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Organizzatori: Pier Paolo Di Fiore e Paolo Pinton

05 Giugno 2008

11:00-13:00 **Registrazione ed Affissione Posters**

13:00-14:30 **Pranzo**

Chairman: Rosario Rizzuto

14:30-15:15 **Irene Moretti** "Role of NFAT transcription factors in skeletal muscle examined by RNAi" Padova

15:15-16:00 **Luca Carli** "Botulinum neurotoxin A promotes slow myosin heavy chain expression in mouse skeletal muscle" Padova

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

16:30-18:30 **Posters** (Numeri Pari)

Chairman: Ruggero Pardi

18:30-19:15 **Paola Aguiari** "High glucose induces adipogenic differentiation of muscle-derived stem cells" Ferrara

19:15-20:00 **Giulia Milan** "FoxO3 and FoxO1 control different atrophy-related genes" Padova

20:15 **Cena**

06 Giugno 2008

08:00-09:00 **Colazione**

Chairman: Carlo Tacchetti

9:00- 9:45 **Agata Tinnirello** "Validation of p130Cas as essential mediator of HER2-induced tumorigenesis" Torino

9:45:00-10:30 **Cristina Florean** "High content analysis of γ-secretase activity reveals variable dominance of presenilin mutations linked to familial Alzheimer disease" Padova

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

11:00-13:00 **Posters** (Numeri dispari)

13:00-14:30 **Pranzo**

Chairman: Luca Scorrano

14:30-15:15 **Carlotta Giorgi** "PML at the Endoplasmic Reticulum-mitochondria contact sites plays a critical role in apoptosis" Milano

15:15-16:00 **Diego De Stefani** "The mitochondrial Voltage-Dependent Anion Channels as master regulators of cell physiology" Ferrara

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

16:30-18:00 **Posters** (Tutti)

Chairman: Paolo Bernardi

18:00-18:45 **Micol Maritan** "Spatio temporal dynamics of cAMP in olfactory sensory neurons" Padova

18:45-19:30 **Alberto Ciolfi** "Regolazione di NFI-A da parte di proteine polycomb/trithorax durante l'emopoiesi normale e leucemica" Roma

20:15 **Cena**

07 Giugno 2008

08:00-09:00 **Colazione**

Chairman: Tullio Pozzan

9:00- 9:45 **Carlotta Costa** "Negative feedback regulation of Rac in leukocytes from mice expressing a constitutively active PI3Kgamma" Torino

9:45-10:30 **Federico Tommaso Bianchi** "Identification of an APP partner with a bionformatic approach and experimental validation" Torino

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

11:15 **Proclamazione miglior poster e miglior tesi di dottorato**

Oral Communications

(abstracts in chronological order)

Role of NFAT transcription factors in skeletal muscle examined by RNAi

Irene Moretti, Elisa Calabria, Stefano Ciciliot, Anne Picard, Schiaffino Stefano, Marta Murgia
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Calcineurin/NFAT signalling has been implicated in nerve activity-dependent fibre type specification in skeletal muscle. The role of the transcription factor NFAT, a major target of calcineurin, has been investigated using an in vivo transfection approach in rat skeletal muscles. NFAT is a family of transcription factors that comprises four members regulated by calcineurin: NFATc1, -c2, -c3, -c4. To clarify the role of each of them, we used an RNAi-based strategy: we designed specific shRNA, capable of generating siRNAs that selectively block the expression of the target NFAT. The effect of NFAT knock-down on specific MyHC promoters was examined by co-transfecting adult muscles with RNAi constructs and promoter-reporter constructs. We found that the activity of MyHC-slow promoter is inhibited by all four NFAT siRNAs. The activity of MyHC-2A and -2X promoter is inhibited by all NFAT siRNAs, except NFATc1 siRNA, and the activity of the of MyHC-2B promoter is inhibited only by NFATc4 siRNA. These results suggest that the expression of a specific myosin can be related to the particular combination of NFATs expressed in the muscle fibre, and the distribution of the endogenous NFAT isoforms in different types of fibres supports this interpretation.

Botulinum neurotoxin A promotes slow myosin heavy chain expression in mouse skeletal muscle

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On the basis of the specific myosin heavy chain isoform that they express, mammalian skeletal muscle fibers are classified as Slow (type I) or Fast (type 2) fibers. Slow fibers show low velocity of shortening and high fatigue resistance, whereas fast fibers show high velocity but lesser resistance. Muscle fibers composition is strongly influenced by nerve activity in adult skeletal muscle; in fact, a reduced neuromuscular activity (for example, spinal cord injury) promotes a slow-to-fast fiber transformation in muscle, whereas an increase of muscle activity cause a shift in the opposite direction (fast-to-slow).
We observed that a blockade of nerve activity, induced by Botulinum Neurotoxin type A (BoNT/A), promotes the expression of the slow isoform of myosin heavy chain (MyHC) in contrast with other neuromuscular inactivity models. In order to correlate the MyHC isoform switch with the muscle fiber denervation by BoNT injection we analysed the expression of the marker N-CAM (neural cell adhesion molecule). N-CAM is an integral membrane glycoprotein which accumulates on muscle fiber membrane after denervation and it is not expressed in muscle under physiological condition. The results obtained show that BoNT induced-slow fibers never express N-CAM, while nearby fibers of the same muscle are positive for N-CAM. This observation would suggest that these fibers are still active and that they could undergone to a compensatory overload that lead them to a slow phenotype shift. Future experiments will be performed to better understand this phenomenon and to investigate if botulinum neurotoxins block preferentially some fibers rather than others.

High glucose induces adipogenic differentiation of muscle-derived stem cells

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Mesenchymal stem cells residing in both adipose and muscle tissue ensure tissue regeneration, but the physiological stimuli controlling their differentiation into adipocytes remain unknown. We demonstrate that high glucose triggers adipogenic differentiation of adipose or skeletal muscle mesechymal primary cell cultures, either in standard culture condition, either in a dynamic culture system (bioreactor). In this differentiation process, two crucial events are ROS production and Protein Kinase C ß activation: in fact, differentiation can be directly induced by oxidizing agents and inhibited by PKCß siRNA silencing. The differentiated adipocytes, when implanted in vivo, form viable and vascularized adipose tissue. The data points out a novel differentiation route triggered by high glucose, concerning not only resident stem cells of the adipose tissue, but also uncommitted precursors present in muscle cells to form adipose depots and show that high glucose per se has an adipogenic potential, thus opening the search for novel signal transduction pathways operating in obesity and diabetes.

To look for further actors playing in this differentiation route, we first tested the effect of endocannabinoid in glucose-induced adipocyte differentiation. Endogenous cannabinoids and cannabinoids increase food intake and promote weight gain in animals by activating central and peripheral receptors. Our results show that endocannabinoids play a role in glucose-induced adipocyte differentiation: agonist treatment in low glucose conditions promotes adipocyte differentiation of mesenchymal stem cells, while antagonist reverts the adipogenic differentiation induced by high glucose.

Furthermore, the promyelocytic leukaemia protein (PML) is a nuclear tumor suppessor able to regulate many cellular functions through interaction with different proteins. Anyway, the role of PML in differentiation has not been elucidated yet. Interestingly, preliminary data show that PML knockout mice display a lower level of glucose-induced adipocyte differentiation than wild type mice, opening for an involvement of this protein in adipocyte differentiation of mesenchymal stem cells.

FoxO3 and FoxO1 control different atrophy-related genes

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FoxO1, 3 and 4 belong to the superfamily of Forkhead transcription factors and are expressed in skeletal muscles. FoxOs are involved in several cellular functions including activation of an atrophy program which leads to muscle loss. FoxO factors are under IGF-1/PI3K/AKT control and in physiological conditions are phosphorylated by AKT and sequestered into the cytoplasm. On the other hand, in many pathological conditions FoxOs are dephosphorylated, translocate into the nucleus and interact with promoters of target genes. Since it is known that FoxO1 and FoxO3 overexpression causes muscle atrophy we asked whether they control the same set of atrophy-related genes or whether every FoxO member regulates a peculiar subset of genes. To address this question we used Chromatin immunoprecipitation (ChIP) approach. First we determined which are the FoxO1 and FoxO3 binding site on promoter of Atrogin-1, the critical atrophy-related ubiquitin ligase. ChIP was performed on endogenous FoxO3 and 1 in atrophying adult muscles during fasting. We show that FoxO3 binds to specific regions of Artogin-1 promoter and induces histones acetylation. Surprisingly these sites are specific for FoxO3, since FoxO1 does not or weakly interacts. Using dual-luciferase-assay, we confirmed the preferential FoxO3-mediated transactivation of Atrogin-1 promoter. All these results suggest that FoxO members potentially control different subset of atrogenes. To identify these genes, we applied ChIP on Chip technique that extends the analyses of protein-DNA binding to promoter regions of whole genome. By different ChIP on Chip experiments we identified the FoxO3-dependent and FoxO1-dependent atrogenes. The overlap of these two groups shed a light into the mechanisms of FoxO-mediated muscle atrophy and define the critical genes that are required for the atrophy program.

Validation of p130Cas as essential mediator of HER2-induced tumorigenesis

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To investigate the role of p130Cas in mammary epithelium, we generated transgenic mice MMTV-p130Cas.In vivo overexpression of p130Cas results in mammary epithelial hyperplasia during development and pregnancy and delayed involution. When MMTV-p130Cas mice were crossed with MMTV-HER2/NeuT mice, double transgenic mice display a significantly shorter latency of tumour formation than the HER2/NeuT parental strain. My aim consists in investigating the role of p130Cas in HER2 tumorigenesis. To assess whether p130Cas is required for HER2-mediated transformation my experimental strategy is based on MEFs derived from p130Cas knock out mice.To investigate the molecular mechanisms downstream HER2 transformation and to point out a essential role for p130Cas in HER2 tumorigenesis, we stably expressed human oncogenic HER2(Valin to Glutamic) in Cas-/- and Cas+/+ MEFs by mean of retroviral transduction. After puromycin selection, four stable cell lines were assayed for anchorage independent ability. Cas+/+ and Cas-/- cell lines, expressing empty vector as control, didn't give rise to colonies, as expected. Strikingly, while Cas+/+ fibroblasts expressing HER2 gave rise to an elevated number of visible colonies after 14 days from soft-agar seeding, Cas-/- cells showed 10fold less colonies than Cas+/+ cells.To further investigate the possible cooperation between p130Cas and HER2, I used three-dimensional(3D)culture of human breast MCF-10A expressing an inducible HER2. The use of MCF-10A cells stably over-expressing p130Cas, in presence of active or inactive HER2, showed that there is a cooperation between HER2 and p130Cas in inducing multiacinar formation as well as invasion. As evaluated in 3D assay, p130Cas alone could be able to induce spontaneous multiacinar formation when overexpressed, but with a lower rate compared to HER2 activated/p130Cas overexpressing cells. Interestingly, the two proteins could cooperate in inducing 3D-invasion as evaluated by laminin staining.Taken together these data indicate that p130Cas is required for HER2-induced transformation and acquirement of a tumorigenic potential.

High content analysis of γ-secretase activity reveals variable dominance of presenilin mutations linked to familial Alzheimer disease

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γ-secretase mediates the intramembranous proteolysis of amyloid precursor protein (APP), Notch and other cellular substrates and is considered a prime pharmacological target in the development of therapeutics for Alzheimer's disease (AD).
We describe here an efficient, new, simple, sensitive and rapid assay to quantify γ-secretase activity in living cells by flow cytometry using two membrane-bound fluorescent probes, APP-GFP or C99- GFP, as substrates for γ-secretase. The principle of the assay is based on the fact that the soluble intracellular domain of GFP-tagged APP (AICD-GFP) is released from the membrane into the cytosol following γ-secretase cleavage. Using this feature, the enzymatic activity of γ-secretase could be deduced from the extent of the membrane retention of the probe observed after plasma membrane permeabilization and washout of the cleaved fraction.
By applying two well-known γ-secretase inhibitors (DAPT and L-685,458), we validated our assay showing that the positional GFP-based probes for γ-secretase activity behave properly when expressed in different cell lines, providing the basis for the further development of a high-throughput and high content screening for AD targeted drug discovery. Moreover, by co-expression of different familiar AD-linked mutated forms of presenilin - the key component of the γ-secretase complex - in cells devoid of any endogenous γ-secretase, our method allowed us to evaluate in situ the contribution of different presenilin variants to the modulation of the enzyme.

PML at the Endoplasmic Reticulum-mitochondria contact sites plays a critical role in apoptosis

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Promyelocytic leukemia protein (PML) is a tumor suppressor implicated in leukemia and cancer pathogenesis. PML epitomizes a multiprotein nuclear structure, the PML-nuclear body (PML-NB), whose proper formation and function depends on PML. Studies in knockout mice and cells unraveled an essential pleiotropic role for PML in multiple p53- dependent and -independent apoptotic pathways. As a result, PML null mice and cells are protected from apoptosis triggered by a number of stimuli.
It is known that PML and the PML-NB can certainly modulate transcriptional pro-apoptotic pathways, in turn triggering apoptosis or reinforcing pro-apoptotic programs. However, it cannot be ruled out that PML might also modulate apoptotic programs or even directly trigger apoptosis through unrecognized biochemical mechanisms, which do not depend on the PML-NB and the nucleus. Indeed, some of PML isoforms display a cytoplasmic localization, where many apoptotic programs are triggered and initially executed. Interestingly, we detected PML isoforms in other cellular compartments than PML-NB, in particular in a region at close contact between endoplasmic reticulum (ER) and mitochondria, called MAM (mitochondria associated membrane). The sites of close interaction between the ER and mitochondria appear to play a key role in the participation of mitochondria to Ca2+ signalling and apoptosis.
The aim of our work is to elucidate if the ER/MAM localization of PML could be essential for apoptotic pathway and if also in that area PML acts as key player to orchestrate proteins network. In this purpose we investigated if the effects of PML in cell death process is correlated with its involvement in calcium homeostasis and if a PML chimera targeted to ER/MAM site can rescue the sensitivity to apoptotic stimulation in PML null cells.

The mitochondrial Voltage-Dependent Anion Channels as master regulators of cell physiology

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Voltage-dependent anion channels (VDACs) are the most abundant proteins located at the surface of the mitochondrion, an organelle having a crucial role in many aspects of cell physiology. VDACs are indeed involved in the regulation of cellular metabolism, calcium signaling, and cell death. While yeasts have only one VDAC, higher eukaryotes possess three distinct isoforms (VDAC1, VDAC2 and VDAC3), but the reasons of such a divergent evolution is not still clear. Here we show that these diverse mitochondrial porins share some common functions, for example by acting as Ca^{2+} channels in the mitochondrial outer membrane, supporting the efficient mitochondrial calcium uptake machinery in a similar way. However, VDAC also shows isoform-specific functions such as regulation of cell death, with VDAC1 acting as a pro-apoptotic protein and VDAC2 displaying an anti-apoptotic activity, probably through the inhibition of BAK oligomerization. Moreover we demonstrate that the different VDAC isoforms can interact with specific partners, leading to the formation of different protein complexes regulating diverse cellular responses. VDAC1 can indeed specifically interact with endoplasmic reticulum Ca^{2+} release channels (IP_3R), through the action of the chaperone GRP75, promoting the efficient calcium transfer from intracellular stores to mitochondrial matrix. VDAC2 instead is involved in the assembly of a macromolecular complex comprising mTOR, Raptor and FKBP38, and its presence is necessary for the nutrient-sensing activity of mTOR, leading to regulation of autophagy. These data underline the critical role of mitochondrial porins in many aspects of cell physiology, showing how the evolution of different isoforms relies on the formation of specific protein-protein interactions that mediates particular cellular events.

Spatio-temporal dynamics of cAMP in olfactory sensory neurons

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A unique aspect in the topographical organization of the olfactory system is the dual role played by the olfactory receptor (OR) of the olfactory sensory neurons (OSN): transduction of chemical signal in the cilia and axonal convergence to the target glomeruli in the olfactory bulb.
Indeed it has been demonstrated that the OR protein is expressed not only on the cilia dendrite , but also on the axon termini-growth cone.
It is not known how the OR can perform such different functions in the same cell.
The intracellular signalling cascade triggered by the OR activation in the cilia involves cAMP and calcium. The intracellular events triggered upon the activation of the OR at the growth cone remain to be clarified. Whether the OR on the axon termini is a functional receptor which acts through cAMP remains to be demonstrated.
The emerging view is that spatio-temporal dynamics of the variations of second messenger concentration could play a crucial role in the specificity of cellular response.
In our project we seek to clarify the dual role of the OR, studying the spatio-temporal dynamics of cAMP in OSN in cultures, using genetically encoded FRET based sensor , for cAMP.
We demonstrate that the machinery required for the generation (adenylyl cyclases) and the hydrolysis (phosphodiesterases) of cAMP is present and functional at the axon termini-growth cone.
Moreover, odor stimulation of the OR at the growth cone level indicates that the OR at the growth cone is a functional receptor, capable of binding odorants and triggering an intracellular signalling cascade, which involves cAMP and calcium, similar to the cilia dendrite.
The OR is not the only determinant in the axonal convergence mechanism: OSN expressing different OR express different level and/or type of axon guidance molecules. It has been proposed that OR- derived cAMP signal could induce a nuclear translocation of the catalitic subunit of the PKA, which in turns can regulate the expression of axon guidance molecules. We demonstrate that, after odor stimulation of the OR at the growth cone, we can detect a nuclear translocation of the catalitic subunit of our PKA probe.

Regolazione di NFI-A da parte di proteine polycomb/trithorax durante l'emopoiesi normale e leucemica

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L'equilibrio funzionale tra le proteine Polycomb (PcGs) e Trithorax (TrxGs) regola il mantenimento della staminalità cellulare e l'espressione dei geni tessuto-specifici. Anomalie nell'attività di proteine PcGs/TrxGs possono modificare l'espressione di geni regolati impedendo la normale maturazione delle cellule staminali/progenitrici e provocare la formazione/espansione di cellule staminali tumorali. Ad esempio tra le TrxGs di mammifero, le traslocazioni del gene MLL causano un'anomala espressione dei geni HOX e sono associate con forme specifiche e aggressive di leucemia.
Ultimamente abbiamo identificato il Nuclear Factor I-A (NFI-A) quale fattore di trascrizione chiave che, insieme al microRNA-223 (miR223), un suo inibitore tradizionale, ed al fattore di trascrizione CEBP/α, regola il differenziamento granulocitario. Più recentemente abbiamo osservato che la regolazione dell'espressione del gene NFI-A è sotto il controllo della proteina polycomb Yin e Yang 1 (YY1). YY1 promuove il reclutamento di Suz12, un componente del complesso repressorio polycomb PRC2 e ciò causa modificazioni epigenetiche della struttura cromatinica del promotore di NFI-A. In particolare abbiamo misurato una diminuzione del segnale attivatorio trithorax Trimetilazione (me3) sulla lisina (K) 4 dell'istone (H) 3 (H3K4me3) ed un aumento del segnale repressorio polycomb H3K27me3 nella regione regolatoria del gene NFI-A. Ciò determina il silenziamento di NFI-A e correla al differenziamento granulocitario in cellule normali e leucemiche.
Queste evidenze indicano una deregolazione dell'espressione di NFI-A da parte di PcGs/TrxGs quale evento epigenetico chiave per la mielopoiesi e potenzialmente implicato nello sviluppo della leucemia mieloide.

Negative feedback regulation of Rac in leukocytes from mice expressing a constitutively active PI3Kgamma

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Polarization of chemotaxing cells depends on positive feedback loops which amplify shallow gradients of chemoattractants into sharp intracellular responses. In particular, reciprocal activation of phosphoinositide 3-kinases (PI3K) and small GTPases like Rac leads to accumulation, at the leading edge, of the PI3K product phosphatidylinositol 3,4,5-trisphosphate (PIP3). Mice carrying a “knock-in” allele of the G protein-coupled receptor (GPCR)-activated PI3Kgamma, encoding a plasma membrane-targeted protein, appeared normal but their leukocytes showed GPCR-uncoupled PIP3 accumulation. In vivo, the mutation increased proliferation and decreased apoptosis, leading to leukocytosis, and delayed resolution of inflammation in wound healing. Mutant leukocytes showed significantly impaired directional cell migration in response to chemoattractants. Stimulated mutant macrophages did not polarize PIP3 and showed a shortened Rac activation due to enhanced PI3K-dependent activation of RacGAPs. Together with the finding that chemoattractants stimulate a PIP3-dependent GAP activation in wild-type macrophages, these results identify a novel molecular mechanism involving PI3K- and RacGAP-dependent negative control of Rac, that limits and fine-tunes feedback loops promoting cell polarization and directional motility.

Identification of an APP partner with a bionformatic approach and experimental validation

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Alzheimer disease (AD) is a neurodegenerative disorder characterized by a progressive and irreversible decline of cognitive function. Altered proteolytic processing of the Alzheimer amyloid precursor protein (APP) is well established as one of the major pathogenetic mechanism underlying AD. However, despite extensive efforts, the normal functions of this protein and their relationships with the abnormal processing remain still elusive. To investigate these issues, we have used a conserved coexpression-based bioinformatic approach, which in theory is capable to reveal the possible functions and the potential interactors of any protein. In good agreement with the literature, this analysis has revealed that APP may play a crucial role in biological processes related to cell-matrix interaction. The top scoring hit of our bioinformatic screening was the heat shock protein Hsp47, a collagen-binding protein that assists the molecular maturation of procollagen. Therefore, we are experimentally addressing the hypothesis that HSP47 is a functional partner of APP. To this aim we are conducting expression and functional studies. Although previous studies have shown that HSP47 is expressed at very low levels in CNS, we have found that Hsp47 is present in primary cultures of hippocampal neurons and astrocytes. Moreover, Hsp47 is upregulated in mouse hippocampus with after kainate-induced seizures, with a kinetic similar to the 770 APP isoform. On the other hand, we have found that HSP47 is capable to counteract the effects on cell migration induced by APP overexpression.In conclusion these results indicate that Hsp47 may functionally interact with APP and it's pathway.

Posters

Role of microRNA-133 in skeletal muscle differentiation

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Terminal differentiation of skeletal muscle cells is a multi-step process that involves irreversible withdrawal from the cell cycle, transcriptional activation of the muscle-specific genes, and fusion of differentiated myocytes into multinucleated myotubes. Several microRNAs are specifically expressed or enriched in skeletal muscle, particularly three of them, miR-206, miR-1 and miR-133 are induced during differentiation of C2C12 myoblasts. The role of miR-133 in myogenic differentiation appears controversial, as, although expressed in adult muscle and terminally differentiated cells, it seems to have a pro-proliferative activity. To asses the activity of miR-133 in myogenic differentiation, we analysed in more details the expression pattern of miR-133 in C2C12 and the effect of its inhibition and overexpression in proliferating and differentiating C2C12. We found that miR-133 is mainly expressed in myotubes and that its inhibition interferes with the differentiation ability of C2C12; specularly, its overexpression enhances myoblast differentiation, suggesting that miR-133 may also play a pro-differentiative role in myogenesis.

Poster 2

Evoluzione molecolare delle metallotioneine nei teleostei antartici: analisi filogenetica basata sulle 3'UTR

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Le metallotioneine (MT) sono proteine a basso peso molecolare, ricche di cisteine e generalmente mancanti di aminoacidi aromatici e di istidine. Sono proteine non enzimatiche chelanti i metalli pesanti e sono *scavengers* delle specie reattive dell'ossigeno (ROS). Le MT sono coinvolte in alcuni processi biochimici cellulari svolgendo il ruolo di "riserva" di ioni Cu^{2+} e Zn^{2+} durante la biosintesi di metalloenzimi e di altre metalloproteine. Tutti i vertebrati possiedono più isoforme di MT. In particolare, nei teleostei sono due isoforme. Nell'ambito della ricerca sull'evoluzione delle MT nei teleostei abbiamo caratterizzato la regione non tradotta all'estremità 3' (3'UTR) del cDNA delle isoforme di nove specie di teleostei antartici, di cui sette appartenenti alla famiglia di Nototheniidae (*Treamatomus hansoni*, *T. newnesi*, *T. eulepidotus*, *T. pennelli*, *T. lepidorhinus*, *Pleuragramma antarcticum* e *Gobbionotothen gibberifrons*), una appartenente alla famiglia di Artedidraconidae (*Histiodraco velifer*) ed un'altra alla famiglia di Bathydraconidae (*Cygnodraco mawsoni*). Dall'analisi filogenetica effettuata impiegando i quattro metodi statistici [Neighbor-Joining (NJ), Minimum Evolution (ME), Maximum Parsimony (MP) e UPGMA] e considerando altre sequenze della 3'UTR di nototenidi disponibili nel *database* di *GenBank*, risulta che tutte le MT vengono raggruppate in due cluster corrispondenti a ciascuna delle due isoforme. Questo risultato suggerisce che in questi pesci antartici esistono almeno due gruppi di geni paraloghi (MT1 e MT2). In particolare, in ciascuno dei due cluster per le specie del genere *Trematomus* si evidenziano relazioni filogenetiche ben supportate. Possiamo ipotizzare che nella 3'UTR si siano accumulate delle mutazioni che possono aver portato alla diversificazione funzionale delle MT in queste specie di teleostei. La caratterizzazione delle 3'UTR di MT di altre specie permetterà di avere un quadro più completo dell'evoluzione molecolare delle MT in questo gruppo.

Verifica ed applicazione in vivo di recenti scoperte nel campo del controllo del ciclo cellulare

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La maggior parte delle cellule che compongono un individuo adulto si trova in uno stato di non proliferazione, che può essere temporaneo, come nella quiescenza, o permanente, come nella senescenza replicativa e nel differenziamento terminale.
Recentemente è stato dimostrato che è possibile promuovere la proliferazione di questi tipi cellulari in vitro mediante la soppressione di inibitori chiave delle chinasi ciclina-dipendenti (CDKI). In particolar modo la sola rimozione dell'inibitore p21 con RNA interferente è in grado di riportare in ciclo cellule muscolari terminalmente differenziate, in assenza di ulteriori stimoli mitogenici (fattori di crescita sierici) (Pajalunga et al. 2007). Allo scopo di verificare in vivo i risultati già ottenuti in vitro, abbiamo introdotto oligonucleotidi di RNA interferenti (siRNA) per gli inibitori p21 e p27 nel muscolo tibiale anteriore murino, sfruttando la tecnica dell'elettroporazione diretta del tessuto. In seguito alla colorazione dei nuclei delle sezioni di tessuto muscolare abbiamo determinato che la soppressione di tali inibitori determina un aumento di cellularità rispetto al muscolo non trattato. Per ottenere una riduzione più efficiente e prolungata nel tempo dei CDKI nel tessuto muscolare abbiamo deciso di produrre virus adeno-associati ingegnerizzati con RNA interferente (shRNA) per i CDKI. Tali virus verranno utilizzati per infettare il muscolo di topi e verificare la riattivazione del ciclo cellulare in vivo. L'importate risultato di consentire il reingresso in ciclo di cellule post-mitotiche attraverso la sola rimozione dei CDKI chiave ha in sè un ampio potenziale applicativo che coinvolge più campi. Si potranno infatti avere applicazioni di questa scoperta sia in campo biotecnologico che nel campo della medicina rigenerativa.

Poster 4

Lipids effect on neurotransmission

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The lipids composition of membranes has an important role in several processes. Notably, the presence of lipids with particular shape can influence the curvature of the membrane needed for its fusion, so that small but localized amount of specific lipids can modify events like neurotransmission, a very regulated form of vesicles fusion.
Lysophospholipids are inverted cone shaped lipids. They can be obtained by the action of a phospholipase A2 on membrane lipids. Some neurotoxins present in snakes venoms (SPANs) are endowed with this enzymatic activity. Neuronal cells cultures treated with SPANs show significant amount of lysophosphatidylcholine(LPC) and oleic acid (analyzed by mass-spectrometry), which demonstrates that PLA2 activity is rilevant in vivo.
LPC alone is able to induce a complete but reversible paralysis of a neuromuscular junction (mouse phrenic nerve-hemidiaphragm preparations are used as a model).
In primary neuronal cell cultures, LPC induces formation of discrete bulges at various sites of neuronal projections. These bulges are characterised by accumulation of vesicular markers and by a rapid increase of intracellular calcium. All these effects can be explained by an exo-endocytotic imbalance induced by LPC as a consequence of its shape. Other lysophospholipids (lysophosphtidylethanolamine, lysophosphatidic acid, lysophosphatidylserine, lysophosphatidylglycerol and lysophosphatidylinositol) are able to induce the same effects, but LPC is the most effective among this class of molecules.
Another class of lipids termed alkilphospholipids has similar shape properties. They are proposed in anticancer therapy, and their chemical structure, with an ether bond, makes them more stable than lysolipids. Using both neuromuscular junction preparations and primary cell cultures, we tested three compounds: miltefosine, perifosine and lysoPAF. We found that they are able to induce paralysis and bulging of neurons. This data suggest that the ability to influence neurotransmission is present in different molecules with an inverted cone shape, thus opening the possibility for a large spectrum of possible drugs or research tools.

Trichoplein, a novel keratin-binding protein, localizes in mitochondria-associated membranes

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Trichoplein is a novel protein that interacts in vitro with keratin and contains a trichohyalin/plectin homology domain (TPHD). 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. The subcellular fractionation of murine liver homogenates indicated that trichoplein is exclusively localized in mitochondria-associated membranes (MAM) and that keratin 8 is almost completely accumulated in this fraction as well. Levels of trichoplein influence mitochondrial morphology, as its overexpression causes fragmentation of the mitochondrial network, which is independent from 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.

Poster 6

Cell-specific regulation of ferroportin expression after phlebotomy

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Expression of Ferroportin, the sole known iron exporter, is mainly regulated, at post-translational level by Hepcidin. Moreover, Ferroportin may be regulated at transcriptional level by several stimuli.
The aim of this study was the analysis of Ferroportin expression under conditions of increased iron demand. To this end, the response to phlebotomy of Haptoglobin-null and wild-type mice was analyzed. Haptoglobin-null mice accumulate iron in proximal tubular cells of kidney and in reticulo-endothelial macrophages, thus representing a good model to study iron mobilization from these cell types.
As expected, phlebotomy caused a strong inhibition of Hepcidin expression. Iron was mobilized from liver in both Haptoglobin-null and wild-type mice and from kidney and spleen in Haptoglobin-null animals. Hepcidin down-regulation correlated with increased Ferroportin protein levels in the liver, kidney and spleen macrophages of both Haptoglobin-null and wild-type mice. However, the increase in Ferroportin protein expression was significantly higher in Haptoglobin-null kidney than in wild-type counterpart. Furthermore, Ferroportin mRNA levels were regulated in a tissue- and cell-specific manner: Ferroportin mRNA levels did not change in liver and kidney, increased in duodenum and spleen macrophages while decreased in spleen erythroid cells of both Haptoglobin-null and wild-type mice.
Phlebotomy leads to tissue hypoxia and iron deficiency which stabilize Hif1α. We hypothesized that Hif could play a role in Ferroportin transcriptional control.
Under hypoxic conditions, Ferroportin mRNA increased in RAW264.7 macrophage cell line, as occured in spleen macrophages after phlebotomy. Ferroportin mRNA induction by hypoxia was blocked by Actinomycin-D in a dose-dependent manner, indicating that it was mediated by transcriptional activation. Furthermore, bioinformatic analysis revealed three putative Hif-binding sites on Ferroportin promoter.
These data suggest that Hif may regulate Ferroportin expression allowing iron stores mobilization and red cell differentiation after erythropoietic stimuli. The analysis of Ferroportin promoter in RAW264.7 cells, as well as in other cell types, is in progress.

Study of the mechanism of action of a Brazilian myotoxin, BthTX-I, in mouse muscle cell line

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Among the accidents with venomous animals, bites by ophidians are the most important, for their frequency and gravity. They occur throughout Brazil and pose an important problem of public health. *Bothrops* snakes account for about 90% of poisonous snake bites in Brazil, bothropstoxin-I (BthTX-I, ~13.7 kDa) being the main myotoxin of this venom. The myotoxins' mechanisms of action are intensively studied in many laboratories worldwide with several aims: discovering the pathogenesis of the diseases in which they are involved, learning more about the physiological processes they affect, identifying appropriate protocols of treatment and inhibitors to be used as therapeutics. My research project is the study of the effects of BthTX-I in different muscle cells in culture and as isolated fibers by Ca^{2+} imaging in order to elucidate its mechanisms of action. Currently, I am using the mouse cell line C2C12 in the undifferentiated myoblast stage and as differentiated cells, the myotubes. After toxin purification and checking its purity by SDS-PAGE, we performed a toxicity test in the ex vivo diaphragm preparation and a vitality test (MTS assay) in C2C12. From the latter we found that myotubes are more susceptible to BthTX-I action whereas myoblasts seem to be resistant. By calcium imaging with the Fura2AM indicator in C2C12 we investigated the role of calcium in the BthTX-I myotoxicity. Our results show that myotoxicity in the myotubes is dose-dependent and that the toxin induced cell lesion is calcium-independent, as BthTX-I can disrupt the plasma membrane without extracellular calcium. This lesion is followed by a massive entry of calcium ions. The mechanism of membrane lesion and the putative acceptor of BthTX-I in the muscle fibers is still unknown. Therefore as future perspective we will try to localize the putative acceptor using pharmacological approaches in differentiated C2C12 to investigate the role of calcium in myotoxicity more deeply.

Poster 8

NapA of Borrelia burgdorferi drives Th17 inflammation in Lyme Arthritis

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Human Lyme arthritis caused by *Borrelia burgdorferi* is characterized by an inflammatory infiltrate, consisting mainly of neutrophils and T cells. The Neutrophil Activating Protein A (NapA) was found to be essential for *B. burgdorferi* persistence within ticks, but its role in immune response in Lyme arthritis was unknown. Here, we report that this virulence factor is a major antigen of the humoral response in patients with Lyme arthritis. We show that T cells from synovial fluid of patients with Lyme arthritis produce interleukin (IL)-17 in response to NapA. NapA is a Toll-Like Receptor-2 agonist able to induce the expression of IL-23 in neutrophils and monocytes, and IL-6, IL-1β and transforming growth factor-β in monocytes. We conclude that NapA of B. burgdorferi represents an important driver of T helper (Th) 17 immune responses, and elicits a synovial Th17 response that might play an important role in the pathogenesis of Lyme arthritis.

Targeting OPA1-dependent mitochondrial cristae remodeling: a novel strategy to drive apoptosis of tumor cells?

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OPA1 is an inner mitochondrial membrane dynamin-related protein that has genetically distinguishable functions in mitochondrial fusion and apoptosis. OPA1 forms oligomers whose disruption is associated with remodelling of the mitochondrial cristae and complete release of cytochrome c, required in the cytosol to fully activate effector caspases during cell death. Inactivating mutations in the GTPase domain of OPA1 impair this antiapoptotic activity, enhancing susceptibility to mitochondrial-dependent apoptosis. Thus, the inhibition of the GTPase domain of OPA1 could represent a novel target to enhance death of cancer cells. Suramin is a known commercially available GTPase inhibitor. In silico modelling showed that suramin can bind to the GTPase cleft of the protein. Since suramin is known for its inhibitory effect on mitochondrial respiratory chain, we sought a micromolar window of concentration that does not affect basal and ADP-stimulated respiration. These same safe concentrations enhanced cytochrome c release induced by the BID, a proapoptotic BH3-only member of the Bcl-2 family, without interfering with the activation of BAX and BAK, the mitochondrial receptors of BID. Interestingly, OPA1 oligomers were apparently targeted by suramin. Suramin seems therefore a good candidate to test whether OPA1 can be targeted to enhance apoptosis of cancer cell. In order to address the specificity of suramin we are testing its effects on the GTPase activity of recombinant OPA1. Finally, we are extending our analysis to novel compounds derived from suramin that display better in silico binding to the GTPase cleft of OPA1.

Poster 10

Exploring the role of the OPA1-dependent cristae remodelling pathway in the mitochondrial alterations of Huntington's Disease

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Huntington's disease (HD) is caused by an abnormal expanded polyglutamine (polyQ) repeat in the huntingtin (Htt) protein, whose functions 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.
Lymphoblasts from heterozygous HD patients were characterized by polarized and coalescent mitochondria, while mitochondrial network in homozygous cells was highly fragmented and distributed through the whole cell volume. The overexpression of the mitochondria-shaping protein OPA1 can correct the morphological alterations in HD lymphoblasts. Morphological changes were accompanied by latent mitochondrial dysfunction in situ and by faster release of cytochrome c induced by recombinant BID in vitro. Oligomers of OPA1 that correlate with the maintenance of cristae structure, appear less represented and disrupt faster following cBID in HD mitochondria, suggesting that by a yet unidentified mechanism mutated Htt interferes with oligomerization of OPA1. These results open the possibility that in the course of HD mitochondria are hypersensitive to apoptotic stimulation and call for a deeper investigation of the molecular interplay between Htt and the inner mitochondrial membrane.

KRIT1 regulates cell adhesion by controlling ICAP1A functions and cytoskeleton dynamics

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Mutations in the KRIT1 gene have been shown to cause Cerebral Cavernous Malformations, which are vascular malformations occurring in 0.1 to 0.5% of the population and predisposing to seizures, intracerebral haemorrhage and focal neurological deficits.
Although KRIT1 functions are not yet understood, it is known that its N-terminal portion contains an NPXY motif that forms the binding site for ICAP1A, a negative regulator of b1 integrin adhesive functions, suggesting that KRIT1 could be involved in the regulation of b1-mediated cell adhesion. Indeed, we demonstrated that KRIT1 overexpression enhances ICAP1A nuclear accumulation, indicating that KRIT1 may promote b1 adhesive functions by sequestering ICAP1A into the nucleus. Furthermore, by taking advantage of MEF cell lines derived from KRIT1-null mouse embryos, we analyzed the effects of KRIT1 absence/overexpression on cell adhesive properties, founding that KRIT1-/- and KRIT1 overexpressing MEF cells behave differently during early phases of adhesion on fibronectin. In particular, the presence of KRIT1 correlates with the formation of focal contacts connected to long actin stress fibers at peripheral cell protrusions, which is a typical b1-mediated adhesive phenotype, further supporting the positive role of KRIT1 on the regulation of b1 adhesive functions. On the other hand, by analyzing KRIT1 subcellular localization in both b1 integrin-null and b1 overexpressing GE11 cell lines, we found that the presence of b1 integrin correlates with KRIT1 nuclear translocation, suggesting that KRIT1 and b1 can reciprocally influence their functions. In addiction, by comparing microarray mRNA expression profiles of KRIT1-/- and KRIT1 overexpressing MEF cells, we found a differential expression of several genes functionally related to actin cytoskeleton dynamics, suggesting that KRIT1 may regulate cell adhesion by acting at multiple levels, including ICAP1A function regulation and modulation of cytoskeleton dynamics.

Poster 12

OA1 signaling pathway: role in melanosome biogenesis and melanoma progression

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OA1 is a pigment cell specific membrane glycoprotein, whose misfunction is responsible for Ocular Albinism type 1, an X-linked disease involved in the visual system. The characteristic phenotype of the disease is the presence of giant melanosomes (macromelanosomes) in RPE (Retinal Pigmented Epithelium) and skin, suggesting that OA1 plays a role in melanosome biogenesis.
The OA1 protein belongs to the GPCR (Gprotein Coupled Receptors) superfamily, but unlike all others known GPCR, it localizes on endolysosomal and melanosomal membranes.
In spite of this knowledge, the physiological function of OA1 is still unclear.
Our results show that OA1 regulates MITF (MIcrophthalmia-associated Transcription Factor), a transcription factor located in the center of multiple signaling pathways, controlling the differentiation, morphology, proliferation, and survival of the melanocyte lineage (i.e. melanoblasts, melanocytes and melanoma). In differentiation, MITF plays a major role in melanogenesis, inducing the expression of key enzymes. In proliferation, it plays a double role as inducer/repressor of cellular proliferation. Furthermore, changes in MITF activity are implicated in melanoma progression.
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 sizable decrease.
In light of these results, our experiments have been directed to understand the OA1 signaling pathways involved in MITF regulation.
Moreover, we performed WB assays in human melanoma cell lines, finding a tight correlation between OA1 and MITF expression. This way can lead to elucidate a putative role of OA1 in melanoma progression.

Contributo della Timidina Chinasi mitocondriale e della Ribonucleotide Reduttasi R1-p53R2 nella sintesi dei precursori del DNA mitocondriale

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In cellule quiescenti, la replicazione del DNA mitocondriale (mtDNA) richiede un apporto bilanciato di dNTP assicurato dall'azione concertata di enzimi citosolici e mitocondriali (mt), sia anabolici che catabolici. La timidina chinasi mitocondriale (TK2), recuperando la timidina presente nell'ambiente extracellulare, è tra i principali enzimi coinvolti nella regolazione del pool del dTTP. p53R2, isoforma stabile di R2, si complessa con R1 per dare una ribonucleotide reduttasi (RNR) attiva in cellule quiescenti non danneggiate. Mutazioni a carico della TK2 causano la sindrome da deplezione del mtDNA; la deficienza genetica di p53R2 determina una severa patologia mt.
In colture quiescenti di fibroblasti di due pazienti mutanti TK2 (T77M/R161K e R152G/K171Del) e di controllo abbiamo misurato le attività specifiche della TK2 e dei suoi enzimi competitori in estratti cellulari, le dimensioni dei pool mitocondriali e citosolici dei dNTP e abbiamo confrontato la capacità di fosforilare timidina radioattiva.
Sebbene l'attività TK2 nei pazienti risulta inferiore al 10% del controllo, l'attività *in situ* sembra essere meno colpita e la dimensione del pool del dTTP non è diversa dai controlli. Non vi sono evidenti variazioni di espressione degli altri enzimi coinvolti nella regolazione del dTTP.
Con lo scopo di osservare il contributo della sintesi *de novo*, abbiamo inoltre silenziato p53R2 mediante RNA interference in cellule quiescenti e misurato gli effetti del silenziamento sulle dimensioni dei pool dei dNTP.

Poster 14

Sintesi e regolazione del pool mitocondriale del dTTP in cellule umane

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La timidina chinasi mitocondriale (mt), TK2, è uno degli enzimi coinvolti nella sintesi del mtdTTP ed è la sola timidina chinasi funzionante in cellule quiescenti. Mutazioni della TK2 causano la forma miopatica di sindrome da deplezione del mtDNA il cui fenotipo è stato attribuito alla mancanza di mtdTTP nel muscolo scheletrico. Di recente è stato dimostrato che l'isoforma R1-p53R2 della RNR è presente in cellule non proliferanti e garantisce una bassa sintesi *de novo* dei dNTP e che mutazioni di p53R2 causano patologie mitocondriali.
Per studiare il ruolo della TK2 e di R1-p53R2 nella sintesi del mtdTTP in fibroblasti umani non proliferanti si è verificata la loro espressione tramite real-time-PCR e western blot ed è stata determinata la loro attività in situ con esperimenti di flusso isotopico. Mediante RNAi e l'impiego di inibitori chimici specifici è stata inoltre calcolata l'influenza reciproca della TK2, della sintesi *de novo* e della timidina fosforilasi sulla regolazione del pool del dTTP in fibroblasti quiescenti.
E' stato così dimostrato che l'apporto di dTTP per la sintesi del mtDNA in cellule non proliferanti è garantito della collaborazione tra le attività di enzimi citoplasmatici e mitocondriali.
Perciò, per il metabolismo dei pool mitocondriali dei dNTP è necessario lo scambio di nucleosidi e nucleotidi tra citoplasma e mitocondri. Ci siamo proposti di individuare il trasportatore di membrana responsabile dell'importo del dTMP nel mitocondrio silenziando mediante RNAi due geni ortologhi di Rim2p, che in *S. cerevisiae* media il trasporto di nucleotidi pirimidinici attraverso la membrana mitocondriale interna. Le sequenze silenziate codificano per due proteine appartenenti alla famiglia dei "mitochondrial carrier" e presentano un'elevata similarità che suggerisce una funzione ridondante per i due geni. Abbiamo quindi deciso di analizzare gli effetti del silenziamento contemporaneo dei due geni negli scambi di deossinucleotidi tra mitocondri e citosol.

Functional interaction between Citron-k and Anillin in cytokinesis

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Abscission is the final stage of the cytokinetic process, during which the intracellular connection between the postmitotic cells is severed. Recent studies have identified a number of proteins essential for abscission, but it is poorly understood how they interact with each other to accomplish this process.
Citron-k and Anillin play important roles in cytokinesis in several systems. The phenotype of Drosophila S2 cells depleted of Citron-k (Dck) and Anillin are extraordinarily similar, suggesting that Dck and Anillin are in the same pathway for the completion of cytokinesis.
We have investigated the functional interaction between Citron-k and Anillin in mammals.
We have demonstrated that Citron-k and Anillin colocalize during cytokinesis in HeLa cells; moreover, Citron-k and Anillin coimmunoprecipitate when overexpressed, indicating that these two proteins can be part of the same molecular complex.
We have found that the overexpression of Citron-k affects Anillin localization and that the knockdown of each protein affects the localization at the midbody of the other. Finally, studies in knock out mice suggest that Citron-k could regulate Anillin protein levels.
We propose that Citron-k and Anillin are closely interacting partners during cytokinesis and that this interaction is required for the final events of cell division.

Poster 16

Metabolic interconnection of mitochondrial and cytosolic deoxyguanosine and 9-β-d-arabinofuranosylguanine nucleotide pools

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Mitochondrial (mt) and nuclear DNA draw their precursors (dNTPs) from two distinct pools separated by the inner mt membrane. We previously demonstrated that mt and cytosolic dTTP pools communicate and the main source of mt dTTP in cycling cells is cytosolic *de novo* synthesis catalyzed by ribonucleotide reductase. In quiescent cells intra-mt phosphorylation of thymidine complements ribonucleotide reduction. Here we analyze the dynamic interrelations of the deoxyguanosine triphosphate (dGTP) pools.
Cultures of $HGPRT^-$ CHO cells and $HGPRT^-$ human fibroblasts were incubated during growth and quiescence with ^{3}H-deoxyguanosine (GdR) in pulse-chase experiments measuring radioactivity incorporated into mt and cytosolic guanine deoxyribonucleotides.
By preventing cytosolic phosphorylation of GdR by deoxycytidine kinase (dCK) and its degradation by purine nucleoside phosphorylase, inhibited by immucillin H, we favoured incorporation of ^{3}H-GdR into the mt pool. A dynamic isotopic equilibrium arose rapidly from the shuttling of deoxynucleotides between mitochondria and cytosol, incorporation into DNA, deoxynucleotide degradation, and was slightly affected when we inhibited DNA synthesis by aphidicolin. Import of deoxynucleotides formed *de novo* in the cytosol was the major source of mt dGTP, with minor contributions by cytosolic dCK and mt deoxyguanosine kinase (dGK). Inhibition of ribonucleotide reduction by hydroxyurea disturbed the equilibrium and greatly decreased dGTP turnover suggesting a close connection between ribonucleotide reduction and pool degradation.
The model of metabolic interconnection of mt and cytosolic deoxyribonucleotides pools established earlier for dTTP can be extended to dGTP pools.
9-β-D-arabinofuranosylguanine (AraG) is a GdR analog used in the treatment of T-cell leukaemia and is phosphorylated both by dCK and by dGK. In pulse and chase experiments with ^{3}H-AraG in CHO cells we investigate its incorporation into mt and cytosolic pools and DNA.

Acetylcholine and its receptors in immunity

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Organization of immune responses requires exchanging information between cells.
In the nervous system the exchange of information between neurons is accomplished by specific adhesion molecules and neurotransmitters. In the immune system the cell-cell contact is mediated by adhesion molecules and various receptors. It has been recently demonstrated that chemokines function as soluble transmitters in the immune synapse (IS) between T and antigen presenting cells (APC). Thus, in immunological as well as in neuronal synapse, information may be mediated by both transmembrane and soluble molecules. Here, we propose to analyze whether acetylcholine (ACh) may function as transmitter in the immune system.
The expression of a functional cholinergic system in lymphocytes is well documented. Lymphocytes express nicotinic (nAChRs) and muscarinic receptors (mAChRs) and also the enzymes responsible for Ach production - the choline-acetyltransferase (ChAT) - and degradation - the acetylcholine-esterase (AChE).
First, we analysed the localization of selected AchRs at the IS between T and B cells. Among the mAchRs, we found that M5 is significantly recruited to the IS. We plan to investigate the signal pathway required for this recruitment. Among the nAchRs, we found that alpha4 and beta2-nAChR subunits are highly expressed in NK cells. We plan to clarify their role in NK-target cell synapses and cytotoxic activity.
We also investigated the role of the cholinergic system in T cell proliferation. T cells, in which the ACh production or the ACh storing in vesicles had been blocked, did not show defects in proliferation.
Finally we analysed the role of the cholinergic system in T cell migration. Treatment with tubocurarine, a nAChRs antagonist, hemicholinium-3, a drug which blocks the reuptake of choline, and AChE, the enzyme responsible for ACh degradation, enhances T cell migration towards chemokines. We plan to clarify the role of nAChRs in T cell migration.

Poster 18

AKT Kinase reducing endoplasmic reticulum Ca^{2+} release through inositol 1,4,5 trisphosphate receptor type 3 protects cells from Ca^{2+}-dependent apoptotic stimuli

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The proto-oncogene AKT (also known as Protein Kinase B, PKB) is a potent inhibitor of apoptosis, and it is activated in many human cancers. A number of recent studies have highlighted the importance of the inositol 1,4,5-trisphosphate (IP3) receptor (IP3R) in mediating calcium (Ca^{2+}) transfer from the Endoplasmic Reticulum (ER) to the mitochondria in several models of apoptosis. AKT is a serine-threonine kinase and recent data indicate the IP3R as a target of its phosphorylation activity.
Here we show that HeLa and Cos 7 cells, lacking the IP3R type 2 and 1 respectively, overexpressing the constitutively active myristoylated/palmitylated AKT1, were found to have a reduced Ca^{2+} release from ER after stimulation with agonist coupled to the generation of IP3; interestingly, in SH-SY 5Y cells, lacking the IP3R type 3, this inhibition mediated by AKT is negligible.
In turn, this affected cytosolic and mitochondrial Ca^{2+} response after Ca^{2+} release from the ER induced either by agonist stimulation or by apoptotic stimuli releasing Ca^{2+} from intracellular stores (such as oxidative stress and arachidonic acid) in HeLa and Cos-7 but not in SH-SY 5Y cells.
Most importantly, this alteration of ER Ca2+ content and release observed in HeLa and Cos 7 cells, reduces significantly cellular sensitivity to Ca^{2+} mediated proapoptotic stimulation.
These results reveal a primary role of AKT in shaping intracellular Ca^{2+} homeostasis, suggests that its action may be targeted to the isoform 3 of the IP3R (which appears to be selectively located close to mitochondria and to mediate Ca^{2+} transfer to this organelle) and may underlie its protective mechanism against some proapoptotic stimuli.

KRIT1A, a protein involved in the human disease Cerebral Cavernous Malformation, is a molecular sensor for oxidative stress

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The Krit1 gene has been associated with Cerebral Cavernous Malformation (CCM), an autosomal dominant disease affecting cerebral microvasculature. This gene encodes for a protein containing an NPXY motif, three ankyrin repeats, and a FERM domain. Although the presence of these structural domains suggests that KRIT1 may behave as a scaffold protein, the biological functions of this protein are poorly understood so far. Recently, we identified a KRIT1 isoform, named KRIT1B, characterized by the alternative splicing of the 15° exon encoding for the lobe F3 of the FERM domain.
By fluorescence microscopy analysis, we observed that KRIT1A and KRIT1B isoforms have a distinct subcellular localization. In particular, while KRIT1A shows a predominant nuclear localization, KRIT1B localizes exclusively in the cytoplasm, suggesting a major role for the FERM domain in KRIT1A nuclear localization. Indeed, by analyzing the subcellular localization of distinct KRIT1A fragments, we found that the FERM domain is sufficient for nuclear localization. Importantly, in the attempt to identify molecular mechanisms involved in CCM pathogenesis, we found that KRIT1A is a target for oxidative stress. Indeed, oxidative stimuli induce KRIT1A tyrosine phosphorylation, which is dependent on the FERM domain integrity and associated with a nucleus-to-cytoplasm translocation of the protein.
Taken together, our findings point to a model where KRIT1A behaves as a molecular sensor for oxidative stress events by undergoing tyrosine phosphorylation and subcellular localization changes, suggesting a potential protective role for KRIT1A against microenvironmental stresses that can affect cerebral vasculature.

Poster 20

Autophagy in Skeletal Muscle

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Autophagy allows cell survival during starvation through the bulk degradation of proteins and organelles by lysosomal enzymes. However, the mechanisms responsible for the induction and regulation of the autophagy program are poorly understood. We have recently shown that the FoxO3 transcription factor, which plays a critical role in muscle atrophy, is necessary and sufficient for the induction of autophagy in skeletal muscle in vivo. FoxO3 controls the transcription of autophagy-related genes, including LC3 and Bnip3, and Bnip3 appears to mediate the effect of FoxO3 on autophagy. This effect is not prevented by proteasome inhibitors. Thus, FoxO3 controls the two major systems of protein breakdown in skeletal muscle, the ubiquitin-proteasomal and autophagic/lysosomal pathways, independently. These findings point to FoxO3 and Bnip3 as potential therapeutic targets in muscle wasting disorders and other degenerative and neoplastic diseases in which autophagy is involved (Mammucari, 2007). Moreover it is known that autophagy is activated soon after birth in neonatal tissues and is essential for survival because mice deficient in Atg5 or Atg7 (two proteins involved in the formation of autophagosomes) die within 1 day after birth (Komatsu et al., 2005). In order to better understand the function(s) of skeletal muscle autophagy, we are currently exploring:
1 - the role of the autophagy in neonatal skeletal muscle. For this purpose we are mating Atg7loxP mice with the MLC1f/Cre mice (in which Cre recombinase expression is driven by Myosin Light Chain 1f promoter).
2 - the role of the autophagy in adult skeletal muscle. For this purpose we are crossing Atg7loxP mice with the HSA/Cre mice (in which Cre recombinase expression is driven by Human Skeletal Actin promoter).
3 – the role of p62/SQSTM1 in skeletal muscle during the autophagy. This protein regulates the formation of ubiquitinated protein aggregates and is removed by autophagy.

Contributo della prolil-isomerasi Pin1 all'attività apoptotica mitocondriale di p53

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L'oncosoppressore p53 è in grado di sopprimere lo sviluppo tumorale poiché viene attivato in seguito a stress causati ad esempio dalla proliferazione incontrollata o dall'ipossia connessa alla crescita tumorale, ed in seguito coordina diverse risposte cellulari antiproliferative. In particolare, l'induzione del processo apoptotico rappresenta il principale determinante della sensibilità cellulare ai farmaci antitumorali. La rilevanza di tali eventi è evidenziata dall'elevata frequenza di mutazioni a carico del gene TP53 nei tumori umani.
Oltre a regolare la trascrizione di numerosi geni effettori p53 è anche in grado di localizzare al mitocondrio, dove stimola il processo apoptotico mediante l'attivazione diretta di alcuni fattori proapoptotici e l'inibizione di proteine antiapoptotiche. Un importante modulatore dell'attività apoptotica di p53 è la prolil-isomerasi Pin1, che induce specifici cambiamenti conformazionali di p53 in seguito alla sua fosforilazione indotta da stress, favorendone la stabilizzazione e l'attività trascrizionale. È stato dimostrato che Pin1 gioca un ruolo anche nella modulazione di alcune proteine che regolano l'apoptosi a livello mitocondriale, pertanto abbiamo voluto verificare se esso possa influenzare anche l'attività apoptotica di p53 al mitocondrio
Abbiamo inizialmente osservato che entrambe le proteine localizzano al mitocondrio in diverse linee cellulari tumorali e che l'espressione dell'una è necessaria all'efficiente localizzazione mitocondriale dell'altra. Abbiamo inoltre dimostrato che l'espressione di Pin1 è necessaria all'attività apoptotica mitocondriale di p53 e che la sua sovraespressione è in grado di promuovere l'apoptosi indotta da p53 indipendentemente dalla transattivazione.
In conclusione, abbiamo evidenziato che la prolil-isomerasi Pin1 svolge un ruolo cruciale nella regolazione della frazione citoplasmatica di p53 e stiamo indagando quale sia il meccanismo alla base di questo effetto.

Poster 22

Convergence of integrins and EGF receptor signalling via PI3K/Akt/FoxO pathway in early gene Egr-1 expression

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The early gene Egr-1, a broadly expressed member of the zing-finger family of transcription factors, is induced in many cell types by a variety of growth and differentiation stimuli, including EGF. Here we demonstrate that Egr-1 expression is mainly regulated by integrin-mediated adhesion. Integrin-dependent adhesion plays a dual role in Egr-1 regulation, either being sufficient "per se" to induce Egr-1, or required for EGF-dependent expression of Egr-1, which occurs only in adherent cells and not in cells in suspension. To dissect the molecular basis of integrin-dependent Egr-1 regulation, we show by FLIM-based FRET that in living cells beta1-integrin associates with the EGF receptor (EGFR) and that EGF further increases the extent complex formation. Interestingly, Egr-1 induction depends on integrin-dependent PI3K/Akt activation, as indicated by the decrease in Egr-1 levels in presence of the pharmacological inhibitor LY294002, the kinase-defective Akt mutant and Akt1/2 shRNAs. Indeed, upon adhesion activated Akt translocates into the nucleus and phosphorylates FoxO1, a Forkhead transcription factors. Consistently, FoxO1 silencing results in Egr-1-increased levels, indicating that FoxO1 behaves as a negative regulator of Egr-1 expression. These data demonstrate that integrin/EGFR cross-talk is required for expression of Egr-1 through a novel regulatory cascade involving the activation of the PI3K/Akt/Forkhead pathway.

Rescue of myopathic collagen VI null mice by genetic inactivation of mitochondrial cyclophilin D

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Considerable progress in understanding the pathogenesis of collagen VI diseases has been made in mice with targeted disruption of the Col6a1 gene, which display an early-onset myopathic syndrome due to lack of collagen VI. Mitochondria in skeletal muscle fibers and in myoblasts from Col6a1$^{-/-}$ mice depolarize in response to oligomycin, an anomalous response that can be corrected by cyclosporin (CsA). This finding suggests that in collagen VI myopathies flickering of the permeability transition pore (PTP, an inner membrane high-conductance channel) is increased and causes depletion of pyridine nucleotides, progressive impairment of respiration, and switch of the F1FO ATP synthase into an ATP hydrolase maintaining the membrane potential at the expense of glycolytic ATP. This interpretation is consistent with the therapeutic effect of treatment of Col6a1$^{-/-}$mice with CsA, which desensitizes the PTP in vivo. To further test the role of the PTP in the pathogenesis of collagen VI myopathies, we have generated Col6a1$^{-/-}$ Ppif$^{-/-}$ mice (Ppif is the unique mouse gene encoding for mitochondrial cyclophilin D, whose inactivation desensitizes the PTP). We will report the striking rescue of Col6a1$^{-/-}$Ppif$^{-/-}$ mice from the myopathy despite their total lack of collagen VI.

Poster 24

Snake PLA2 neurotoxins enter neurons, bind specifically to mitochondria and open their transition pores

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Snake presynaptic neurotoxins endowed with phospholipase A2 (PLA2) activity (SPANs) are potent inducers of paralysis through inhibition of the neuromuscular junction. SPANs were recently shown to induce exocytosis of synaptic vesicles following the hydrolysis of phospholipids into lysophospholipids and fatty acids and the sustained influx of Ca^{2+} from the medium. It was also shown that these toxins are able to penetrate spinal cord motorneurons and cerebellar granule neurons and to selectively bind to mitochondria. As a result of this interaction mitochondria depolarize and undergo a profound shape change from elongated to round and swollen. Throughout calcium retention capacity assays we show that SPANs facilitate opening of the mitochondrial permeability transition pore (PTP), an inner membrane high-conductance channel. The relative potency of SPANs was similar for PTP opening and for PLA2 activity, suggesting a causal relationship, which is also supported by the effect of the PLA2 products on PTP opening. These findings contribute to define the cellular events that lead to SPAN intoxication of nerve terminals and suggest that mitochondrial impairment is an important determinant of their toxicity.

Identification and characterization of microRNAs involved in tumour progression and metastasis

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MicroRNAs (miRNAs) are single-stranded small non coding RNAs, generated from the processing of endogenous primary miRNA transcripts. Mature miRNAs modulate gene expression via the association with the 3'untranslated region (3'UTR) of specific target mRNAs and the induction of translational repression or even mRNA degradation. An oncogenic- or tumour suppressor-like role has been characterized so far for several miRNAs. The aim of our work is the identification of miRNAs involved in tumour progression and metastasis formation in melanomas. Our cellular model is represented by the poorly metastatic human melanoma parental cell line A375P and four different variants (MA-1, MA-2, MC-1, MC-2) with higher metastatic potential, derived from the parental cells by repeated passages in mouse. To identify potential metastasis-promoting miRNAs, expression for 365 miRNAs was evaluated in the A375P parental cells and in one variant, MA-2, by quantitative Real Time-PCR (qRT-PCR). 8 differentially expressed miRNAs were identified and validated by qRT-PCR. We are now investigating the functional roles of four of them (miR214, miR137, miR187 and miR512-3p) by downmodulating or overexpressing miRNA expression in a transient or stable manner. We are analyzing cell growth and survival, *in vitro* migration, invasion and *in vivo* metastasis formation. MicroRNA functions are mediated by the regulation of target genes. Therefore, we are also using bioinformatic algorithms to identify potential targets that could provide a mechanism for the involvement of our miRNAs in tumour progression; then, we plan to validate some of them by reporter assay.

Poster 26

Differenze genere specifiche nel cuore, in risposta ad allenamento, nel ceppo murino C57BL/6J

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Nonostante un`aumentata conoscenza dei meccanismi di adattamento cardiaco in risposta ad un incremento del carico di lavoro, ci sono differenze significative tra i due sessi, che sono ancora poco elucidate.
Topi adulti, maschi e femmine del ceppo C57BL/6J all`eta` di dieci mesi, sono stati sottoposti ad esercizio fisico del nuoto per sei settimane, ad un allenamento di 3h /giorno, 5g /settimana.
Con analisi ecocardiografica si e` riscontrato un aumento del diametro in diastole ed in sistole della camera ventricolare sinistra, nel gruppo di genere maschile con conseguente ridotta contrattilità e frazione di eiezione. Il gruppo di topi di genere femminile ha invece mantenuto inalterata la funzionalità cardiaca dopo allenamento.
Sono seguite analisi istologiche e biochimiche per valutare la presenza di fibrosi, infiltrazione infiammatoria e il numero di cellule apoptotiche nel tessuto cardiaco.
Dimostriamo che, in risposta allo stress da allenamento del nuoto, il miocardio dei topi maschi adulti C57BL/6J rimodella in maniera differenziale rispetto i topi femmina.

MR imaging of infiltrating monocytes

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Infiltration of inflammatory cells is a common characteristic of pathologies like arthritis, ischemia, multiple sclerosis and autoimmune diseases. Non-invasive visualization of infiltrating cells could facilitate diagnosis and assessment of drug treatment.
Tracking of individual cells by MRI requires the intracellular accumulation of contrast agent. In vitro labeling of cells permits incorporation of large amounts of iron oxide and consequently high detection sensitivity but it remains controversial whether labeled cells would respond normally to stimuli. This question was addressed in an established experimental system for acute inflammatory processes, the murine air pouch model. In this system an air pouch is generated on the back of mice. By flushing the compartment with buffer, large numbers of infiltrated cells can be collected and analysed ex vivo.
Bone marrow derived macrophages (BMDM) were differentiated in vitro, labeled with Endorem (SPIO) and unlabeled cells were eliminated by magnetic enrichment. Purified and enriched BMDM were injected intravenous into the tail vein of isogenic mice presenting a carrageenan-induced inflammation in the air pouch. Endorem labeled macrophages were detected by fluorescent microscopy as well as by MR imaging ex vivo in the cell populations eluted from carrageenan pouches of mice injected i.v. with in vitro labeled BMDM. This strongly suggests that Endorem labeled macrophages can still respond to chemokine gradients.
Direct in vivo and ex vivo MR detection of organs was attempted in mice and we observed non-specific trapping of labelled BMDM in small brain capillaries and massive accumulation in lungs and liver was evident.

Poster 28

Smad2 and 3 Transcription Factors are critical for myostatin signaling and cooperate with FoxO3 to control muscle mass

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Myostatin is an important negative modulator of muscle growth during myogenesis. However, the role of the myostatin pathway in adulthood and the transcription factors involved in the signaling are unclear. Using genetic tools we perturbed this pathway in adult myofibers, in vivo, to characterize the downstream targets and their ability to activate an atrophy program. Mutants of myostatin receptors are expressed specifically in skeletal muscles and the effects on fiber size and on activation of atrogin-1 and MuRF1 promoters are monitored. Inhibition of the pathway induces muscle growth although it does not block muscle loss during catabolic conditions. We further characterize that the pathway is not sufficient to activate an atrophy program but requires AKT inhibition and FoxO activation. In fact, FoxO inhibition either by RNAi or by expressing constitutively active Akt completely prevents myostatin effects on muscle loss. Finally, we determined that Smad2 and Smad3 are the transcription factors downstream of myostatin and that Smads need to cooperate with FoxO3 to activate an atrophy program. These findings point to myostatin inhibitors as good drugs to promote muscle growth during rehabilitation of patients but useless to prevent activation of an atrophy program during disease.

K+-selective channels in mitochondria

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K+-selective channels have been found to be present in the inner membrane of mitochondria. The initial input for the project came with the discovery of the Shaker-family voltage-dependent Kv1.3 channel in the mitochondria of lymphocytes and lymphoma-derived (Jurkat) cells (Szabò et al., 2005). Work in press shows that it plays an important role in apoptosis. Using a biochemical approach, we have now identified it also in the mitochondria of MCF-7 (a breast cancer line) and PC3 (considered to be a prostate cancer line) cells.
We have now identified the Intermediate conductance Ca2+-activated K+-selective channel KCa3.1 (IK) in electrophysiological recording from the inner membrane of mitochondria isolated from HCT116 (Human Colon Tumor 116) cells. Its presence has been confirmed by Western blots. We are currently looking for the IK in the mitochondria of other cell lines. It appears not to be present in the mitochondria of C-26 cells, a murine colon cancer line, even though the IK is expressed on plasma membrane.
Both channels are predominantly expressed in the plasma membrane of cells, and thus constitute examples of proteins with multiple cellular locations. Various K+ channels, including the ones just mentioned, are upregulated in rapidly proliferating cells (e.g. lymphocytes) and in many cancer types. The physiological role of the mitochondrial population remains to be clarified.

Poster 30

Genome-wide discovery of functional STAT3 binding sites

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STAT3 is a transcription factor playing a crucial role in inflammation, immunity and oncogenesis. Direct targets so far identified are in limited number, not sufficient to explain its crucial functions. To improve our understanding of STAT3 transcriptional network we developed a computational approach for the discovery of STAT3 functional binding sites. We generated a Positional Weight Matrix (PMW) from 54 functional validated STAT3 binding sites and used a log-likelihood ratio scoring function to identify potential binding sites of predicted affinity. Theoretical affinity was experimentally confirmed by EMSA competition assays.
To identify those sites more likely to be bound *in vivo* and thus to be functional, phylogenetic footprint was carried out between *H.Sapiens* and eight different vertebrate species using a sequence alignment-based method and a whole-genome comparison. The results obtained were crossed with a list of genes differentially expressed in MEFs plus or minus STAT3, obtained by microarray analysis. Predicted binding sites present in putative regulatory regions of differentially regulated genes were assigned a score based on the degree of species conservation and on predicted affinity.
In order to validate the method, the top ten STAT3 binding sites thus identified, plus other seven candidates selected on the basis of their biological function, were subjected to Chromatin Immunoprecipitation (ChIP) analysis using chromatin from wild type or STAT3-/- MEFs either untreated or treated with Oncostatin M to activate STAT3. A high percentage of sites was found to be bound by STAT3 *in vivo* (16 out of 18 tested) indicating a very low rate of false positive predictions. Moreover, expression analysis revealed a good correlation between STAT3 *in vivo* occupancy and levels of corresponding mRNAs. Therefore, our computational approach can provide a potent tool to identify novel STAT3 transcriptional targets.

Production of recombinant human ECP and Der p 10 allergen in eukaryotic expression system

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In the last years, a remarkable increase in allergic pathologies was highlighted. In industrialized countries, 20-25% of the total population suffers for IgE mediated allergic diseases and it has been reported that european house dust mite (*Dermatophagoides pteronyssinus*) is the origin of main environmental allergens responsible of pathologies as allergic rhinitis, asthma and atopic dermatitis in sensitized population. Actually, allergy immunotherapy is performed with natural allergen extracts; biologically-derived complex mixtures of several proteins, having variable composition difficult to standardize and possible cause of cross reactivity. The development of recombinant allergens through genetic engineering promote the progressive replacement of the natural extracts in immunotherapy procols, allowing the production of specific, pure, easy to characterize and more tolerable products. In the present study human recombinant ECP (Eosinophil Cationic Protein) and recombinant Der p 10 allergen derived from european house dust mite were produced. ECP is an important eosinophil protein and the serum ECP assay is used for monitoring allergic and other inflammatory diseases. Recombinant ECP and Der p 10 were produced in eukaryotic expression system and was characterized by dot blot, western blot, ELISA or RAST Inhibition assay. Recombinant proteins will offer a new opportunity in inflammatory deseases monitoring, diagnosis and immunotherapy of allergic pathologies.

Poster 32

p53 and endoplasmic reticulum-mitochondria crosstalk: new light on the induction of the p53 dependent apoptotic pathway

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P53 is a multifunctional protein and exerts its major role as tumor suppressor promoting apoptosis in response to death stimuli. P53 transcription-dependent proapoptotic mechanism, has been widely characterized, whereas few is known about its transcription-independent activity. Recent studies indicate that under some circumstances a fraction of p53, translocates to mitochondria and induces cell death, demostrating that mitochondrial targeted p53 is sufficient to trigger apoptosis.

Mitochondria have a central role in the regulation of intracellular Ca^{2+} homeostasis, being endowed with a complex array of transporters and enzymes regulated by this ion. The function of Ca^{2+} in apoptosis is a complex network, and in response of different death stimuli, Ca^{2+} itself can induce apoptosis directing the interplay between mitochondria and endoplasmic reticulum (ER). The aim of this study is to elucidate the mitochondrial pathway in which p53 acts as a key player with particular attention to Ca^{2+} homeostasis that has been measured exploiting the aequorin technology. Interestingly the overexpression of p53 wild type and of a p53 mutant, lacking the nuclear import sequence (p53-NLS), promotes a higher increase of mitochondrial Ca^{2+} uptake after agonist stimulation. The same mitochondrial effect is detected in mef (murine embryonic fibroblasts) where p53 has been activated by anticancer drug Adriamycin. In agreement with this observation the mitochondrial membrane potential is significantly increased in p53 overexpressing cells.

Moreover, in these cells the measurement of ER Ca^{2+} levels reveals a higher state of store filling. A possible ER target of p53 is the Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA) which transfers Ca^{2+} from the cytosol to the ER. Indeed we could demonstrate a p53-SERCA interaction.

This study gives a novel insight into the possible mechanism involving p53, mitochondria and the ER in the apoptosis mediated by Ca^{2+}.

miR31 and miR146a in breast cancer cell lines

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MicroRNAs are small non-coding RNAs that regulate target gene expression post-transcriptionally, binding to the 3' UnTranslated Region (3' UTR) of target mRNAs and triggering translation repression or mRNA decay. Recent works suggest a role for miRNAs in the initiation and progression of human malignancies and indicate that they can act as tumor suppressors or oncogenes. We compared the expression of 156 miRNAs in normal versus transformed versus transformed and invasive mammary epithelial cell lines by quantitative Real Time-PCR (qRT-PCR). We found deregulated expression of miR31, highly expressed in normal but not in tumor cells and miR146a, highly expressed only in invasive cells. Expression of these miRNAs is being investigated in more breast cancer. In order to investigate their function we expressed them permanently in empty cell lines following lentivirus infections: HeLa and MCF10A cells for miR146a and MCF7 cells for miR31. We are now knocking down their expression by using anti-miRs. EGF- or serum-dependent migration was inhibited in miR146a-expressing MCF10A or HeLa cells. In order to identify miR31 and miR146a target genes we performed bioinformatic analyses using three different algorithms and microarray experiments. For each algorithm we obtained a long list of target genes that we shortened by looking for common miRNA-regulated genes: CXCL12 and ZFAND6 for miR31 and, for instance, IRAK1 and TRAF6 for miR146a. By performing a whole genome microarray analysis of miR146a-expressing HeLa or MCF10A cells we identified some miR146a-regulated genes involved in cell cycle or intracellular signalling cascades in HeLa cells and in stress response and DNA damage in MCF10A cells. We crossed the bioinformatic PicTar analysis with the microarray outcome and found only a common gene but some modulated genes and some putative target genes predicted by PicTar belong to the same biological classes. These results suggest a role in migration and invasion for miR146a.

Poster 34

a-Latrotoxin as a tool to uncover part of the mechanism of NMJ (neuro muscular juncrion) repair after damage coupled with acute inflammation

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The aim of the project is to unravel part of the interactions among muscle, nerves and Schwann cells at the damaged neuromuscular junction (NMJ). At present, little is known about the role of the perisynaptic Schwann cells on the NMJ recovery following nerve injury. Under normal conditions, PSCs cap the presynaptic motor nerve terminal; following total or partial denervation PSCs become activated, change shape and participate in the removal of cell debris and regeneration of the nerve terminal. The messages involved in their activation and motility are not known. Up to now, nerve terminals were damaged by mechanical disruption, an activity that cannot be standardized and controlled at the molecular level. The intramuscular injection of the black widow spider vertebrate-specific toxin, a-Latrotoxin (a-LTX), represents our experimental model of controlled nerve terminal damage coupled to acute inflammation. I am currently characterizing the neurotoxic effects of this toxin both on some primary neuronal cultures and on neuromuscolar junction preparations. Upon intoxication spinal cord motorneurons and cerebellar granule neurons show membrane enlargements, bulges, that are the result of unbalanced exo-endocytic processes. NMJs from mouse tibial muscles injected with a-LTX appear alterated, with lose of contacts between pre- and postsynaptic elements. I will characterize these morphological changes at different times after toxin injection, using a large panel of antibodies specific for synaptic proteins under the confocal microscope.

Importance of Ras localization in the sites of close interaction between the Endoplasmic Reticulum and mitochondria

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Ras proteins are small GTPases that cycle between inactive GDP-bound and active GTP-bound conformation. These proteins, through the activation of many effector pathways, regulate many cellular responses, including proliferation, survival and differentiation and activating Ras mutations have been detected in 30% of human cancers. Traditionally, Ras proteins are defined as plasma membrane-bound proteins, but recent evidences demonstrate that they can translocate from plasma membrane to intracellular compartment, but the meaning of this translocation is mostly unknown. In our work we demonstrate that, in resting condition, Ras proteins localize significantly in the Mitochondrial Associated Membrane (MAM) i.e. sites of close interaction between the Endoplasmic Reticulum (ER) and mitochondria, in the ER and in the mitochondria. Interestingly we could show t hat the MAM localization is enriched in the presence of Ras mutation. Therefore we focused our attention on possible ER proteins interacting with Ras. We could demonstrate that Ras interacts with the Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA) which transfers Ca^{2+} from the cytosol of the cell to the lumen of the ER. To explain the meaning of this interaction we analyzed the effect of HRAS overexpression on intracellular calcium homeostasis demonstrating that HRAS alters mitochondrial and ER calcium homeostasis. The future aim of our work will be to investigate the importance of the ER localization of Ras protein in the regulation of cell cycle and tumor development.

Poster 36

In vitro study of the TTF1 role in the differentation of a multipotent neural cell line

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TTF-1/NKX2.1 is a homeodomain-containing tissue-specific transcription factor involved in the organogenesis of the thyroid gland, lung and ventral forebrain. Whereas the role of TTF1 in the thyroid and lung development is already known, its function in the brain is still to be clarified. Homozigous mutant mice have demonstrated that the loss of TTF1 causes a ventral to dorsal molecular respecification within the basal telencephalon. MNS 70 is a multipotent neural cell line able to generate neurons, astrocytes and oligodendrocytes in vitro. To better elucidate the role of TTF1 in brain development, in this study we propose to investigate if the TTF1 over-expression is capable of driving the MNS70 differentiation to a specific lineage (neuronal, glial, ecc…) or if it is necessary to obtain a determinate neuronal subpopulation (i.e. interneurons or telencephalic cholinergic neurons). We characterized the differentation of MNS 70 and we found that, at the culture conditions that we used, they differentiate to neurons (45%) and to glial cells (18%). Furthermore, we cloned the TTF1 gene downstream the CMV promoter in a expression vector (pCMV-TTF1). We costrasfected pCMV-TTF1 together with a construct bearing the puromycin resistance (since MNS70 cells are already neomycin resistent) and we selected many clones that were stably resistent. Theese clones were screened for the expression of TTF1 protein by immunofluorescence. Only one of theese showed TTF1 expression. Theese data were confirmed by western blot, where only clone U showed a positive band of 42 Kd. We are currently performing in vitro differentiation experiments in order to assess if specific lineage/s or neuronal subpopulation/s is obtained when TTF-1 overexpression occurs in MNS-70 cells.

Index

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