Associazione di Biologia Cellulare e del Differenziamento

Mechanisms of Signal Transduction

Organisers

Sara Sigismuns (Chair) - IFOM, Milan

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Programme & Abstracts

Padua, 16-17 May 2014

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Programme

Friday, 16 May

12:00 **Registration**

13:00-14:00 **Lunch**

14:00 **Welcome**

14:00-15:00 **Opening Lecture**

Harald Stenmark (Oslo, Norway)

Phosphoinositide and Rab dependent membrane contact sites and their role in endosome motility and neurite outgrowth

15:00-17:15 **Session 1: Intracellular Transport and Communication in the Regulation of Cell Signaling**

Chairs: Simona Polo & Emilio Hirsh

15:00-15:15 *Emiliana Tognon (Milan)*
Increased V-ATPase expression to support Notch signaling during stem cells establishment in *Drosophila*

15:15-15:30 *Caterina Lucano (Milan)*
A novel role of the endocytic adaptor proteins Eps15 and Eps15L1 in the regulation of Notch signaling

15:30-15:45 *Filippo Acconcia (Rome)*
Clathrin heavy chain-based pathway is involved in the regulation of 17β-estradiol-induced cell proliferation

15:45-16:00 *Alexia Conte (Milan)*
Defining the interplay between EGFR endocytosis and signaling through predictive modeling and wet-lab experiments

16:00-16:15 *Michol Savio (Milan)*
Identifying DUBs in the EGFR pathway

16:15-16:30 *Eelco van Anken (Milan)*
Acute and chronic stress signaling from the endoplasmic reticulum

16:30-16:45 *Sowmya Lakshminarayanan (Padua)*
Genome wide screen to identify ER-mitochondria tether proteins using FRET biosensors

16:45-17:00 *Riccardo Filadi (Padua)*
Presenilin-2 is a master regulator of endoplasmic reticulum-mitochondria tethering

17:00-17:15 *Gaia Gherardi (Padua)*
The role of the Mitochondrial Calcium Uniporter in the control of skeletal muscle mass

17:15-17:45 **Coffee break**

17:45-19:15 **Poster Session**

19:15-19:45 **election of next vice-chair**

20:00 **Dinner**

Saturday, 17 May

8:30-9:30 **Opening Lecture**

Nuno Raimundo (Goettingen, Germany)

Mitochondrial signaling - the energy factory takes control of the boardroom

9:30-10:30 **Session 2: Mitochondrial Signaling and Calcium Homeostasis**

Chairs: Rosario Rizzuto & Eelco Van Anken

9:30-9:45 *Maria Patron (Padua)*
MICU1 and MICU2 finely tune the mitochondrial Ca2+ uniporter by exerting opposite effect on MCU activity

9:45-10:00 *Renata Tisi (Milan)*
Characterization of ER-involving hypotonic shock-induced calcium release in the budding yeast

10:00-10:15 *Lena Pernas (Padua)*
Toxoplasma effector MAF1 mediates recruitment of host mitochondria and impacts the host response

10:15-10:30 *Tatiana Varanita (Padua)*
The multifunctional mitochondrial inner membrane protein Optic Atrophy 1 controls cellular damage *in vivo*

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

11:00-12:30 **Session 3: Cell Signaling in Cancer and Disease**

Chairs: Paola De Filippi & Letizia Lanzetti

11:00-11:15 *Miriam Martini (Turin)*
Loss of PI3K-C2A promotes tumorigenesis and aneuploidy in breast cancer

11:15-11:30 *Chiara Malinverno (Milan)*
RAB5A in the control of mammary epithelial morphogenesis and motility

11:30-11:45 *Silvia Grasso (Turin)*
p140Cap limits ERBB2 breast cancer progression by regulating apical basal cell polarity

11:45-12:00 *Chiara Recchi (London, United Kingdom)*
The Tumor Suppressor OPCML regulates EMT and cell motility in ovarian cancer through its interaction with Receptor Tyrosine Kinases

12:00-12:15 *Roberto Coppo (Turin)*
ESDN/DCBLD2/CLCP1 coordiantes melanoma progression

12:15-12:30 *Valentina Masola (Padua)*
Heparanase is a player in the renal fibrosis by regulating TGF-β expression and activity

12:30 **Closing Remarks**

13:00-14:00 **Lunch**

Oral Presentations

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

Increased V-ATPase expression to support Notch signaling during stem cells establishment in *Drosophila*

E. Tognon, A. Fumagalli, T. Vaccari
IFOM - Istituto FIRC di Oncologia Molecolare, Milano Italy

Owing to the ability to control trafficking, concentration, degradation and recycling of signal tranducers, the endosome represent a major intracellular hub for signaling regulation. Recently, we and others have uncovered a role for the vacuolar H^+ ATPase (V-ATPase), the proton pump that acidifies the endo-lysosomal system, in promoting Notch, Wnt and mTor signaling. V-ATPase function could thus be modulated to regulate signaling in appropriate tissue contexts. To test this, we surveyed expression of V-ATPase subunit genes in vivo during *Drosophila* development. Among them, we focused on the *vha16-1* gene, which codes for a constitutive membrane-embedded component of the pump, required for proton translocation. We find that *vha16-1* expression is increased in developing sensory organ precursors (SOPs). These are a model of multi-potent epithelial stem cells. SOPs are established by a series of Notch signaling events. The first of these determines abundance and spacing of SOPs as part of a process called lateral inhibition. We show that elevated *vha16-1* expression is required for lateral inhibition. In fact, downregulation of *vha16-1* expression results in an increase in SOPs. We observe that *vha16-1* expression is upregulated by Wnt signaling and downregulated by Notch signaling, consistent with the possibility that V-ATPase is part of the established gene circuitry of SOP development. We are currently exploring the mechanism by which V-ATPase levels control activation of Notch signaling. Overall, our study indicates that developmental regulation of V-ATPase is required to sustain Noch signaling decisions during stem cell development.

A novel role of the endocytic adaptor proteins Eps15 and Eps15L1 in the regulation of Notch signaling

C. Lucano[1], P.P. Di Fiore[1, 2, 3], N. Offenhäuser[1]
[1]IFOM, the FIRC Institute for Molecular Oncology Foundation, Milan, Italy
[2]Istituto Europeo di Oncologia, Milan, Italy
[3]Dipartimento di Scienze della Salute, Univ. degli Studi di Milano, Milan, Italy

Notch signaling is an evolutionary conserved signaling pathway that regulates multiple aspects of development and cell renewal. Endocytosis plays a critical role in Notch signaling regulation, both in the signal-sending and in the signal-receiving cell. Eps15 and Eps15L1 are two endocytic adaptors, involved in clathrin-dependent and independent endocytosis of tyrosine kinase receptors. Double Knockout (DKO) mice for Eps15/15L1 die between 9.5 and 11.5 dpc and show a Notch loss of function phenotype, mirrored by downregulation of Notch target genes. Based on these observations we decided to test whether Eps15 and Eps15L1 can regulate Notch signaling by regulating Notch receptors activity in the signal-receiving cell or Notch ligands activity in the signal-sending cell.
Using an *in vitro* coculture/transactivation assay we observed no reduction in Notch activity after knockdown (KD) of Eps15 or Eps15L1 in the signal-receiving cell. However, after KD of Eps15 or Eps15L1 in the signal-sending cell a 40-50% reduction in Notch activity was observed, even though no further decrease of the signal was observed after the combined Eps15/L1 KD. This observation was true for the four Notch ligands Dll1, Dll4, Jag1 and Jag2.
To understand the molecular mechanism underlying this regulation, we set up a FACS based Dll1 internalization assay. While we observed a strong reduction in Dll1 endocytic constant following KD of Epn1 and Mindbomb1, two known endocytic regulators of Notch ligands, no reduction was observed after KD of Eps15, Eps15L1 or both. Moreover, we assessed that localization of Dll1 in detergent resistant membrane was not altered following Eps15/L1 KD. At the moment we are setting up a quantitative Dll1 recycling assay and a structure/function rescue with Eps15 mutants in order to understand other possible mechanisms through which Eps15/L1 can regulate Notch ligands activity.

Clathrin heavy chain-based pathway is involved in the regulation of 17β-estradiol-induced cell proliferation

P. Totta, V. Pesiri, M. Marino, F. Acconcia
Dept of Sciences, Section Biomedical Sciences and Technology, Univ. Roma Tre, Rome, Italy

The sex hormone 17β-estradiol (E2) regulates a plethora of physiological effects including cell proliferation by binding to the estrogen receptor α (ERα), which as a transcription factor drives E2-sensitive gene transcription and as an extra-nuclear localized signalling molecule triggers the membrane-dependent activation of diverse kinase cascades (*e.g.,* ERK/MAPK; PI3K/AKT pathways). While E2 determines all the molecular events necessary for cells to proliferate, it also induces ERα degradation, which has the main function to synchronize gene expression with the E2-driven effects and contemporarily to limit the possibility of a cell hyper-response to the hormone. Although ERα degradation proceeds through the 26S proteasome, we have recently identified a role for lysosomes in controlling ERα content, thus suggesting an endocytic control of ERα intracellular trafficking. In order to begin unravel the role of membrane trafficking intracellular pathways in ERα signalling, here we applied to breast cancer cells (MCF-7 cells) a siRNA screening library for membrane trafficking related proteins and studied E2-dependent ERα degradation. Results indicate that many proteins involved in membrane trafficking modulate E2-induced ERα degradation. In particular, depletion of many of the structural and functional actors involved in the clathrin-based intracellular trafficking act by fastening ERα degradation. Accordingly, depletion of clathrin heavy chain (CHC) in MCF-7 cells fastens E2-induced ERα breakdown and prevents E2-induced breast cancer cell proliferation. Moreover, in MCF-7 cells E2 rapidly triggers the physical association of ERα with CHC. These discoveries suggest a role for clathrin-mediated internalization route in ERα signalling and indicate that clathrin-mediated endocytic pathway could play an intrinsic role in E2:ERα signalling to breast cancer cell proliferation.

ESDN/DCBLD2/CLCP1 coordiantes melanoma progression

R. Coppo[1,2], F. Orso[1,2,3], D. Dettori[1,4], E. Quaglino[1,2], E. Grassi[1,2], S. Osella-Abate[5], L. Nie[6], M.M. Sadeghi[6], D. Taverna[1,2,3]

[1]Molecular Biotechnology Center (MBC), [2]Dept of Molecular Biotechnology and Health Sciences, [3]Center for Molecular Systems Biology, [4]Human Genetics Foundation (HuGeF), [5]Dept of Medical Sciences, Univ. of Turin, Turin, Italy

[6]Veterans Affairs Connecticut Healthcare System, West Haven, Connecticut, USA

Malignant melanoma is currently the fifth most common cancer in the white population and it is fatal in its metastatic stage. Hence, it is essential to understand the molecular mechanisms driving melanoma progression and set up new therapeutic interventions. Scattered data show that the Endothelial and Smooth muscle cell-Derived Neuropilin-like molecule (ESDN) can control cell proliferation and movement of stromal or tumor cells. We investigated the role of ESDN in melanoma progression, in particular in the tumor microenvironment. Precisely, we injected melanoma cells in ESDN$^{-/-}$ mice and we evaluated how the absence of ESDN in stromal cells could influence melanoma progression. While no effect was found on primary tumor growth, increased extravasation and lung metastasis formation was observed in ESDN$^{-/-}$ mice compared to wild type controls. To understand the causes of these defects, we analyzed blood vessels structure, permeability and perfusion in ESDN$^{-/-}$ mouse lungs and primary tumors. We observed an alteration of blood vessel size, permeability and perfusion in ESDN$^{-/-}$ mice that could suggest a role of ESDN in endothelial cells during melanoma cell extravasation. Furthermore, bioinformatics analyses suggest a putative function of ESDN as a cell junction and adhesion molecule. We are currently isolating murine lung endothelial cells in order to understand the biological mechanism of ESDN during the extravasation process. In addition, we are evaluating a potential contribution of immune cells by performing bone marrow transplants prior to melanoma cell injection and dissemination. In conclusion, our data suggest a functional role for ESDN in melanoma progression.

Identifying DUBs in the EGFR pathway

M. G. Savio[1,2], N. Wollscheid[1] and S. Polo[1,2]
[1]IFOM, Fondazione Istituto FIRC di Oncologia Molecolare, Milan, Italy
[2]Dipartimento di Scienze della Salute, Università degli Studi di Milano, Italy

The Epidermal Growth Factor Receptor (EGFR) is a receptor tyrosine kinase, which upon ligand activation undergoes endocytosis. The internalized receptor is either down-regulated *via* the multivesicular body pathway (MVB) or recycled back to the plasma membrane. The ubiquitination of the receptor serves as a critical signal for the sorting of the receptor. Deubiquitinating enzymes (DUBs) are an important protein family, which opposes ubiquitin ligases and can therefore regulate endocytosis, trafficking and degradation of the EGFR. The human genome encodes for around 90 active DUBs. So far, only a few DUBs have been implicated to function in EGFR endocytosis and degradation. AMSH and UBPY (USP8) were the first two to be studied in details. However, the extent to which inhibition of these enzymes affected degradation of EGFR is limited, suggesting the existence of other DUBs that are effective in controlling EGFR turnover.
We undertook a genome-wide small interfering RNA approach, targeting all predicted DUBs. These siRNAs were screened for their ability to alter degradation kinetics of EGFR and signaling pathways downstream of it. We identified several DUBs slowing EGFR degradation upon EGF stimulation while others hastening the process.
We focused in particular on USP25 and USP9X. USP25 recognizes the EGFR as a direct target, and its ablation accelerated the receptor degradation rate. On the contrary, the absence of USP9X caused a delay in EGFR turnover. USP9X seemed to be important to control the level of ubiquitination of different endocytic adaptors, namely Epsins 1/2 and Eps15, thus indirectly affecting EGFR internalization.

Acute and chronic stress signaling from the endoplasmic reticulum

Milena Vitale, Andrea Orsi, Eelco van Anken
San Raffaele Scientific Institute, Università Vita-Salute, Milan, Italy

Proteins destined to travel along the secretory pathway fold and assemble in the endoplasmic reticulum (ER). If the ER folding capacity becomes insufficient to handle the protein folding load, the unfolded protein response (UPR) is invoked to restore ER homeostasis. Most studies in the field focus on the acute UPR, which can be induced with drugs that perturb productive folding in the ER, such as tunicamycin, while chronic UPR activation lies at the basis of several pathologies. We decided to develop a system to integrate both the acute and chronic UPR. To this end, we inducibly expressed the secretory IgM heavy chain (μ_s) in HeLa cells. We found that μ_s activates both the IRE1 and PERK branches of the UPR in a dose and time dependent manner, with high induction during the acute phase and intermediate induction later, mimicking the chronic UPR. The UPR stress tranducers clustered in the ER membrane only in the acute phase. Co-expression of the λ light chain abolished the UPR, since μ_s + λ can fold to become IgM. Since binding to λ makes the CH1 domain of μ_s 'invisible', we tested a mutant of μ_s lacking the CH1 (μ_Δ), and found that the UPR was not invoked upon μ_Δ expression. Conversely, the CH1 domain was sufficient to activate the UPR when fused to the VL domain of λ and co-expressed with μ_Δ. Our data identify the CH1 domain as a potent UPR activation module, a feature which likely is key in B to plasma cell differentiation. Moreover, we established a framework to study the UPR by a proteostatic challenge and to follow its transitioning from an acute to a chronic response.

Genome wide screen to identify ER-mitochondria tether proteins using FRET biosensors

S. Lakshminarayanan[1], A. Serafini[1], M. Giacomello[1], L. Scorrano[1,2]
[1]Dept of Biosceince and Biotechnology, Univ. of Padova, Italy
[2]Venetian institute of Molecular Medicine, Padova, Italy

Regulation of signaling cascades is essential for cellular homeostasis since the same signal can bring out different outcomes depending on its strength, duration and subcellular localization. Spatial organization of the intracellular organelles and the inter-organelle communication is often involved in the control of such signaling cascades: for example, the juxtaposition between Endoplasmic Reticulum (ER) and plasma membrane modulate in-flux of calcium directly from extracellular space to the ER in response to the signals perceived by proteins located on the plasma membrane. In recent years the inter-organelle communication between ER and mitochondria that occurs in specific membrane domains known as Mitochondrial Associated Membranes (MAMs) has gained importance because of its impact on cell survival and death. This tether has been already implied for its role on the transport of phospholipids and recent evidences highlight its involvement in direct transfer of calcium from ER to mitochondria thus contributing to the maintenance of calcium homeostasis in both the organelles. Mfn2 was recently described as an essential part of the tether. Mfn2 KO MEF cells showed defects both in the ER and mitochondrial morphology. Mfn2 mutants targeted either to mitochondria or to ER were able to correct the morphology of the individual organelles but unable to form the tethers, proving the requirement of Mfn2 on the surface of ER apart from its known localization on the mitochondria. The fact that Mfn2 KO cells have residual tether suggests that other proteins, independent of Mfn2, are involved in maintaining the contacts between the two organelles.

Presenilin-2 is a master regulator of endoplasmic reticulum-mitochondria tethering

R. Filadi[1], E. Greotti[1], T. Pozzan[1,2,3], P. Pizzo[1]
[1]Dept of Biomedical Sciences, Univ. of Padua, Padua, Italy
[2]Venetian Institute of Molecular Medicine (VIMM), Padua, Italy
[3]CNR, Italian National Research Council (CNR), Italy

The organization and the mutual interactions between the endoplasmic reticulum (ER) and mitochondria modulate key aspects of cell pathophysiology, ranging from lipid metabolism and Ca^{2+} homeostasis to cell death. Juxtaposition between these organelles is modulated by several proteins, the majority of which are found enriched in mitochondria associated membranes (MAMs). Presenilin-2 (PS2), whose mutations are responsible for familial Alzheimer's disease (FAD), has also been found enriched in MAMs (Area-Gomez E. et al, 2009).
We have recently demonstrated that PS2 (especially FAD-PS2 mutants) favours ER-mitochondria tethering, thus increasing mitochondrial Ca^{2+} uptake upon release of the ion from the ER (Zampese E. et al., 2011). We here demonstrate that PS2 and mitofusin 2 (Mfn2), a previously characterized ER-mitochondria tether (De Brito O.M. and Scorrano L., 2008), functionally and physically interact at the level of MAMs. Importantly, Mfn2 and PS2 need each other to modulate ER-mitochondria connections and their Ca^{2+} cross-talk. Noteworthy, FAD-PS2 mutants are more efficient in engaging Mfn2, more prone at localizing within MAMs and more potent in modulating organelles' tethering and Ca^{2+} cross-talk. The PS2/Mfn2-dependent increase in ER-mitochondria coupling is confirmed also by quantitative Electron Microscopy and is observed not only in FAD experimental models over-expressing different mutated PS2, but also in human fibroblasts from patient carrying the PS2-N141I mutation, thus a condition independent from any artefact due to PS2 over-expression.
The outcomes of the stronger FAD-PS2-linked effects on ER-mitochondria coupling are under investigation to clarify their involvement in AD pathogenesis and progression.

The role of the Mitochondrial Calcium Uniporter in the control of skeletal muscle mass

G. Gherardi[1], A. Raffaello[1], G. Pallafacchina[1,2], I. Zamparo[1], A. Braga[1], D. De Stefani[1], L. Zentilin[3], M. Giacca[3], C. Mammucari[1], R. Rizzuto[1,2]
[1]Dept of Biomedical Science, Univ. of Padova, Padova, Italy
[2]Institute of Neuroscience, Consiglio Nazionale delle Ricerche, Padova, Italy
[3]Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

The second messenger Ca^{2+} plays a key role in mitochondrial function: cytosolic Ca^{2+} transients, generated by physiological stimuli, elicit mitochondrial Ca^{2+} ($mtCa^{2+}$) uptake which stimulates aerobic metabolism. Accordingly, high amplitude increases in mt [Ca^{2+}] are detected in skeletal muscle mitochondria during contraction. The highly selective channel responsible for Ca^{2+} entry into mitochondria is the Mitochondrial Calcium Uniporter (MCU), whose molecular identity has been described three years ago. More recently the role of MICU1 and MICU2 has been disclosed. MICU1 and MICU2 are direct modulators of the pore-forming subunit (MCU) with opposite effects on channel activity, and form a regulatory dimer. Importantly, mutations of MICU1 have been identified in individuals with a disease phenotype characterized by proximal myopathy, learning difficulties and a progressive extrapyramidal movement disorder. Here we show that MCU is both required and sufficient for muscle mass maintenance and that MCU exerts a protective effect against atrophy. Thus we suggest that MCU plays a crucial role in muscle trophism and therefore represents a possible target of clinical intervention.

Mitochondrial signaling - the *energy factory* takes control of the boardroom

N. Raimundo
Institute for Cellular Biochemistry, University Medical Center Goettingen, Germany

Cellular metabolism is experiencing a *Renaissance*. While mitochondria are classically described as the "cellular energy factory", their roles as signaling platforms start to be unveiled. Furthermore, it is nowadays established that mitochondria are part of a complex organelle network that has fundamental roles in cellular physiology and disease.
We focus on the molecular mechanisms mediating communication between mitochondria and the rest of the cell, in particular the nucleus and two other metabolic organelles, lysosomes and peroxisomes. The importance of interactions between mitochondria, peroxisomes and lysosomes (henceforth, MitoPexLyso) is evidenced by disease phenotypes, as primary defects in one of these organelles often result in secondary perturbations of the other two.
Therefore, we are exploring the hypothesis that MytoPexLyso organelles form a highly interdependent network, and that mitochondrial, peroxisomal or lysosomal diseases are not simply single-organelle dysfunctions but rather imbalances in the MitoPexLyso network. The mechanisms mediating MytoPexLyso interdependence and the implications of this network for cell biology and metabolic diseases are virtually unknown.
We employ genomics and imaging approaches to identify signaling pathways that respond to perturbations in the MitoPexLyso network, in order to understand the molecular mechanisms of metabolic diseases and define therapeutic targets.

MICU1 and MICU2 finely tune the mitochondrial Ca2+ uniporter by exerting opposite effect on MCU activity

M. Patron[1], D. De Stefani[1], V. Checchetto[2], A. Raffaello[1], E. Teardo[2], D. Vecellio Reane[1], M. Mantoan[1], V. Granatiero[1], I. Szabò[2], R. Rizzuto[1]
[1]Dept of Biomedical Sciences, Univ.of Padova and CNR Neuroscience Institute, Padova, Italy
[2]Dept of Biology, University of Padova, Padova, Italy

Mitochondrial calcium accumulation was recently shown to depend on a complex composed of an inner membrane channel (MCU, MCUb) and regulatory subunits (MICU1, MCUR1, EMRE). A fundamental property of MCU is low activity at resting cytosolic Ca2+ concentrations, preventing deleterious Ca2+ cycling and organelle overload. We here demonstrate that these properties are ensured by a regulatory heterodimer composed of two proteins with opposite effects, MICU1 and MICU2, that both in purified lipid bilayers and in intact cells stimulate and inhibit MCU activity, respectively. Both MICU1 and MICU2 are regulated by calcium through their EF-hand domains, thus accounting for the sigmoidal response of MCU to [Ca2+] in situ and allowing tight physiological control. At low [Ca2+], the dominant effect of MICU2 largely shuts down MCU activity; at higher [Ca2+], the stimulatory effect of MICU1 allows the prompt response of mitochondria to Ca2+ signals generated in the cytoplasm.

Characterization of ER-involving hypotonic shock-induced calcium release in the budding yeast

M. Rigamonti , F. Belotti, E. Martegani, R. Tisi
Dept Biotechnology and Biosciences, Milano-Bicocca Univ., Milan, Italy

In the budding yeast, calcium is an important second messenger involved in controlling many fundamental cellular processes, such as nutrient or stress response, cell cycle progression, survival upon exposure to pheromones. Hypotonic shock in *Saccharomyces cerevisiae* triggers an increase in cytosolic Ca^{2+} concentration that involves both calcium influx and calcium release from internal stores. The response is still present in strains carrying the deletion of all known calcium channels, leading to suppose the involvement of still unknown calcium channels.
Computational data (JBC 2002 277:31079–88) suggested to consider Yor365c and Yal053w, two putative TRP ion channels mainly localized in the ER compartment. Yal053w was formerly named Flc2 due to its similarity to a flavin carrier from *Candida albicans*, and to the two closest homologs to this last in budding yeast, Flc1 and Flc3. Our data suggest that calcium release in response to hypotonic shock is mediated by Flc2/Yal053w, but does not require any similar proteins (Yor365c, Flc1 or Flc3), suggesting a specific function for Flc2, previously undiscovered.
FLC2 deletion also impinges on sensitivity to Calcofluor white, suggesting damage to the cell wall, and exacerbates *mid1Δ cch1Δ* mutant sensitivity to tunicamycin, revealing stress on the ER. Both phenotypes could be related to a defect in calcium homeostasis in the ER.
Furthermore, deletion of *CLS2*, encoding for a protein required for calcium homeostasis in the ER, induced a much higher calcium release from internal stores upon hypotonic shock.
We suggest that hypotonic shock induces a calcium signal involving calcium efflux from the ER compartment, for the first time described to directly participate in calcium release in budding yeast.

Toxoplasma effector MAF1 mediates recruitment of host mitochondria and impacts the host response

L. Pernas[1], Y.A. Ankomah[2], A.J. Shastri[1], S.E. Ewald[1], M. Treeck[1], J.P. Boyle[2], J.C. Boothroyd[1]
[1]Dept Microbiology & Immunology, Stanford Univ. School of Medicine, Stanford, USA
[2]Dept Biological Sciences, Univ. of Pittsburgh, Pittsburgh, USA

Recent information has revealed the functional diversity and importance of mitochondria in many cellular processes including orchestrating the innate immune response. Intriguingly, several infectious agents, such as *Toxoplasma, Legionella* and *Chlamydia,* have been reported to grow within vacuoles surrounded by host mitochondria. Although many hypotheses have been proposed for the existence of host mitochondrial association (HMA), the causes and biological consequences of HMA have remained unanswered.
Here we show that HMA is present in Type I and III strains of *Toxoplasma* but missing in Type II strains, both *in vitro* and *in vivo*. Analysis of F1 progeny from a Type II x III cross revealed that HMA is a Mendelian trait that we could map. We use bioinformatics to select potential candidates and experimentally identify the polymorphic parasite protein involved, mitochondrial association factor 1 (MAF1). We show that introducing Type I (HMA^+) *MAF1* allele into Type II (HMA^-) parasites results in conversion to HMA^+ and deletion of *MAF1* in Type I parasites results in a loss of HMA. We observe that the loss and gain of HMA are associated with alterations in the transcription of host cell immune genes and the *in vivo* cytokine response during murine infection. Lastly, we use exogenous expression of MAF1 to show that it binds host mitochondria and thus MAF1 is the parasite protein directly responsible for HMA.
Our findings suggest that association with host mitochondria may represent a novel means by which *Toxoplasma* tachyzoites manipulate the host. The existence of naturally occurring HMA^+ and HMA^- strains of *Toxoplasma, Legionella* and *Chlamydia* indicates the existence of evolutionary niches where HMA is either advantageous or disadvantageous, likely reflecting trade-offs in metabolism, immune-regulation and other functions of mitochondria.

The multifunctional mitochondrial inner membrane protein Optic Atrophy 1 controls cellular damage *in vivo*

T. Varanita[1], M.E. Soriano Garcìa Cuerva[1], V. Romanello[1,3], T. Zaglia[1,3], R. Menabò[3], V. Costa[2], S. Cogliati[1], R. Slack[4], M. Mongillo[1,3], M. Sandri[1,3], F. Di Lisa[3], L. Scorrano[1,2]
[1]Dulbecco Telethon Institute, Venetian Institute of Molecular Medicine, Padova, Italy
[2]Dept of Biology, Univ. of Padova, Padova, Italy
[3]Dept of Biomedical Sciences, University of Padova, Padova, Italy
[4]Center for Neuroscience, University of Ottawa, Canada

Mitochondrial morphological changes have been implied in apoptosis and autophagy in several in vitro models, but their relevance to tissue adaptation to damaging insults is unexplored. Here we show that controlled mild overexpression of Optic atrophy 1 (OPA1), an inner mitochondrial membrane protein that controls cristae remodeling and mitochondrial respiratory efficiency, protects multiple tissues from apoptotic, necrotic and atrophic stimuli. Targeted insertion of one copy of OPA1 isoform 1 in a permissive X chromosome locus does not interfere with mouse development, but it protects from ischemic damage in the heart and the brain, as well as from muscular atrophy and hepatocellular apoptosis. Mechanistically, OPA1 stabilizes the cristae, increasing mitochondrial respiratory efficiency, blunting cytochrome c release in vitro and in vivo as well as mitochondrial dysfunction. Our results indicate that the OPA1-dependent cristae remodeling pathway is an essential determinant of the cellular response to tissue damage in vivo.

Loss of PI3K-C2A promotes tumorigenesis and aneuploidy in breast cancer

M. Martini[1], F. Gulluni[1], M.C. De Santis[1], A. Ghigo[1], E. Ciraolo[1], J.P. Margaria[1], U. Ala[1], M. Compagno[1,2], R. Chiarle[1,2], E. Hirsch[1]
[1]Dept of Molecular Biotechnology and Health Sciences, Univ. of Torino
[2]Dept of Pathology, Harvard Medical School, Boston

PI3K signaling axis is one of the most frequently deregulated pathways in human cancer impacting on cell growth, survival and metabolism. Whereas the majority of efforts have focused on class I PI3K, increasing evidence is pointing to the importance of class II enzymes in cell proliferation and survival.
We generated a mouse strain lacking PI3K-C2α expression and found that the mutation is embryonic lethal. Pik3c2a-/- MEFs displayed strongly reduced proliferative capacity, accompanied by increased apoptosis compared to wt. Heterozygous MEFs also displayed haploinsufficiency and gene dosage dependency. Cell-cycle analysis of Pik3c2a-/- MEFs showed a significant delay in the progress from prophase to anaphase. PI3K-C2α was specifically enriched at the metaphase spindle, interacting with transforming acidic coiled-coil protein 3 (TACC3)/colonic, hepatic tumor overexpressed gene (ch-TOG)/clathrin complex to stabilize K-fibers during early mitosis. Loss of PI3K-C2α resulted in reduced spindle length, altered microtubule (MT) stability and increased metaphase plate width. Karyotype analysis revealed high levels of aneuploidy in Pik3c2a-/- cells compared to controls. The effects of *PI3KC2A* down-regulation were also investigated in a mouse model of breast cancer. Heterozygous loss of PI3K-C2α resulted in an initially delayed tumor onset followed by a faster growth rate compared to wt. Tumors with low PI3K-C2α showed increased sensitivity to anti-MT agents, like Paclitaxel. Expression profiles of breast cancer patients showed that reduced levels of *PIK3C2A* transcripts correlate with tumor aggressiveness, highlighting the role of PI3K-C2α in cancer development.
We demonstrated that loss of PI3K-C2α plays a crucial role in promoting genomic instability, altering chromosome congression during cell division. These findings will eventually validate PI3K-C2α as a new prognostic tool that can be exploited to tailor more effective therapies for aggressive breast cancers.

RAB5A in the control of mammary epithelial morphogenesis and motility

C. Malinverno[1], M. Bergert[2], A. Locatelli[1], S. Corallino[1], A. Palamidessi[1], E. Frittoli[1], Y. Rolland[1], A. Ferrari[2], G. Scita[1,3]
[1]IFOM Foundation, Institute FIRC of Molecular Oncology, Milan, Italy
[2]ETH Zurich, Laboratory of Thermodynamics in Emerging Technologies, Zurich, Switzerland
[3]Dipartimento di Scienze della Salute, Università degli Studi di Milano, Milan, Italy

RAB5A, a master regulator of endocytosis, promotes a tumor mesenchymal invasive program. In *Drosophila*, however, loss-of-function of RAB5 leads to hyperproliferation, pointing to a tumor suppressor function.
To rationalize the complex role of RAB5A in tumor development, we investigated its impact on MCF-10A, an immortalized non-transformed mammary epithelial cell line that mimics morphogenesis of mammary gland when culture on 3D reconstituted basement membrane. We generated inducible MCF-10A cells expressing either RAB5A-WT or its dominant negative form (RAB5A-S34N). We found that the expression of RAB5A-S34N is sufficient to sustain MCF-10A cells proliferation in the absence of EGF, through the secretion of a diffusible growth-promoting factor. Conversely, the expression of RAB5A-WT delayed cell cycle progression of cells grown in 2D, albeit it promoted the formation of hyperproliferative acini when grown in 3D. Thus, RAB5 may either be implicated in growth factor independent growth or promote proliferation in 3D. In keeping with these latter findings, clinical data and *in vitro* studies demonstrated that RAB5A is required for invasion and metastasis, suggesting its involvement in tumor progression.
To further explore this latter role, we tested MCF-10A cells motility. We demonstrated that RAB5A-WT expression does not affect single cell migration, but specifically enhances collective locomotion. Indeed, RAB5A expressing cells showed increased coordination and coherence of epithelial cell sheet motility, probably related to both a tightening of cell-cell contact and an increase in the area and persistence of cell protrusions at the leading front.
We are currently dissecting the molecular mechanisms through which RAB5A altered function or expression impact on both mammary gland morphogenesis and tumor progression.

p140Cap limits ERBB2 breast cancer progression by regulating apical basal cell polarity

S. Grasso[1], I. Russo[1], V. Salemme[1], S. Cabodi[1], J. Staaf[2], P. Provero[1], E. Turco[1], P. Di Stefano[1], P. Defilippi[1]
[1]Dept of Mol Biotech and Health Sciences, Torino Univ, Torino, Italy
[2]Dept of Oncology, Lund Univ. and Skåne University Hospital, Lund, Sweden

SRCIN1/p140Cap is a scaffold protein that behaves as a tumor suppressor protein in breast cancer cells, impairing in vivo tumor growth and metastasis formation. Our data show that the SRCIN1 gene co-amplifies in about 50% of ERBB2 breast tumors and its copy number gain correlates with increased patient survival. Double transgenic mice, over-expressing both rat ERBB2 (NeuT) and p140Cap, show a significant delayed insurgence and decreased growth of ERBB2-driven tumors. Here we studied the PAR polarity complex (Par3-Par6-aPKC) and we show that in 3D cultures of primary cancer cells, p140Cap restores the apical basal polarity destroyed by ERBB2, thanks to its ability to counteract ERBB2 binding to the Par6-aPKC complex, and to favor an accurate re-assembly and localization of the PAR complex at the Tight Junctions. Moreover, p140Cap impairs ERBB2-dependent inappropriate activation of aPKC and STAT3. p140Cap associates with members of the PAR complex, including Par3, aPKC and TIAM1. The association of Tiam1 with p140Cap results in Rac inactivation in the NeuT cells. Interestingly, activity of Cdc42, another component of PAR complex, is also inhibited. Overall these data show that p140Cap expression restores apical-basal polarity in NeuT cells, through re-localisation of the PAR complex and inhibition of Rac and Cdc42 GTPase activity, thus counteracting the ERBB2-induced disruption of the proper mammary morphogenetic program.

The Tumor Suppressor OPCML regulates EMT and cell motility in ovarian cancer through its interaction with Receptor Tyrosine Kinases

J. Antony[1,2], H. Lu[1], A. Patterson[1], S. Roy-Nawathe[1], E. Zanini[1], H. Gabra[1] , C. Recchi[1]
[1] Ovarian Cancer Action Research Centre, Imperial College London, London, United Kingdom
[2] NUS Graduate School for Integrative Sciences and Engineering, National Univ. of Singapore, Singapore

Ovarian cancer is a lethal gynecological malignancy that spreads to vital organs before any symptoms appear. The Opioid-binding Protein/Cell adhesion Molecule-Like (OPCML), a glycosylphosphatidylinositol (GPI) anchored protein belonging to the IgLON family, is silenced in over 80% of ovarian cancer cases. The OPCML tumour suppressor gene inhibits tumour cell growth *in vitro* and dimishes tumorigenesis *in vivo* by negatively regulating a spectrum of Receptor Tyrosine Kinases (RTKs) such as EPHA2, FGFR1, FGFR3, HER2 and HER4. However, the role of OPCML in tumour biology remains to be fully elucidated.
AXL, a TAM family RTK, is a clinically relevant target in ovarian cancer and is involved in cross talk between oncogenic signaling and Epithelial to Mesenchymal Transtition (EMT). By mammalian-2-hybrid, co-immunoprecipitation and immunofluorescence microscopy we found that OPCML associates with AXL and redistributes it to detergent resistant fractions. Since EMT represents a series of events that gives cancer cells the ability to migrate and invade, thereby promoting metastasis, we have investigated whether OPCML may be involved in regulating the EMT program in ovarian carcinoma. We found a down regulation of EMT drivers and different parameters of migration upon expression of OPCML in ovarian cancer cells. As expected, EMT markers are up-regulated upon activation of AXL by its ligand Gas6. However, this effect is completely abrogated in presence of OPCML. Also, silencing AXL in control cells reduces migration to the same level of OPCML expressing cells. Furthermore, silencing AXL in OPCML-expressing cells does not further reduce migration, thus suggesting that OPCML and AXL function in the same pathway.
OPCML can interact with numerous cell surface receptors to alter signal transduction cascades and potentially exert its tumor suppressor effects at a system level, thus making it a promising clinical therapeutic agent.

ESDN/DCBLD2/CLCP1 coordiantes melanoma progression

R. Coppo[1,2], F. Orso[1,2,3], D. Dettori[1,4], E. Quaglino[1,2], E. Grassi[1,2], S. Osella-Abate[5], L. Nie[6], M.M. Sadeghi[6], D. Taverna[1,2,3]
[1]Molecular Biotechnology Center (MBC), [2]Dept of Molecular Biotechnology and Health Sciences, [3]Center for Molecular Systems Biology, [4]Human Genetics Foundation (HuGeF), [5]Dept of Medical Sciences, Univ. of Turin, Turin, Italy
[6]Veterans Affairs Connecticut Healthcare System, West Haven, Connecticut, USA

Malignant melanoma is currently the fifth most common cancer in the white population and it is fatal in its metastatic stage. Hence, it is essential to understand the molecular mechanisms driving melanoma progression and set up new therapeutic interventions. Scattered data show that the Endothelial and Smooth muscle cell-Derived Neuropilin-like molecule (ESDN) can control cell proliferation and movement of stromal or tumor cells. We investigated the role of ESDN in melanoma progression, in particular in the tumor microenvironment. Precisely, we injected melanoma cells in ESDN$^{-/-}$ mice and we evaluated how the absence of ESDN in stromal cells could influence melanoma progression. While no effect was found on primary tumor growth, increased extravasation and lung metastasis formation was observed in ESDN$^{-/-}$ mice compared to wild type controls. To understand the causes of these defects, we analyzed blood vessels structure, permeability and perfusion in ESDN$^{-/-}$ mouse lungs and primary tumors. We observed an alteration of blood vessel size, permeability and perfusion in ESDN$^{-/-}$ mice that could suggest a role of ESDN in endothelial cells during melanoma cell extravasation. Furthermore, bioinformatics analyses suggest a putative function of ESDN as a cell junction and adhesion molecule. We are currently isolating murine lung endothelial cells in order to understand the biological mechanism of ESDN during the extravasation process. In addition, we are evaluating a potential contribution of immune cells by performing bone marrow transplants prior to melanoma cell injection and dissemination. In conclusion, our data suggest a functional role for ESDN in melanoma progression.

Heparanase is a player in the renal fibrosis by regulating TGF-β expression and activity

V. Masola[1,2], G. Zaza[2], M.F. Secchi[1], G. Gambaro[3], A. Lupo[2], M. Onisto[1]
[1]Dept of Biomedical sciences, Univ. of Padova, Italy
[2]Renal Unit, Dept of Medicine, Univ.-Hospital of Verona, Italy
[3]Division of Nephrology and Dialysis, Columbus-Gemelli Hospital, Catholic Univ., School of Medicine, Rome, Italy

Epithelial-mesenchymal transition (EMT) of tubular cells is one of the mechanisms which contribute to renal fibrosis and transforming growth factor-β (TGF-β) is one of the main triggers. Heparanase (HPSE) is a endo-β-D-glucuronidase that clives heparan-sulphate and takes part to extracellular-matrix remodeling thus regulating the bioavailability of growth factors (FGF-2, TGF-β). HPSE controls FGF-2-induced EMT in tubular cells and is necessary for the development of diabetic nephropathy in mice.
The aim of this study was to investigate whether HPSE can modulate the expression and the effects of TGF-β.
First we proved that the lack of HPSE prevents the increased synthesis of TGF-β by tubular cells in response to pro-fibrotic stimuli such as FGF-2, AGE and albumin.
Second, HPSE does not prevent EMT induced by TGF-β although it slows its onset. Indeed TGF-β induces EMT in wt and in HPSE-silenced tubular cells; however in HPSE-silenced cells the acquisition of a mesenchymal phenotype does not develop as quickly as in wt cells, supporting the hypothesis that HPSE facilitates the TGF-β biological activities. Additionally, TGF-β induces an autocrine loop to sustain its signal whereas the lack of HPSE jeopardizes this autocrine loop.
Overall these data confirm that heparanase is a key player in renal fibrosis since it interacts with the regulation and the effects of TGF-β. HPSE is needed for the pathological TGF-β overexpression in response to pro-fibrotic factors such as albuminuria, AGE and FGF-2. Furthermore HPSE modulates TGF-β-induced EMT, the lack of HPSE delays tubular cell transdifferentiation, and impairs TGF-β autocrine loop.

Posters

(presenting authors are shown underlined)

P1

Codon 12 and codon 13 mutations in K-RAS differentially affect colorectal carcinoma cells

M.R. Saladino[1], M.M. Barreca[1], D. Gomez Matallanaz[2], C. Raso[2], W. Kolch[2] , I. Albanese[1]
[1]Dept Biological Chemical and Pharmaceutical Sciences and Technologies, Univ. of Palermo, Palermo, Italy
[2]Dept Systems Biology Ireland, Univ. College of Dublin, Dublin, Ireland

The RAS proteins are involved in one of the most conserved signal transduction pathways that regulates proliferation, differentiation and apoptosis in all animals, and their activating mutations have oncogenic effects. K-RAS mutations, usually point mutations in codons 12 or 13, are common in colorectal carcinomas. However, molecular epidemiological studies suggest that mutations in different codons or different mutations in the same codon of K-Ras may have diverse biological consequences. They may also lead to a different response to therapies based on Cetuximab, an anti-EGFR monoclonal antibody. K-RAS mutations are in fact considered to be a resistance factor to this drug, but it has been reported that tumors with K-RAS mutations in codon 13 might be less resistant to Cetuximab than tumors with codon 12 mutations. To shed more light on the molecular mechanisms responsible for the different effects of K-RAS mutations, we have established and analyzed HT-29 clones stably transfected with cDNAs codifying K-RASG12V (clone K12) and K-RASG13D (clone K13) under the control of a inducible promoter. The expression of the two mutated isoforms of K-RAS induces different effects on the growth rate and on the cell cycle, and also on the level of expression of several proteins controlling proliferation and cell survival. Thus, for example, K-RASG13D and K-RASG12V have a differential effect on the pro-apoptotic (MST2-RASSF1A-LATS1) and the anti-apoptotic (MST2-RAF-1) pathways and K-RASG13D, in our system, appears to have a significant cytotoxic effect. Finally, we observed that Cetuximab affects the cell cycle and proliferation of the K13 clone expressing K-RASG13D but not the HT29 cells and the K12 cells induced to express K-RASG12V. These results support the hypothesis that tumors with different K-RAS mutations could respond differently to therapies.

P2

Molecular characterization of non-clathrin endocytosis of the EGFR

E. Barbieri[1], G. Caldieri[1], G. Nappo[1], P.P. Di Fiore[1,2,3], S. Sigismund[1]
[1]IFOM, Fondazione Istituto FIRC di Oncologia Molecolare, Milan, Italy
[2]Dipartimento di Scienze della Salute, Univ. degli Studi di Milano, Milan, Italy
[3]Istituto Europeo di Oncologia, Milan, Italy

Epidermal Growth Factor Receptor (EGFR) can be internalized through different routes depending on cell context and ligand concentration. Indeed, we have previously shown that internalization via clathrin-mediated endocytosis (CME), active at low dose of EGF, targets receptors to recycling and potentiates signaling. In contrast, internalization via the poorly characterized non-clathrin-dependent pathway (NCE) is essential for degradation of the receptor in the presence of high ligand concentrations and thus safeguards cells against excessive EGFR signaling. Since NCE appears to be a critical negative regulator of EGFR it is possible that loss of this route could lead to aberrant EGFR signaling in cancer.
In order to obtain a molecular characterization of NCE of the EGFR and study its impact on receptor signaling and degradation we used a dual approach. 1) Candidate gene approach: selecting players, which are already known to play a role in major clathrin independent endocytic routes and testing the effect of their ablation on EGFR-NCE. 2) Unbiased proteomic approach: to identify the molecular components of NCE a biochemical purification of NCE vesicles coupled with quantitative mass spectrometry was performed in our lab. The NCE components so identified were analyzed for their effect on EGFR-NCE through RNAi-based assays. This analysis provided us with a list of potential new regulatory proteins of NCE and some non-regulatory cargoes. Of note, some of the NCE regulatory proteins are localized in the endoplasmic reticulum (ER), such as the membrane-shaping factors Reticulon 3 (RTN3). This suggests a tight crosstalk between NCE and ER, and the mechanism underlying this connection is currently under investigation. Importantly, we found that RTN3 KD affects not only EGFR internalization but also its degradation and signaling downstream its activation, demonstrating that the NCE pathway is indeed a critical regulator of EGF-dependent cellular response.

P3

A functional interplay between Pyk2 and Src family kinases links Ca^{2+} signaling to protein tyrosine phosphorylation in thrombin-stimulated platelets

I. Canobbio, G. Guidetti, D. Manganaro, M. Torti
Dept Biology and Biotechnology, Univ. of Pavia, Italy

Platelets express several tyrosine kinases, including members of the Src family SFKs (Src and Lyn) and of the focal adhesion family (FAK and Pyk2). Both SFKs and Pyk2 are regulated by intracellular $Ca^{2+}$and are required for thrombin-induced aggregation and thromboxane A_2 production. However, the hierarchy of protein tyrosine kinases activation linking agonist-induced increase of cytosolic Ca^{2+} to protein tyrosine phosphorylation is poorly understood. In this study, we investigated the interplay between intracellular Ca^{2+}, Pyk2 and SFKs in the regulation of tyrosine phosphorylation in thrombin-stimulated platelets.
We have demonstrated that thrombin causes the time-dependent tyrosine phosphorylation of several proteins in human and mouse platelets. This event is mediated by intracellular Ca^{2+} increase and SFKs activation. Analysis of SFKs activity in human and mouse platelets with an anti-pY416Src antibody reveals that Ca^{2+} is upstream SFKs activation. Accordingly, SFKs phosphorylation is directly promoted by the Ca^{2+} ionophore A23187. Thrombin and A23187 also trigger the SFKs and Ca^{2+} dependent phosphorylation and activation of the focal adhesion kinase Pyk2. However, in platelets from Pyk2 KO mice, stimulation of SFKs by thrombin and A23187 is strongly reduced. Finally, in Pyk2 KO platelets, thrombin-induced aggregation is reduced to a level comparable to that of wild type platelets treated with PP2.
These results indicate that in thrombin-stimulated platelets intracellular Ca^{2+} increase promotes Pyk2 stimulation, which initiates SFKs activation and establishes a positive loop which reinforces the Pyk2/SFKs axis to allow the subsequent massive tyrosine phosphorylation of multiple platelet substrates.

P4

Analysis of the cooperation between Pim1 and c-Myc in regulating the translational machinery in prostate cancer cells

L. Chellini, S. Caldarola, F. Ladisa, F.Loreni
Dept Biology, Tor Vergata Univ., Rome, Italy

A common feature in different kinds of cancer is the alteration in the signalling networks that control protein synthesis, able to promote abnormal cell growth and cancer cell progression. Prostate cancer (PCa) is a neoplasia where deregulation of translation appears to play an important role. Two proteins, the kinase Pim1 and the transcriptional factor c-Myc, both overexpressed in PCa, sinergyze to induce and maintain transformation. Both have a role in translation: c-myc controls the transciption of initiation factors involved in translation (eIF4F) and induce the phosphorylation of 4E-BP1, whereas Pim1 can phosphorylates 4E-BP1 and S6K1 in a mTORC1 independent manner. Moreover Pim1 can stabilize and increase the transcriptional activity of c-myc. In order to address the molecular mechanism at the basis of the sinergy between Pim1 and c-Myc in inducing PCa, we will analyze the impact of their overexpression or depletion on the translational machinery (analyzing the general protein synthesis and translational signalling pathways). Our results could reveal new informations about the cooperation of these two proteins in PCa, showing us new druggable targets for a therapeutic approach.

P5

ERK8 (MAPK15) mediates BCR/ABL induced autophagy and proliferation

D. Colecchia[1,2], M. Rossi[1,3], F. Sasdelli[1,2], A. Strambi[1,2], M. Chiariello[1,2]
[1]Istituto Toscano Tumori-Core Research Laboratory, Signal Transduction Unit, AOU Senese, Siena Italy
[2]Istituto di Fisiologia Clinica, Sede di Siena, CNR, Italy
[3]Università degli Studi di Siena, Italy

Chronic myeloid leukemia (CML) is a myeloproliferative disorder caused by BCR/ABL oncogene formation. Thanks to its constitutively active tyrosine kinase activity, BCR/ABL is indeed able to mimic growth factors stimulation by activating many signaling pathways leading to increased proliferation, decreased apoptosis, reduced growth factor-dependence. Although tyrosine kinase inhibitors (TKI) have clearly revolutionized therapy for this disease, approximately 20% of patients in chronic phase fail to respond to both imatinib and a subsequent second-generation TKI, there is still need for supplementary or alternative options to integrate the current pharmacological approach. In this context, autophagy has been demonstrated as necessary for BCR/ABL-induced leukemogenesis as well as to protect cancer cells from apoptosis induced by antineoplastic drugs such as Imatimib. Based on these evidences, an inhibitor of autophagy, hydroxychloroquine has been already successfully used to potentiate TKI-induced cell death in Ph chromosome-positive cells, including primary CML stem cells.
We have recently described a role for ERK8 in the regulation of the autophagic process, and demonstrated the feasibility of pharmacologically interfering with autophagy by modulating the activity of this MAP kinase.
Here, we show that BCR/ABL induced autophagy in our cellular model system and that ERK8 was able to mediate this effect. Moreover, not only artificial depletion by RNA interference of the endogenous MAP kinase inhibited BCR/ABL-dependent autophagy, but also we demonstrate that it was also possible to pharmacologically interfere with this process. Ultimately, based on the role of autophagy in BCR/ABL-dependent transformation, we showed that ERK8 is also required for cell proliferation and cellular transformation induced by this oncogene, therefore establishing ERK8 as a novel potential and feasible therapeutic target for human CML.

P6

p130Cas as a new scaffold protein regulating ErbB2 degradation

B. Bisaro, S.M. Colombo, M. Sciortino, M.P. Camacho-Leal, S. Cabodi
Dept Molecular Biotechnology and Health Sciences, Univ. Torino, Italy

Overexpression of the ErbB2/HER2 receptor tyrosine kinase occurs in up to 25% of human breast cancers correlating with aggressive disease and poor clinical outcome. Several efficacious targeted therapies, including antibodies and kinase inhibitors, have been developed but resistance to these agents is common. New therapeutic agents are actually in clinical development targeting the endocytic recycling and intracellular trafficking of membrane overexpressed ErbB2. Although it has been demonstrated that inhibition of Hsp90 can induce ErbB2 ubiquitination followed by its downregulation, the mechanisms underlining ErbB2 downregulation are still obscure. We have previously demonstrated that p130Cas over-expression in combination with ErbB2 promotes breast cancer transformation and progression. Here we demonstrate that the co-immunoprecipitation of p130Cas and ErbB2 protects the receptor from degradation. Conversely, lowering p130Cas expression in both SKbr3 and BT474 breast cancer cells, induces ErbB2 downregulation. Interestingly, our preliminary data indicate that p130Cas protects ErbB2 downregulation by impairing the autophagy process rather than affecting ErbB2 endocytic recycling. The results of this ongoing research might help to identify ErbB2 patients who will benefit from the ErbB2 targeted therapies according to the levels of expression of p130Cas.

P7

MICU1 and MICU2 finely tune the mitochondrial Ca^{2+} uniporter by exerting opposite effect on MCU activity

M. Patron[1], D. De Stefani[1], V. Checchetto[2], A. Raffaello[1], E. Teardo[2], D. Vecellio Reane[1], M.Mantoan[1], V. Granatiero[1], I. Szabò[2], R. Rizzuto[1]
[1]Dept of Biomedical Sciences, Univ. of Padova and CNR Neuroscience Institute, Padova, Italy
[2]Dept of Biology, Univ. of Padova, Padova, Italy

Mitochondrial calcium accumulation was recently shown to depend on a complex composed of an inner membrane channel (MCU, MCUb) and regulatory subunits (MICU1, MCUR1, EMRE). A fundamental property of MCU is low activity at resting cytosolic Ca^{2+} concentrations, preventing deleterious Ca^{2+} cycling and organelle overload. We here demonstrate that these properties are ensured by a regulatory heterodimer composed of two proteins with opposite effects, MICU1 and MICU2, that both in purified lipid bilayers and in intact cells stimulate and inhibit MCU activity, respectively. Both MICU1 and MICU2 are regulated by calcium through their EF-hand domains, thus accounting for the sigmoidal response of MCU to $[Ca^{2+}]$ in situ and allowing tight physiological control. At low $[Ca^{2+}]$, the dominant effect of MICU2 largely shuts down MCU activity; at higher $[Ca^{2+}]$, the stimulatory effect of MICU1 allows the prompt response of mitochondria to Ca^{2+} signals generated in the cytoplasm.

P8

Novel and unconventional signaling roles of β arrestins in chemokine-induced MAPK activation during neutrophil migration

M. Delfini[1], M. Fabbri[2], R. Molteni[2], R. Pardi[1]
[1]Univ. Vita-Salute San Raffaele, Milan, Italy
[2]Ospedale San Raffaele, Milan, Italy

Leukocytes directional migration is a key process of inflammatory responses that is achieved thanks to the ability of immune cells to sense even shallow gradient of chemoattractants. Responses to such stimuli are usually mediated by G protein-coupled receptors, whose signal is regulated by beta arrestins (B-Arr). These proteins, classically described as receptor desensitization and internalization mediators, have recently emerged as scaffolds for the recruitment of signaling proteins. Despite their high sequence homology, B-Arr 1 and 2 isoforms display conserved differences in key regions suggesting non-redundant roles. We focus our attention on the study of one of these functional regions, the proline-rich domain (PRD), which may serve as a docking site for transducers. To find proteins interacting with B-Arr, we have performed an overlay screening assay of 153 different SH3 domains that revealed over 20 putative B-Arr interactors, some of which isoform-specific. Identification of MLK3 as a B-Arr2 specific interactor leads to study the MAPK signaling pathway downstream to this MKKK during directional migration. As cellular model we have produced reversibly immortalized mouse neutrophil progenitors that can be differentiated in bona fide PMN. We found that stimulation with an inflammatory chemokine causes phosphorilation of p38, a downstream effector of MLK3. Down regulation of either B-Arr2 or MLK3 leads to increased P-p38 in a non-synergistic way, thereby suggesting that they both have a role in the same pathway. In vitro functional migration assays also show that p38 is a fundamental component of the cellular propulsion machinery. Moreover, down regulation of either B-Arr2 or MLK3 leads to increased leukocyte mobility which is coupled with a reduced directionality of the leukocyte. Taken together our results suggest that B-Arr2 and MLK3 prevent a full chemokine-dependent activation of p38, critical for a proper directional cell migration towards chemotactic cues.

P9

The role of mTORC1 and TCA cycle in renal cyst formation and transformation

L. Drusian[1,2], M. Pema[1,2], V. Mannella[3], M. Chiaravalli[1], G. Musco[3], A. Boletta[1]
[1]Division of Genetics and Cell Biology, Dibit San Raffaele Scientific Institute, Milan, Italy
[2]PhD Program in Biology and Biotherapy of Cancer, Università Vita-Salute San Raffaele, Milan, Italy
[3]Center for Translational Genomics, Dulbecco Telethon Institute at DIBIT- HSR Scientific Institute, Milan,Italy

The mTOR pathway has been implicated in different types of cancer, including renal cell carcinoma (RCC). In several syndromes the kidney is affected by cyst formation, considered initial benign lesions of the renal tubule which progressively evolve into cystadenomas and then transform to RCCs. The molecular determinants of these manifestations are largely unknown. We have developed a mouse model recapitulating progressive cyst formation followed by transformation into cystadenomas by homozygous inactivation of the *Tsc1* gene in the collecting ducts of the kidney. *Tsc1$^{flox/flox}$:KspCre* mice display cyst formation at P20, cystadenomas at P50 and transformation to carcinomas at P80. Based on previous hypothesis these renal lesions are likely the result of a progressive and degenerative process.
Biochemical analysis performed at P20, P50 and P80 on total kidney lysates show increased phosphorylation of p70S6K and S6Rp, two downstream targets of the mTORC1 cascade, in *Tsc1$^{flox/flox}$:KspCre* compared to controls indicating that mTORC1 is upregulated.
We performed metabolomic profiling by NMR spectroscopy on cortical kidney lysates from *Tsc1$^{flox/flox}$:KspCr*e and control mice. The analysis revealed unexpected increased levels of TCA cycle metabolites, such as fumarate in *Tsc1$^{flox/flox}$:KspCre* mice at P50 and P80 compared to control kidneys. To determine if the storage of fumarate is caused by reduced expression of the *Fh1* (fumarate hydratase) enzyme, we performed qRT-PCR analysis. *Tsc1$^{flox/flox}$:KspCre* mice kidneys display low expression of *Fh1* and other TCA cycle enzyme compared to control.
Consistent with the *in-vivo* data, both *Tsc1$^{-/-}$* and *Tsc2$^{-/-}$* MEFs show an increased amount of fumarate and a downregulation of TCA cycle enzyme expression as compared to controls. Rapamycin treatment restores both. We are currently investigating the molecular mechanism.

P10

Epistatic control of oncogene-induced responses by the COP9 signalosome, a modifier of cullin-ring E3 ubiquitin ligases

E. Galimberti, M. Panattoni, R. Pardi
Vita-Salute University San Raffaele School of Medicine, Milan-ITA

Hepatocellular carcinoma (HCC) is one of the most frequent tumors worldwide. The genetic changes that cause hepatocyte transformation are not yet discovered. Aberrant activation of oncogenes can lead both to tumorigenic transformation and to cell cycle arrest due to an activation of DNA damage and stress pathways (defined as oncogene-induced responses, OIR). OIR acts as a barrier against tumorigenic transformation. The purpose of my work is to investigate the role of the COP9 signalosome, a modifier of Cullin-Ring ubiquitin Ligase (CRL) complexes whose substrates are key regulators of cell cycle progression and DNA repair, during the OIR. This activity is carried out by COPS5, the catalytic subunit of the complex, which controls CRL activities by removing the modifier Nedd8 from the Cullin component of the complex. A recent work from our group demonstrated that, in regenerating hepatocytes, the COP9 signalosome is a repressor of replicative stress responses. Moreover COP9 was found overexpressed in a subset of HCC, so we believe that this complex has a permissive role towards the overcoming of cell cycle checkpoints in tumorigenic transformation. To test this hypothesis we generated a cellular model of OIR in which hepatocyte progenitors cells (MLP-29) were manipulated to obtain an overexpression of myc, an oncogene that is frequently overexpressed in HCC. This causes an increase of DNA damage markers in the cells including γ-H2AX, 53BP1 and RPA70, proving that high levels of myc can lead to an OIR in diploid liver cells. Furthermore we will manipulate these cells to obtain a deregulated expression of COPS5 and other COP9 subunits in order to investigate the effects of the gain or loss of function of the complex on the OIR. Finally we are currently testing the effects of other oncogenes overexpressed in HCC, including E2F1, E2F2 and oncogenic β-catenin, to address whether they generate an OIR and if such response involves the COP9 signalsome.

P11

MicroRNAs ad modulators of mitochondrial pathogenesis

F. Grespi[1], S. Cagnin[1,2], G. Lanfranchi[1,2], L. Scorrano[1,3]
[1]Dept of Biology, Univ. of Padova, Padova, Italy
[2]CRIBI Biotechnology Centre, Univ. of Padova, Padova, Italy
[3]Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Padova, Italy

MicroRNAs are a class of small non-coding regulatory RNAs. They are about 22 nucleotides long and they inhibit translation of target mRNAs thanks to their binding to partially complementary sequences in the 3' untranslated regions (UTRs). MicroRNAs thus are key post-transcriptional regulators of many cellular processes, such as apoptosis, proliferation, differentiation and metabolism. Interestingly, miRNAs are emerging as regulators of functions of organelles, including mitochondria. These organelles are extremely dynamics, thanks to the tightly regulated equilibrium between fission and fusion. Opa1 not only mediates fusion, but it preserves cristae structure. While the former depends on Mfn1, prevention of the so-called cristae remodeling, was identified as a key mechanism in cytochrome *c* release and apoptosis. Indeed, pathogenic Opa1 mutations render cells more susceptible to cell death and massive apoptosis of retinal ganglion cells (RCG) is probably one main causes of Autosomal dominant optic atrophy (ADOA), as substantiated by retinal nerve fiber thinning in post-mortem histopathology of two ADOA patients. ADOA is a progressive ocular disorder characterized by a high degree of inter- and intra-familial phenotypic variability. Identification of pathology modulators will allow their application in pathology treatment. Intriguingly, mitochondrial fusion and fission can be regulated by miRNAs. This opens the interesting possibility that these non-coding RNAs might participate in modulation of penetrance of diseases affecting mitochondrial shape. Our preliminary data, demonstrate that miRNAs can affect protein levels of Opa1, thus leading to an impairment of mitochondria morphology and cell death, as seen in mouse fibroblasts upon expression of miRNAs candidates. For this reason, we hypothesize that the differential penetrance of ADOA is mediated by miRNAs and this feature can be exploited therapeutically to counteract ADOA severity and progression.

P12

STAT3/ERp57 Interplay in human prostate cancer

R. Cocchiola[1,2], C. Grillo[1,2], D. Romaniello[1,2], I. Marrocco[1,2], F. Altieri[1,2], G. Perugia[3], S. Chichiarelli[1,2], M. Eufemi[1,2]
[1]Dept of Biochemical Sciences, Sapienza Univ. of Rome
[2]Cenci-Bolognetti Foundation
[3]Dept of Gynecological-Obstetric and Urologic Sciences

Prostate cancer (Pca) is a common cause of death and remains incurable in the androgen-refractory phase. Progression from normal prostate epithelium to an androgen-responsive tumour, and finally to hormone-refractory carcinoma is a multistep process, usually accompanied by alterated balance between Androgen Receptor signaling pathway and other pathways as STAT3 and EGF.
The involvement of STAT3 (Signal Transducer and Activator of Transcription 3) protein in tumoral process is scientifically known. The activated form of STAT3, phosphorylated on its tyrosine 705 residue, mediates cellular response of cytokine, growth factor and oxidative stress pathways as transcription factor of specific target genes. In prostate cancer (PCa) STAT3 plays a role as hub protein of multiple signaling pathways.
The aim of this study is to investigate the function of STAT3 and its post-translational modifications (pY^{705} activated form, pS^{727} and acK^{685} increased trascriptional activity, glutC response to oxidative stress) and the role of ERp57 in the STAT3 signaling pathway involved in PCa with different Gleason score (6, 7, 8 and 9). ERp57, also known as PDIA3, ERp60, ERp61 or GRP57, belongs to the Proteins Disulfide Isomerase family and is an endoplasmic reticulum (ER) resident thiol disulfide oxidoreductase associated with STAT3 in the cytosol and in the nuclear STAT3-containing enhanceosome. It is a necessary cofactor for the regulation of STAT3 transcriptional activity. Our studies were conducted on FFPE (Formalin Fixed Paraffin-Embedded) tissues of normal and tumoral samples obtained from radical prostatectomy with Gleason Score 6, 7, 8 and 9. The preliminary data indicate that in the tumors with lower Gleason values, STAT3 is activated by cytokine stimulation, typical of inflammatory responses. On the contrary, at high Gleason values is evident the activation of other pathways involving oxidative stress Stat3/ERp57/Ref1-mediated.

P13

PI3K Class II α controls spatially restricted endosomal PtdIns3*P* and Rab11 activation to promote primary cilium function

F. Gulluni[1], I. Franco[1], C.C. Campa[1], J.P. Margaria[1], E. Ciraolo[1], M. Martini[1], Y. Posor[2], V. Haucke[2], M. Falasca[3], G.R. Merlo[1], E. Hirsch[1]
[1]Molecular Biotechnology Center, Dept of Molecular Biotechnology and Health Sciences, Univ. of Torino, Torino, Italy
[2]Leibniz Institut fu¨r Molekulare Pharmakologie, Berlin, Germany
[3]Centre for Diabetes, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary Univ. of London, London, UK

Multiple phosphatidylinositol (PtdIns) 3-kinases (PI3Ks) can produce PtdIns3*P* to control endocytic trafficking, but whether enzyme specialization occurs in defined subcellular locations is unclear. Here, we report that PI3K-C2α is enriched in the pericentriolar recycling endocytic compartment (PRE) at the base of the primary cilium, where it regulates production of a specific pool of PtdIns3*P*. Loss of PI3K-C2α-derived PtdIns3*P* leads to mislocalization of PRE markers such as TfR and Rab11, reduces Rab11 activation, and blocks accumulation of Rab8 at the primary cilium. These changes in turn cause defects in primary cilium elongation, Smo ciliary translocation, and Sonic Hedgehog (Shh) signaling and ultimately impair embryonic development. Selective reconstitution of PtdIns3*P* levels in cells lacking PI3K-C2α rescues Rab11 activation, primary cilium length, and Shh pathway induction. Thus, PI3K-C2α regulates the formation of a PtdIns3*P* pool at the PRE required for Rab11 and Shh pathway activation.

P14

Involvement of mitochondrial dynamic in the regulation of angiogenesis

S. Herkenne, L. Scorrano
Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Padova, Italy
Dept of Biology, Univ. of Padova, Padova, Italy

During cell life, mitochondria not only synthesize most of the ATP, but they are also involved in TCA, fatty acid b-oxidation, gluconeogenesis, Ca2+ signaling and redox homeostasis. Moreover, mitochondria act as crucial amplifiers of apoptotic signals by releasing cytochrome c and other proapoptotic molecules. Mitochondrial functions are regulated by their dynamic, resulting from a balance between mitochondrial fusion protein (Optic Atrophy 1 (OPA-1), mitofusin 1 (MFN1) and MFN2) and fission protein (Dynamin-related protein-1 (DRP-1). Surprisingly, the role of mitochondrial dynamics in angiogenesis, process responsible for formation of new blood vessels from preexisting ones, has not been addressed. Interestingly, proteins involved in mitochondrial fission (DRP-1 and calcineurin) are downregulated in endothelial cells stimulated with different angiogenics factors (VEGFA, bFGF and tumor-conditionned medium). Moreover, in the case where angiogenesis is inhibited, DRP-1 protein level is increased. In addition, in vitro, migration, proliferation and permeability are increases in DRP—knockdown endothelial cells compared to control cells.
Taken together, these data suggest that mitochondrial fission plays a crucial role in the regulation of angiogenesis *in vitro*. Our further studies will attempt to investigate the role of DRP-1 in developmental, pathological and tumoral angiogenesis.

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P15

The GTPase activating protein RN-tre controls focal adhesion turnover and cell migration

A. Palamidessi, E. Frittoli, N. Ducano, N. Offenhauser, S. Sigismund, H. Kajiho, D. Parazzoli, A. Oldani, M. Gobbi, G. Serini, P.P. Di Fiore, G. Scita, L. Lanzetti
[1]IFOM, Fondazione Istituto FIRC di Oncologia Molecolare, Milano, Italy
[2]Dipartimento di Oncologia, Università degli Studi di Torino, Istituto per la Ricerca e la Cura del Cancro, Candiolo, Torino, Italy
[3]Dipartimento di Biochimica e Farmacologia Molecolare, "Mario Negri" Istituto per la Ricerca Farmacologica, Milano, Italy
[4]Dipartimento di Oncologia Sperimentale, Istituto Europeo di Oncologia, Milano, Italy
[5]Dipartimento di Scienze della Salute, San Paolo Universita' degli Studi di Milano, Milan, Italy

Background: Integrin-mediated adhesion of cells to the extracellular matrix (ECM) relies on the dynamic formation of focal adhesions (FAs), which are biochemical and mechanosensitive platforms composed of a large variety of cytosolic and transmembrane proteins. During migration, there is a constant turnover of ECM contacts that initially form as nascent adhesions at the leading edge, mature into FAs as actomyosin tension builds up, and are then disassembled at the cell rear, thus allowing for cell detachment. While the mechanisms of FA assembly have largely been defined, the molecular circuitry that regulates their disassembly still remains elusive.
Results: Here, we show that RN-tre, a GTPase-activating protein (GAP) for Rabs, including Rab5 and Rab43, is a novel regulator of FA dynamics and cell migration. RN-tre localises to FAs and to a pool of Rab5-positive vesicles mainly associated with FAs undergoing rapid remodelling. We found that RN-tre inhibits endocytosis of b1, but not b3 integrins and delays the turnover of FAs ultimately impairing b1-dependent, but not b3-dependent chemotactic cell migration. All these effects are mediated by its GAP activity and rely on Rab5.
Conclusions: Our findings identify RN-tre as the Rab5-GAP that spatio-temporally controls FA remodelling during chemotactic cell migration.

P16

Future therapeutic strategies for malignant pleural mesothelioma based on energy metabolism characterization

A.G. Manente, G. Pinton, S. Zonca, L. Moro
Dept Pharmaceutical Sciences, Univ. of Piemonte Orientale "A. Avogadro", Novara, Italy

Malignant Pleural Mesothelioma (MPM) is a rare but increasingly prevalent, highly aggressive form of cancer associated with exposure to asbestos fibers. Recent advances in understanding the disease's complex biology have led to moderate improvements in the effectiveness of the standard therapies; accordingly the unmet need for novel treatments is high.
The goal of our study was to characterize the metabolic status of MPM cells, with different histotype, in order to identify new therapeutic targets and improve the current therapies.
Reprogramming of energy metabolism is one of the hallmarks of cancer. In normal conditions, cells rely on mitochondrial oxidative phosphorylation to provide energy for cellular activities, while the general enhancement of the glycolytic machinery in various cancer cells is well described.
We investigated both the oxidative phosphorylation and the glycolytic activity in MPM derived cell lines. Moreover, we analyzed the expression and the activity of glucose/lactate transporters in both normoxic and hypoxic conditions, with the final goal to test the therapeutic efficacy of their selective inhibitors.
In summary, we identified alterations in the mitochondrial energy metabolism in MPM cells that lead to a marked dependence on the glycolytic pathway. Our findings provide an innovative rationale to use inhibitors of enzymes involved in the glycolytic process and/or membrane transporters, alone or in combination with standard chemotherapic agents as a future strategy for clinical management of this form of tumor.

P17

Honey and wound healing: role of aquaporins

E. Ranzato, S. Martinotti, B. Burlando
DiSIT- Dip Scienze e Innovazione Tecnologica, University of Piemonte Orientale, Alessandria, Italy

Honey is a natural, sweet substance produced by honeybees of the genus Apis and the group of Meliponinae.
Honey has been recognized for millennia as a topical antimicrobial agent in wound care. Honey's therapeutic properties are largely attributed to its antimicrobial and anti-inflammatory activities. Although honey has been used for centuries in wound care, only now it is being integrated into modern medical practice.
Honey works differently from antibiotics, which attack the bacteria's cell wall or inhibit intracellular metabolic pathways. Honey is hygroscopic, meaning it draws moisture out of the environment and thus dehydrates bacteria. Its sugar content is also high enough to hinder the growth of microbes, but the sugar content alone is not the sole reason for honey's wound healing properties.
Honey is increasingly used as a wound dressing in clinical settings, but the relationships between its healing properties and the wound repair mechanism are still largely unexplored. Therefore, we started a project to investigate the potential wound healing effects of honey on skin cells.
So, to get better insight into the honey's mechanisms of action, we have investigated the role of aquaporin (AQP), which play a pivotal role in the process of cell migration and wound repair. Keratinocytes were subjected to gene silencing for AQP-1, AQP-4 and AQP-5, and then scratch wounded in the presence or absence of honeys.
The results show that the siRNA for AQP-1 and AQP-4 does not significantly alter the wound closure rate but inhibits the honey's stimulatory effects. In contrast, siRNA for AQP-5 inhibits the un-stimulated cells, but does not prevents the wound closure induced by honey exposure.

P18

Physiological and cellular functions of Eps15 and Eps15L1 in mammals

C. Milesi[1], P.P. Di Fiore[1, 2, 3], N. Offenhäuser[1]
[1]IFOM, the FIRC Institute for Molecular Oncology Foundation, Milan, Italy
[2]Istituto Europeo di Oncologia, Milan, Italy
[3]Dipartimento di Scienze della Salute, Universita' degli Studi di Milano, Milan, Italy

Eps15 and Eps15L1 are two endocytic adaptor proteins involved in clathrin and non-clathrin mediated endocytosis of membrane receptors.
Eps15 knockout (KO) mice are healthy and fertile without obvious defects, while Eps15L1-KO mice die after birth, likely due to neural defects. Double knockout mice (DKO), instead, present a more severe phenotype: they are midgestation lethal and exhibit neural and cardiovascular defects.
To investigate whether Eps15 and Eps15L1 play a cell-autonomous function in the vascular system, we generated syngenic lung endothelial cells of all four genotypes and endothelial-specific DKO (eDKO) mice. Despite reduced VEGFR signaling in DKO endothelial cells, eDKO mice displayed only a mild vascular defect and thus did not phenocopy the complete DKO vascular phenotype.
Thus, the vascular phenotype in DKO embryos is non-cell autonomous and might be caused by 1) defective instructive cues from somites and/or the nervous system, 2) altered signaling from stromal cells or 3) defective tissue organization (i.e. by defective ECM deposition, cell-ECM interaction and/or cell-cell adhesion).
Since we also observed that tissues from Eps15L1-KO and DKO embryos are fragile, we now want to test whether a general defect in tissue organization exists in these mice.
For this purpose we will perform rheological analysis on tissues from adult conditionally deleted Eps15L1-KO mice and we will look whether ECM composition is affected, by Second Harmonic Generation Microscopy (SHG) and Western Blotting/Immunohistochemical analysis for matrix proteins. Moreover we will use KO fibroblasts to see whether altered ECM composition is due to a defect in Golgi trafficking, using lectin staining and VSVG trafficking assays. In addition we will explore whether cell-cell and/or cell-adhesion matrix is affected by immunofluorescence analysis, adhesion assays using the xCELLigence system and by integrin-R internalization assays.

P19

The transcriptional induction of the RhoU GTPase gene by the non-canonical Wnt/Planar Cell Polarity pathway is mediated by the Sp1 transcription factor

V. Orecchia[1], D. Schiavone[1,2], I. Molineris[1], P. Provero[1], V. Poli[1]
[1]Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Turin, Italy
[2]Present address : MRC Laboratory of Molecular Biology, Cambridge, UK

RhoU is a small GTPase originally identified as a Wnt-1 target gene implicated in Wnt-1-induced cell migration. We previously demonstrated that Wnt-1-mediated RhoU induction occurs transcriptionally and does not involve the canonical β-catenin-dependent pathway but is dependent on JNK activity, typical of the non-canonical Wnt/Planar Cell Polarity (PCP) pathway *(Schiavone et al, Biochem. J., 2010)*.
In order to identify transcription factors involved in the poorly understood PCP pathway, we analyzed serial 5' deletions of the Wnt1-responsive TATA-less mouse RhoU promoter, and identified a region between positions -366 and -167 that is required for both basal and Wnt-inducible promoter activity. This region is also equally responsive to Wnt-4 and Wnt-5a, respectively considered as canonical and non-canonical Wnt members, which can activate RhoU expression similarly to Wnt-1. Bioinformatic analysis indicated that the transcription factor Sp1 displays high total affinity for the RhoU promoter. We thus assessed Sp1 *in vivo* binding by ChIP analysis. Sp1 binding to the RhoU promoter was detected upon stimulation with all three Wnt factors tested, and abolished by treatment with the Sp1-inhibitor mithramycin. Sp1, a well known regulator of housekeeping genes, has also been implicated in inducible transcription via JNK and MAPK-mediated phosphorylation. Accordingly, we demonstrated that both Wnt-mediated Sp1 phosphorylation and binding to the RhoU promoter are dependent on JNK activity. Interestingly, we observed that by wound healing assays that this signaling is important to maintain the migratory ability of tumor cells displayind constitutive activation of PCP pathway, e.g. MDA-MB-231 cells. These results identify Sp1 as a crucial transcription factor in Wnt-mediated RhoU regulation and suggest that this factor may play a more general role in mediating the transcriptional effects of the PCP pathway downstream of JNK kinases.

P20

miR-214 and miR-148b: a miRNA network controlling melanoma progression

F. Orso[1,2,3], L. Quirico[1,2], E. Penna[1,2], A.R. Elia[1], M. Stadler[4], D.Taverna[1,2,3]
[1]Molecular Biotechnology Center (MBC)
[2]Dept Molecular Biotechnology and Health Sciences
[3]Center for Complex Systems in Molecular Biology and Medicine
[4]Friederich Miescher Institute, Basel, Switzerland

MicroRNAs are small non-coding, single-stranded RNAs that act as negative regulators of gene expression and growing evidences demonstrate that they are hallmarks of tumor progression. Since dissemination of primary tumor cells and metastasis formation are the main cause of cancer-related mortality, it is crucial to identify miRNA regulators of tumor progression. We demonstrated that miR-214 is upregulated in malignant melanomas and it coordinates metastasis dissemination by increasing migration, invasion, extravasation and survival of melanoma cells via a novel pathway involving TFAP2A and TFAP2C as well as multiple adhesion molecules, including ALCAM. Recently, we proved that the downregulation of miR-148b by miR-214, via TFAP2, contributes to miR-214-induced metastatization by regulating ALCAM. We then expanded our analysis and unravelled a regulatory network through which miR-214 regulates not only its own target genes (TFAP2C, TFAP2A), but also miR-148b target genes (ITGA5, PI3KCA, ROCK1). In fact, we demonstrated that the overexpression of miR-214 caused a reduction of miR-148b expression level and a consequent derepression of miR-148b target genes such as PI3KCA, ITGA5 and ROCK1 whereas the opposite was observed following miR-214 downregulation. These data suggest that miR-214 can be considered a pro-metastatic hub and its inhibition could be relevant for targeted therapy. For this purpose, we developed optimized sponges to efficiently inhibit miR-214 action. We showed that miR-214 sponges are able to counteract miR-214 and consequently miR-148b action in tumor cell dissemination and metastasis formation. More recently, we proved that a combinatorial therapy based on miR-214 downregulation and miR-148b overexpression results in a more effective inhibition of metastatization than the modulation of a single gene. Our study demonstrates that the alteration of a microRNA network can be highly relevant for the development of targeted therapies.

P21

Flavonoids affect cAMP release by human retinal pigment epithelial cells

B. Pavan, A. Capuzzo and G. Forlani
Dept of Life Science and Biotechnology, University of Ferrara, Ferrara, Italy

The concentration of the intracellular second messenger 3',5'-cyclic adenosine monophosphate (cAMP) increases as a consequence of either receptor-mediated signals or the internal modulation of adenylate cyclase (AC) by various downstream effectors. Thereafter, ATP-dependent efflux mediated by several multidrug resistance proteins (MRPs), including MRP4, MRP5, and MRP8, combined with receptor desensitization and phosphodiesterase degradation activity, is implicated in temporal resolution of cAMP signal regulation. Secondary metabolites of plants, such as flavonols are known to interfere with ABC transporters in mammalian cells. We investigated the effect of the treatment with anthocyanins (such as cyanidin, and cyanidin-3-glucoside extracted from brown rice) and flavonols (quercetin, and quercetin-3-glucoside from red onion) on cAMP efflux.
Cultured human retinal pigment epithelial (HRPE) cells grown in 24-well plates were stimulated for a time course with 1 μM adrenaline or 10 μM forskolin (FSK), in the presence and absence of increasing concentrations of anthocyanins or flavonols, then intracellular and extracellular cAMP levels were determined. Either compound significantly inhibited cAMP efflux from intact cells after adrenaline stimulation, but only quercetin inhibited cAMP efflux induced by FSK. Conversely, cyanidin-3-glucoside inhibited adrenaline-stimulated AC activity in cell membrane preparations, while quercetin was inactive. The treatment with these plant-derived compounds might therefore produce different biological effects by affecting different components of cAMP signalling cascade, which may be useful therapeutic targets in their own right.

P22

Opa1 dependent cristae remodeling controls mitochondrial matrix pH and oxidative stress by favoring ATPase activity

R. Quintana-Cabrera[1,2], Luca Scorrano[1,2]
[1]Dulbecco-Telethon Institute, Venetian Institute of Molecular Medicine, Padova, Italy
[2]Dept of Biology, Univ. of Padova, Padova, Italy

Mitochondria are highly dynamics organelles undergoing continuous cycles of fusion and fission that determine their morphology. The inner membrane pro-fusion protein Opa1 is also a central player in mitochondrial cristae remodeling, favoring respiratory efficiency and supercomplex assembly. However, whether Opa1 influences key bioenergetic parameters of mitochondria remains still unclear.
Here we show that Opa1 regulates mitochondrial pH and redox status by impinging on the mitochondrial ATPase. The genetically encoded ratiometric pH sensor mt-SypHer revealed less matrix acidification upon inhibition of complex III with antimycin A in adult fibroblasts (MAFs) from Opa1 overexpressing ($Opa1^{Tg}$) mice. Using genetic and pharmacological approaches, we found that cristae stabilization by Opa1 promotes ATPase dimerization, which accounts for the higher activity of the enzyme in $Opa1^{Tg}$ MAFs. Conversely, reduced dimerization in cyclophilin D knock-out cells or pharmacological inhibition of ATPase prevented Opa1 effects on matrix pH. Of note, when respiration was blocked, ATPase activity prevented ROS accumulation and loss of mitochondrial membrane potential in $Opa1^{Tg}$ but not wt cells. In line with these results, $Opa1^{Tg}$ MAFs presented lower cell death rates under forced ATP production by mitochondria.
In sum, here we describe a novel role for Opa1 in the regulation of matrix pH and redox status, indicating that Opa1 is a key regulator of mitochondrial bioenergetics.

P23

Protein kinase CK2: a new target to overcome imatinib-resistance in CML cells

V. Salizzato, C. Borgo, L. Cesaro, L.A. Pinna, A. Donella-Deana
Dept of Biomedical Sciences, University of Padova, Padova, Italy

Chronic myeloid leukemia (CML) is a malignant myeloproliferative disorder of hematopoietic system, driven by the fusion protein Bcr-Abl, a constitutively active tyrosine kinase. Despite the great efficacy of the Bcr-Abl inhibitor imatinib, resistance to this drug is recognized as a major problem in therapy failure. Protein kinase CK2, a pro-survival and constitutively active Ser/Thr kinase, is frequently deregulated in cancer cells. To dissect the mechanisms of CK2-related imatinib-resistance, KCL22 CML cells, either sensitive (S) or resistant (R) to imatinib, were analyzed evaluating a potential cross-talk of CK2 with Bcr-Abl and/or its downstream molecules with particular attention to MAPK and Akt/mTOR pathways. We highlighted that protein-level and activity of CK2 and Bcr-Abl are similar in S- and R-KCL22 cells, and that they are interacting proteins. R-KCL22 cells, whose mechanism of resistance is not due to *BCR-ABL1* gene amplification, mutations or P-glycoprotein expression, are characterized by a higher phosphorylation degree of ERK1/2-T202/Y204, Akt-S473 and rpS6-S235/6 as compared to sensitive cells. In R-KCL22 cells high imatinib concentrations only partially inhibit the phosphorylation of rpS6, the common downstream effector of MAPK and Akt/mTOR pathways. At variance, CK2 down-regulation almost abrogates rpS6 phosphorylation, with a parallel substantial decrease of protein synthesis as compared to control. Furthermore, CX-4945, a selective CK2-inhibitor currently in clinical trials, strongly reduces KCL22 cell viability, activating apoptosis. Combined treatments with CX-4945, imatinib and/or U0126, an inhibitor of MAPK pathway, promote a synergistic reduction of R-KCL22 cell viability. Collectively, our results identify CK2-dependent signaling as a player in CML imatinib-resistance and propose CK2 inhibitors as promising drugs for combined therapies in CML treatment.

P24

The transcriptional repressor PRDM1 as a new player in breast cancer cell invasion

M. Sciortino[1], F. Orso, B. Bisaro, M. delP. Camacho Leal, S. Cabodi
[1]Molecular Biotechnology Center (MBC); Dept. of Molecular Biotechnology and Health Sciences; Univ. of Torino; Torino, Italy

The ability of ErbB2-positive human mammary epithelial cells grown in 3D to invade is strictly dependent on the over-expression of the adaptor protein p130Cas. To identify the gene expression changes specifically involved in p130Cas–dependent invasion of ErbB2 transformed cells, microarray analysis of coding and non coding genes was previously performed.
Among the differentially expressed genes in p130Cas/ErbB2-dependent invasion, the transcriptional repressor PRDM1 (also known as BLIMP1) was found up-regulated both at RNA and protein levels. Few data are available on the involvement of PRDM1 in cancer progression. Therefore, the mechanisms underlying its expression, regulation and functional role in breast cancer were analyzed. We first assessed whether the activation of PI3K/Akt and Erk1/2 MAPK signalling pathways, previously demonstrated to be crucial in promoting p130Cas/ErbB2-dependent invasion, impinges on PRDM1 increased expression. By inhibiting these two cascades at different levels we showed that high levels of PRDM1 are downstream to activation of Erk1/2 MAPK-mTOR pathway.
PRDM1 gene is a putative target of the miR-23b that is down-regulated in p130Cas/ErbB2 invasive cells. By over-expressing miR-23b into invasive cells, we found that PRDM1 gene was down-regulated, thus indicating a transcriptional regulation between PRDM1 and miR-23b. The functional role of this interplay in terms of invasion was then investigated. Interestingly, transient miR-23b over-expression in p130Cas/ErbB2 cells strongly reduces their ability to migrate and invade. The molecular mechanisms through which PRDM1 and mir23b affect invasion are currently under investigation. These data suggest an important role of PRDM1 in mediating p130Cas/ErbB2-dependent invasive behavior, getting new insights into the role of PRDM1 in mammary carcinoma.

P25

Abnormal keratinocyte proliferation in mice with a conditional deletion of rictor in the epidermis

B. Tassone[1], S. Saoncella, E. Calautti
Dept Biotechnology, Univ. of Turin

mTOR (mammalian target of rapamycin) is a serine-threonine protein kinase that regulates diverse cellular processes such as growth, metabolism, proliferation, survival and differentiation. mTOR nucleates two distinct protein complexes characterized by their different sensitivity to rapamycin, namely mTORC1 and mTORC2.
Rictor is an adaptor protein essential for mTORC2 assembly. mTORC2 phosphorylates both Akt and some PKC isoforms at their Hydrophobic Motifs (HM) and Turn Motifs (TM), thereby contributing to kinase activation and stability. Because both Akt and PKC signaling regulate epidermal cell biology, and *rictor* knockouts mice die before stratified epithelia formation, we generated mice with a conditional deletion of *rictor* in the epidermis to investigate the role of rictor/mTORC2 in epidermal homeostasis and disease. At the molecular level, *rictor*-deficient keratinocytes lack Akt phosphorylation at the HM (Ser473), have reduced Akt expression and diplay impaired phosphorylation/expression levels of both FoxOs and mTORC1 signaling components. Moreover *rictor*-deficient keratinocytes display abnormalities in the actin cytoskeleton dynamics. Interestingly, *rictor*-deficient keratinocytes show features consistent with impaired differentiation, both *in vitro* and *in vivo*. Notably, we found that *rictor*-deficient mice are less prone to epidermal hyperplasia induced by acute treatment with the TPA tumour promoter, indicating that *rictor* depletion impairs proliferative responses that favour neoplastic transformation. These findings identify mTORC2 as an important hub for the integration of signals at the switch between keratinocyte proliferation and differentiation.

P26

Role of the endocytic protein Epsin3 in tumorigenesis and physiology of the mammary gland

C. Iavarone[1], F. Tettamanzi[1], S. Confalonieri[1], M. Vecchi[1], S. Sigismund[1], P.P. Di Fiore[1,2,3]
[1]IFOM, the FIRC Institute for Molecular Oncology Foundation, Milan, Italy
[2]Dipartimento di Scienze della Salute, Università degli Studi di Milano, Milan, Italy
[3]European Institute of Oncology, Milan, Italy

Epsin3 (Epn3) belongs to the Epsin family of endocytic adaptor proteins. While the other family members Epn1 and Epn2 are ubiquitously expressed, Epn3 is exclusively expressed in gastric cells and in wounded or pathologically altered tissues, suggesting that Epn3 might exert a specific function. Recent data from our lab pointed to a novel oncogenic role of Epn3, showing that 30% of breast tumors overexpresses Epn3 and its upregulation positively correlates with markers of aggressive disease and poor prognosis. Moreover, alterations in Epn3 expression levels in breast cancer cell lines influence their tumorigenic potential *in vitro* and *in vivo.* Interestingly, Epn3 overexpression enhances E-cadherin internalization and activates the epithelial-to-mesenchymal transition (EMT) program, conferring a more invasive phenotype to normal and cancer cell lines. However, it remains unclear which is the exact physiological role of Epn3 and whether the observed functions are acquired upon gene amplification/overexpression in a tumoral context or they also underlie cell physiological processes. To address this issue, we will undertake two main approaches: first *in vitro*, using normal and cancer mammary cell lines, in which we will silence and overexpress Epn3 in order to perform morphogenetic assays; second *in vivo*, taking advantage of an Epn3-KO mouse model, already available in our lab. This model will allow us to unveil the effects of Epn3 ablation on the development and morphology of the mammary gland and it will provide us with an *ex vivo* system to analyze Epn3 functions in a physiological setting. Another mouse model available in our lab, Epn3-KI, in which the transgene is specifically expressed in the mammary tissue, will be exploited to identify alterations in the development and morphology of the mammary gland induced by Epn3 overexpression. Results will be presented.

P27

Lysosomal function is involved in 17β-estradiol-induced estrogen receptor α degradation and cell proliferation

P. Totta, V. Pesiri, M. Marino, F. Acconcia
Dept of Sciences, Section Biomedical Sciences and Technology, Univ. Roma Tre, Rome, Italy

The homeostatic control of the cellular proteome steady-state is dependent either on the 26S proteasome activity or on the lysosome function. The sex hormone 17β-estradiol (E2) controls a plethora of biological functions by binding to the estrogen receptor α (ERα), which is both a nuclear ligand-activated transcription factor and also an extrinsic plasma membrane receptor. Regulation of E2-induced physiological functions (*e.g.,* cell proliferation) requires the synergistic activation of both transcription of estrogen responsive element (ERE)-containing genes and rapid extra-nuclear phosphorylation of many different signalling kinases (*e.g.,* ERK/MAPK; PI3K/AKT). Although E2 controls ERα intracellular content and activity *via* the 26S proteasome-mediated degradation, biochemical and microscopy-based evidence suggests a possible cross-talk among lysosomes and ERα activities. Here, we studied the putative localization of endogenous ERα to lysosomes and the role played by lysosomal function in ERα signalling. By using confocal microscopy and biochemical assays, we report that ERα localizes to lysosomes and to endosomes in an E2-dependent manner. Moreover, the inhibition of lysosomal function obtained by chloroquine demonstrates that, in addition to 26S proteasome-mediated receptor elimination, lysosome-based degradation also contributes to the E2-dependent ERα breakdown. Remarkably, the lysosome function is further involved in those ERα activities required for E2-dependent cell proliferation while it is dispensable for ERα-mediated ERE-containing gene transcription. Our discoveries reveal a novel lysosome-dependent degradation pathway for ERα and show a novel biological mechanism by which E2 regulates ERα cellular content and, as a consequence, cellular functions.

P28

Defining the role of glycerophosphoinositols in the regulation of actin cytoskeleton: involvement of the tyrosine phosphatase shp1

A. Varone, C. Amoruso, S. Mariggiò, D. Corda
Institute of Protein Biochemistry, CNR, Napoli, Italy

The glycerophosphoinositols are cellular products of phospholipase A_2 IVα activity on the membrane phosphoinositides. When added exogenously, the glycerophosphoinositols can enter cells and have multiple effects, such as modulation of actin cytoskeleton organisation in fibroblasts and lymphocytes, and reduction of invasive potential of metastatic cells. Attempts to identify the molecular targets of the glycerophosphoinositols through a proteomic approach have led to the identification of more than 100 proteins that are involved in various aspects of cell regulation. Among these, we focussed on the tyrosine phosphatase Shp1. We studied Shp1 in the context of glycerophosphoinositol 4-phosphate-induced membrane ruffle formation in NIH3T3 fibroblasts, where inhibition of the enzymatic activity of Shp1 completely abolished glycerophosphoinositol 4-phosphate-mediated reorganisation of the actin cytoskeleton. Furthermore, a role for Shp1 has been demonstrated in glycerophosphoinositol-mediated inhibition of tumour cell invasion. In A375MM melanoma cells, glycerophosphoinositol treatment results in inhibition of extracellular matrix degradation, and this activity is suppressed when an inactive mutant of Shp1 is expressed, while it is essentially unaffected by expression of the native enzyme. This approach led to the definition of Shp1 as the first direct glycerophosphoinositol target that has been identified to date, thus defining the cascade involved in glycerophosphoinositols-mediated control of the actin cytoskeleton.

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P29

Regulation of mitochondrial Ca^{2+} uptake by SIRT3 mediated deacetylation

D. Vecellio Reane, A. Raffaello, R. Rizzuto
Dept of Biomedical Sciences, Univ. of Padua, Padua, Italy

The ability of mitochondria to uptake calcium plays a fundamental role in the regulation of cell metabolism, cell survival and other cell-type specific functions. A public database of protein modifications reported several post-translational modifications of the uniporter components, among which lysine acetylation is particularly consistent. SIRT3 is a mitochondrial NAD-dependent deacetylase that has been shown to regulate mitochondrial metabolism in conditions of nutrient adaptation and stress. Given the crucial role of mitochondrial calcium on cell metabolism, we asked whether the acetylation state of the calcium uniporter complex plays a modulatory role on mitochondrial calcium uptake and, thus, on the metabolic state of the cell. First, we found that SIRT3 overexpression causes a decrease of mitochondrial calcium uptake upon stimulation, without any alteration of mitochondrial morphology or membrane potential. Thus, we selected one lysine in MCU protein sequence (K331) and one in MICU1 protein sequence (K440) that were shown to be acetylated and conserved among the species. The substitution of MCU lysine with an arginine (MCU^{K331R}), an aminoacid that can not be acetylated, does not compromise its activity, whereas MICU1 mutant ($MICU1^{K440R}$) causes a reduction of its activity compared to MICU1 wild type. Furthermore, co-trasfection of SIRT3 and MICU1 wild type causes a decrease of MICU1 ability to act as positive modulator of the mitochondrial calcium uniporter. In conclusion, we showed that mitochondrial acetylation levels modulate mitochondrial calcium uptake. Indeed, SIRT3 regulates MICU1 and deacetylated MICU1 appears to be less effective to modulate the mitochondrial calcium uniporter. Further studies will be carried on to understand the mechanism responsible for the regulation of MICU1 activity by its acetylation state and the upstream signals that control SIRT3 activity.

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Finding the key factors in the role of erp44 in the signal integration at the endoplasmic reticulum-golgi interface

E.D. Yoboue[1,2], T. Anelli[1,2], S. Sannino[1,2], G. D'Angelo[3], A. Luini[3], P. Pinton[4], A. Rimessi [4], R. Sitia[1,2]
[1]San Raffaele Scientific Institute, Division of Genetics and Cell biology, Protein transport and secretion Unit
[2]Univ. Vita-Salute San Raffaele
[3]Institute of Protein Biochemistry, Italian National Research Council (CNR), Naples, Italy
[4]Dept of Morphology Surgery and Experimental Medicine, Section of Pathology, Oncology and Experimental Biology, Univ. of Ferrara, Italy

At least one third of the human genome encodes secretory proteins. As a result, a large flux of vesicles is continuously exchanged between the endoplasmic reticulum (ER) and Golgi complex, allowing the release of native proteins and the retrieval of non-native conformers (quality control) and ER residents. These events must be tightly regulated and integrated with other signaling pathways emanating from the ER and the Golgi.
Indeed, the ER and the Golgi are emerging as key signaling hubs. The ER is a main actor in Ca^{2+} signaling, redox homeostasis and Unfolded Protein Response (UPR). The later increases the folding capacity of the ER to the physiological demands. At the Golgi level, KDEL-Receptors (KDELR) which mediate the retrieval of ER residents, also act as signaling devices. Thus, the binding to the KDELR of few ER chaperones, which are able to reach the Golgi, triggers signaling cascades which are crucial for the activation of ER/Golgi vesicular transport, and metabolism. Thus this pathway may adapt vesicular transport to the biosynthetic production in the ER. So, identifying the molecules that regulate and integrate these processes is of key relevance for scientific knowledge, therapy and biotechnology.
Recent findings from our group suggest that ERp44, a multifunctional chaperone cycling between the ER and cis-Golgi, may act as a molecular relay. ERp44 is very abundant in all professional secretory cells, playing key roles in the QC of antibodies and other secreted proteins. It also regulates ER redox homeostasis (by binding the oxidase Ero1α and the peroxiredoxin Prx4) and Ca^{2+} release, by interacting with the IP3-Receptor 1 (IP3R1) at mitochondrial-associated ER membranes (MAM). An additional interesting property of ERp44 is its strong ability to activate the KDELR signaling
I will present our preliminary data and strategies which will lead us to decipher the molecular mechanisms and the physiological consequences of the signaling properties of ERp44.

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A localized autophagic filter prevents entry of mitochondria carrying pathogenic Opa1 mutations in retinal ganglion cell axons

M. Zaninello[1] and L. Scorrano[1,2]
[1]Venetian Institute of Molecular Medicine (VIMM), Padova, Italy
[2]Dept of Biology, Univ. of Padua

Mutations in proteins that control mitochondrial shape result in neurodegenerative diseases like Autosomal Dominant Optic Atrophy (ADOA), associate to mutated Optic Atrophy 1 (Opa1) and caused by retinal ganglion cell (RGC) loss. Intense research on Opa1 elucidated its multiple functions in mitochondrial fusion, apoptosis and metabolism, but the pathomechanisms of ADOA remain unknown. Here we show that an autophagic filter reduces axonal mitochondria in RGCs expressing pathogenic Opa1. Mutated Opa1 triggers a loop of mitochondrial dysfunction and localized autophagosome accumulation at the axonal hillock. Pharmacological or genetic inhibition of autophagy restores axonal mitochondrial entry and rescues RGCs from excess apoptosis caused by mutated Opa1. Thus localized autophagy contributes to define axonal mitochondria and pathogenesis of ADOA.

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The role of the endocytic protein numb in tumor cell migration

M. Zobel[1], A. Disanza[1], F. Senic-Matuglia[1], G. Scita[1,2], P.P. Di Fiore[1,2,3]
[1]IFOM - The FIRC Institute of Molecular Oncology Foundation, Milan
[2]Dept of Health Sciecens, Univ. of Milan, Milano
[3]European Institute of Oncology, Milano

NUMB is a cell fate determinant that controls signaling output by intervening in the context of asymmetric cell division. NUMB, however, was originally identified as an adaptor protein regulating signaling emanating from various plasma membrane (PM) receptors, including NOTCH, Receptor Tyrosine Kinases (RTKs), such as EGFR and c-MET, and integrins, acting at different trafficking levels. Moreover NUMB binds to key players of Clathrin-mediated endocytosis, regulating the internalization of various receptors and it has been found to localize in recycling endosomes where it may regulate the delivery of cargos back to the PM. We identified Numb as a negative regulator of Circular Dorsal Ruffles (CDRs) formation downstream of c-MET and PDGFR. This is accompanied by increase in mesenchymal mode of motility and cell invasion. CDRs formation depends on the recycling back of active Rac to spatial restricted sites of plasma membrane via Arf6. We found that Numb is enriched in Arf6 recycling compartment and inhibits MHC I and Rac recycling. These evidences suggest that Numb might act as a negative regulator of Arf6 dependent recycling. Since, as for most of small GTPases, GEFs are the primary regulatory target, we focused our attention on ARF6 GEFs and in particular on EFA6 A-D and Cytohesin (Arno) subfamilies, reported to control actin remodeling. Among these GEFs, only EFA6 B binds Numb via its PTB domain. Numb may regulate Arf6 dependent recycling by interacting and possibly modulating its GEF EFA6B. If it were true the down-regulation of EFA6 B would abrogate the increase in CDRs formation induced by down-regulation of Numb. Preliminary experiments demonstrated that removal of EFA6 B by itself does not alter CDRs formation while it inhibits the increase of CDR brought by Numb down-regulation. These results further suggest that EFA6 B may be the candidate GEF interacting with Numb during the process of CDR formation.