ortopedico Rizzoli, Bologna, Italy; [6]Department of Evolutionary Biology, University of Siena, Italy; [7]Debiopharm SA, Lausanne, Switzerland

Ullrich congenital muscular dystrophy (UCMD) and Bethlem myopathy (BM) are inherited disorders caused by mutations of genes encoding the extracellular matrix protein collagen (col) VI. Mice lacking colVI (*Col6a1*$^{-/-}$) display a myopathic phenotype associated with ultrastructural alterations of mitochondria and sarcoplasmic reticulum, mitochondrial dysfunction with abnormal opening of the permeability transition pore (PTP), and increased apoptosis of muscle fibers. Treatment with cyclosporin (Cs) A, an immunosuppressive drug that desensitizes the PTP by binding to cyclophilin (Cyp) D, was shown to rescue myofiber alterations in *Col6a1*$^{-/-}$ mice and in UCMD patients, suggesting a correlation between PTP opening and pathogenesis of colVI dystrophies. Inactivation of the gene encoding for CypD rescues the disease phenotype of colVI deficiency. In the absence of CypD, *Col6a1*$^{-/-}$ mice show rescue from mitochondrial dysfunction and ultrastructural defects, and normalized incidence of apoptosis. These findings (i) demonstrate that lack of CypD is equivalent to its inhibition with CsA at curing the mouse dystrophic phenotype; (ii) establish a cause-effect relationship between CypD-dependent PTP regulation and pathogenesis of the colVI dystrophy; (iii) validate CypD and the PTP as pharmacological targets for the therapy of colVI myopathies. Thereby, we have investigated the therapeutic effects of the selective cyclophilin inhibitor D MeAla3 EtVal4 cyclosporin (Debio 025) in *Col6a1*$^{-/-}$ mice. Debio 025 does not block the immune response, yet it desensitizes the PTP in vivo. Treatment with Debio 025 prevents mitochondrial dysfunction and normalizes the apoptotic rates and ultrastructural lesions of *Col6a1*$^{-/-}$ mice. These findings provide an important proof of principle that colVI dystrophies can be treated with Debio 025; and represent an essential step toward a therapy of UCMD and BM because Debio 025 does not expose patients to the potentially harmful effects of immunosuppression.

Muscle control of motor neuron degeneration and survival in neuromuscular diseases

Michela Aucello[1], Gabriella Dobrowolny[1], Emanuele Rizzuto[1],Feliciano Protasi[3], Giorgio Fanò[3], Marco Sandri[2], Antonio Musarò[1]
[1]Dip. Istologia ed Embriologia Medica, Univ. di Roma La Sapienza, Italia
[2]Dulbecco Telethon Institute, Padova, Italia
[3]Ce.S.I. Univ. G. d'Annunzio, Chieti, Italia

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by a selective degeneration of motor neurons, atrophy, and paralysis of skeletal muscle. 20% of familial ALS results from a toxic gain of function associated with dominant mutations in the SuperOxide Dismutase1 gene, which alters the anti-oxidant capacity of the SOD1 enzyme. Notably, restriction of SOD1 mutant expression selectively to post-natal motor neurons failed to produce detectable sign of pathology or motor-neuron disease (Lino et al., 2002), suggesting that other cell types may be involved in ALS disease. To define whether skeletal muscle is a direct target of SOD1-mediated toxicity and to determine the specific contribution of oxidative stress to muscle wasting in ALS disease, we generated transgenic mice in which the SOD1 mutant gene (SOD1G93A) was selectively expressed in skeletal muscle under the transcriptional control of muscle specific promoter (MLC) (Dobrowolny et al., 2008). We show that muscle-restricted expression of mutant SOD1G93A induces pathological alterations in skeletal muscle such as muscle atrophy, associated with a reduction in muscle strength and mitochondrial dysfunction. The analysis of molecular pathways associated with muscle atrophy revealed that accumulation of ROS served as signaling molecules to initiate autophagy. Interestingly, the spinal cord of muscle-specific expression of SOD1G93A transgenic mice contained elevated levels of several markers associated with pre-symptomatic signs of ALS, such as microglial cell activation, pro-inflammatory cytokines and mitochondria modifications. Our data demonstrate that skeletal muscle is a primary target of SOD1G93A - mediated toxicity, underscoring the contribution of skeletal muscle to the pathogenesis of ALS. The characterisation of this new mouse model promises to significantly advance our understanding of the possible pathogenic mechanisms that lead to ALS in humans.

Effect of SOD1 expression in cell and animal model of familial amyotrophic lateral sclerosis

Elisa Onesto, Mariarita Galbiati, Arianna Zito, Daniela Sau, Valeria Crippa, Angelo Poletti
Department of Endocrinology, Physiopathology and Applied Biology, CEND, CIMND, Univesità degli Studi di Milano, Italy

The Amyotrophic Lateral Sclerosis (ALS) is an adult onset neurodegenerative disease characterized by the progressive loss of motor neurons that induces muscle weakness and atrophy. Some familiar forms of ALS (fALS) are linked to a mutant superoxide dismutase type 1 (SOD1) that acquires neurotoxic properties that lead to motor neuron degeneration. Since fALS is not a cell-autonomous disease, involving multiple motoneurons and also their target, muscle cells, we generated two models of fALS. Motor neuronal (NSC34) and muscle (C2C12) cells, transfected with wild type (wt) or mutant (G93A) SOD1, have been used to compare biochemical behavior of wt and G93A-SOD1 in the two cell types. In NSC34, G93A-SOD1 appears to be excluded from the nucleus and aggregates; whereas in muscle cells there are no differences between wt and G93A-SOD1. Moreover, using filter retardation and western blot assay, we observed the presence of insoluble species and high molecular weight species in NSC34 expressing G93A-SOD1, while in C2C12 these forms appear only when the proteasome is inhibited with MG132. These aggregates may be consequence of altered turnover related to mutant SOD1 misfolding; then we studied proteasome activity using the proteasome reporter system (YFPu). Our data showed YFPu accumulation only in NSC34 expressing mutant SOD1, indicating that this protein interferes with Ubiquitin-Proteasome-Pathway. These data suggest that the degradative system in muscle cells is more efficient than that of motor neurons; this different rate of degradation may explain the selective death of motor neuron observed in ALS. Grants: Telethon-Italy (#GGP06063, GGP07063), Italian Ministry of Labour, Health and Social Affairs, University of Milan-FIRST, FONDAZIONE CARIPLO 2008-2307.

Akt activity in Endoplasmic Reticulum and mitochondria: a Ca^{2+}-dependent pathway controlling cell fate

Saverio Marchi, Carlotta Giorgi, Alessandro Rimessi, Veronica Granatiero, Martina Marinello, Elisa Rizzieri, Chiara Agnoletto, Rosario Rizzuto, Paolo Pinton
Department of Experimental and Diagnostic Medicine, Section of General Pathology, and Interdisciplinary Center for the Study of Inflammation (ICSI), University of Ferrara

The proto-oncogene Akt (also known as Protein Kinase B, Pkb) is a serine-threonine kinase and a potent inhibitor of apoptosis, activated in many human cancers. Its activation leads to a rapid translocation to different subcellular compartments, such as Endoplasmic Reticulum (ER) and mitochondria.
Recent data indicate the inositol 1,4,5-trisphosphate (IP_3) receptor (IP_3R), mediating calcium (Ca^{2+}) transfer from the ER to the mitochondria, is a target of Akt phosphorylation activity.
Here we show that constitutively active form of Akt reduces Ca^{2+} release from ER both after stimulation with agonist, coupled to the generation of IP3, and apoptotic stimuli, releasing Ca^{2+} from intracellular stores (such as arachidonic acid). This alteration of ER Ca2+ release reduces significantly cellular sensitivity to apoptosis. Interestingly, in SH-SY 5Y cells, lacking the IP_3R type 3, this inhibition mediated by Akt is negligible, suggesting that its action is specifically targeted to the isoform 3 of the IP_3R.
At the same time, activation of Akt pathway leads to a rapid accumulation of Akt itself in mitochondria. At this level, overexpression of Akt is able to "turn off" mitochondria, decreasing Mitochondrial Membrane Potential (MMP), Ca^{2+} uptake and ATP content. Interestingly, we demonstrate the interaction of Akt with the mitochondrial adenine nucleotide translocator ANT that could be responsible of the mitochondrial alteration induced by Akt.
These results reveal a primary role of Akt in shaping intracellular Ca^{2+} homeostasis, regulating the fate of a cell at different subcellular compartments.

Melanosome biogenesis: role of OA1 signaling pathway

Paola Falletta[1,2], Maria Bono[1,2], Massimilano Monticone[3], Paola Bagnato[1,2], MariaVittoria Schiaffino[4], Colin R. Goding[5], Carlo Tacchetti[1,2], Caterina Valetti[1,2]
[1]MicroScoBio Research Center, Department of Experimental Medicine, University of Genoa, Genoa, Italy
[2]Italian Foundation for Cancer Research (FIRC) Institute of Molecular Oncology (IFOM), Milan, Italy
[3]Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy
[4]San Raffaele Scientific Institute, DIBIT, Milan, Italy
[5]Signalling and Development Laboratory, Marie Curie Research Institute, Oxted, Surrey, United Kingdom

The OA1 protein is a pigment cell specific membrane glycoprotein that belongs to the GPCR (*Gprotein Coupled Receptors*) superfamily. Unlike all other known GPCRs, it localizes almost exclusively in intracellular compartments, i.e. endolysosomal and melanosomal membranes, and it is not expressed on melanocytes plasmamembrane under physiological conditions.
Absence or misfunctions of OA1 bring to Ocular Albinism type 1, an X-linked disease involved in the visual system. The phenotypic characteristic of this disease is the presence of giant melanosomes (macromelanosomes) in retinal pigmented epithelium (RPE) and skin. These observations suggest that OA1 could play a role in melanosome biogenesis. In spite of this knowledge, the physiological function of OA1 is still poorly understood.
Our results show that OA1 regulates MITF (*MIcrophthalmia-associated Transcription Factor*), a transcription factor involved all along the life of pigmented cells. MITF is located in the center of multiple signaling pathways, controlling the differentiation, morphology, proliferation, and survival of the melanocyte lineage (melanoblasts and melanocytes). Furthermore, changes in Mitf activity are implicated in melanoma progression. Mitf plays a major role in melanocytes differentiation, by inducing the key enzymes of melanogenesis.
We demonstrate that OA1 regulates MITF at transcriptional level in two distinct cell systems: the rate of production of MITF is sustained by the presence of OA1. In cells depleted for OA1, the mRNA and protein levels of MITF show a notable decrease, leading to impaired transcription of a key melanogenesis enzyme GP100. We further demonstrate that OA1 is involved in the cAMP-signaling pathway, sustaining the MITF transcription under differentiative conditions.

p130Cas adaptor as a crucial regulator of breast cancer tumorigenesis

Brigitte Bisaro[1], Maria del Pilar Camacho-Leal[1], Maura Montani[2], Rodica Cojoca[1], Guido Forni[1], Alessandro Mautino[1], Guido Tarone[1], Augusto Amici[2], Emilia Turco[1], Sara Cabodi[1], Paola Defilippi[1]
[1]Molecular Biotechnology Center, University of Torino, Italy
[2]Department of Molecular Cellular and Animal Biology, University of Camerino, Italy

p130Cas is an adaptor protein originally described as a major substrate of oncoprotein v- crk and v-src. Our previous work in MMTV-p130Cas transgenic mice, has shown that p130Cas plays a role in the mammary gland development, inducing hyperplasia. Moreover, our data also implicated p130Cas in HER2-dependent breast cancer.
To further assess the role of p130Cas in breast tumorigenesis and its potential theraupetic efficacy, we down-regulated p130Cas by RNAi in cells over-expressing the HER2 oncoprotein. p130Cas silencing affects the ability of Her2-transformed cells to grow in soft agar, migrate and invade as well as to form metastasis after intra tail vein injection. In addition, preclinical studies performed by nipple injection in the mammary gland of NeuT mice of p130Cas siRNA or of AAV viral particles expressing p130Cas shRNAs, decrease spontaneous tumour formation.
As an additional model, we silenced p130Cas in mesenchymal breast cancer cells, characterized by high levels of Cyclooxygenase-2 (Cox2), an enzyme implicated in inflammatory process and found over-expressed in various type of aggressive cancers. p130Cas down-regulation caused decreased Cox2 expression and major defects in cell proliferation, migration and invasion. The inhibition of p130Cas also induced profound morphological and biochemical changes leading to a mesenchymal-epithelial transition. In vivo, p130Cas silencing severely impaired tumour formation in xenografts. Taken together, these results demonstrate that p130Cas is a crucial regulator of both transformation and invasion of breast cancer cells, highlighting its potential use as a novel therapeutic target in human tumours.

La mesalazina inibisce la via di segnalazione proliferativa di β-catenina agendo attraverso l'induzione di μ-protocaderina in cellule di cancro colo-rettale

Sandra Parenti[1], Fabrizio Ferrarini[1], Roberta Zini[1], Lorena Losi[2], Brenno Canovi[3], Sergio Ferrari[1], Alexis Grande[1]
[1]Dipartimento Scienze Biomediche, Università di Modena e Reggio Emilia
[2]Dipartimento di Anatomia Patologica, Università di Modena e Reggio Emilia
[3]SOFAR s.p.a Milano

Numerosi studi indicano che la mesalazina (5-ASA) è un promettente candidato per la chemioprevenzione del cancro colo-rettale a causa della sua capacità di ridurre la proliferazione, aumentare l'apoptosi, attivare i punti di controllo del ciclo cellulare e i sistemi di riparo del DNA evitando allo stesso tempo i temibili effetti collaterali causati da prolungate somministrazioni di farmaci antinfiammatori non steroidei. Una recente osservazione ha suggerito che, almeno in parte, questi effetti potrebbero essere mediati dalla capacità del 5-ASA di inibire la via di trasduzione del segnale pro-proliferativa della β-catenina interferendo con la traslocazione nucleare di questo fattore trascrizionale. L'obiettivo del nostro studio è stato di caratterizzare i meccanismi molecolari responsabili di questo effetto del 5-ASA. A tale fine abbiamo trattato con 5-ASA linee cellulari di adenocarcinoma del colon analizzandone gli effetti attraverso saggi di Immunofluorescenza, DNA microarray, RT–PCR quantitativa, e Co-immunoprecipitazione. I risultati ottenuti hanno evidenziato che il 5-ASA induce l'espressione di una proteina chiamata μ-protocaderina che appartiene alla superfamiglia delle caderine ed è in grado di sequestrare la β-catenina sulla membrana plasmatica delle cellule trattate impedendone la traslocazione nucleare quindi la sua attività trascrizionale. Ulteriori studi di DNA microarray hanno evidenziato che l'espressione della μ-protocaderina subisce una notevole riduzione durante la trasformazione / progressione di tali neoplasie, suggerendo che il gene che codifica per tale proteina sia probabilmente un onco-soppressore. Queste osservazioni suggeriscono che la μ-protocaderina potrebbe esssere utilizzata come marcatore biologico per monitorare la risposta chemiopreventiva al 5-ASA.

Peptide Aptamers targeting mutant p53 induce apoptosis in tumor cells

Andrea Bisso[1,2], Elisa Guida[1,4], Cristina Fenollar-Ferrer[3], Marco Napoli[1,2], Claudio Anselmi[3], Javier E. Girardini[1,2], Paolo Carloni[3] and Giannino Del Sal[1,2]
[1]LNCIB, Area Science Park, Padriciano, Trieste
[2]Dipartimento di Scienze della Vita, University of Trieste, Trieste, Italy
[3]SISSA, International School for Advanced Studies and CNR-INFM-DEMOCRITOS Modeling Center for Research in Atomistic Simulation, Trieste
[4]present address: Instituto de Neurociencias de Alicante CSIC-UMH - Unit of Developmental Neurobiology, San Juan de Alicante, Spain

Most tumors are characterized by impairment of the p53 pathway, mostly because of mutations of the p53 gene (TP53): indeed, at least 50% of human tumors carry mutations in TP53. Interestingly, the majority of the TP53 alterations are missense mutations leading to the expression of full length point mutants that not only lost wild-type tumor-suppressive functions, but also paradoxically accumulate to high levels in tumor cells and actively collaborate with tumor progression through the acquisition of novel properties. Indeed, p53 mutants were shown to favor tumorigenesis and their expression has been associated with enhanced tumorigenic potential in mice, increased proliferation, and resistance to drugs commonly used in anticancer therapy. For these reasons, mutant p53 represents an attractive target for the development of selective anticancer therapies. The interactions and activities of selected proteins can be specifically modulated by binding of peptide aptamers (PAs). PAs consist of a short variable peptide domain usually expressed in the context of a protein scaffold and they are selected from high-complexity libraries to specifically target proteins and modulate their activity.
Here we report the identification and characterization of short PAs able to bind to different p53 mutants, whereas not to wt p53. The identified PAs specifically interfere with mutant p53 transcriptional functions and are able to trigger apoptosis selectively in tumor cells expressing mutant p53. Of note, ablation of endogenous mutant p53 almost completely abolishes PA-induced cell death, confirming the requirement of mutant p53 for PAs pro-apoptotic functions. Moreover, by molecular modeling we defined a region on mutant p53 that is predicted to be recognized by PAs.
These PAs could provide a potential strategy to inhibit the oncogenic functions of mutant p53 and improve mutant p53-targeted cancer therapies.

Endocitosi clatrina-indipendente dell'EGFR

Gilda Nappo[1], Sara Sigismund[1], Simona Polo[1, 3], Pier Paolo Di Fiore[1, 2, 3.]
[1]IFOM, Istituto FIRC di Oncologia Molecolare, Milano, Italia
[2]Istituto Europeo di Oncologia, Milano, Italia
[3]Dipartimento di Medicina Chirurgia e Odontoiatria, Università degli Studi di Milano, Milano

Il recettore per l'EGF (EGFR) puo' essere internalizzato attraverso due vie, a seconda della concentrazione di ligando. A basse concentrazioni di EGF, il recettore non e' ubiquitinato ed e' internalizzato esclusivamente attraverso una via di endocitosi dipendente da clatrina, chiamata CME (clathrin-mediated endocytosis). A concentrazioni saturanti di ligando, invece, parte del recettore viene internalizzato attraverso una via che non dipende da clatrina, chiamata NCE (non-clathrin endocytosis). In queste condizioni il recettore e' efficientemente ubiquitinato [1]. Le due vie di internalizzazione sono associate a due diverse funzioni: l'internalizzazione via clatrina e' coinvolta nel riciclo del recettore alla membrana plasmatica e nella traduzione del segnale dai compartimenti intracellulari; al contrario, la via indipendente da clatrina porta i recettori alla degradazione [2].
Il meccanismo molecolare coinvolto nell'NCE dell'EGFR non e' noto. Le uniche conoscenze disponibili indicano che questo pathway e' insensibile all'RNA interference di clatrina ed e' invece bloccato dal trattamento con agenti che bloccano il colesterolo di membrana. Per questa ragione, l'NCE e' anche definito come "internalizzazione dipendente dai rafts", regioni della membrana plasmatica arricchite in colesterolo.
Al fine di identificare i componenti molecolari dell'NCE dell'EGFR, stiamo utilizzando un approccio di proteomica su larga scala. A questo scopo, abbiamo messo a punto un protocollo per la purificazione biochimica di vescicole di endocitosi specifiche per l'EGFR. I risultati dell'intera procedura verranno presentati.

[1] S. Sigismund et al., Proc Natl Acad Sci U S A 102, 2760 (2005).
[2] S. Sigismund et al., Dev Cell, 15, 209-19 (2008).

Membrane trafficking in neuronal development

Eugenio F. Fornasiero[1], Dario Bonanomi[1,5], Annette Gärtner[4], Simon Halegoua[2], Gregorio Valdez[2], Andrea Menegon[1], Fabio Benfenati[3], Carlos G. Dotti[4], Flavia Valtorta[1]
[1]San Raffaele Scientific Institute, Division of Neuroscience, Neuropsychopharmacology Unit
[2]State University of New York at Stony Brook, Stony Brook NY, USA
[3]University of Genova and IIT Central Laboratories, Genova, Italy
[4]VIB - Catholic University of Leuven, Leuven, Belgium
[5]The Salk Institute for Biological Studies, La Jolla CA, USA

The establishment and maintenance of an organized architecture are essential processes for proper neuronal activity and ultimately for the functioning of the whole brain. The plasmalemma is the neuron's largest organelle undergoing continuous reconfiguration during the first steps of neuronal development. For this reason, spatially-regulated plasma membrane traffic together with polarized exocytosis and endocytosis are tightly regulated, fundamental processes for the development of neurons. In the present work we address the identity and regulation of the membrane paths during neuronal growth and axonal remodelling of young hippocampal neurons in vitro. At early developmental stages the membrane is delivered, retrieved and rearranged in a constitutive manner. At later stages, during synaptogenesis, a program of maturation leads to a change in plasma membrane dynamics corresponding to the emergence of depolarization-induced synaptic vesicle exo-endocytosis. We propose that the control of bulk membrane retrieval accounts for the efficient remodelling of the axonal plasma membrane and growth cone during axonal outgrowth and that its down regulation during and after synapse formation has a role in the preservation of synaptic vesicle specificity.

Impaired motor coordination and cortical hypomyelination in Hemopexin-null mice

Noemi Morello[1], Elisabetta Tonoli[2], Lorenzo Silengo[1], Alessandro Vercelli[2], Fiorella Altruda[1], Emanuela Tolosano[1]
[1]Molecular Biotechnology Center, University of Torino, Torino, Italy
[2]Dept of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Torino, Italy

Hemopexin is a plasma protein with the highest heme binding affinity. We have recently reported that Hemopexin-null mice show dysregulated iron homeostasis in the central nervous system as they accumulate iron in oligodendrocytes in the basal ganglia. Iron loading is not associated to an adequate increase in ferritins, thus resulting in oxidative stress. As iron levels in oligodendrocytes may affect myelinogenesis and, consequently, motor behaviour, we analyzed myelin distribution and motor performance in Hemopexin-null mice at 2 and 12 months of age.
Myelin-specific Black-Gold stain showed a reduction in cortical myelinated fibers in Hemopexin-null mice compared to wild-type controls both at 2 and 12 months of age. These results were confirmed by biochemical analysis showing a reduction of the myelin binding protein (MBP) expression in the cortex and basal ganglia of Hemopexin-null mice compared to age-matched wild-type controls. Motor coordination was analyzed with the rotarod test. Hemopexin-null mice were unable to perform efficiently on the rotarod starting from five months of age. Motor impairment worsened with age, the rotarod mean score being reduced of 16% and 31% in Hemopexin-null mice compared to wild-type animals at 6 and 12 months, respectively.
These data demonstrate that hemopexin affects motor performances, likely interfering with myelination and may be explained either by an effect of Hemopexin on oligodendrocyte differentiation or by an effect on myelin synthesis in fully differentiated oligodendrocytes. To discriminate between these possibilities, quantification of oligodendrocytes at different developmental stages is currently under investigation.
Taken together, our data suggest that modulation of Hemopexin expression in brain might be an important factor in the pathogenesis of human neurodegenerative disorders characterized by myelin deficits.

The expression study of the heat shock protein 70 cytoplasmic subgroup in *Tetrahymena thermophila*

Ting Yu, Sabrina Barchetta, Antonietta La Terza, Cristina Miceli
Dip. Biologia MCA, Univ. di Camerino, Italia

Heat shock proteins (HSPs) are good candidates for expression regulation and cell stress response studies due to their fast and high induction ability under various stress conditions. Although several HSPs genes have been reported as good phylogenetic markers in ciliates, the expression and functional studies on HSPs were never carried out comprehensively. A complete survey on Tetrahymena macronuclear genome database indicates the presence of total 14 predicted HSP70 genes. Five genes with high sequence similarity cluster into one subgroup, and the orthologs of this subgroup from other organisms are generally localized to cytoplasm and are thought to be critical molecules during cell stress responses. We performed a series of experiments,mainly by real-time PCR, to understand the transcription profiles of this HSP70 subgroup. Our results suggest that, although highly similar in their gene sequences,the 5 genes showed very different transcription patterns after the cells being heat-shocked with varied time and temperatures. An inspection of the un-translated regions of the 5 isoforms suggested a possible mechanism for their distinct transcription patterns, and a more elaborate experimental analysis by constructing reporter vector with regulating sequences is ongoing at this moment. This study is the first to report the expression pattern of a complete subgroup under the HSP70 subfamily, and provides many relevant hints for further clarification of the mechanism of transcription regulation in these eukaryotes.

The Par/aPKC complex controls the vectorial migration of medaka macrophages *in vivo*

Carolina L. Crespo[1], Raffaella Molteni[1], Barbara Clissi[1], Philipp Keller[2], Jochen Wittbrodt[3], Ruggero Pardi[1]
[1]Leukocyte Biology Unit, S. Raffaele University School of Medicine, Milan, Italy
[2]Cell Biology and Biophysics Unit, EMBL, Heidelberg, Germany
[3]Heid elberg Institute for Zoology-Univ. of Heidelberg, Heidelberg, Germany

The establishment and maintenance of cell polarity is a requirement for leukocyte migratory response to inflammatory cues. A conserved polarity complex consisting of Par3, Par6 and atypical PKC (aPKC) in conjunction with small GTPases of the Rho Family temporally and spatially controls polarization in several cell types. We used a Medaka (*Oryzias latipes*) transgenic line as a model system to explore the functional role of this signaling complex *in vivo* during wound-triggered macrophage polarization and directed migration. By using a transient transgenesis approach, we overexpressed specifically in macrophages the orl_Par3 dominant-negative deletion mutant known to interfere with Par3/aPKC binding. Compared with control cells, orl_Par3 mutant-expressing macrophages display a prolonged "sensing time" with non-polarized status and, as an outcome, a delayed response to the injury. Closer inspection of cell morphologies reveals increased number of unpolarized pseudopodes and elongated phenotypes in mutant-expressing cells. Accordingly, blocking PKCξ signaling with a myr-PKCξ pseudosubstrate inhibitor impairs macrophage directional motility when compared with untreated cells. RhoA signaling is contributing as well to stabilize macrophage asymmetry as abnormal cell elongation and concomitant reduction in cell speed occurs in the presence of a Rho Kinase pharmacological inhibitor. Collectively, our data support the hypothesis that the Par/aPKC complex and its effector GTPases are involved in regulating the directional migration of myeloid cells responding to inflammatory cues *in vivo*.

Physical activity counteracts muscle wasting and increases life span in tumor-bearing mice

Emanuele Berardi[1,2], Dario Coletti[1,2], Paola Aulino[1], Veronica Cardillo[1], Mario Molinaro[1,2], Sergio Adamo[1,2]
[1]Dept Histology and medical Embryology, Sapienza University of Rome
[2]Interuniversity Institute of Myology

Cachexia, a muscle wasting syndrome associated to many chronic diseases, is a bad prognostic factor, interferes with therapy and worsens quality of life. We investigated the effects of physical activity by wheel running on adult Balb/C mice bearing a colon carcinoma (C26) as a model of cancer cachexia. Histological and molecular analysis performed on cachectic muscle of tumor-bearing mice showed an increased number of damaged myofibers which correlated with necrotic events. In tumor-bearing mice we found, by WB analysis, up-regulation of Pax7 and MyoD signals, both in uninjured and injured muscles, which is not followed by expression of later regenerative hallmarks. By flow cytometry we found that satellite cell proliferation was increased in tumor-bearing mice. The above suggests activation of satellite cells in cachexia which results in an abortive attempt to maintain muscle homeostasis. Spontaneous physical activity counteracted cachexia at multiple levels, while having some effects on the damage of specific muscles. Exercised mice showed: rescued body weight loss, maintenance of muscle mass and resistance to fatigue, reduced protein degradation and increased food intake. All together these responses resulted in a significant increase in survival of exercised tumor-bearing mice as compared to unexercised tumor-bearing mice. Our data highlight the importance of physical activity for an integrated approach aimed against cancer cachexia.

Smad2 and 3 transcription factors control muscle mass in adulthood

Roberta Sartori[1,2,3], Giulia Milan[1,2,3], Maria Patron[1], Cristina Mammucari[1,3], Bert Blaauw[1], Reimar Abraham[1], Marco Sandri[1,2,3]
[1]Venetian Institute of Molecular Medicine, Padova, Italy
[2]Dulbecco Telethon Institute, Padova, Italy
[3]Department of Biomedical Sciences, University of Padova, Padova, Italy

Loss of muscle mass occurs in a variety of diseases, including cancer, chronic heart failure, aquired immunodeficiency syndrome,
diabetes, and renal failure, often aggravating pathological progression. Preventing muscle wasting by promoting muscle growth has been proposed as a possible therapeutic approach. Myostatin is an important negative modulator of muscle growth during myogenesis, and myostatin inhibitors are attractive drug targets. However, the role of the myostatin pathway in adulthood and the transcription factors involved in the signaling are unclear. Moreover, recent results confirm that other transforming growth factor-β (TGF-β) members control muscle mass. Using genetic tools, we perturbed this pathway in adult myofibers, *in vivo*, to characterize the downstream targets and their ability to control muscle mass. Smad2 and Smad3 are the transcription factors downstream of myostatin/TGF-β and induce an atrophy program that is muscle RING-finger protein 1 (MuRF1) independent. Furthermore, Smad2/3 inhibition promotes muscle hypertrophy independent of satellite cells but partially dependent of mammalian target of rapamycin signaling.
Thus myostatin and Akt pathways cross-talk at different levels. These findings point to myostatin inhibitors as good drugs to promote muscle growth during rehabilitation, especially when they are combined with IGF-1-Akt activators

Study of the mechanism of intoxication of snake presynaptic PLA2 neurotoxins

Marco Paoli[1], Michela Rigoni[1], Eva Milanesi[1], Paola Caccin[1], Andrea Rasola[1], Paolo Bernardi[1], Grielof Koster[2], Ornella Rossetto[1], Antony D. Postle[2], and Cesare Montecucco[1]
Dip. Sc. Biom. Sperim., Univ. di Padova, Padova

Snake presynaptic PLA2 neurotoxins (SPANs) paralyze the neuromuscular junction by hydrolyzing synaptic terminal phospholipids into lysophospholipids and fatty acids, which alter the membrane curvature and permeability of the presynaptic membrane of the nerve terminals, inducing influx of extracellular calcium and and massive synaptic vesicles exocytosis. Here, we show that SPANs are capable of penetrating inside cultured neurons and of binding selectively to mitochondria. As a result of this interaction mitochondria depolarize and undergo a profound shape rearrangement from elongated to round and swollen. We show that SPANs facilitate mitochondrial permeability transition pore (mPTP) opening, and a correlation between neurotoxins PLA2 activity and mPTP opening was found, suggesting a causal relationship. Using high sensitivity mass spectrometry, the PLA2 activity of four SPANs was determined for the first time on cultured neurons. The production of lyso derivatives of phosphatidylcholine, phosphatidyletanolamine and phosphatidylserine was monitored as a function of time for each PLA2 neurotoxin, revealing the outer layer of the plasma membrane as main site of action. The hydrolytic activity of notexin and beta-bungarotoxin levels off after 40 minutes of intoxication, comparable to the time required to cause neuromuscular junction paralysis in hemidiaphragm nerve-muscular preparation, whilst taipoxin and textilotoxin activity is maintained linear up to two hours from intoxication. Together, these findings show that the outer layer of the plasma membrane is the main target of SPANs hydrolytic activity, and that, once they enter into the cytosol, their activity is strongly reduced and possibly concentrated on mitochondria.

Role of Nuclear Factor I-A in hematopoietic lineage commitment and differentiation

Linda Marie Starnes[1,2,6] Antonio Sorrentino[3,6], Elvira Pelosi[3], Monica Ballarino[4], Ornella Morsilli[3], Mauro Biffoni[3], Marco Gabbianelli[3], Alessandro Fatica[4], Irene Bozzoni[4], Cesare Peschle[5], Clara Nervi[1,2]
[1]Department of Histology and Medical Embryology, University "La Sapienza", Rome, Italy
[2]San Raffaele Biomedical Park Foundation, Rome, Italy
[3]Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy
[4]Department of Genetics and Molecular Biology, University "La Sapienza", Rome, Italy
[5]IRCCS MultiMedica, 20099 Milan, Italy
[6]L.M.S and A.S contributed equally to this study

Hematopoietic lineage commitment and differentiation is governed largely by a selective combination of transcription factors (TFs). The TF Nuclear Factor I-A (NFI-A) is a member of the NFI TF family that are known for their positive and negative transcriptional regulatory roles in a cell type and promoter specific context. NFI-A has a major role in brain development, and shows a unique pattern of expression in the developing mouse embryo. NFI-A was previously noted by our group as a relevant target of the myeloid regulator microRNA-223, whereas nothing is known on its role in normal erythro-granulopoiesis. Here we have identified NFI-A as being necessary for directing hematopoietic progenitors (HPCs) to the erythroid (E) or granulocytic (G) lineage. In cord blood CD34+ HPCs placed in hematopoietic unilineage culture differentiation systems, we demonstrated a lineage specific expression pattern of NFI-A: during E differentiation it is strongly upregulated whereas during G differentiation is markedly downregulated. Using lentiviral vectors encoding NFI-A and siNFI-A for expression in myeloid cell lines and in CD34+ HPCs, we showed that NFI-A is required for E differentiation and its overexpression enhances E differentiation under suboptimal erythropoietin concentrations. Conversely, the silencing of NFI-A during unilineage G differentiation is required as its overexpression blocks G differentiation. Using an (E+G) bilineage culture system exogenous manipulation of NFI-A was found to direct HPCs to the E or G fate. Finally, a dual and opposite transcriptional action of NFI-A was identified by activating the β-globin promoter and repressing G-CSF receptor expression. Altogether, these results indicate that in early hematopoiesis the NFI-A expression level acts as a novel factor directing HPCs into either the E or G lineage.

Tissue-specific human-mouse conserved co-expression networks for prediction of mammalian genes functional properties

Ala Ugo, Bracco Chiara, Piro Rosario, Damasco Christian, Provero Paolo, Di Cunto Ferdinando
Molecular Biotechnology Center, Department of Genetics, Biology and Biochemistry, University of Turin, Turin, Italy

Genes and proteins of living organisms deploy their functions through a complex series of interactions. Phylogenetic conservation of co-expression relationships has been proposed as a very strong criterion to identify functionally relevant links among genes. In particular, genome-wide conserved co-expression analysis has revealed its capability in many biological areas. Previous studies based on public available gene-expression data predicted unknown gene functions (Miozzi et al, 2008) and identified many putative disease-relevant genes (Ala et al, 2008).
In order to increase the power of the approach, we have built two species-specific databases that represent the collection of all CEL files for microarray experiments downloadable from GEO, performed on Affymetrix Human U133 Plus 2.0 Array and Affymetrix Mouse 430 2.0 Array. The two databases contain nearly 5200 human and 2300 mouse experiments.
Biological features of every samples have been related to three parameters (healthy or disease, tissues or cell lines, development stage) and to the translation of biological tissues description in accurate MeSH terms.
These features allow queries to retrieve expression and co-expression patterns that help to make functional predictions more specific.
We built twenty conserved co-expression networks from these databases. Each network is constructed from specific queries based on generic (all the embryonic or all tumor cell lines) or tissue-specific collection of samples. Their analysis highlights the more fundamental genes interactions and gene behavior peculiarities observed in specific tissues or among a subset of different conditions.
Furthermore, the enrichment evaluation of various biological features (GO and OMIM terms, KEGG pathways and HPRD interactome links) has revealed the robustness of information contained in these networks.
These preliminary results strongly suggest that the inclusion of tissue-specificity in conserved co-expression analysis allows the identification of functional correlations that are missed by generic approaches.

I microRNA regolati da E1A accoppiano il differenziamento all'uscita dal ciclo cellulare

Matteo Marzi[1], Eleonora Puggioni[2], Valentina Dall'Olio[3], Loris Bernard[4], Marco Crescenzi[2], Fabrizio Bianchi[1], Francesco Nicassio[1], Pier Paolo Di Fiore[1,4,5]
[1]Fondazione IFOM, Milan, Italy
[2]Istituto Superiore di Sanità, Rome, Italy
[3]Cogentech, Milan Italy
[4]Istituto Europeo di Oncologia, Milan, Italy
[5]Dipartimento di Medicina, Chirurgia ed Odontoiatria, Universita' degli Studi di Milano, Milan, Italy

La proliferazione e il de-differenziamento sono due elementi chiave del processo di tumorigenesi. Il nostro studio si basa sulla capacita' dell'oncogene adenovirale E1A di superare il blocco proliferativo di miotubi terminalmente differenziati. In precedenza questo modello ha fornito indicazioni su un gruppo di geni correlati al cancro e capaci di predire il rischio di metastasi in database indipendenti di carcinoma alla mammella (Nicassio F et al., JCI 2005).
Attraverso l'integrazione di profili di espressione di mRNA e di microRNA, abbiamo ora svelato una profonda interazione tra il livello di controllo trascrizionale e post-trascrizionale.
L'azione di E1A e' stata largamente correlata alla sua capacita' di inibire le funzioni anti-proliferative della famiglia di proteine Rb, con conseguente attivazione dei fattori trascrizionali E2F. Al contrario mostriamo ora che sui microRNA E1A ha prevalentemente un effetto Rb-E2F indipendente e che la maggior parte dei microRNA vengono indotti durante il processo di differenziamento e down-regolati al reingresso nel ciclo cellulare da E1A.
L'analisi degli mRNA target mostra che questi miRNA hanno un chiaro effetto inibitorio sui geni proliferativi Rb-E2F dipendenti. Si viene pertanto a configurare, nella regolazione dello stato post-mitotico, un loop regolatorio, sia in condizioni fisiologiche (differenziamento) che patologiche (E1A).
Sorprendentemente l'analisi di i) un modello 3D di morfogenesi di acini mammari, ii) di linee cellulari derivate da tumori primari alla mammella e iii) di diversi modelli isogenici di trasformazione, rivela che le stesse famiglie di microRNA identificate nel modello funzionano da regolatori negativi del ciclo cellulare anche in altri contesti fisiologici e potrebbero avere un ruolo di oncosoppressori.

Poster Abstracts

P1

L'endocitosi mediata da clatrina nella regolazione del destino dell'EGFR

Veronica Algisi[1], Sara Sigismund[1], Pier Paolo Di Fiore[1,2,3]
[1]IFOM, Istituto FIRC di Oncologia Molecolare, Milano, Italia
[2]Istituto Europeo di Oncologia, Milano, Italia
[3]Dip.di Medicina, Chirurgia ed Odontoiatria, Univ. degli Studi di Milano, Milano, Italia

Il recettore per il fattore di crescita epidermale (EGFR) può essere internalizzato attraverso differenti processi di endocitosi a seconda della concentrazione di ligando. A basse concentrazioni, il recettore è internalizzato quasi esclusivamente attraverso endocitosi mediata da clatrina (CME, Clathrin-Mediated Endocytosis). Ad alte dosi di ligando, una parte dei recettori viene ubiquitinata, internalizzata mediante una via endocitica indipendente dalla clatrina (NCE, Non-Clathrin Endocytosis) e destinata alla degradazione via lisosoma [1,2].
I recettori che entrano attraverso CME possono subire due diversi destini. Una parte dei recettori raggiunge gli endosomi ed e' poi riciclata sulla membrana plasmatica. Questo permette una prolungata trasduzione del segnale dai compartimenti intracellulari. Una parte dei recettori e' invece destinata alla degradazione [2].
Una serie di evidenze raccolte nel nostro laboratorio indicano l'esistenza di due popolazioni di vescicole di clatrina, legate ai due diversi destini che il recettore segue una volta internalizzato. Queste vescicole sono caratterizzate dalla presenza di diversi adattatori molecolari. In questo studio, abbiamo svolto un'analisi dettagliata di queste proteine mediante tecniche di RNA interference, saggi biochimici e live imaging.

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[2] S. Sigismund et al., Dev Cell, 15, 209-19 (2008).

P2

Constitutively active Stat3 synergizes with the Neu oncogene in mammary tumorigenesis

Isaia Barbieri[1], Sara Pensa[1], Tania Pannellini[2], Paolo Provero[1], Christine J. Watson[3], Piero Musiani[2], Valeria Poli[1].
[1]Molecular Biotechnology Center, University of Turin, Turin, Italy
[2]CeSI Anatomia Patologica, University G. D'Annunzio, Chieti, Italy
[3]Department of Pathology, University of Cambridge, Cambridge, UK

The transcriptional activator Stat3 is constitutively activated in about 70% of human tumors where its inactivation induces growth arrest and apoptosis. Although Stat3 is able to regulate several aspects of tumor genesis, the molecular mechanisms are not fully understood. To investigate the role of STAT3 in mammary tumor genesis, we intercrossed MMTV-Her2Neu transgenic mice (Neat), which develop multimodal mammary Aden carcinomas at high multiplicity, with mice expressing a constitutively active form of STAT3 (STAT3C). NeuT;STAT3C mice develop faster growing, more invasive, less differentiated and less apoptotic tumors. Interestingly, tumor-derived cell lines from NeuT;STAT3C mice show increased invasive capacities. NeuT/STAT3C cells form more dynamic cell-cell contacts, displace actin from the membrane while forming abundant actin stress fibres and are more metastatic in vivo. By comparing the gene expression profiles of NeuT/STAT3C and NeuT/STAT3WT cells, we identified a few genes known to be involved in the cross-talk between cells and extracellular environment which are upregulated in both STAT3C-expressing cells and tumors. We focused our attention on Cten, a peculiar member of the Tensins family. Tensins are cytoplasmic proteins that link the actin cytoskeleton to integrin-based adhesion sites. Although Cten lacks the N-terminal actin binding domain and could contribute to form more dynamic cell-matrix contacts. Cten silencing in NeuT;STAT3C results in significant inhibition of their migration potential and partially reverted the defects in cell-cell junctions.
Cten is upregulated in response to EGF stimulation and required for EGF induced migration of human mammary epithelial cells. We demonstrated that Cten is upregulated also upon IL6 stimulation in a STAT3-dependent manner.
Thus, constitutively active STAT3 can synergize with the Neu oncogene to promote cell survival and trigger cytoskeleton and cell junctions reorganization, leading to enhanced motility and invasion/metastases, and Cten may represent a key target for these activities. The molecular mechanisms through which STAT3 controls Cten expression are currently under investigation

P3

Effects of overexpression of spermine oxidase (SMO) in brain in physiological and pathological conditions

Gabriella Bellavia, Manuela Cervelli, Paolo Mariottini
Dip. Biologia , Univ Roma Tre, Rome, Italy

Spermine oxidase (SMO) is an enzyme involved in the polyamine homeostasis in animal cells, oxidizing spermine to produce spermidine, aminopropanal and H2O2. Polyamines (PA) have important roles in protein synthesis, cell division and cell proliferation, and the PA uptake, synthesis and degradation are finely tuned. Increases in polyamine metabolism have been implicated in several neuro-pathological conditions, including excitotoxicity. In order to define the role of SMO enzyme in brain physiology and pathology we have generated two mouse models conditionally overexpressing SMO, based on the Cre-loxP system, in either the nervous system (Dach-SMO line) or in total body (Total-SMO line). The rationale of generating the Dach-SMO mouse line was to analyse the enzyme overexpression effect in cortical neurons with no pleiotropic effect due to other organs. On the other hand, Total-SMO line allows us to analyse SMO overexpression in all the brain regions and in both neuronal and glial cells. We have analysed by RT-PCR the levels of SMO transcript and of the other enzymes involved in polyamine metabolism (SSAT, APAO and ODC) in both transgenic models. In parallel, we have also measured SMO enzymatic activity and PA contents in the same samples. As expected, SMO activity resulted higher in brain than in other organs in both transgenic lines. We have also studied in these mice the effect of SMO-overexpression in excitotoxic conditions, by kainic acid (KA) injection experiment. We have analyzed by immunohistochemistry the expression of different markers of brain injury. In particular, we have examined neuronal degeneration and astrocyte and microglia activation. It resulted that both transgenic SMO mouse lines are more sensitive to KA than their syngenic littermates, suggesting a role of this enzyme in the KA-induced stress probably mediated by its reaction products.

P4

Regulation of FoxO3 transcription factor during muscle atrophy

Enrico Bertaggia[1], Luisa Coletto[1], Gabriella Dobrowolny[2], Antonio Musarò[2], Marco Sandri[1]
[1]Dulbecco Telethon Institute at Venetian Institute of Molecular Medicine, Padova, Italy
[2]Institute Pasteur Cenci-Bolognetti, Department of Histology and Medical Embryology, CE-BEMM and IIM, Sapienza University of Rome, Rome, Italy

FoxO proteins are transcription factors that control cell cycle progression, DNA repair, muscle atrophy, stress resistance and apoptosis. These divergent functions are carefully regulated by post-translational modifications including phosphorylation, ubiquitination and acetylation.
In response to oxidative stress FoxO can translocate into the nucleus. The mechanisms which regulate FoxO relocalization include monoubiquitination, acetylation and phosphorylation. Acetylation occurs after oxidative stress and negatively modulate FoxO activity by interfering FoxO interaction on target promoters. We recent studied the role of oxidative stress in activation of FoxO3 and of an atrophy program by using transgenic mice that express SOD1G93A specifically in skeletal muscles. We studied the consequence of acetylation on FoxO3 transcriptional activity in adult skeletal muscle. Furthermore, we have generated different FoxO3 mutants which mimic acetylation or prevent acetylation. The different mutants show different capability to transactivate Atrogin-1 promoter and to induce an atrophy program when they are expressed in adult muscles. Moreover acetylation and ubiquitination are tightly coordinated to modulate FoxO activity in atrophying muscles. Our findings shed light on the complex and elaborated regulation of FoxO transcription factors during different catabolic conditions.

P5

Cyclodextrins enhance the solubility and the turnover of aggregation prone proteins

Elena Bolzoni[1], Isabella Palazzolo[3], Paola Rusmini[1], Ada De Luigi[2], Marten Beeg[2], Mario Salmona[2], Angelo Poletti[1]
[1]Dipartimento di Endocrinologia, Fisiopatologia e Biologia Applicata, Centro di Eccellenza sulle Malattie Neurodegenerative, Università di Milano, Milano, Italy
[2]Istituto di Ricerca Mario Negri Milano
[3]NIND, NIH, Bethesda, MD, USA

Cyclodextrins (Cdx) are cyclic oligosaccharides validated as artificial chaperones for the proper protein refolding. Natural chaperones like the Heat Shock Proteins (Hsp) are involved in the quality control of the proteins, preventing misfolding, assisting the refolding or addressing to the degradation systems. It was demonstrated that the Hsps overexpression, also prevented aggregation of aberrant proteins, maintaining them in a soluble state, competent for a rapid degradation. Protein aggregation in insoluble inclusions is the hallmark of many degenerative diseases. We focused our attention on two diseases characterized by motoneuron death, Amyotrophic Lateral Sclerosis (ALS) and Spinobulbar Muscular Atrophy (SBMA). In our model of ALS we transiently expressed mutant SOD1 (SODG93A), an enzyme found mutated in 10% familial ALS cases (fALS), in immortalized motoneurons (NSC34). Our models of SBMA are based on the expression of the androgen receptor with an abnormal expansion of the polyglutamine tract (ARPolyQ) which characterize all cases of SBMA. ARPolyQ was, therefore, transiently transfected in NSC34 or stably expressed in PC12 cells, in response to doxycycline. The protein quality control system in our models was found impaired, causing proteasome saturation and ineffective clearance that possibly led to protein aggregation. We, therefore, tested the effect of Cdx on SODG93A and ARPolyQ. We investigated their role in decreasing the aggregation and the proteasome impairment using a YFPu reporter system. We found evidence that Cdx were effective in decreasing the mutant proteins inclusions keeping them soluble. Moreover, this compound enhanced the turnover of both SODG93A and ARPolyQ, increasing the protein degradation and desaturating the proteasome, even in presence of the inhibitor MG132. Cdx may, therefore, enhance alternative degradation pathways, such as autophagy and may be an interesting candidate to counteract the toxicity mediated by misfolded proteins.

P6

New role of the PML tumor suppressor in adipogenic differentiation

Angela Bononi[1], Carlotta Giorgi[1], Barbara Zavan[2], Sara Leo[1], Letizia Ferroni[2], Sonia Missiroli[1], Federica Poletti[1], Alice Roffi[1], Giovanni Abatangelo[2], Guy A. Rutter[3], Paolo Pinton[1]
[1]Department of Experimental and Diagnostic Medicine, Interdisciplinary Center for the Study of Inflammation (ICSI), University of Ferrara, Ferrara, Italy
[2]Department of Histology, Microbiology and Medical Biotechnology, Histology Unit, Faculty of Medicine, University of Padova, Padova, Italy
[3]Department of Cell Biology, Division of Medicine, Faculty of Medicine, Imperial College London, London, UK

Promyelocytic leukemia protein (PML) is a tumor suppressor implicated in leukemia and cancer pathogenesis. PML localizes to the PML-nuclear body (PML-NBs), a subnuclear macromolecular structure of which it is the essential component. PML has been demonstrated to participate in a lot of cellular processes, from cellular proliferation to senescence and apoptosis, which are facilitates through the partially or temporarily interaction of PML with many proteins. Disruption of the PML gene results in defective myeloid development and increased tumor susceptibility on treatment with carcinogens. However, the physiological role of PML in the development and homeostasis of tissues outside of the haemopoietic compartment is still unclear.
Some evidences seem to suggest a role of PML in regulating adipogenic differentiation and insulin release. Indeed, FoxO1 (Forkhead bOX-containing protein, O sub-family) after nuclear translocation induced by oxidative stress has been demonstrated to engage PML loci in β cells. Similarly, the sterol regulatory element-binding proteins (SREBPs), a family of transcription factors implicated in maintenance of cellular lipid homeostasis, have been seen predominantly a nuclear localization very similar to PML-NBs.
Here we show that PML plays an essential role in adipogenic differentiation. Deletion of PML impaired high glucose dependent adipogenic differentiation of Muscle-Derived Stem Cells *in vitro* and reduced fat accumulation *in vivo*. Moreover, in PML null mice, a higher expression of mRNA transcripts of genes involved in insulin secretion (Maf-A, NeuroD, Pdx-1 and GLUT-2) was revealed. Despite this, no differences in insulin release between wild type and PML null mice were observed, as well as for insulin mRNA levels, and insulin tolerance or glucose utilization *in vivo*. Thus, we propose that PML plays a novel interesting role in the regulation of the complex transcriptional pathway that promotes adipocyte differentiation.

P7

P53 localization at ER and mitochondria is essential for apoptosis induction modulating calcium homeostasis

Massimo Bonora[1], Carlotta Giorgi[1,2], Roberta Siviero1, Chiara Agnoletto[1], Sonia Missiroli[1], Federica Poletti[1], Simone Pattergnani[1], Alessandro Rimessi[1], Giannino Del Sal[3], Rosario Rizzuto[1], Paolo Pinton[1]
[1]Department of Experimental and Diagnostic Medicine, Section of General Pathology, Interdisciplinary Center for the Study of Inflammation (ICSI) and Emilia Romagna Laboratory BioPharmaNet, University of Ferrara, Ferrara, Italy
[2]Vita-Salute San Raffaele University, Center of Excellence in Cell Development, and IIT Network, Research Unit of Molecular Neuroscience, Milan, Italy
[3]Laboratorio Nazionale Consorzio Interuniversitario Biotecnologie (LNCIB), Area Science Park, and Dipartimento di Biochimica Biofisica e Chimica delle Macromolecole, Università di Trieste, Trieste, Italy

P53 is a notorious proapoptotic protein involved in apoptosis induction or cell cycle arrest after a wide group of citotoxic stimuly. Recently has been show that p53 translocates to mitochondria during induction of apoptosis even if this mechanism is not fully clarified. Throw biochemical cell fractioning we show that p53 translocates at Endoplasmic Reticulum (ER) in MEF and mitochondria of HCT116 wt after doxorubicin and alpha amanitin treatment respectively while in HeLa cells after p53 overexpression. All these events result in apoptotic induction. Aequorin measurements reveal that early p53 translocation to ER (after 6h of treatment) induces increase in ER Calcium steady state, reflecting in a augmented mitochondrial calcium uptake, fundamental event in calcium dependent apoptotic stimuli. ImmunoCoIP assay shows the ability of p53 to interact with the Sarco-Endoplasmic Reticulum Ca2+-ATPase (SERCA).SERCA transfers calcium from the cytosol of the cell to the lumen of the ER at the expense of ATP hydrolysis. Our hypothesis is thus that p53 is able to increase SERCA activity and in turn mitochondrial calcium uptake. Moreover, during the early phase of p53 activation, in HCT116wt as well as in HeLa cells, p53 promotes strong ROS production together with increase in mitochondrial membrane potential and network integrity. Longer p53 activation instead results in reduced mitochondrial calcium uptake in organelles depolarized and fragmented. On the contrary, in Bax overexpressing cells, mitochondria are immediately less prone to accumulate calcium, suggesting for p53 a mechanism different from the already proposed interaction with Bcl-2 family members at mitochondria.
Our results suggest a model by which p53 could modulate calcium storage in the ER and, at the same time, mitochondrial physiology throw mitochondrial ROS production, creating a two-ways control on apoptosis induction.

P8

Role of PPP4R2 in neuronal differentiation and apoptosis

Ylenia Bosio[1], Paola Camera[1], Gaia Berto[1], Chiara Ambrogio[2], Lorenzo Silengo, Ferdinando Di Cunto[1]
[1]Dip.Genetica,biologia e biochimica,Molecular Biotechnology Center, Univ. di Torino
[2]CERMS, Dip. Scienze biomediche e oncologia umana, Univ. di Torino

Spinal muscular atrophy (SMA) is caused by reduced levels of the protein Survival of Motor Neuron and consequent loss of motor neurons, leading to progressive muscular and respiratory paralysis. SMN is an essential protein, required for the regulation of RNA methabolism and splicing. Therefore, it is not clear why reduced levels of this protein lead to a phenotype restricted to motor neurons.
Recently it has been shown that SMN is specifically involved in neuronal apoptosis and neuritogenesis; therefore, the elucidation of the other proteins that may functionally interact with SMN in a neuronal context is a very important issue.
PPP4R2 is a 50 kDa regulatory subunit of the protein phosphatase 4, a ubiquitous serine/threonine phosphatase that plays pleiotropic roles. It has been recently found that PPP4R2 interacts with the SMN complex and cooperates with SMN in the maturation of the splicing machinery. This finding raises the interesting possibility that PPP4R2 might be involved in the neuronal-specific functions of SMN.
We have found that PPP4R2 shows a unique localization in neuronal cells. It becomes enriched in neurites of differentiating PC12 cells and of the motorneuron-like NSC34 cells. Moreover in differentiating hippocampal neurons, the protein displays a very dynamic behaviour, with strong concentration in the developing axon .
PPP4R2 knockdown impairs differentiation of all these cell types, with strong effects on the axon and axonal-like processes. Consistently, even the overexpression of PPP4R2 mutants leads to distinct phenotypes in differentiating neuronal cells. Finally, we found that PPP4R2 can cooperate with SMN in specifically regulating apoptosis of neuronal cells.
Altogether, our data suggest that PPP4R2 is a new SMN partner that modulates differentiation and survival of neuronal cells.

P9

Development and characterization of an *ex vivo*- Muscle Engineered Tissue (X-MET) for cell/gene therapy

Silvia Carosio[1,2], Laura Barberi[1], Emanuele Rizzuto[1,2], Carmine Nicoletti[1], Mario Molinaro[1], Zaccaria Del Prete[2], Antonio Musarò[1]
[1]Dip. Istologia ed Embriologia Medica, Univ. di Roma La Sapienza, Italia
[2]Dip. Meccanica ed Aeronautica, Univ. di Roma La Sapienza, Italia

Cell-based therapies for severe muscle disease still struggle by the difficulty in obtaining a sufficient number of autologous myoblasts and by their inefficient incorporation into the host muscle. Poor survival of injected cells, minimal migration from injection site and rapid senescence of the surviving population have failed to produce satisfactory protocols of muscle regeneration that might be considered for therapeutic purposes. A role for stem cells in adult mammalian regeneration has been implicated by recent studies, demonstrating the homing of bone marrow-derived haematopoietic stem cells to sites of injury and subsequent differentiation into multiple tissue types. However, transplantation of bone marrow-derived stem cells into injured/pathological cardiac and skeletal muscle had so far a limited impact on muscle cell replacement.
To overcome this problem, remarkable progress have been recently done in tissue engineering technology toward the goal of creating organoids in vitro from cells and cellular scaffolding. Tissue engineering represents, therefore, the novel scientific approach that attempts to mimic neo-organogenesis to "produce" ex-vivo living tissue.
Conventional muscle-tissue engineering techniques employ artificial scaffolds, which interfere with the ability to measure and control contractile properties during tissue growth and do not mimic the complex architecture that characterizes skeletal muscle tissue.
In contrast, here we propose the generation of a 3-dimensional skeletal muscle tissue construct, named Engineered Tissue Muscle (X-MET), without the support of any scaffold and of any specific chemical layer.
The final goal of the project is to transplant the X-MET in recipient pathological models of both skeletal and cardiac muscle and verify whether the transplanted organoid is able to work in vivo and to rescue the muscle function.

P10

Trichoplein/mitostatin, a novel protein at the crossroad between mitochondria and intermediate filaments

Cristina Cerqua[1], Vasiliki Anesti[1], Kai Stefan Dimmer[1], Raffaele Baffa[2], Luca Scorrano[1,3]
[1]Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Padova-Italy
[2]Kimmel Cancer Center and Department of Pathology, Thomas Jefferson University, Philadelphia, PA, USA
[3]Dept of Cell Physiology and Metabolism, University of Geneva Medical School, Geneva, Switzerland

Trichoplein/mitostatin is a novel protein that interacts in vitro with the intermediate filament keratin and contains a trichohyalin/plectin homology domain (TPHD). It has been recently described as a tumour suppressor gene frequently deleted in bladder and prostate cancers. Fractionation experiments indicated that a large fraction of trichoplein is retrieved on mitochondria. We therefore explored the possibility that trichoplein participates in mitochondrial dynamics and morphology. Fusion to GFP of different fragments of trichoplein showed that the first 111 aa are sufficient for a punctuate distribution that partially overlaps with mitochondria. Subcellular fractionation experiments indicated that trichoplein is exclusively localized in mitochondria-associated membranes (MAM) and that keratin 8 is almost completely accumulated in this fraction as well. Like other proteins that reside in MAMs, even trichoplein seems to regulate ER-mitochondria interactions, since high levels of trichoplein dissociate the two organelles. Levels of trichoplein influence mitochondrial morphology, as its overexpression causes fragmentation of the mitochondrial network, which is independent of Drp-1, a protein that regulates mitochondrial fission. Since mitochondrial fragmentation is commonly associated with apoptosis, we are investigating a possible role of trichoplein in the death cascade. Preliminary results show that levels of trichoplein correlate with spontaneous apoptosis.

P11

NapA of *Borrelia burgdorferi* drives Th17 inflammation in Lyme arthritis

Gaia Codolo[1,2], A. Amedei[3], F. Bossi[4], A.C. Steere[5], E. Papinutto[1,6], C.T. Baldari[7], G. Zanotti[6], C. Montecucco[2], F. Tedesco[4], M.M. D'Elios[3,8], M. de Bernard[1,9]
[1]Venetian Institute of Molecular Medicine, Padova, Italy
[2]University of Padua, Department of Experimental Biomedical Sciences, Padova, Italy
[3]University of Florence, Department of Internal Medicine, Florence, Italy
[4]University of Trieste, Department of General Pathology, Trieste, Italy
[5]Center for Immunology and Inflammatory Diseases, Division of Rheumatology, Allergy and Immunology, Massachusetts General Hospital, Harvard Medical School, Boston, United States
[6]University of Padua, Department of Chemistry, Padua, Italy
[7]University of Siena, Department of Evolutionary Biology, Siena, Italy
[8]University of Florence, Department of Internal Medicine, Florence, Italy
[9]University of Padua, Department of Biology, Padua, Italy

This study was undertaken to evaluate the role of the innate and acquired immune responses elicited by the protein NapA of *Borrelia burgdorferi* in Lyme arthritis, which is characterized by an inflammatory infiltrate, consisting mainly of neutrophils and T cells. In the past years Th1 cells were proposed to play a central role in Lyme arthritis. More recently Th1 cells were shown not to be essential in inducing Lyme arthritis in mice suggesting that other mediators and T cells are involved. In particular the attention was focused on Th17 cells.
We analized the cytokine profile of synovial fluid T cells, from patients with Lyme arthritis, specific for NapA and we observed that these cells produced interleukin (IL)-17 in response to NapA. In agreement with these data, NapA was found to induce the expression of IL-23 in neutrophils and monocytes, and IL-6, IL-1β and transforming growth factor (TGF)-β in monocytes, all cytokines crucial for the differentiation of the Th17 subset. Finally, we demonstrated *in vivo* that NapA is able to recruit lymphocytes in rat synovia. Accordingly, it was found to promote the release of monocytes- and lymphocytes-attractive chemokines from endothelial cells and macrophages. Collectively our results suggest that NapA might be one of the major bacterial products of *Borrelia burgdorferi* responsible for triggering and sustaining inflammation within synovia, with a strong ability to recruit monocytes and lymphocytes from the blood. Moreover, we found that NapA is able to drive the expression of IL-6, IL-1β, IL-23, and TGF-β by innate immune cells and, in virtue of such an activity, to elicit a synovial T helper 17 response which, in turn, is expected to play a crucial role in the pathogenesis of Lyme arthritis.

P12

Role of FOXA in mitochondrial citrate carrier gene expression and insulin secretion

Paolo Convertini[1], Vito Iacobazzi[1,2], Vittoria Infantino[1], Faustino Bisaccia[3], Alessandra Castegna[1], Ferdinando Palmieri[1,2]
[1]Department of Pharmaco-Biology, Laboratory of Biochemistry and Molecular Biology, University of Bari, Bari, Italy
[2]CNR Institute of Biomembranes and Bioenergetics, Bari, Italy
[3]Department of Chemistry, University of Basilicata, Potenza, Italy

The FOXA subfamily of forkhead box (FOX) transcription factors includes three members, FOXA1, FOXA2, and FOXA3 that play an important role in the control of glucose metabolism by regulating multiple target genes in liver, pancreas, and adipose tissue [1]. The mitochondrial citrate carrier (CIC), also known as the tricarboxylate carrier, is an integral protein of the mitochondrial inner membrane that is essential for fatty acid and sterol biosynthesis. Furthermore, CIC has been found to be involved in the control of glucose-stimulated insulin secretion [2]. CIC mRNA and/or protein levels are high in liver, pancreas and kidney. Recently, we have begun to investigate the transcriptional regulation mechanisms of CIC. In this study, we have investigated the transcriptional role of the FOXA site present in the CIC gene promoter. We have shown that wild-type CIC FOXA site confers transcriptional activation of the luciferase gene reporter, particularly in cells overexpressing FOXA1. We have also demonstrated that FOXA overexpression and silencing increases and reduces CIC transcript and protein levels, respectively. In addition, FOXA1 silencing in pancreatic beta cells decreases not only CIC mRNA and protein but also the cytosolic citrate amount and glucose-stimulated insulin secretion. This finding is consistent with the recent report that FOXA1-deficient mouse islets exhibit a severe defect of glucose-stimulated insulin secretion [3]. On the basis of our results, the effect of FOXA1 on glucose-stimulated insulin secretion can be also explained by its transcriptional regulation of CIC gene expression.

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P13

Morphological changes in Huntington's Disease Mitochondria sustain their increased susceptibility to apoptotic stimuli

Veronica Costa[1,2], Dmitry Lim[1,3], Ernesto Carafoli[1,3], Mauro Piacentini[4], Walter Malorni[5], Luca Scorrano[1,2]
[1]Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Padova, Italy
[2]Department of cellular Physiology and Metabolism, University of Geneve, Geneve, Switzerland
[3]Departments of Biochemistry and Experimental Veterinary Science, University of Padua, Padua, Italy
[4]Department of Biology, University of Rome "Tor Vergata", Rome, Italy
[5]Department of Drug Research and Evalutation, Istituto Superiore di Sanità, Rome, Italy

Huntington's disease (HD) is a neurodegenerative disease caused by an abnormal expanded polyglutamine (polyQ) repeat in the huntingtin (Htt) protein, whose function remain unclear. Alterations of mitochondrial function have been suggested to play a central role in the pathogenesis of HD. The underlying molecular mechanisms has not been yet elucidated; however, recent reports indicate a dramatic mitochondrial ultrastructural reorganization, resembling cristae remodelling, in lymphoblasts from HD patients. We therefore explored the relationship between mitochondrial shape and function in different HD models. Lymphoblasts from heterozygous HD patients displayed polarized and clumped mitochondria, while mitochondrial network in homozygous cells was highly fragmented and distributed through the whole cell volume. Levels of mitochondria-shaping proteins were not altered, but the pro-fission protein Drp1 was retrieved on HD mitochondria, suggesting a role for the activation of the fission machinery in the observed fragmentation. Overexpression of the pro-fusion OPA1 and Mfn1 and inhibition of the fission machinery completely corrected the fragmentation. A superimposable morphological phenotype was observed in a striatal neuron cell line from a knock-in mouse model of Huntington's Disease (Hdh111). Morphological changes were accompanied by latent mitochondrial dysfunction in situ and by faster release of cytochrome c induced by recombinant BID in vitro, in line with the faster disruption of OPA1 oligomers. As a consequence, HD lymphoblasts were more sensitive to intrinsic apoptotic stimuli. Thus, alterations in pathways controlling morphology of mitochondria are likely to sustain the increased apoptotic susceptibility of HD cells.

P14

Development of a coexpression-based predictor for Drosophila positional candidates

Christian Damasco[1], Lorenzo Silengo[1], Maurizio Gatti[2], Paolo Provero[1], Ferdinando Di Cunto[1]
[1]Molecular Biotechnology Center, Università di Torino
[2]Istituto di Biologia e Patologia Molecolari del CNR, Roma

In the last few years, microarray technology produced a huge amount of expression data for several model organisms. Therefore, the development of processing tools aimed to extract useful functional information from published primary data has become a very important computational biology subject.
We previously developed data-mining approach named CLOE (Pellegrino et al., 2004) capable to extract functional informations from meta-analysis of microarray datasets of different species, based on the phylogenetic conservation of co-expression. This approach allows to make high confidence predictions about proteins function and interaction and to find relationships between genes and diseases (Ala et al., 2008). We have investigated the possibility of massively using it to identify previously unknown gene-function associations in Drosophila Melanogaster. Our first goal is the definition of the best positional candidate gene for mitotic mutations mapped to wide genomic loci by classical genetic approaches.
The positional candidate predictor is actually based on microarray data that have been collected from the Stanford Microarray Database (SMD) and Gene Expression Omnibus (GEO). All the probes were completely re-mapped to the most significant biological database, Entrez Gene, Ensembl and Flybase.
For every probes referred to genes that compose a locus isolated to have a role in mitosis, we generate a list of all the other probes, ordered by Pearson correlation coefficient. For the functional statistical analysis, we introduce the information available from high-throughput RNAi screening performed in Drosophila, defining a subset of reference genes sharing a similar mitotic phenotype. The basic mechanism of the predictor is to assign to every gene a functional score, based on ranks of the reference genes in the corresponding list.
The systematic use of our approach on artificial loci centered on known mitotic genes (leave-one-out cross-validation) shows a very good performance. We are currently testing other subsets of reference genes obtained from Drosophila RNAi screenings for other biological functions.

P15

Novel insights into the human p53 network from a genome-scale protein interaction profile of Drosophila p53

Andrea Lunardi[1,2*], Giulio Di Minin[1,2*], Paolo Provero[3], Marco Dal Ferro[1], Giannino Del Sal[1,2], Licio Collavin[1,2]
[1]Laboratorio Nazionale CIB (LNCIB), AREA Science Park, Padriciano, Trieste, Italy
[2]Dipartimento di Scienze della Vita, Università degli Studi di Trieste, Trieste, Italy
[3]Dipartimento di Genetica, Biologia e Biochimica, Università degli Studi di Torino, Torino, Italy
*AL and GDM 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, greatly expanding the current Drosophila p53 interactome.
Looking for evolutionary conservation of these interactions, we found that 37 out of 41 mammalian orthologs tested bound to one or more p53-family members. An RNAi based functional assay for modulation of the p53 pathway returned five positive hits, validating the biological relevance of these interactions.

P16

Structural and functional differences between KRIT1A and KRIT1B isoforms: a framework for understanding CCM pathogenesis

Floriana Francalanci[1], Maria Avolio[1], Elisa De Luca[1], Dario Longo[3], Valeria Menchise[4], Paolo Guazzi[2], Francesco Sgrò[1], Marco Marino[1], Luca Goitre[1], Fiorella Balzac[1], Lorenza Trabalzini[2], Saverio Francesco Retta[1]

[1]Molecular Biotechnology Centre, Department of Genetics, Biology and Biochemistry, University of Torino, Italy.
[2]Department of Molecular Biology, University of Siena, Italy
[3]Department of Chemistry IFM, University of Torino, Italy
[4]Institute of Biostructure and Bioimaging, National Research Council of Napoli, Italy

KRIT1 is a disease gene responsible for Cerebral Cavernous Malformations (CCM). It encodes for a protein containing distinct protein-protein interaction domains, including three NPXY/F motifs and a FERM domain. Previously, we isolated KRIT1B, an isoform characterized by the alternative splicing of the 15th coding exon and suspected to cause CCM when abnormally expressed. Combining homology modeling and docking methods of protein-structure and ligand binding prediction with the yeast two-hybrid assay of in vivo protein-protein interaction and cellular biology analyses we identified both structural and functional differences between KRIT1A and KRIT1B isoforms.

We found that the 15th exon encodes for the distal b-sheet of the F3/PTB-like subdomain of KRIT1A FERM domain, demonstrating that KRIT1B is devoid of a functional PTB binding pocket. As major functional consequence, KRIT1B is unable to bind Rap1A, while the FERM domain of KRIT1A is even sufficient for this function. Furthermore, we found that a functional PTB subdomain enables the nucleocytoplasmic shuttling of KRIT1A, while its alteration confers a restricted cytoplasmic localization and a dominant negative role to KRIT1B. Importantly, we also demonstrated that KRIT1A, but not KRIT1B, may adopt a closed conformation through an intramolecular interaction involving the third NPXY/F motif at the N-terminus and the PTB subdomain of the FERM domain, and proposed a mechanism whereby an open/closed conformation switch regulates KRIT1A nuclear translocation and interaction with Rap1A in a mutually exclusive manner.

As most mutations found in CCM patients affect the KRIT1 FERM domain, the new insights into the structure-function relationship of this domain may constitute a useful framework for understanding molecular mechanisms underlying CCM pathogenesis.

P17

Bimodal pattern of chemokine induced Rap1 activation: dissecting the molecular mechanisms

Antonella Giammarresi[1,2], Monica Fabbri[1], Raffaella Molteni[1], Ruggero Pardi[1]
[1]Unit of Leukocyte Biology Vita-Salute University School of Medicine DIBIT-Scientific Institute San Raffaele, Milano, Italy
[2]Dipartimento Di Medicina Sperimentale (Dimes) Sez. Anatomia Umana, Università degli Studi di Genova, Centro IFOM Di Oncologia Cellulare E Strutturale, Genova, Italy

The multi-step leukocyte extravasation process is governed by adhesion molecules and chemotactic factors dynamically interplaying in the presence of shear forces. Responsiveness to chemotactic ligands is mediated by G protein-coupled receptors (GPCRs) which are finely regulated by a well characterized family of cytosolic proteins, beta-arrestin 1 and 2. Recent evidence indicates that, in addition to playing a regulatory role in GPCR desensitization and internalization, beta-arrestins may contribute to GPCR signaling by functioning as scaffolds for the recruitment of signaling proteins into complexes with agonist-occupied receptors. On this basis, we investigated the physiological role of beta-arrestin 2 in chemokine-driven dynamics associated with leukocyte extravasation, with special interest to the activation of the Rap1 small GTPase, recently emerged as pivotal regulator of integrin function. The analysis of KC (Keratinocyte-derived Chemokine) the Rap1 activation profile in RBL (Rat Basophilic Leukemia) cells expressing mCXCR2 shows a bimodal kinetic, with the first peak at 30"/1' and the second at 5' after stimulation. RNA interference-mediated depletion of beta-arrestin 2 specifically inhibits the Rap1 activation thereby suggesting that the formation of Rap1-GTP is regulated by beta-arrestin 2. In order to elucidate the GEFs and GAPs involved in the GTPase activation we down regulated C3G (Rap1GEF) and Spa1 (RapGAP): the former doesn't seem to be involved as C3G depletion has no effect in KC-dependent Rap1-GTP formation, while Spa-1 has probably a role in the early activation peak. Since this oscillatory chemokine-induced Rap1 activation is present on other myeloid cell lines and fresh PMN's we are now translating our research to these more appropriated cells.

P18

Comparison of mesenchymal precursor cells isolated from adult human tissues

Letizia Gulino, Ivana Manini, Barbara Gava, Enrico Pierantozzi, Vincenzo Sorrentino
Center for Stem Cells, Molecular Medicine Section, Department of Neuroscience, University of Siena, Siena

Mesenchymal precursor cells (MCs) have been derived from different sources from the adult human tissues. We established cell culture of human adult MCs from bone marrow (BM-MSCs), adipose tissue (ADAS-MCS), pancreatic islets (PID-MCs), cardiac tissue (HD-MCs) and skin (SK-MCs). Cells were characterized by their proliferative potential and colony efficenty. MCs were characterized by FACS analysis osteogenic and adipogenic differentiation potential were quantified by spectrophotometer analysis. No significant differences were observed in the expansion potential or in the percentage of CD73+, CD90+ and CD105+ among all cell types. Only in HD-MCs the percentage of CD49b+ and CD54+ cells was statistically different compared to the other cells. All MCs expressed the embryonic marker Nanog. MCs generated from different human adult tissues had a finite proliferative potential and were able to differentiate in tissues of mesoderm origin. However BM-MCs and ADAS-MCs had the higher differentiation capability while the lowest differentiation potential was observed for Sk-MCs.

P19

Alternative genes control with alternative polyadenylation sites

Antonio Lembo, Ivan Molineris, Paolo Provero
Molecular Biotechnology Center, Department of Genetics, Biology and Biochemistry, University of Turin, Turin, Italy

Many human genes are characterized by alternative transcription end sites, and there is evidence that this phenomenon is relevant to their post transcriptional regulation. In particular proliferating cells have been shown to express transcripts with shorter 3' UTRs, lacking miRNA binding sites and other regulatory elements. To study this phenomenon in cancer by we analyze public Affymetrix gene expression datasets using custom probeset definitions so as to evaluate the differential expression of those portions of the transcripts located downstream of predicted alternative polyadenylation sites. For each gene there are three probset definition, meant as coordinate couples where probes match are searched: 1. from the beginning of the gene to polyadenylation site 2. from the polyadenylation site to the end of the gene 3. from the beginning to end of the gene While this study would be ideally conducted on exon arrays, we decidedto base our preliminary analysis on the HGU-133 chips since a much larger number of cancer-related samples are available in public databases. Preliminary results show evidence of the use of different transcription end sites between breast cancer samples characterized by different tumor aggressiveness. We plan to analyze the prevalence of regulatory sites in the alternatively used transcript regions to identify relevant miRNAs and other trans-acting regulatory elements, and to extend the analysis to other types of cancer.

P20

Role of PKCθ in skeletal muscle regeneration

Luca Madaro, Piera Smeriglio, Mario Molinaro, Marina Bouchè
Dip. Istologia ed Embriologia Medica, Univ. La Sapienza, Roma, Italia

Skeletal muscle is a dynamic tissue able to respond to several stimuli, such as atrophy and hypertrophy and to regenerate following muscle damage. Satellite cells (the muscle stem cells, SCs) are involved in muscle regeneration and growth. Following muscle damage, SCs are able to give rise to myoblasts, which then merge together to form new muscle fiber or fuse to pre-existing myofibers. An initial immunological response is required to remove damaged fibers and to contribute to a favourable microevironment for SCs activation/differentiation; finally, extra-cellular matrix remodeling is required to restore muscle functionality. PKCθ is the predominantly expressed Protein kinase C isoform in skeletal muscle. It has been shown that PKCθ is involved in myofiber development, neuromuscular cross talk and metabolic homeostasis (lack of PKCθ induces insulin resistance). In this work we investigate the role of PKCθ in skeletal muscle regeneration, comparing WT C57BL6 mice with PKCθ KO C57BL6 mice, following Tibialis anterior (TA) freeze injury at different periods of time after damage. Histological analysis of Hematoxylin/Eosin-stained TA transverse sections, showed an impaired reorganization of regenerating muscle at 4 and 7 day post injury, and a reduced Cross-sectional Area (CSA) of regenerating, eMyHC (embryonic Myosin) positive, fibers in PKCθ KO mice, as compared to WT . Accordingly, immunofluorescence and western blot analyses showed a reduction in eMyHC expression in mice lacking of PKCθ. Semi-quantitative RT-PCR analysis showed a delay in the expression of SCs activated markers (MyoD, myogenin, Myf5) but not of the early marker, Pax7. These data demonstrate that lack of PKCθ impairs skeletal muscle regeneration. Whether PKCθ activity is required for SCs activation/differentiation, for the inflammatory response or for extra-cellular matrix remodeling, is still to be investigated.

P21

L'ormone tiroideo T3 promuove la sopravvivenza e la funzionalità di isole pancreatiche di ratto *ex vivo*

Claudia Mangialardo[1], Cecilia Verga Falzacappa[1,2], Giulia Moriggi[1], Antonio Stigliano[1], Vincenzo Toscano[1], Silvia Misiti[1]
[1]II Facoltà di Medicina e Chirurgia,Sapienza Università di Roma
[2]DEM,Fondazione per il Diabete, l'Endocrinologia ed il Metabolismo, Roma

Il trapianto di isole di Langherans rappresenta un'innovativa strategia terapeutica nel trattamento della patologia diabetica. Data la scarsa sopravvivenza delle isole post-trapianto negli ultimi anni molti studi si sono concentrati su fattori in grado di migliorare lo status dell'isola prima dell'impianto. Abbiamo recentemente dimostrato che l'ormone tiroideo T3 è in grado di promuovere la funzionalità e la vitalità β cellulare in linee cellulari immortalizzate, suggerendo che esso possa essere considerato un candidato per l'espansione della massa insulare ex vivo. Isole pancreatiche ottenute mediante collagenasi sono state coltivate in presenza e non dell'ormone tiroideo T3(10^{-7}M) e analizzati i diversi aspetti dell'isola. La vitalità cellulare è stata valutata mediante l'incorporazione di due fluocromi che evidenziano le cellule vitali e mediante l'analisi del core cell damage.Mediante saggio TUNEL è stata inoltre valutata la capacità della T3 di contrastare l'apoptosi indotta. I risultati ottenuti hanno dimostrato che il trattamento con l'ormone tiroideo T3 di colture primarie di isole pancreatiche di ratto ne preserva l'integrità strutturale, incrementando la vitalità β cellulare e contrastando così il processo fisiologico di morte cellulare che colpisce l'isola già dopo pochi giorni di coltura e che rappresenta uno dei principali limiti nel successo del trapianto. Abbiamo inoltre dimostrato che la funzionalità, in termini di secrezione insulinica, aumenta in seguito al trattamento ormonale.In conclusione le nostre evidenze suggeriscono l'ormone tiroideo T3 come potenziale candidato per rendere isole pancreatiche qualitativamente e quantitativamente adatte all'impianto e resistenti al rigetto.

P22

Ruolo dei geni DEPDC-1A/1B nel controllo della transizione G2/M

Stefano Marchesi[1], Francesca Montani[2], Serena Bologna[1,4], Carmela Mazzoccoli[1,5], Matteo Marzi[1], Fabrizio Bianchi[1], Francesco Nicassio[1], Pier Paolo Di Fiore[1,2,3.]
[1]Fondazione IFOM, via Adamello 16, Milano
[2]Dipartimento di Oncologia Sperimentale, Istituto Europeo di Oncologia, Milano
[3]Dipartimento di Medicina, Chirurgia ed Odontoiatria, Universita' di Milano, Milano
[4]Istituto Scientifico San Raffaele, Milano
[5]Centro di Riferimento Oncologico della Basilicata, Rionero in Vulture (PZ)

La progressione del ciclo cellulare è un processo estremamente complesso e regolato, la cui accuratezza è monitorata da diversi sistemi di controllo: i meccanismi di checkpoint. Il checkpoint che regola la transizione G2/M è quello maggiormente coinvolto nel controllo dell'integrità del genoma, e di consequenza è il primo meccanismo cellulare attivato in risposta a danno al DNA. Abbiamo identificato le proteine DEPDC-1A e DEPDC-1B come nuove componenti dei pathway che regolano la transizione G2/M. A livello strutturale, entrambe le proteine sono caratterizzate dalla presenza di un dominio Rho-GAP, suggerendo un possibile ruolo nella modulazione dell'attività di una o più Rho-GTPasi.
Abbiamo scoperto che il silenziamento dei due geni (mediante RNA interference) in cellule sincronizzate causa un ritardo nella transizione G2/M, osservato mediante analisi di immunofluorescenza e confermato con esperimenti di timelapse; inoltre, a livello molecolare, il knock down di DEPDC-1A/1B provoca un aumento del livello di fosforilazione di ERK-1/2. Entrambi i fenotipi possono essere recuperati trattando le cellule con un inibitore di MEK-1/2 (U0126), rinforzando l'idea di un'interazione genetica tra DEPDC-1A/1B e il pathway delle MAP kinasi.
In parallelo, stiamo investigando la possibile relazione funzionale tra le due proteine e le Rho-GTPasi: esperimenti preliminari hanno mostrato come l'iper-attivazione di alcune Rho-GTPasi causi un fenotipo mitotico simile a quello ottenuto con il silenziamento di DEPDC-1A/1B, supportando l'ipotesi che queste ultime modulino negativamente l'attività delle Rho-GTPasi. Mediante saggi biochimici di binding (GST pulldown) e di attività (GAP assay) stiamo cercando di caratterizzare l'interazione molecolare tra le due proteine di interesse e alcune Rho-GTPasi candidate.
In conclusione, il nostro studio propone le proteine DEPDC-1A e DEPDC-1B come nuovo e sorprendente link tra le Rho-GTPasi e il checkpoint G2/M, ipotizzando un nuovo ruolo di queste importanti proteine di signaling nella regolazione del ciclo cellulare.

P23

Autophagy controls muscle mass and force

Eva Masiero[1,2,4], Cristina Mammucari[4], Lisa Agatea[2], Bert Blaauw[2,3], Masaaki Komatsu[5], Carlo Reggiani[3], Stefano Schiaffino[2,4], Marco Sandri[1,2,4]
[1]Dulbecco Telethon Institute
[2]Venetian Institute of Molecular Medicine, Padova, Italy
[3]Department of Human Anatomy and Physiology, University of Padova
[4]Department of Biomedical Science, University of Padova, Padova, Italy
[5]Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

The ubiquitin-proteasome and autophagy-lysosome pathways are the two major routes for protein and organelle clearance in eukaryotic cells. In skeletal muscle both systems are under Akt-FoxO regulation and their excessive activation induces severe muscle loss. Conversely altered autophagy has been observed in various myopathies with accumulation of inclusions and vacuoles. However the role of autophagy in skeletal muscle has not been determined by specific loss-of-function approaches. Here we specifically deleted Atg7 gene in skeletal muscle and we analysed the contribution of autophagy to homeostasis of organelle and proteins and its role in muscle wasting. Genetic ablation of Atg7 resulted in profound muscle atrophy because of increased expression of atrophy-related genes. Physiological studies revealed an important decrease in absolute and specific force which is age-dependent. Morphological analysis showed accumulation of abnormal mitochondria, sarcoplasmic reticulum distension, disorganization of sarcomere and formation of aberrant concentric membranous structure. Autophagy inhibition exacerbated muscle loss during denervation and fasting and induced sarcolemmal instability which resulted in myofiber death. Thus maintenance of autophagy flux is important to preserve muscle mass and to maintain myofiber integrity. Our results suggest that inhibition or alteration of autophagy can contribute to muscle degeneration in some muscular dystrophies characterized by accumulation of abnormal mitochondria and inclusions.

P24

The synapsins gene family and neural differentiation in basal chordates

Luca Moronti, Mario Pestarino, Simona Candiani
Dept of Biology, Lab of Developmental Neurobiology, University of Genoa, Genoa, Italy

Synapsins are a family of neuron-specific phosphoproteins that have been implicated in synaptogenesis and in the regulation of neurotransmitter release by associating with synaptic vesicles. We have analyzed the molecular evolution of synapsin gene family. In higher vertebrates, synapsins are encoded by three distinct genes, while protochordates, as well as several invertebrates, possess a single synapsin gene. Then, studying homologous genes in basal chordates, devoid of genome duplication, could help a better understanding in the complex functions of these proteins. Invertebrate and vertebrate synapsins transcripts share similarity among three highly conserved domains known as A, C and E domain, that play distinct and overlapping roles in the synapsin-dependent regulation of neurotransmitter release. One possible hypothesis on the evolution of synapsin proteins is that new domains are added at different stages of evolution probably to cope up with the increased complexity in protein functions, and we attempt to correlate the emergence of domain E with the beginning of the genesis of nervous system organization. We report the cloning and characterization of synapsin in two basal chordates: the ascidian Ciona intestinalis and the amphioxus Branchiostoma floridae. Using several synapsin genes identified in the recently sequenced genomes of several metazoans we define the genomic organization. Such analysis reveals extensive conservation of Syn-locus in several metazoans. Moreover, developmental expression study underline that synapsin in basal chordates is a neuronal-specific marker, which is expressed in several cell types of PNS and many, if not all, CNS neurons. Finally, we demonstrate that protochordate synapsin is restricted to the post-mitotic phase of CNS development and probably is a good marker of early postmitotic neurons.

P25

Intracellular Ca2+ homeostasis and mitochondria in oligodendrocytes during oxidative stress and their role in apoptotic cell death

Simone Patergnani[1], Massimo Bonora[1], Angela Bononi[1], Fulvio Celsi[1], Carlotta Giorgi[1,2], Saverio Marchi[1], Sonia Missiroli[1], Federica Poletti[1], Alessandro Rimessi[1], Paolo Pinton[1]
[1]Department of Experimental and Diagnostic Medicine, Section of General Pathology, Interdisciplinary Center for the Study of Inflammation (ICSI) and Emilia Romagna Laboratory BioPharmaNet, University of Ferrara, Ferrara, Italy

The project address mitochondrial calcium (Ca2+) signalling and its regulation, with the aim of understanding how these events partecipate in the pathogenesis of multiple sclerosis (MS) and can be targeted by novel drugs. Ca2+ is an important and complex messenger in the mitochondria, regulating processes as diverse as metabolic stimulaiton and pro-apoptotic structural altaerations. In response to a large number of stimuli, Ca2+ flows into the cell, and reaches mitochondria. Amplitude and timing of the Ca2+flux detemines an effect that can range from a number of cell activities (Contraction, secretion, etc.)to its death. Dynamics and final outcomeof mitochondrial Ca2+ signal depend on their 3D structure and other regulatory influences (kinase, redoz state). the project utilizes a variety of experimental appproaches and will focus on three related, complementary parts aimed at obtaining a deeper insight into the complex relationship between mitochondrial Ca2+ hommeostasis and apoptosis in oligodendrocytes, with special attention to identify alterations occusrring in MS. The three subtopics are: 1)Ca2+ homeostasis in oligodendrocytes. 2) Understending the role of ROS as target of ROS-mediated oligodendrocyte loss. 3) Understending the role of different effector proteins on mitochondrial responses to ROS and during inflammatory process. Different aspect of this study highlight a terapeutical perspective: 1)the analysis of mitochondrial Ca2+ signalling in oligodendrocytes may give new pharmacological targets 2)the analysis of mitochondrial signalling and apoptosys to get deeper insight into the pathogenesis o MS.

P26

Swapping of VDAC domains to investigate structure-function relationships

Simona Reina[1], Vanessa Palermo[2], Cristina Mazzoni[2], Francesca Guarino[1], Angela Messina[1], Vito De Pinto[1]
[1]Dip. Sc. Chimiche, Università di Catania
[2]Dip. Biologia cellulare e dello sviluppo, Università di Roma "La Sapienza"

The outer mitochondrial membrane (OMM) is the boundary structure of the mitochondria, and thus plays a crucial role in mediating interactions between mitochondrial systems and the other cell compartments. OMM is permeable to various ions and metabolites due to the abundant presence of the mitochondrial porin, also known as the Voltage-Dependent Anion Selective Channel (VDAC). VDAC interacts with several mitochondrial and cytosolic proteins, including kinases and cytochrome c.
In higher eukaryotes three genes encode for VDAC. The knowledge of VDAC isoforms is mainly restricted to VDAC1 and VDAC2. On the contrary VDAC3 has been poorly studied since it does not show pore-forming activity in cellular assays or in reconstitution experiment.
In this work we investigated the effect of the substitution of the N-terminal sequence of the human VDAC3 with the homologous sequences of human VDAC1 and VDAC2. The activity of the chimeric proteins was monitored in the Saccharomyces cerevisiae strain BY4742 where the endogenous VDAC1 was deleted. Results obtained in complementation assay, chronological ageing and oxidative stress resistance measurements outline the importance of the N-terminal moiety of VDAC1 and VDAC2 in the function of the protein and of the whole mitochondria.

P27

Dissection of the factors contributing to protective immune response to Flu vaccine in mice

Vanessa Ronconi[1,2], Stefania Crotta[2], Marianna Taccone[2], Barbara Baudner[2], Wack Andreas[2]
[1]Dip. Scienze Biomediche Sperimentali, Univ. di Padova, Italia
[2]Novartis Vaccines Research Center, Siena, Italia

Influenza is still a significant cause of morbidity and mortality, with seasonal epidemics occurring worldwide. Therefore, improved vaccines that induce a broader and more potent immune response are needed to provide protection to the populations most at risk. Formulation of vaccines with potent adjuvants is an attractive approach for enhancing the performance of vaccines composed of subunit antigens and offers the opportunity to drive the immune response into a desired Th profile. In the present study we investigated the different types of immune responses induced, Th1 and Th2, for their ability to confer protection against viral infection. The MF59 adjuvant increases humoral and cellular immune responses to Flu antigens, inducing Th2-associated IgG subclasses and cytokines. The addition to MF59 of CpG oligonucleotides does not lead to a further increase of the immune response, but modifies it towards a Th1 biased profile as well as the infection with sublethal doses of influenza virus. Both Th1 and Th2 immune responses induce effective neutralizing antibody titers, as demonstrated by passive immunization experiments with sera from vaccinated or pre-exposed mice into naïve recipients. In addition, they provide protection from lethal challenge with homologous influenza virus. To investigate the role of Th1- and Th2-induced cells, we are developing an adoptive transfer model of Flu-specific CD4 T cells into naïve mice. This model will allow to assess either direct protective effector functions and whether T cell help is a limiting factor for the extent and quality of vaccine induced Ab response. Our investigations have significant implications for the development of new and improved Flu vaccines against pandemic and inter-pandemic influenza virus strains. They offer the opportunity to establish which type of immune response is more effective in the protection against viral infection and open the possibility to drive it into a desired direction by choosing appropriate adjuvants or combinations thereof.

P28

Novel ancient myosins in mammalian skeletal muscles

Alberto C Rossi[1,2], Cristina Mammucari[1,2], Carla Argentini[1,2], Carlo Reggiani[3], Stefano Schiaffino[1,2]
[1]Department of Biological Sciences, University of Padova, Italy
[2]Venetian Institute of Molecular Medicine (VIMM), Padova, Italy
[3]Department of Anatomy and Physiology, Univ. of Padova, Italy

The mammalian genome contains three ancient sarcomeric myosin heavy chain (*MYH*) genes, *MYH14/7b*, *MYH15* and *MYH16*, in addition to the two clusters of skeletal and cardiac *MYH*s. *MYH16* is expressed in jaw muscles of carnivores and primates, however the expression pattern of *MYH14* and *MYH15* is not known. Here we report that in rat and mouse, MYH14 transcripts are detected by RT-PCR in heart, slow muscles and extraocular muscles (EOM), whereas MYH15 transcripts are detected exclusively in EOM. However, MYH14 protein is expressed only in a minor fiber population present is both orbital and global layers of EOM, corresponding to slow-tonic fibers, and in the bag fibers of muscle spindles. In contrast, MYH15 protein is present in most fibers of the orbital layer of EOM and in the extracapsular region of bag intrafusal fibers. MYH14 is initially expressed in all EOM fibers and after birth becomes progressively confined to the slow-tonic fibers, while MYH15 is first detected in the orbital layer of EOM at postnatal day 7. *MYH14/7b* orthologs are present in all vertebrate classes but *MYH15* orthologs are not present in the fish genome. The Xenopus MYH15 ortholog is exclusively expressed in cardiac muscle, whereas the chicken ortholog is expressed in both embryonic and adult heart and in developing but not adult skeletal muscle, except for the orbital layer of EOM. These findings point to striking evolution of *MYH15* gene function among vertebrate classes.

P29

Anastrozole and ER+/PgR+ breast cancer patients: Gene expression change and response prediction

Vijay Singh[1,4], Maurizia Mello Grand[1], Maria Scatolini[1], Chiara Ghimenti[1], Lea Regolo[2], Alberto Zambelli[2], Alberto Costa[2], Silvia Marsoni[3], Paolo Provero[4], Giovanna Chiorino[1]
[1]Fondazione "Edo ed Elvo Tempia Valenta per la lotta contro i tumori – ONLUS", Biella, Italy
[2]Fondazione Salvatore Maugeri, Pavia, Italy
[3]Southern Europe New Drug Organization, Milano, Italy
[4]Molecular Biotechnology Centre, University of Turin, Italy

Anastrozole is a highly specific and efficient inhibitor of the aromatase enzyme, leading to profound oestrogen deprivation in post-menopausal women. Although the proven efficacy of Anastrozole for ER+/PgR+ cancer patients, this therapy still fails for approximately 20% of patients. Recent advances in genomic medicine have made it possible to analyze the gene expression profiles of tumours with relatively easy experimental high-throughput techniques. Interesting results have already been obtained in regard to gene expression profiles predictive of prognosis. Less information is available on gene profiles predictive of response to neoadjuvant therapies in general, and to anti-aromatase inhibitors in particular. We analysed the gene expression profiling of tru-cut biopsies and matched surgical specimens treated for three month with Anastrozole. The main objective of the project is to study the molecular changes induced by Anastrozole in post-menopausal ER+/PgR+ breast cancer patients and delineate a predictive signature within the expression profiles of the whole genome in breast cancer biopsies collected before treatment, by their association to clinical-pathological response to treatment. GO analysis of the genes differentially expressed in responders only, showed an enrichment in processes linked to down regulation of cell cycle and to stimulation of the immune response; on the contrary, non responders show an increase in mechanisms of induction of T-Cell anergy by MHC antigen presentation without IL-2 production. Moreover, non responders show an up regulation of network processes related to androgen receptor nuclear signalling and an increase of the pathway of synaptic transmission. We identified a predictive signature of 55 genes whose basal expression is different in responders compared to non-responders and also correlates with response data. Our gene expression signature showed a good predictive value when applied on a totally independent dataset, described in the literature.

P30

Defining the role of the adaptor protein p130Cas in mammary normal and cancer stem cells

Giusy Tornillo, B. Bisaro, P. Defilippi, S. Cabodi
MBC, Department of Genetics, Biology and Biochemistry, University of Torino, Italy

Mammary stem cells have been identified as a self-renewing multi-potent population that generates ductal, alveolar and myoepithelial cells within the mammary gland. Recent data suggest that cancer arises upon mutations in either stem or progenitor cells that acquire the ability to self-renew.
We have previously demonstrated that MMTV-p130Cas transgenic mice that over-express the p130Cas adaptor protein in the mammary gland, display epithelial hyperplasia and increased proliferation suggesting a potential role for p130Cas in the maintenance of stem/progenitor cell compartment. Moreover, p130Cas and Her2Neu oncogene synergise in in vivo mammary transformation.
To explore the relevance of p130Cas in mammary stem/progenitor cells (MaSC), we first performed FACS analyses on primary mammary epithelial cells isolated from MMTV-p130Cas and WT mice and found that p130Cas over-expression leads to amplification of cells displaying the stem\progenitor cell marker ALDH1. To investigate p130Cas involvement in MaSC self-renewal, we used an in vitro system in which mammary stem cells can be propagated in culture as floating spherical colonies termed mammospheres. Both primary and secondary mammospheres derived from MMTV-p130Cas mice are significantly bigger than those from WT, suggesting that p130Cas might enhance the amount of fast proliferating progenitors. Colony forming assays are underway to confirm this hypothesis.
To address the functional role of p130Cas in breast cancer stem cells, MCF-7 breast cancer cell-line that harbour a cancer stem subpopulation was used as experimental model. We show that MCF7-derived mammospheres express higher levels of p130Cas mRNA than adherent MCF-7 cells. Further, silencing of p130Cas in MCF-7 cells inhibits mammosphere growth. Consistently, p130Cas over-expression leads to an increase in mammosphere size.
Therefore, these preliminary results show that p130Cas might be a player in normal and cancer stem biology.

P31

Distinctive patterns of cytokine response in cultures of human airway epithelium infected by influenza viruses with different receptor specificity

Barbara Tuccino[1,2], Elisabetta Monaci[2], Alessandro Muzzi[2], Mikhail Matrosovich[3], Stefania Crotta[2]
[1]Dip. Scienze Biomediche Sperimentali, Univ. di Padova, Italia
[2]Novartis V&D, Siena, Italia
[3]Institute of Virology, Philipps University, Marburg, Germany

To test the hypothesis that virus interactions with sialic acid receptors may play a role in innate antiviral immunity, we used recombinant viruses and differentiated cultures of human airway epithelial cells (HAE). The hemagglutinin of the pandemic virus A/Hong Kong/1/68 (H3N2) (HK) differs from its putative avian precursor by 7 amino acid sobstitutions. We generated the complete recombinant virus rHK and its HA variants with amino acid reversions back to the ancestral avian sequence (rHK-5aa_I62R, N81D, K92N, S193N, G144A_Human (2-6); rHK-R2_**L226Q**, **S228G** and rHK-7aa_I62R, N81D, K92N, S193N, G144A, L226Q, S228G_Avian (2-3)). Thanks to substitutions L226Q and S228G, rHK-R2 and rHK-7aa had an avian-virus-like receptor-binding specificity. We infected HAE cultures with the viruses and collected samples from the apical and basolateral sides of the cultures at different times post infection. Virus titers were determined in MDCK cells and concentrations of chemokines and pro- and anti-inflammatory mediators were measured by a multiplex bead assay. Concentrations of most cytokines progressively increased at the apical side of the cultures in the course of the infection. Many cytokines, including T-cell-attracting chemokines, were induced to similar levels by different viruses. Some mediators were induced strongly by rHK-R2 and rHK-7aa than by rHK and rHK-5aa. Avian-like viruses stimulated a higher release of potent chemo-attractants of innate immune cells (G-CSF and IL-8), shedded adhesion molecules (CD25, VCAM-1, ICAM-1), pro-apoptotic factors (TRAIL). Avian-like viruses typically induced similar or higher levels of cytokines at the apical side compared to human-like viruses, which were stronger inducers of basolaterally secreted mediators. These data provide the first direct experimental evidence that receptor specificity of influenza viruses can significantly affect patterns of innate immune responses in human airway epithelium. Further studies are warranted to determine the role of the observed effects in the host range and pathogenicity of influenza viruses in humans.

P32

Transcriptional modulation of microRNA-223 gene expression during lineage specification of hematopoietic cells

Laura Vian[1,2], Francesco Fazi[1,2], Linda Marie Starnes[1,2], Serena Racanicchi[3], Marinella Di Carlo[3], Teresa Mangiacrapa[2], Cristina Rofani[1,2], Francesco Grignani[3], Clara Nervi[1,2]
[1]Department of Histology and Medical Embryology, University of Rome "Sapienza", Rome, Italy
[2]San Raffaele Bio-medical Park Foundation, Rome, Italy
[3]General Pathology Section, Department of Clinical and Experimental Medicine, University of Perugia, Italy

MicroRNAs (MiRs) are endogenously expressed noncoding small RNAs controlling key biological processes such as development, cell differentiation, proliferation and apoptosis by repressing specific target genes. MiRs tissue and time specific expression is modulated by unique combinations of *cis*-acting regulatory factors/elements. We described a crucial role during myelopoiesis for miR223. MiR223 expression is regulated by two putative promoter regions, both presenting DNA binding sites for hematopoietic lineage-specific transcription factors including C/EBPα, NFIA, PU.1, C/EBPβ, TAL1, GATA1 and LMO2, whose activity is required for the correct execution of the hematopoietic program. Here we investigated the transcriptional regulatory circuits modulating miR223 gene expression in relation to lineage specification and terminal differentiation of myeloid precursor cell lines into monocytic, granulocytic and erythroid lineages. By northern blot and qRT-PCR, we found that miR223 expression is increased during monocytopoiesis, strongly up-regulated during granulopoiesis and negatively regulated during erythroid differentiation. Immunoblot, ChIP and promoter assays showed that the recruitment of: i) C/EBPβ and PU.1 myeloid transcription factors on the distal promoter region of miR223 gene may drive its expression during monocytic differentiation; ii) C/EBPα to miR223 proximal regulatory region may be related to its strong induction during granulopoiesis; ii) TAL1/LMO2 and GATA1 at their sites on proximal promoter region of miR223 gene may repress its expression during erythroid differentiation. Thus, miR223 expression levels during hematopoietic lineage differentiation appear modulated by the coordinate function of regulatory lineage specific transcription factors that, by acting together mediate specific biological responses through the usage of two miR223 gene promoters, further underling the role of miR223 for the correct execution of the hematopoietic program.

P33

Role of hemopexin and FLVCR in heme homeostasis

Francesca Vinchi, Lorenzo Silengo, Fiorella Altruda and Emanuela Tolosano
Dept of Genetics, Biology and Biochemistry and Molecular Biotechnology Center, University of Turin, Turin, Italy

Heme is a crucial molecule which is required for aerobic life. Heme, a ubiquitous iron-containing compound, serves as the prosthetic group of hemoproteins, such as hemoglobin, myoglobin and cytochromes. However, free heme can be highly cytotoxic, due to its hydrophobicity and pro-oxidant properties. Therefore heme content is tightly controlled both at cellular and systemic level. This project is aimed at understanding the role of the plasma heme scavenger Hemopexin (Hx) and the heme exporter Feline leukemia virus subgroup C cellular receptor (FLVCR) in maintaining heme homeostasis. We are focusing on macrophages and hepatocytes, the main cell types involved in heme handling, by using both in vitro and in vivo models.
We analyzed heme uptake and catabolism in Raw264.7 cells treated with the heme-Hx or heme-Albumin complex. Heme uptake was significantly higher with heme-Albumin than with heme-Hx. Accordingly, Heme oxygenase-1 expression and activity, as well as Ferritins expression, were strongly induced by heme-Albumin, but only slightly increased by heme-Hx. Furthermore, the heme-Albumin complex induced Ferroportin expression both at an mRNA and a protein level, whereas the heme-Hx complex had no effect.
Conversely, on HepG2 cells, Hx mediates heme uptake with a significantly higher efficiency than Albumin. Once in hepatic cells, heme may be exported, catabolyzed or used to build new hemoproteins. The contribution of catabolism and recycling after Hx-mediated heme import in hepatocytes, is currently under investigation.
These data demonstrate that Hx prevents heme uptake by macrophages, thus suggesting that they become involved in extracellular heme catabolism only under conditions of massive hemolysis when Hx buffering capacity is overwhelmed, whereas the specific function of Hx is to mediate heme uptake and recycle in hepatocytes. The role of FLVCR in heme export from hepatocyte will be evaluated in vitro by silencing FLVCR in hepatoma cell lines and in vivo by analyzing liver-specific FLVCR conditional knockout mice under normal or heme overloaded conditions.