XV Congresso Nazionale

Associazione Italiana di Biologia e Genetica Generale e Molecolare

Centro Congressi "Beniamino Andreatta"
Università della Calabria
Arcavacata di Rende (CS)
27-28 settembre 2013

http://AIBG2013.azuleon.org

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Si ringrazia l'Università della Calabria
per aver contribuito alla realizzazione del Congresso

Programma

Venerdì, 27 settembre

8:00 **Apertura registrazioni**

13:00 ***Cocktail di benvenuto***

14:00-14:30 **Benvenuto**

14:30-16:30 **Sessione I**
Organizzazione cellulare e risposte allo stress
Chairs: Graziella Pellegrini, Claudio Brancolini

14:30-14:50 L. Rossi, P. Iacopetti, C. Ghezzani, A. Salvetti (Università di Pisa)
A prohibitin-2 homolog regulates planarian stem cell mitochondria cristae organization and is required for the maintenance of rapidly dividing progeny and tissue specific progenitors

14:50-15:10 S. Sannino, D. Fasano, L. Nitsch, C. Zurzolo, S. Paladino (Università Federico II, Napoli)
Lipid rafts regulate signalling and trafficking of p75NTR in thyroid cells

15:10-15:30 G. Amodio, O. Moltedo, V. Pecoraro, P. Remondelli (Università di Salerno)
Sec23a as the key molecular target of ER Stress dependent modulation of COPII assembling

15:30-15:50 M. Rusconi, G. Buoli Comani, I. Rivolta, A. Panariti, S. Pelucchi, R. Meneveri, A. Piperno, D. Barisani (Università Bicocca, Milano)
Hypoxia and regulation of iron-related genes expression

15:50-16:10 J. Maiuolo, S. Bulotta, C. Verderio, R. Benfante, N. Borgese (Università di Catanzaro)
Selective activation of ATF6 mediates endoplasmic reticulum proliferation triggered by a membrane protein

16:10-16:30 L. Mezzasoma, C. Antognelli, A. Gambelunghe, G. Muzi, V.N. Talesa (Università di Perugia)
Reactive oxygen species induce apoptosis in bronchial epithelial BEAS-2B cells by inhibiting the antiglycation glyoxalase I defence: involvement of superoxide anion, hydrogen peroxide and NF-κB

16:30-17:00 ***Coffee break***

17:00-19:00 **Sessione II**
Regolazione dell'espressione genica: dalla trascrizione alla degradazione
Chairs: Paola Riva, Aldo Pagano

17:00-17:20 G. Fiume, A. Rossi, A. de Laurentiis, C. Falcone, A. Pisano, E. Vecchio, M. Pontoriero, E. Iaccino, A. Scialdone, F. Fasanella Masci, S. Mimmi, S. Ceglia, C. Palmieri, G. Scala, I. Quinto (Università di Catanzaro)
Eukaryotic translation initiation factor 4H is under the transcriptional control of p65/NF-κB

17:20-17:40 L. Patrucco, S. Zucchelli, S. Gustincich, P. Carninci, D. Sblattero, C. Santoro, D. Cotella (Università di Novara)
Exploiting the biotechnological potential of SINEUPTM, novel approach to improve protein synthesis mediated by long non-coding RNAs

17:40-18:00 V.G. D'Agostino, M. Amadio, C. Tiedje, V. Adami, M. Gaestel, A. Provenzani (Università di Trento)
Identification of inhibitors of HuR-RNA complex formation by a novel high throughput biochemical assay

18:00-18:20 F. Rusconi, L. Paganini, E. Toffolo, A. Maroli, S. De Biasi, M. Sala, E. Battaglioli (Università di Milano)
Neuro-LSD1: a mammalian epigenetic modulator of neuronal excitability

18:20-18:40 P. Zavattari, M. Antonelli, L. Moi, M. Badiali, F. Giangaspero (Università di Cagliari)
Searching for methylome alterations in human low-grade gliomas as potential diagnostic and prognostic epigenetic biomarkers

18:40-19:00 A. Malena, M. Pennuto, C. Tezze, D. Borgia, A.P. Russell, G. Soraru̇, L. Vergani (Università di Padova)
Androgen-dependent impairment of myogenesis in Spinal and Bulbar Muscular Atrophy

19:00 Trasferimento in hotel

20:30 ***Cena a Buffet presso Pinacoteca di Palazzo Arnone e successiva visita guidata nel Centro Storico di Cosenza***

Sabato, 28 settembre

8:30-10:30 Sessione III
Genomica strutturale e funzionale
Chairs: Elena Battaglioli, Angelo Poletti

8:30-8:50 E. Maestri, M. Gullì, M. Marmiroli, S. Graziano, L. Pagano, M.L. Savo Sardaro, N. Marmiroli (Università di Parma)
Toxicogenomics with model organisms: a new approach for studying the effects of chemicals and contaminants

8:50-9:10 F. Piva, S. Milantoni, T. Armeni, G. Principato (Università di Ancona)
ExportAid: un database di elementi di RNA che regolano l'export di trascritti nei mammiferi

9:10-9:30 D. Barbagallo, S. Piro, A.G. Condorelli, L.G. Mascali, F. Urbano, N. Parrinello, A. Monello, L. Statello, M. Ragusa, A.M. Rabuazzo, C. Di Pietro, F. Purrello, M. Purrello (Università di Catania)
miR-296-3p, miR-298-5p and their downstream networks are causally involved in the higher resistance of mammalian pancreatic α cells to cytokine-induced apoptosis as compared to β cells

9:30-9:50 L. Goitre, V. Cutano, E. Trapani, A. Morina, R. Canzoneri, B. Pergolizzi, S. Gianoglio, A. Moglia, L. Cianfruglia, T. Armeni, S. Delle Monache, M. Tognon, M. Fontanella, E. Baldini, L. Trabalzini, S.F. Retta (Università di Torino)
Cerebral Cavernous Malformations: the CCM Italia research network offers new therapeutic perspectives

9:50-10:10 M. Maurutto, E. Agostoni, L. Floreani, P. Vatta, M. Tripodi, A. Fuso, F. Persichetti, S. Gustincich (International School for Advanced Studies - SISSA)
LINE-1 copy number variation in Alzheimer's disease

10:10-10:30 V. Pennucci, R. Zini, R. Norfo, P. Guglielmelli, E. Bianchi, S. Salati, G. Sacchi, Z. Prudente, E. Tenedini, S. Ruberti, S. Rontauroli, C. Paoli, T. Fanelli, C. Mannarelli, E. Tagliafico, A.M. Vannucchi, S. Ferrari, and R. Manfredini on behalf of AGIMM investigators (Università di Modena)
Abnormal expression of WT1-as, MEG3 and ANRIL long non-coding RNAs in primary myelofibrosis and their clinical correlates

10:30-10:50 ***Coffee break***

10:50-13:10 **Sessione IV**
Meccanismi molecolari in sistemi complessi
Chairs: Maria Teresa Fiorenza, Simona Paladino

10:50-11:10 A. Di Rocco, S. Carulli, E. Tenedini, E. Bianchi, E. Tagliafico, R. Manfredini, G. Pellegrini, M. De Luca (Università di Modena)
Isolation of human keratinocyte stem cells and high-throughput screening approach for their characterization

11:10-11:30 A. Cariboni, K. Davidson, S. Chauvet, V. Andre', R. Maggi, Y. Yoshida, F. Mann, J. Parnavelas , C. Ruhrberg (University College London, UK)
Novel roles for the class3 semaphorins and their receptors in the development of GnRH neurons

11:30-11:50 C. Montaldo, S. Mattei, A. Baiocchini, N. Rotiroti, F. Del Nonno, L.P. Pucillo, A.M. Cozzolino, C. Battistelli, L. Amicone, G. Ippolito, V. Van Noort, A. Conigliaro, T. Alonzi, M. Tripodi, C. Mancone (Università di Roma)
Hepatocytic vitronectin is an early molecular signature of liver fibrosis in hepatitis C infections with hepatic iron overload

11:50-12:10 R. Sirianni, A. Chimento, I. Casaburi, M. Bartucci, M. Patrizii, R. Dattilo, P. Avena, S. Andò, V. Pezzi (Università della Calabria)
L'attivazione selettiva di GPER inibisce la proliferazione e attiva l'apoptosi in cellule di Leydig tumorali

12:10-12:30 C. Donati, F. Cencetti, G. Fibbi, P. Bruni (Università di Firenze)
Role of S1P in vascular regeneration and its possible implications in systemic sclerosis

12:30-12:50 T. Chioccarelli, G. Cacciola, L. Altucci, A. Viggiano, S. Fasano, R. Pierantoni, G. Cobellis (II Università di Napoli)
Nuclear size as estrogen-responsive chromatin quality parameter of mouse spermatozoa

12:50-13:10 C. Corrado, L. Saieva, S. Raimondo, A. Flugy, G. De Leo, R. Alessandro (Università di Palermo)
Crosstalk between chronic myelogenous leukemia (CML) and bone marrow stromal cells: role of interleukin 8 and CML derived- exosomes

13:10-14:00 ***Pranzo***

14:00-16:00 **Sessione V**
Basi molecolari e cellulari dei tumori
Chairs: Laura Moro, Riccardo Alessandro

14:00-14:20 C. Bartolacci, C. Andreani, L. Pietrella, C. Garulli, C. Kalogris, M.E. Zabaleta, V. Gambini, M. Tilio, C. Lasconi, C. Curcio, B. Belletti, C. Marchini, A. Amici (Università di Camerino)
DNA vaccines against HER2 control Δ16HER2-driven tumorigenicity

14:20-14:40 G. Di Minin, A. Bellazzo, M. Dal Ferro, G. Chiaruttini, R. Bulla, S. Piazza, S. Bicciato, G. Del Sal, L. Collavin (Università di Trieste)
Mutant p53 interacts with the tumor suppressor DAB2IP to reprogram the behavior of cancer cells exposed to inflammatory cytokines

14:40-15:00 B. Costa, E. Da Pozzo, S. Daniele, A. Bertamino, I. Gomez-Monterey, P. Campiglia, D. Sorriento, C. Del Giudice, G. Iaccarino, E. Novellino, C. Martini (Università di Pisa)
Reactivation of p53 by inhibition of interaction with its natural inhibitor MDM2 in glioblastoma multiforme cells

15:00-15:20 F. De Amicis, C. Guido, M. Santoro, I. Perrotta, S. Panza, D. Tramontano, S. Aquila, S. Andò (Università della Calabria)
PR-B binding to the sp1 sites of the phosphatase tensin homologue deleted from chromosome 10 (PTEN) gene promoter induces autophagy in breast cancer cells

15:20-15:40 M. Marzagalli, L. Casati, R.M. Moretti, M. Montagnani Marelli, P. Limonta (Università di Milano)
Dissecting the role of ERβ in melanoma growth and its cross-talk with the tumor epigenome

15:40-16:00 W. Bruno, P. Origone, C. Martinuzzi, M. Arvigo, F. Barbieri, L. Mastracci, D. Comandini, G. Bianchi-Scarrà (Università di Genova)
IGF family elements and tumorigenesis in GastroIntestinalstromal tumors (GISTs) lacking KIT / PDGFRA mutations

16:00-16:20 Chiusura lavori e *coffee break*

16:20 Assemblea soci A.I.B.G.

20:30 *Cena Sociale presso Grand Hotel San Michele di Cetraro (CS)*

Comunicazioni Orali

A prohibitin-2 homolog regulates planarian stem cell mitochondria cristae organization and is required for the maintenance of rapidly dividing progeny and tissue specific progenitors

L. Rossi, P. Iacopetti, C. Ghezzani, A. Salvetti
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Prohibitins play a pivotal role in the regulation of mitochondria architecture. Recently we have identified a Prohibitin 2 homologue (*DjPhb2*) that is specifically expressed in planarian stem cells (neoblasts). Planarians are well known for their astonishing regenerative capabilities and are a model for studying molecular mechanisms involved in stem cell biology. Thanks to the presence of neoblasts, planarians continuously replace aged cells in a physiological turn-over that allows them to have a very long lifespan. Neoblasts are not an homogeneous population and recent data indicate that some of them express lineage-specific factors thus resulting in fate-determined sub-populations. *DjPhb2* silencing by RNA interference (RNAi) produces alterations in neoblast mitochondria morphology, impairs regeneration and leads animals to die. Interestingly, *DjPhb2* silenced planarians loose the rapidly proliferating transit neoblast progeny but not a subpopulation of low-proliferation rate neoblasts, positive for *DjPiwi1* marker and behaving as quiescent totipotent stem cells. According to what demonstrated for rapidly proliferating cells, such as pluripotent embryonic stem cells, the preferred metabolic state of neoblasts is glycolytic and the disruption of mitochondrial ATP production is tolerated. On the contrary, several lines of evidence demonstrate that proper mitochondrial cristae morphology is required for proliferation and differentiation of stem cells. Indeed, even if quiescent stem cells do not use mitochondria for ATP production, they still need prohibitins to maintain a functional mitochondrial compartment as mitochondria cannot arise de novo when stem cells differentiate into their rapidly dividing progeny. Here, we propose a model for *DjPhb2* function in regulating mitochondria architecture that is dispensable for quiescent stem cells but is fundamental for pluripotent transit stem cell population proliferation and differentiation into specific progenitor cells.

Sessione I
Organizzazione cellulare e risposte allo stress

Lipid rafts regulate signalling and trafficking of p75^{NTR} in thyroid cells

S. Sannino, D. Fasano, L. Nitsch, C. Zurzolo, S. Paladino
Dept Molecular Medicine and Medical Biotechnology, Federico II Univ., Napoli, Italy

Localization and trafficking of receptors play a key role in their signalling capability. Mislocalisation and/or altered trafficking of receptors frequently correlate with the activation of proliferative pathways leading to development of cancer. Recently in papillary thyroid carcinomas it has been observed neo-expression of p75^{NTR} in association with the presence of BRAF V600E. However, it is unknown its role in the development and progression of thyroid cancer. We investigated the signal transduction of p75^{NTR} in thyroid cells and how its signalling can be regulated by its localization. Lipid rafts, which are areas of membranes enriched in sphingolipids and cholesterol, are believed to function in cellular signalling by recruiting specific molecules in a unique lipid environment, thus increasing the efficiency of signal transduction. By non-ionic detergent extraction assays and purification on sucrose density gradients we found that in FRT (Fischer Rat Thyroid) cells, both at steady-state and upon NGF stimulation, p75^{NTR} partitions into lipid rafts with a kinetic comparable to that of ERK 1/2 activation, indicating that lipid rafts regulate the p75^{NTR} signalling. Surprisingly, either cholesterol or sphingolipid depletion leads to a drastic increase of ERK1/2 phosphorylation, while these treatments reduced the amount of the receptor insoluble to detergent extraction. Thus, all these data indicate that lipid rafts modulate the signalling of p75^{NTR} by down-regulating its signal transduction. How do they accomplish this task? Interestingly, by immunofluorescence and biochemical assays, we found that cholesterol depletion leads to an intracellular accumulation of the receptor, indicating that lipid rafts can regulate its internalization and recycling to the plasma membrane.

Sec23a as the key molecular target of ER Stress dependent modulation of COPII assembling

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Exit from the Endoplasmic Reticulum (ER) of newly synthesized proteins is mediated by COPII vesicles that bud from the ER at the ER Exit Sites (ERESs). Disruption of ER homeostasis causes accumulation of unfolded and misfolded proteins in the ER. This condition is referred as ER stress. Several evidences suggest a link between the ER stress and the vesicular trafficking within of the early secretory pathway. Previously, we demonstrated that ER Stress rapidly impairs the anterograde transport to the Golgi complex and the formation of COPII vesicles. Sec23a is one of the component of the COPII vesicles coat and its GTPase activating function on Sar1 is one of the key mechanisms of COPII assembly. Interestingly, we found that ER Stress reduces the association to the ER membrane of Sec23a. Concomitantly, FRAP and FLIP analysis of Sec23a revealed that ER stress accelerates its recycling kinetics on ER membrane. The reduced permanence of Sec23a at the ERES could be the mean through which ER Stress modulates COPII assembling and vesicular trafficking. Moreover, we found that Sec23a is mono-ubiquitinated in mammalian cells in two different sites of its second β-barrel domain and that the induction of ER stress reduces the amount of mono-ubiquitinated Sec23a. Noteworthy, this modification is a mono-ubiquitination on two different cysteines of the β-Barrel domain that is implicated in the binding of Sec23a to the ER membrane. Emerging evidences have demonstrated that ubiquitination on cysteines is implicated in many processes including signal transduction and membrane translocation. The regulatory function of cysteine ubiquitination, its localization in the β-Barrel domain and its impairment during ER stress strictly support the idea that Sec23a is the molecular target of ER Stress-dependent modulation of vesicular trafficking and that the regulation of Sec23a ubiquitination is the molecular mechanism involved in this phenomena.

Sessione I
Organizzazione cellulare e risposte allo stress

Hypoxia and regulation of iron-related genes expression

M. Rusconi, G. Buoli Comani, I. Rivolta, A. Panariti, S. Pelucchi, R. Meneveri, A. Piperno, D. Barisani
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Iron absorption and transport are tightly regulated by physiological and pathological conditions. Most of these stimuli act through the modulation of the expression of hepcidin, a small peptide produced by the liver than regulates ferroportin, the only known iron exporter. Hepcidin expression is regulated by iron, but also by inflammation and hypoxia. In hypoxia an increase in erythropoiesis is required and thus hepcidin has to be downregulated, but the mechanisms involved have not been clarified yet. To further evaluate these mechanisms we induced hypoxia (10% oxygen) for 12 and 24 hours in C57B6 mice and liver and duodenum were obtained at the end of the treatment. RNA was extracted, retrotranscribed and mRNA levels of iron-related genes evaluated by q-PCR. In the liver, hepcidin mRNA was reduced as compared to controls both at 12 and 24 hours. Since one of the major pathways that regulates hepcidin expression is that of the BMPs, we evaluate mRNA levels of ID1, another target of BMP pathway. ID1 was significantly downregulated, but only after 24 hours of hypoxia. This pathway is also regulated by matriptase 2, which cleaves one essential cofactor of BMPRs. Differently from what expected, matriptase mRNA was reduced at 24 hours of hypoxia. No difference was observed in ferroportin mRNA levels in the liver, supporting its post-transcriptional regulation. In the duodenum no variation in ferroprtin mRNA was observed, whereas an increase in DMT1 (apical iron transporter) mRNA level was detected. In conclusion hypoxia quickly downregulates hepcidin expression and ID1, suggesting a possible involvement of the BMPR and SMAD in the response to this stimulus rather than an effect mediated by an hypoxia responsive element.

Sessione I
Organizzazione cellulare e risposte allo stress

Selective activation of ATF6 mediates endoplasmic reticulum proliferation triggered by a membrane protein

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It is well known that the Endoplasmic Reticulum (ER) is capable of expanding its surface area in response to both cargo load and to increased expression of resident membrane proteins. While the response to increased cargo load, known as the Unfolded Protein Response (UPR) is well characterized, the mechanism of the response to membrane protein load has been unclear. As model system to investigate this phenomenon, we have used a HeLa-TetOff cell line inducibly expressing a tail-anchored construct, consisting in an N-terminal, cytosolic GFP moiety, anchored to the ER membrane by the tail of cytochrome b5 (GFP-b(5)tail). After removal of doxycycline, GFP-b(5)tail is expressed at moderate levels (1-2% of total ER protein), which nevertheless induce ER proliferation, assessed both by EM and by a 3-4-fold increase in phosphatidylcholine (PC) synthesis. We assessed a possible participation of each of the three arms of the UPR, and found that only the ATF6 arm was selectively activated after induction of GFP-b(5)tail; peak ATF6alpha activation preceded the increase in PC synthesis, and silencing of ATF6alpha abolished the ER proliferation response. In contrast, knockdown of Ire1 was without effect. To investigate whether the well-known SREBP pathway is also involved in the response, we probed for SREBP cleavage with specific antibodies, but found that GFP-b(5)tail expression was without effect on this pathway. Thus, ATF6 activation appears to be both necessary and sufficient for the ER proliferation response. Surprisingly, upregulation of known ATF6 target genes was not observed under our conditions. We propose that a sensing mechanism operates within the lipid bilayer to trigger the selective activation of ATF6.

Sessione I
Organizzazione cellulare e risposte allo stress

Reactive oxygen species induce apoptosis in bronchial epithelial BEAS-2B cells by inhibiting the antiglycation glyoxalase I defence: involvement of superoxide anion, hydrogen peroxide and NF-kB

L. Mezzasoma[1], C. Antognelli[1], A. Gambelunghe[2] , G. Muzi[2] V.N. Talesa[1]
[1]Dipartimento di Medicina Sperimentale e Scienze Biochimiche, Univ. degli Studi di Perugia, Italia
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Reactive oxygen species (ROS) are implicated in the regulation of apoptosis through a number of distinct mechanisms depending on cell type and stimulation conditions. Glyoxalase I (GI) metabolizes methylglyoxal (MG) and MG-derived advanced glycation end products (AGEs) known to cause apoptosis. This study examined the possible role of GI among the mechanisms of ROS-driven apoptosis in human bronchial epithelial BEAS-2B cells exposed to wood dust and signaling pathways by which these reactive species regulate GI expression. Our results showed that wood dust generated distinct ROS (superoxide anion, and hydrogen peroxide) by selectively inhibiting the enzymatic activity of superoxide dismutase or glutathione peroxidase and catalase enzymes. These ROS caused a dramatic inhibition of the antiglycation GI enzyme, leading to the intracellular accumulation of the pro-apoptotic AGE, argpyrimidine (AP) and programmed cell death via a mitochondrial pathway. Pre-treatment with N-acetyl-L-cysteine (NAC), a ROS scavenger, prevented these events. Hence, ROS-induced apoptosis in BEAS-2B cells occurred via a novel mechanism relying on GI inhibition and AP accumulation. We interestingly found that superoxide anion and hydrogen peroxide induced a diverse apoptosis level by differently inhibiting GI via NF-kB pathway. Since maintenance of an intact epithelium is a critically important determinant of normal respiratory function, the knowledge of the mechanisms underlying its disruption may provide insight into the genesis of a number of pathological conditions commonly occurring in wood dust occupational exposure. Our findings suggest that the antioxidant NAC may merit investigation as a potential preventive agent in wood dust exposure-induced respiratory diseases.

Eukaryotic translation initiation factor 4H is under the transcriptional control of p65/NF-κB

G. Fiume, A. Rossi, A. de Laurentiis, C. Falcone, A. Pisano, E. Vecchio, M. Pontoriero, E. Iaccino, A. Scialdone, F. Fasanella Masci, S. Mimmi, S. Ceglia, C. Palmieri, G. Scala, I. Quinto
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Background
Protein synthesis is timely regulated at the step of translation initiation, allowing the fast, reversible and spatial control of gene expression. Initiation of protein synthesis requires at least 13 translation initiation factors to assemble the 80S ribosomal initiation complex. Loss of translation control may lead to cell malignant transformation as consequence of increased rate of protein synthesis and translation activation of mRNA species that are relevant for cell proliferation and survival. Here, we asked whether translational initiation factors could be regulated by NF-κB transcription factor, a major regulator of genes involved in cell proliferation, survival, and inflammatory response.
Results
We found that the p65 subunit of NF-κB activates the transcription of eIF4H gene, which is the regulatory subunit of eIF4A, the most relevant RNA helicase in translation initiation. Moreover, we found that eIF4H gene expression and protein content correlated with the level of p65 DNA binding activity in different cancer cell lines, such as breast cancer (MDA-MB-231, MCF7), neuroblastoma (SH-SY5Y), glioblastoma (U251, D54) and B-cell lymphoma (MC3, DeFew), supporting the role of p65 as transcriptional activator of eIF4H in different cellular contexts. In addition, we observed that the p65-dependent transcriptional activation of eIF4H increased the eIF4H protein cellular content augmenting the rate of global protein synthesis.
Conclusion
The altered expression of a spliced isoform of eIF4H was previously shown to be strictly associated with tumor cell behaviour and increased translation rate of peculiarly structured 5'cap mRNAs, including those of proliferative functions, such as cyclin D1. In this scenario, the evidence of NF-kB-dependent transcriptional control of eIF4H could expand the spectrum of action of NF-kB inhibitors in chemotherapy as these agents could inhibit the global protein synthesis by repressing the eIF4H gene expression.

Sessione II
Regolazione dell'espressione genica: dalla trascrizione alla degradazione

Exploiting the biotechnological potential of SINEUP™, novel approach to improve protein synthesis mediated by long non-coding RNAs

L. Patrucco[1], S. Zucchelli[1,2,3], S. Gustincich[2,3], P. Carninci[3,4], D. Sblattero[1], C. Santoro[1,3], D. Cotella[1]
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Most of the mammalian genome is transcribed, generating a vast repertoire of transcripts that includes protein-coding mRNAs, long non-coding RNAs (lncRNAs) and repetitive sequences, such as SINEs (short interspersed nuclear elements). We recently reported that some lncRNAs containing an inverted SINE B2 and a short sequence antisense to a target mRNA can increase translation of the sense mRNA (Carrieri et al, 2012) and termed this mechanism SINEUP™ (*SINE*-mediated *UP*-regulation of translation). Artificial constructs can be designed to target virtually any sense RNA to improve protein manufacturing. We aim to exploit this feature as a biotechnological tool to increase the production of recombinant proteins in mammalian serum-free cell cultures. We used our established CHO-S cell lines expressing recombinant antibodies (Di Niro et al, 2007) and transfected them with SINEUP™ plasmids. Preliminary results showed that SINEUP™ can significantly increase the production of secreted antibodies (up to 30%), without affecting the expression of the target mRNA. We conclude that SINEUP™ provides an additional layer of control over protein synthesis which can be combined with existing tools.

COMUNICAZIONI ORALI

Identification of inhibitors of HuR-RNA complex formation by a novel high throughput biochemical assay

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[2]Dept of Drug Sciences, Univ. of Pavia, Pavia, Italy
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The RNA-binding protein HuR/ELAVL1 binds to AU-rich elements (AREs) promoting the stabilization and translation of a number of mRNAs into the cytoplasm, dictating their fate. We applied the AlphaScreen technology using purified human HuR protein, expressed in a mammalian cell-based system, to characterize its binding performance *in vitro* towards a ssRNA probe corresponding to the AREs of TNFα 3'UnTranslated Region. We analyzed the binding properties in saturation and time course experiments, including competition assays. The method revealed to be a successful tool for determination of HuR binding kinetic parameters in the nanomolar range, with calculated *Kd* of 2.5±0.60 nM, k_{on} of $2.76*10^6 \pm 0.56$ M^{-1} min^{-1}, and k_{off} of 0.007±0.005 min^{-1}. We also tested the HuR-RNA complex formation by fluorescent probe-based RNA-EMSA. To evaluate the application in high throughput studies, we calculated the Z-factor of the assay in 384-well plate format obtaining a value of 0.84 and an averaged coefficient of variation between controls of 8%, indicating that this biochemical assay fulfills criteria of robustness for a targeted screening approach. By screening 2000 molecules we identified one compound (D1) that, in breast cancer MCF-7 cells, decreases the level of secreted TNFα protein with a concomitant reduction of TNFα mRNA polysomal loading. Interestingly, the expression of total TNFα mRNA does not change upon treatment, suggesting a post-transcriptional mechanism of action of D1. Consistently with our *in vitro* data, the number of TNFα mRNA copies is lower in HuR immunoprecipitates of treated samples compared to the controls. Summarizing, we show here a method to identify small molecules able to interfere with HuR binding to its cognate mRNAs both *in vitro* and in cells. Moreover given its versatility, this tool could be applied to other RNA-Binding Proteins recognizing different RNA, DNA, or protein species, opening new perspectives in the identification of small-molecule modulators of RNA binding proteins activity.

Sessione II
Regolazione dell'espressione genica: dalla trascrizione alla degradazione

Neuro-LSD1: a mammalian epigenetic modulator of neuronal excitability

F. Rusconi[1*], L. Paganini[1*], E. Toffolo[1], A. Maroli[1], S. De Biasi[2], M. Sala[1], E. Battaglioli[1]
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LSD1, Lysine Specific Demethylase 1, a histone H3-Lys4 specific demethylase, is an epigenetic co-repressor ubiquitously expressed, ad highly conserved from yeast to human. Only in the mammalian brain, splicing event including mini-exon E8a introduces alongside LSD1, neuro-LSD1-8a dominant negative isoform unable, upon phosphorylation, to repress target genes transcription. In neurons, relative amount of LSD1/nLSD1-8a/phospho-nLSD1-8a fine-tunes transcriptional plasticity. We describe the neuro-specific alternative splicing mechanism regulating nLSD1-8a levels in physiological and pathological conditions. In the mouse brain, homeostatic response to pharmacologically induced *Status Epilepticus*, entails nLSD1-8a down-regulation, suggesting that inclusion of exon E8a is sensitive to neuronal activation. We have evidences that in mice, deletion of nLSD1-8a protects from epilepsy and that LSD1 participate in the control of neuronal excitability; meanwhile, in human neurodevelopmental disorders featuring epilepsy such as Rett syndrome, alternative splicing de-regulation of nLSD1-8a correlates to circuitry hyper-excitability.

COMUNICAZIONI ORALI

Searching for methylome alterations in human low-grade gliomas as potential diagnostic and prognostic epigenetic biomarkers

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Low-grade brain gliomas (LGG) are slow-growing tumors originating from glial cells, ie cells serving as support network and regulation of neurons. LGG are more frequent brain neoplasms in children and young adults. According to WHO classification the most frequent histotypes are pilocytic astrocytoma (WHO grade I), ganglioglioma (WHO grade I) pleomorphic xantoastrocytoma (WHO grade II) and diffuse gliomas (WHO grade II). LGG increase slowly in size and tend to invade and infiltrate the brain, being able to determine over time permanent neurological deficits. Also, they tend to become more aggressive over the years (increasing grade of malignancy). The identification of clinically relevant molecules could allow to improve the diagnosis and to develop innovative therapies against specific molecular targets. Similar to what happened for Genome-wide association studies (GWAS), it is now believed that Epigenome-wide association studies (EWAS) can reveal pathways involved in the etiology of many diseases, including cancer, given the important role that epigenetic factors play regulating gene expression, DNA repair control systems, cell cycle and apoptosis control, migration and cell proliferation control. We conducted a study on 20 DNA samples extracted from the tumor tissue of LGG patients and 4 control brain tissue, using the Illumina BeadChip 27k, interrogating more than 27,000 CpG loci, located in the vicinity of promoters and transcription start sites of 14,475 coding sequences. We performed a differential methylation analysis by using statistical tools in R environment. Functional bioinformatic annotations analyses were conducted through DAVID package. Our preliminary results have highlighted alterations in the methylation state of genes belonging to pathways such as the signaling of immune system, cellular oxidation and hemostasis, receptor systems of neuroactive ligands. We are currently evaluating possible differences in the methylation pattern of tumors with different brain locations, different age at onset or different tumor progression stages.

Sessione II
Regolazione dell'espressione genica: dalla trascrizione alla degradazione

Androgen-dependent impairment of myogenesis in Spinal and Bulbar Muscular Atrophy

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Spinal and bulbar muscular atrophy (SBMA) is an inherited neuromuscular disease caused by expansion of a polyglutamine (polyQ) tract in the androgen receptor (AR). SBMA is triggered by the interaction between polyQ-AR and its natural ligands, testosterone and dihydrotestosterone (DHT). SBMA is characterized by the loss of lower motor neurons and skeletal muscle fasciculations, weakness, and atrophy. To test the hypothesis that the interaction between polyQ-AR and androgens exerts cell-autonomous toxicity in skeletal muscle, we characterized the process of myogenesis and polyQ-AR expression in DHT-treated satellite cells obtained from SBMA patients and age-matched healthy control subjects. Treatment with androgens increased the size and number of myonuclei in myotubes from control subjects, but not from SBMA patients. Myotubes from SBMA patients had a reduced number of nuclei, suggesting impaired myotube fusion and altered contractile structures. The lack of anabolic effects of androgens on myotubes from SBMA patients was not due to defects in myoblast proliferation, differentiation or apoptosis. DHT treatment of myotubes from SBMA patients increased nuclear accumulation of polyQ-AR and decreased the expression of interleukin-4 (IL-4) when compared to myotubes from control subjects. Following DHT treatment, exposure of myotubes from SBMA patients with IL-4 treatment rescued myonuclear number and size to control levels. This supports the hypothesis that androgens alter the fusion process in SBMA myogenesis. In conclusion, these results provide evidence of an androgen-dependent impairment of myogenesis in SBMA that could contribute to disease pathogenesis.

Toxicogenomics with model organisms: a new approach for studying the effects of chemicals and contaminants

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Nanotechnology is a rapidly growing industry and engineered nanomaterials (ENMs) are applied in several areas such as electronics, biomedicine, pharmaceuticals, cosmetics, food production. The scarce knowledge about uptake, interaction with cells and toxicity, is hindering the full application of ENMs, as shown by recent decisions of the European Commission and European Food Safety Authority. The aim of this work was to develop a toxicogenomics approach for risk assessment of ENMs, focusing on quantum dots cadmium sulfide nanoparticles (CdS NPs) using Arabidopsis thaliana (L.) Heynh and the yeast Saccharomyces cerevisiae as model systems. Two mutant lines of Arabidopsis have been selected as resistant to lethal concentrations of CdS NPs, and the phenotypes and genotypes have been extensively characterised. The global gene expression profile in the two mutants has been analysed using Affymetrix GeneChip Arabidopsis ATH1 Genome Array, showing differences in the panel of induced and repressed genes. The approach in yeast is based on a collection of 6000 haploid strains carrying deletions in genes which are not essential for survival. Resistance (or hypersensitivity) to CdS NPs exhibited by a specific strain suggests that the gene deleted in that strain plays a role in the phenotype. Genes identified in the two model systems are currently being characterised. The same approach based on yeast strains collection has been applied to detection of the effects of different drugs and chemicals, identifying genes putatively involved in resistance and sensitivity. The comparison in this case has been perfomed with the response obtained in human liver cell lines (HepG2), assessed through RealTime PCR microfluidic cards for evaluating gene expression of candidate genes. These new high-throughput toxicogenomics approaches provide data which contribute to building networks of genes and proteins involved in response to xenobiotics in different organisms.

Sessione III
Genomica strutturale e funzionale

ExportAid: un *database* di elementi di RNA che regolano l'*export* di trascritti nei mammiferi

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Una delle sfide dell'era post-genomica consiste nel decifrare i linguaggi che regolano i vari processi molecolari. Ad esempio il linguaggio della trascrizione, del *folding* dell'RNA e delle proteine, dello *splicing* e dell'*export* dell'RNA. Tale studio è complicato dal fatto che ogni linguaggio ha molte parole possibili, il loro significato può dipendere dal contesto, esse non hanno una posizione fissa nel gene e possono essere sovrapposte a parole di altri linguaggi. Tale complessità, ad esempio, rende ancora impossibile predire gli schemi di *splicing* di un gene [Piva et al. 2012]. Un processo accoppiato allo *splicing*, e ancora poco compreso, è quello che regola *export*, ritenzione e *import* dell'RNA tra il nucleo e il citoplasma. La conoscenza delle sue regole permetterebbe di comprendere i motivi per cui diversi trascritti escono dal nucleo con diversa efficienza, quelli che non subiscono *splicing* non possono uscire spontaneamente, alcuni *long non-coding* RNA pur non subendo *splicing* escono dal nucleo e viceversa alcuni di quelli che subiscono *splicing* sono trattenuti nel nucleo. Inoltre tale studio chiarirebbe in che modo i trascritti virali senza introni riescano ad essere esportati al citoplasma. Per far luce su questi fenomeni abbiamo raccolto dalla letteratura e annotato tutte le sequenze di RNA note regolare l'*export* nucleocitoplasmatico di trascritti nei mammiferi. Con tali dati abbiamo creato **ExportAid** (www.introni.it/ExportAid/ExportAid.html), un *database* di elementi di RNA che regolano l'*export* di trascritti nei mammiferi. Il *database*, liberamente accessibile, contiene circa 400 elementi. L'utente può interrogare il *database* anche inserendo la propria sequenza, ottenendo così gli eventuali allineamenti verso le sequenze contenute in **ExportAid**.

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Sessione III
Genomica strutturale e funzionale

miR-296-3p, miR-298-5p and their downstream networks are causally involved in the higher resistance of mammalian pancreatic α cells to cytokine-induced apoptosis as compared to β cells

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Background
The molecular bases of mammalian pancreatic α cells higher resistance than β to proinflammatory cytokines are poorly defined. MicroRNAs are master regulators of cell networks, but only scanty data are available on their transcriptome in these cells and its alterations in diabetes mellitus.
Results
High-throughput real-time PCR showed a marked difference of microRNA transcriptome between murine pancreatic α (αTC1-6) and β (βTC1) cells at steady state. We also characterized the alterations of αTC1-6 microRNA transcriptome after treatment with proinflammatory cytokines. We focused our study on miR-296-3p and miR-298-5p, which were: (1) specifically expressed at steady state in αTC1-6, but not in βTC1 or INS-1; (2) significantly downregulated in αTC1-6 after treatment with cytokines in comparison to untreated controls. These microRNAs share more targets than expected by chance and were co-expressed in αTC1-6 during a 6–48 h time course treatment with cytokines. The genes encoding them are physically clustered in the murine and human genome. By exploiting specific microRNA mimics, we demonstrated that experimental upregulation of miR-296-3p and miR-298-5p raised the propensity to apoptosis of transfected and cytokine-treated αTC1-6 with respect to αTC1-6, treated with cytokines after transfection with scramble molecules. Both microRNAs control the expression of IGF1Rβ, its downstream targets phospho-IRS-1 and phospho-ERK, and TNFα. Our computational analysis suggests that MAFB controls the expression of miR-296-3p and miR-298-5p.
Conclusions
Altogether, microRNA profiling, functional analysis with synthetic mimics and molecular characterization of modulated pathways strongly suggest that specific downregulation of miR-296-3p and miR-298-5p, coupled to upregulation of their targets as IGF1Rβ and TNFα, is a major determinant of mammalian pancreatic α cells resistance to apoptosis induction by cytokines.

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COMUNICAZIONI ORALI

Sessione III
Genomica strutturale e funzionale

Cerebral Cavernous Malformations: the CCM Italia research network offers new therapeutic perspectives

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Cerebral Cavernous Malformation (CCM), also known as Cavernous Angioma or Cavernoma, is a major cerebrovascular disease characterized by abnormally enlarged and leaky capillary cavities (caverns) that may occur in either sporadic or hereditary forms and predispose to a plethora of serious clinical symptoms, including recurrent headaches, seizures, neurological deficits, stroke, and intracerebral hemorrhage. CCM lesions may be single or multiple (even hundreds), ranging in size from a few millimeters to a few centimeters. Symptomatic disease typically begins in the third through fifth decades of life, although symptomatic lesions have been described in all age groups with no sex predominance. Diagnosis is made by magnetic resonance imaging (MRI). The CCM disease has been recognized as a common clinical entity: its prevalence in the general population ranges between 0.3% and 0.5%, accounting for approximately 24 million people worldwide, with a major impact on quality of life and significant socio-economical consequences. Nevertheless, knowledge and risk awareness of this disease is still poor within the society and very low even among medical doctors. To date, there are not direct therapeutic approaches, besides the surgical removal of accessible lesions in patients with recurrent hemorrhage or intractable seizures. To find insights and facilitate breakthroughs into CCM pathogenesis mechanisms and their translational implementation, we established the CCM_Italia multidisciplinary research network (http://www.ccmitalia.unito.it/), which is composed of clinical and research centers located in distinct Italian regions. Through the integrated cooperation of clinicians and researchers with complementary expertise and interests related to distinct aspects of the CCM disease, we contributed significantly to the characterization of molecular mechanisms underlying CCM disease, providing fundamental insights into the development of novel, safe and effective therapeutic strategies.

LINE-1 copy number variation in Alzheimer's disease

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The human genome presents an average of one million Long INterspersedElement (LINE) sequences with less than one hundred of them able to retrotranspose. They seem to represent the highest source of inter-individual variation, since truncated LINEs provide transcription initiation sequences as well as exon and splice junctions to their host genes. Transcription of full-length LINEs occurs during neurogenesis providing the machinery for retrotransposition and leading to a large repertory of intra-individual genomic differences among brain regions. Alzheimer's disease (AD) is considered a complex, progressive and irreversible neurodegenerative disease of the brain and represents the most common form of dementia in the elderly. Here we assess the role of retrotransposons in AD by studying their copy number variation in post-mortem brains of AD individuals and mice models. To this purpose we have taken advantage of TaqMan assays, performing qPCRs on tissue samples from AD patients and TgCRND8 mice, a genetic animal model of AD.

Sessione III
Genomica strutturale e funzionale

Abnormal expression of WT1-as, MEG3 and ANRIL long non-coding RNAs in primary myelofibrosis and their clinical correlates

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Long non-coding RNAs (lncRNAs) are emerging as key regulators of gene expression in normal and cancer cells by recruiting chromatin remodeling complexes. Despite their characterization in several tumor types, little is known about the role of lncRNAs in malignant hematopoiesis. In particular, lncRNAs expression has never been investigated in cells from primary myelofibrosis (PMF) patients. PMF is a Philadelphia negative chronic Myeloproliferative Neoplasm (MPN) that originates from deregulated clonal proliferation of hematopoietic stem cell associated with overproduction of mature blood cells. Molecular basis underlying MPN pathogenesis were partially unraveled in 2005-2006 with the identification of somatic mutations of JAK2 and MPL, after which many other mutations were identified. Recently, several new molecular pathogenetic mechanisms were proposed, such as the aberrant expression of coding and non-coding RNAs. In order to identify other molecular abnormalities harbored by PMF patients, we investigated the expression of CDKN2B-antisense (ANRIL), MEG3 and WT1-antisense lncRNAs, previously described as potentially involved in hematological malignancies, in CD34+ cells from PMF patients. The results evidence that the majority of PMF samples displays a co-upregulation of WT1 and its antisense RNA compared to controls. These samples also show an increased MEG3 expression. In these patients, we found a correlation with high Dynamic International Prognostic Scoring System (DIPPS) plus score and elevated number of circulating CD34+ cells. Moreover, the expression pattern of CDKN2B/ANRIL distinguishes a group of patients characterized by an upregulation of CDKN2B, and, among these, a subgroup with downregulated ANRIL. Of note, this group of patients was characterized by a higher grade of bone marrow fibrosis and by the presence of JAK2V617F mutation. Our results suggest that a deregulated expression of these lncRNAs could play a role in PMF pathogenesis and progression.

Isolation of human keratinocyte stem cells and high-throughput screening approach for their characterization

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In the last three decades, regenerative medicine has opened new horizons for the in vitro reconstruction of epithelial tissues and gene therapy treatment of skin disorders involving the use of adult keratinocyte stem cells (KSCs). Although the ability to identify and isolate these cells represents an important prerequisite for the development of these approaches, molecular markers and their precise in vivo localization are still lacking. In order to define genes involved in the control of stemness and commitment of KSCs, we developed a non-invasive, stem cell-preserving magnetic micro beads based method in order to obtain a KSCs enriched population for high throughput screening experiments. After 3T3 murine fibroblast feeder layer depletion from our keratinocyte cultures, we isolated a subpopulation of basal epithelial cells on the basis of the different expression levels of the a6β4 integrin. By using different approaches, including clonal analysis and p63 bright cells quantification, we clearly showed that a6β4 integrin bright cells have greater growth potential and clonogenic capacity compared to the remaining cell fraction and they include the KSCs population. Comparing gene expression profile of a KSCs-enriched and a terminally differentiated cell population coming from the same original primary cell culture we defined a set of genes most probably involved in stemness maintenance. Ongoing gene profiling on single clone type will allow us to validate this gene signature and to start functional studies on selected genes. Extending this approach to different ectodermal derived tissues will provide a genome wide signature of the molecular pathways underlying self-renewal, commitment and differentiation of KSCs.

Sessione IV
Meccanismi molecolari in sistemi complessi

Novel roles for the class3 semaphorins and their receptors in the development of GnRH neurons

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Gonadotropin releasing hormone (GnRH) neurons are a small group of scattered hypothalamic neuroendocrine cells that control reproductive functions in all mammals and many vertebrates. Despite their position in the adult hypothalamus, during development they originate in the nasal placode and migrate along the vomeronasal nerve to reach the forebrain and attain their final position in the hypothalamus. Failure of GnRH neurons to migrate lead to Hypogonadotropic Hypogonadism (HH) and Kallmann Syndrome (KS), genetic disorders, whose mutated loci are largely unknown. We have previously demonstrated that class 3 semaphorin (SEMA) 3A controls the positioning of the vomeronasal nerve and therefore the migration of GnRH neurons via Neuropilin (NRP1-2) receptors (Cariboni et al., Hum Mol Gen 2011). Predicted by our findings on mouse models, mutations of the SEMA3A gene have been subsequently identified in patients with KS (Hanchate et al., PLoS Genet 2012). In the search for additional SEMA3-mediated signalling pathways involved in this developmental process we found that PLEXIND1, which is the SEMA3E receptor, is expressed by GnRH neurons with a pattern of expression that is temporally complementary to NRP1. Specifically, we found that in the nasal compartment GnRH neurons express high levels of NRP1 and low levels of PLEXIND1, whereas once entering the forebrain they stop expressing NRP1 and express high levels PLXND1. Accordingly mice lacking PLXND1 show a reduction in the total number of GnRH neurons, and an increased number of caspase-positive cells in the forebrain where GnRH neurons normallu project to the medial preoptic area. This results in decreased size of their gonads and reduced fertility, both in mice lacking PlexinD1 or its SEMA3E ligand. Predicted by our findings on mouse models, we found mutations of the SEMA3E gene in two siblings affected by KS, strongly supporting a role of SEMA3E/PlexinD1 genetic pathway in the aethiopathogenesis of KS/HH.

Hepatocytic vitronectin is an early molecular signature of liver fibrosis in hepatitis C infections with hepatic iron overload

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HCV-induced iron overload has been shown to promote liver fibrosis, steatosis and hepatocellular carcinoma. The zonal-restricted histological distribution of pathological iron deposits has hampered the attempt to perform large-scale *in vivo* molecular investigations. Diagnostic and prognostic markers are not yet available to assess iron overload-induced liver fibrogenesis and progression in HCV infections. By means of Spike-in SILAC proteomic approach, we unveiled a specific membrane protein expression signature of HCV cell cultures in the presence of iron overload. Computational analysis of proteomic data set highlighted the hepatocytic vitronectin expression as the most promising specific biomarker for iron-associated fibrogenesis in HCV infections. The robustness of our *in vitro* findings was challenged in human liver biopsies by immunohistochemistry and yielded two major results: *i)* hepatocytic vitronectin expression is associated to liver fibrogenesis in HCV-infected patients with iron overload; *ii)* hepatic vitronectin expression was found to discriminate the transition between mild to moderate fibrosis. Our findings provide molecular evidence on the pathological effects of viral-induced iron overload and allow for hypothesizing on new diagnostic tools and anti-fibrotic approaches.

Sessione IV
Meccanismi molecolari in sistemi complessi

L'attivazione selettiva di GPER inibisce la proliferazione e attiva l'apoptosi in cellule di Leydig tumorali

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Gli Estrogeni sono in grado di legare anche un recettore accoppiato ad una proteina G denominato GPER e l'attivazione di tale recettore può determinare, in maniera cellulo-specifica, l'incremento o l'inibizione della proliferazione di diversi tipi cellulari tumorali. Obiettivo di questo studio è stato quello di investigare l'espressione di GPER nella linea tumorale di cellule di Leydig di ratto R2C, valutare gli effetti della attivazione selettiva di GPER sulla proliferazione delle R2C e definire i meccanismi molecolari indotti dalla sua attivazione. Abbiamo dimostrato che le cellule R2C esprimono GPER e che quando questo recettore viene attivato utilizzando uno specifico ligando denominato G-1, induce un decremento significativo della proliferazione associato all'attivazione di fenomeni apoptotici. La riprova che l'induzione dell'apoptosi sia dipendente dall'attivazione di GPER è che l'utilizzo di uno specifico siRNA silenziante l'espressione di GPER, determina l'abrogazione dell'incremento di Bax, del decremento di Bcl-2, del rilascio del citocromo c, dell'attivazione di PARP-1 e dell'inibizione della proliferazione delle R2C indotta da G-1. Tali eventi sono mediati da una attivazione rapida ma sostenuta nel tempo delle ERK1/2. Abbiamo inoltre osservato che il trattamento con G-1 è in grado di inibire la crescita di tumori xenograft ottenuti iniettando cellule R2C in topi nudi CD1. Inoltre, la somministrazione *in vivo* di G-1 non alterava la morfologia testicolare come fanno altri farmaci comunemente utilizzati nella terapia dei tumori delle cellule del Leydig come il cisplatino che danneggia in maniera severa la struttura cellulare compromettendo la fertilità dei pazienti. Tali osservazioni indicano che l'utilizzo di molecole in grado di attivare il GPER in maniera specifica possono rappresentare una valida terapia alternativa al cisplatino consentendo di salvaguardare la fertilità in pazienti con tumori delle cellule del Leydig.

Role of S1P in vascular regeneration and its possible implications in systemic sclerosis

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Recent studies showed that smooth muscle (SM) progenitors can contribute to neointima formation and repair after injury. Mesoangioblasts (MB) are progenitor mesenchymal cells isolated from explants of dorsal aorta. Sphingosine 1-phosphate (S1P) is a lipid mediator that regulates a wide number of biological processes. We previously demonstrated that S1P acts as mitogen and anti-apoptotic agent in MB. A microarray study performed to establish transcriptional profiles of human MB treated with S1P highlighted the transcription factor GATA6 as target gene regulated by this sphingolipid. GATA6 up-regulation induced by S1P was responsible for the enhanced expression of SM-specific contractile proteins. GATA6 appeared to be critical in the pro-differentiating action of TGFβ. Inhibition of TGFβ-induced S1P formation abrogated GATA6 up-regulation, supporting the view that S1P axis plays a physiological role in mediating the pro-myogenic effect of TGFβ. Differentiation of MB in a 3D matrigel culture was impaired when S1P formation was inhibited. Co-cultures of human MB with endothelial cells (N-MVEC) differentiated into "neovascular-like" networks. Conditioned medium (CM) of N-MVEC stimulated MB migration at the same extent of S1P alone. When S1P production was blocked in N-MVEC, the chemo-attractant properties of CM was reduced. Interestingly, employment of endothelial cells isolated from skin biopsies of patients affected by systemic sclerosis (SSc-MVEC) in 3D matrigel culture highlighted that the addition of CM of MB ameliorated in vitro morphogenesis, in line with the view that a soluble factor released by MB supports SSc-MVEC angiogenesis. qPCR data highlighted that SSc-MVEC exhibit a pattern of S1PR and enzymes of S1P metabolism which drives an anti-angiogenic behaviour. This study individuates novel players in the transcriptional regulation of MB differentiation into SM cells and suggests a possible role for S1P axis to favour vascular regeneration in SSc.

Sessione IV
Meccanismi molecolari in sistemi complessi

Nuclear size as estrogen-responsive chromatin quality parameter of mouse spermatozoa

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Recently, we have investigated the endocannabinoid involvement in chromatin remodeling events occurring in male spermatids. Indeed, we have demonstrated that genetic inactivation of the cannabinoid receptor type 1 (*Cnr1*) negatively influences chromatin remodeling mechanisms, by reducing histone displacement and indices of sperm chromatin quality (chromatin condensation and DNA integrity). Conversely, *Cnr1* knock-out (*Cnr1*$^{-/-}$) male mice, treated with estrogens, replaced histones and rescued chromatin condensation as well as DNA integrity. In the present study, by exploiting *Cnr1*$^{+/+}$, *Cnr1*$^{+/-}$ and *Cnr1*$^{-/-}$ epididymal sperm samples, we show that histone retention directly correlates with low values of sperm chromatin quality indices determining sperm nuclear size elongation. Moreover, we demonstrate that estrogens, by promoting histone displacement and chromatin condensation rescue, are able to efficiently reduce the greater nuclear length observed in *Cnr1*$^{-/-}$ sperm. As a consequence of our results, we suggest that nucleus length may be used as a morphological parameter useful to screen out spermatozoa with low chromatin quality.

Crosstalk between chronic myelogenous leukemia (CML) and bone marrow stromal cells: role of interleukin 8 and CML derived- exosomes

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Chronic myelogenous leukemia (CML) is a myeloproliferative disorder characterized by the t(9:22)(q34:q11) reciprocal translocation, resulting in the expression of the chimeric Bcr–Abl oncoprotein with constitutive tyrosine kinase activity. Exosomes (Exo) are small vesicles of endosomal origin and of 40-100 nm diameter released by many cell types including cancer cells. Several data indicate that Exo play an important role in cell-to-cell communication and tumor-stroma interaction, thus potentially affecting cancer progression. It is well known that stromal microenvironment contributes to disease progression through the establishment of a bi-directional crosstalk with cancer cells. In the bone marrow (BM), stromal cells are able to sustain the growth and survival of leukemic cells by protecting malignant cells from chemotherapy- induced death; on the other hand, leukaemia cells induce changes in the bone marrow stroma composition. Our hypothesis is that CML exosomes could have a functional role in this crosstalk. We demonstrate that treatment of BM-derived-HS5 cells with LAMA84-released Exo induced a significant increase of Interleukin 8 (IL8), as well as an augmented LAMA84 cell adhesion to stromal monolayer and LAMA84 migration towards HS5 conditioned medium. To better investigate the possible role of IL8 in the modulation of leukemia phenotype, we treated CML cells with recombinant IL8 (rIL8). Addition of rIL8 to LAMA84 cells increases the adhesion of leukemic cells to stromal cells and triggers survival pathways, as demonstrated by colony formation assay in methocult and activation of signal transduction pathways by western blot. Inhibition of IL8 receptors, CXCR1 and CXCR2, with SB225002 on LAMA84 cells reverts the effects described previously, confirming a role of IL8 in this crosstalk. In conclusions our data show that LAMA84-derived Exo modulate bone marrow microenvironment, increasing the production of the IL8 by stromal cells; moreover IL8 is able to affect leukemia cell proliferation and survival in a paracrine fashion.

Sessione V
Basi molecolari e cellulari dei tumori

DNA vaccines against HER2 control Δ16HER2-driven tumorigenicity

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Direct involvement of the HER2 receptor in human breast cancer has been widely described but emerging evidence suggests that the real transforming form of this oncoprotein is its splice variant Δ16HER2. A mouse model transgenic for the human Δ16HER2 isoform, developing invasive mammary cancer with early onset and 100% penetrance, is currently available. From these mice, a cell line, we named CAM6, was established and selected as representative of its tumor of origin. CAM6 cells give rise to HER2-positive tumors that grow with a short latency time when injected in syngenic mice and can be exploited as models to investigate Δ16HER2 behavior in terms of oncogenicity and response to therapies. In this study we identified src as key regulator of Δ16HER2-positive CAM6 cells' growth. Indeed, we demonstrated that it is possible to control CAM6 proliferation and viability by pharmacological interference with this critical signaling node downstream of Δ16HER2. Moreover, we showed that electroporation with DNA vaccines encoding the extracellular and transmembrane domains of human wild-type HER2 (HuHuT), of its splice variant (Δ16HuHuT) or of the human/ rat chimeric form (HuRT), triggered a protective immune response toward transplanted Δ16HER2-positive tumors. Such protection relies mainly on the antibody production. Furthermore, these specific anti-HER2 antibodies inhibited CAM6 growth and are able to commit them to apoptosis. To correlate mechanisms of antibodies action with their tumor-inhibitory potential, we showed that treatment with anti-HER2 antibodies caused a reduction in the basal level of src phosphorylation. In conclusion, DNA vaccines conceived against HER2 are effective in controlling Δ16HER2-driven tumorigenicity by induction of antibodies which directly impair the signaling pathway downstream of Δ16HER2.

Mutant p53 interacts with the tumor suppressor DAB2IP to reprogram the behavior of cancer cells exposed to inflammatory cytokines

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Inflammation is a significant factor both in cancer initiation and evolution. In particular, there is convincing evidence for a pro-oncogenic role of the inflammatory cytokine TNFα. The tumor suppressor DAB2IP is a cytoplasmic adaptor protein that plays a crucial role in signaling by TNFα, as it promotes activation of Ask1/JNK and limits activation of NF-kB to elicit a growth-restraining cellular response to this cytokine. Mutation of the tumor suppressor p53 is extremely frequent in tumors, and there is strong evidence that missense p53 mutants (mutp53) acquire oncogenic properties that drive cancer metastasis, proliferation, and survival (gain of function). We observed that cancer cells expressing mutp53 respond with a pro-invasive behavior when treated with inflammatory cytokines, and in particular TNFα. This is a facet of mutant p53 gain of function that is still relatively unexplored, but may have relevant clinical implications. We discovered that mutant p53 interacts with DAB2IP in metastatic breast cancer cell lines. We found that mutp53 fuels NF-kB activation by TNFα, while at the same time dampens activation of Ask1/JNK, thus sustaining migration and survival of cancer cells exposed to inflammation. This activity largely depends on inhibition of DAB2IP functions, mediated by formation of a cytoplasmic complex with mutp53. We explored the transcriptional impact of this molecular axis, and found that cancer cells bearing mutp53 respond to chronic TNF stimulation with a gene expression program that can potentially modify the tumor microenvironment. This novel interaction represents an additional mechanism by which mutant p53 can influence tumor evolution, with implications for our understanding of the complex role of inflammation in cancer.

Sessione V
Basi molecolari e cellulari dei tumori

Reactivation of p53 by inhibition of interaction with its natural inhibitor MDM2 in glioblastoma multiforme cells

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Background: the genotoxic modalities in use for the treatment of Glioblastoma Multiforme (GBM) are ineffective to activate p53 due to an oncogenic block exercised on p53 pathway [1]. This block is explicated frequently through the overexpression of the p53 natural inhibitor MDM2 [2]. The oncoprotein MDM2 binds to p53 and mediates its degradation via proteasome. The aim of this work was to investigate whether the inhibition of p53/MDM2 interaction was able to reactivate p53 in GBM.
Methods: human GBM cell lines with wt-p53 and MDM2-overexpressing were used as model of GBM. The MDM2 antagonist ISA27 was employed to inhibit p53/MDM2 interaction [3]. At subcellular level, the reactivation of p53 was investigated by analyzing the levels of p53, p53/MDM2 complex and mRNA of p53 target genes. The ISA27 effects on cell cycle progression and cell death induction were investigated by DNA content analysis and evaluation of apoptosis markers. The gene silencing of p21 by siRNA was performed to assess whether ISA27 effects on cell cycle and apoptosis were abrogated.
Results: The results showed that inhibition of p53/MDM2 interaction effectively stabilizes the intracellular p53 levels in the models *in vitro* and *in vivo* of GBM. The reactivation of the p53 function causes a drastic reduction in the survival/growth of tumor cells, via irreversible cell cyle block and apoptosis induction. Interestingly, this approach was not toxic in normal cell models, showing selectivity of action on tumor cells. In conclusion, these results suggest that inhibition of the interaction between p53 and MDM2 can be an effective strategy to reactivate the tumor suppressor function of p53 in GBM.
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PR-B binding to the sp1 sites of the phosphatase tensin homologue deleted from chromosome 10 (PTEN) gene promoter induces autophagy in breast cancer cells

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The tumor suppressor activity of the phosphatase and tensin homolog on chromosome 10 (*PTEN*) is subject of intense efforts. Herein, we report that progesterone (OHPg), through PR-B, increases *PTEN* at the protein and mRNA level. By plasmids encoding deleted constructs of the *PTEN* gene promoter, transiently transfected in MCF-7 and T47D breast cancer cells, we identified a region between -1118 bp to – 916 bp responsive to OHPg/PR-B. Mitramycin prevented the OHPg-induced *PTEN* promoter transactivation indicating an Sp1 mediation. ChIP and EMSA analyses showed an increase of PR-B, Sp1, SRC1, CBP and RNA polymerase II recruitment upon OHPg at *PTEN* promoter and PR-B knockdown abolished the complex formation. The functional outcome of *PTEN* regulation by PR-B was a decrease in cell survival due to OHPg-induced autophagy. The OHPg effect on cell survival was reversed by PR-B or *PTEN* siRNA addressing a cooperation between these two factors. Analysis of autophagy-related markers such as PI3KIII, Beclin 1, AMBRA and UVRAG indicated for the first time the ability of OHPg/PR-B to induce their expression. The PR-B/*PTEN* signaling was able to induce Beclin 1 promoter and to influence the initial stage of autophagy since it inhibited the Beclin1/Bcl2 complex formation. Biochemical (MDC) and ultrastructural methods (TEM) results confirm that OHPg/PR-B exerts the autophagic process which was mediated by Beclin 1 as evidenced by specific siRNA. The persistence of such effects in T47D and in ectopically-PR-B expressing HeLa cells, underscores a novel mechanism through which OHPg/PR-B acts on breast cancer, revealing a unique role for PR-B/*PTEN* in regulation of autophagy and an interesting connection in tumor suppressor signalling.

Sessione V
Basi molecolari e cellulari dei tumori

Dissecting the role of ERβ in melanoma growth and its cross-talk with the tumor epigenome

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The incidence of melanoma cases is increasing worldwide and its prognosis is still poor. Clinical observations demonstrate that estrogen receptor-β (ERβ) is expressed in human melanoma tissues and its level of expression decreases with tumor progression, suggesting its cancer suppressive activity. Moreover, epigenetic alterations (DNA methylation, histone modifications) were proposed to play a key role in melanocyte malignant transformation. Data from this Laboratory indicate that ERβ is expressed in human melanoma cells (BLM, A375). Based on this observation, experiments were performed to clarify the effects of ERβ activation (by means of specific agonists: E2, DPN, KB1, KB2, KB4) on melanoma cell growth and on the melanoma epigenome. In BLM melanoma cells, we found that: 1) ERβ agonists significantly and specifically inhibit cell proliferation; 2) upon activation, ERβ translocates from the cytoplasm into the nucleus (by immunofluorescence analysis) confirming the classical activity of steroid receptors; 3) global DNA is hypomethylated with respect to normal melanocytes (by digestion of genomic DNA using sensitive DNA methylation restriction enzymes followed by CG-sites PCR); 4) the acetylation level of histone H4 (H4K16Ac) and the methylation level of histone H3 (H3K4me3) are significantly higher than the corresponding histone modifications in normal melanocytes (by fluorescent Western blot); 5) ERβ activation increases global DNA methylation as well as the levels of H4K16Ac and of H3K4me3. Conclusions: in human melanoma cells, ERβ is associated with a significant antitumor effect and its activation affects the melanoma epigenome, suggesting that its activity might be mediated by a modification of the expression of oncogenes/tumor suppressor genes. It is suggested that ERβ might be considered as a molecular target for novel therapeutic strategies in melanoma. *(Supported by Fondazione Banca del Monte di Lombardia; KB compounds were kindly provided by Karo Bio AB)*

IGF family elements and tumorigenesis in GastroIntestinalstromal tumors (GISTs) lacking KIT / PDGFRA mutations

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About 15% of GISTs patients (wild-type GISTs) lack gain-of-function mutations in KIT or PDGFRA genes and the specific target therapy (Imatinib). Recent studies suggest that aberrations of IGFs pathway could be implicated in GIST tumorigenesis (1). IGF1R induces signaling through the MAP and PI3 kinases, overlapping with KIT downstream cascades. High expression of IGF2 is reported and correlates with a high rate of metastatic relapse, particularly in WT-GISTs, however the mechanism regulating IGF2 overexpression is still to be clarified. We evaluated the expression of p-ERK as a key downstream component in 11 wt-GISTs selected out of 120 GISTs and speculated that the activation of alternative signaling pathways bypassing KIT kinase activity might play a role. We studied two siblings affected by GIST from a family displaying Cerebrotendinous Xanthomatosis, carrying the germline CYP27A1 mutation that correlates also with the impairment of vitamin D metabolites, known to have anticancer properties connected to IGF1 levels. The siblings carried two different somatic mutations in KIT and PDGFRA. In order to understand the causative proneness shared by the siblings and to identify upstream pathway-activation independently on the KIT / PDGFRA mutation, we are going to study in these two cases and in 20 WT-GISTs recruited by the Genoa GIST Unit: 1) IGFs expression and methylation status of IGF2 (to evaluate a self-sustaining loop of IGF activation and a mechanism of dysregulation that might exacerbate a competitive binding with a defective IGF1); 2) a panel of alternative genes potentially involved in GIST tumorigenesis by next generation sequencing (NGS). Clarifying the relationship between IGF family elements and GIST tumorigenesis could provide an effective treatment approach.

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Abstracts

L'esercizio fisico in animali non allenati determina Unfolded Protein Response nel fegato

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La Unfolded Protein Response (UPR) è una risposta adattativa che si verifica nelle cellule in seguito a stress che coinvolgono il reticolo endoplasmatico ruvido (RER). Le condizioni alla base dell'UPR sono numerose e vanno dalla presenza di mutazioni che impediscono il corretto avvolgimento delle proteine che subiscono la maturazione nel RER, all'azione di farmaci o di agenti inquinanti. L'UPR si riscontra nel diabete, in numerose patologie neurodegenerative e in situazioni di abuso (alcoool, droghe). Più recentemente si è osservata UPR anche in condizioni di ipossia. Scopo dell'UPR è in primo luogo l'attenuazione della sintesi proteica e del ciclo cellulare, quindi l'aumento della sintesi di proteine volte a risolvere il problema (es. con l'eliminazione delle proteine malavvolte) e infine, in caso lo stress sia sufficientemente grave, l'attivazione dell'apoptosi. È noto che l'esercizo fisico, causando una mobilizzazione del calcio, determina UPR nel muscolo scheletrico; tale condizione contribuisce positivamente all'adattamento del muscolo stesso. Tuttavia, l'esercizio fisico non viene sempre svolto in maniera corretta e tale da essere salutare. In particolare abbiamo appuntato la nostra attenzione sull'esecuzione di un esercizio intenso in un animale non allenato (lo "sportivo della domenica"). Confrontando l'attivazione dell'UPR nelle cellule epatiche di 4 gruppi di ratti (sedentari; allenati; sedentari sottoposti ad un esercizio intenso "una tantum" 1 h prima del sacrificio; allenati sacrificati 1 h dopo l'ultima sessione di allenamento - per confronto) abbiamo riscontrato una precoce attivazione dell'UPR proprio nel gruppo di animali non allenati sottoposti ad esercizio. Riteniamo che alla base di tale risposta potenzialmente dannosa vi sia il ridotto apporto sanguigno ai visceri, a favore del muscolo scheletrico, che potrebbe aver determinato condizioni di ipossia sufficienti da attivare l'UPR, con induzione della proteina pro-apoptotica CHOP.

Glyoxalase I inhibition induces apoptosis in irradiated MCF-7 cells via a novel mechanism involving Hsp27, p53, NF-kB

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BACKGROUND: Glyoxalase I (GI) metabolizes methylglyoxal (MG) and MG-derived advanced glycation end products (AGEs), known to cause apoptosis. Radiotherapy is an important modality widely used in cancer treatment. Exposure of cells to ionizing radiation (IR) results in a number of complex biological responses, including programmed cell death. The present study was aimed at investigating whether, and through which molecular mechanism, GI was involved in IR-induced apoptosis.

METHODS: Apoptosis was evaluated by TdT-mediated dUTP nick-end labeling (TUNEL) and DNA fragmentation assays by agarose gel electrophoresis. Real-time TaqMan polymerase chain reaction, western blot and spectrophotometric methods were used to study transcript or protein levels, or specific activity, respectively.

RESULTS: IR induced a dramatic reactive oxygen species (ROS)-mediated inhibition of GI, leading to Hsp27-identified argpyrimidine protein accumulation that, in a mechanism involving p53 and NF-kB, triggered an apoptotic mitochondrial pathway. GI inhibition occurred both at functional and transcriptional level, this latter occurring via ERK1/2 MAPK and ERα modulation.

CONCLUSION: IR-induced ROS-mediated GI inhibition resulted in a mitochondrial apoptotic pathway via a novel mechanism involving Hsp27, p53 and NF-kB in irradiated MCF-7 cells.

Bioactive paper platform for colorimetric phenols detection

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Polyphenols, as food antioxidants, are of great interest due to their health benefits as they decrease the risks of cancer and coronary cardiopathy (1). Moreover they influence the quality and organoleptic characteristics of foods (2). Lastly, some neurotransmitters are phenolic compounds. Hence the need to work out a sensitive, portable and inexpensive detection methods to monitor these compounds (3). We developed a disposable paper-based bioassay for the detection of phenolic compounds; the assay was successfully applied for the determination of polyphenols in a real matrix such as wine. The colorimetric quantification of the analyte is based on an enzymatic assay. The tyrosinase enzyme has been immobilized on a filter paper by simple over-spotting with 3-methyl-2-benzothiazolinone hydrazone (MBTH), that allows the detection of phenols by forming stable colored adducts with their enzymatic oxidation products. The color intensity of the adduct (developed after 5 min of reaction) was found to increase proportionally with the increase of the phenolic substrate concentrations. Analyte detection can be achieved by eye and quantification can be simply obtained by using a camera phone and an image analysis software. The response, characteristics of the sensor were determined using l-3,4-dihydroxyphenyl-alanine (l-DOPA), an archetype substrate of tyrosinase, as the analyte. This disposable paper-based biosensor relies on a rapid and simple method, without need of sophisticated instrumentation and trained personnel and could be extremely useful in remote locations or developing countries which does not have ready access to laboratory facilities and where simple, sensitive and low cost bioassays are essentials.

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Glyoxalase II promotes *in-vitro S*-glutathionylation

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The glyoxalase pathway is involved in cellular detoxification of α-ketoldehydes and includes two enzymes. Glo I (Glyoxalase I) that catalyses the formation of SLG (S-D-lactoylglutathione) from MG (methylglyoxal) and GSH (glutathione), and Glo II (Glyoxalase II) that catalyses the hydrolysis of SLG in D-lactate, regenerating the GSH used as co-factor during the first reaction. As the reaction catalysis proposed the release from glyoxalase II active site of unprotonated glutathione (GS^-), we hypothesized a role of glyoxalase II on S-glutathionylation. S-glutathionylation is a reversible post-translational modification of protein cysteine residues by the addition of GSH. S-glutathionylation can be promoted either by oxidative stress to protect proteins from redox alteration or can be regulated under basal conditions to affect proteins changes in structure, activity or sub-cellular localization. To date, three potential catalysts of protein glutathionylation are been proposed: GSTπ, glutaredoxin 1 and flavoprotein sulfhydryl oxidase, but other enzymes may be implicated in this catalysis. In this work we studied Glo II as a new potential candidate to promote S-glutathionylation. To demonstrate its active involvement in protein glutathionylation were used actin, malate dehydrogenase and GAPDH purified proteins, which are known to be glutathionylated, for *in vitro* experiments. Proteins were incubated at different time points with GSSG, GSH, SLG or SLG+Glo II and glutathionylation levels are been tested with immunoblotting using monoclonal antibody anti PSSG. In this work we shows that Glo II, using its natural substrate SLG, allows a rapid and specific protein-SSG formation, leading enzymatic regulation of S-glutathionylation in proteins of different origin and cellular compartmentalization.

Functional analysis of CDKN2A 5'UTR variants associated with melanoma predisposition: an internal ribosomal entry site provides a novel mechanism for the regulation of p16 translation

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Germline mutations in the CDKN2A/p16 tumor suppressor predispose to melanoma (MM). A number of variants with unknown functional significance are found in the 5'UTR during routine screening. In addition, given its critical role in cell homeostasis, there is much interest in understanding the molecular regulators of p16 expression. We studied a panel of 19 germline p16 5'UTR variants, identified in MM patients from our population and GenoMEL partners, through luciferase assays coupled to quantitation of luciferase mRNA in transfected MM cell lines. We found three that can be considered of clinical significance, due to their negative impact on the post-transcriptional dynamics of the CDKN2A/p16 mRNA. Further, we report here that p16 belongs to the expanding group of proteins whose translation is influenced by sequence/structural features of the5' UTR mRNA that are endowed of cellular Internal Ribosome Entry Site (IRES) activity. To study the potential for p16 5'UTR to drive cap-independent translation we developed a dual-luciferase assay using a bicistronic vector, where wildtype, missense or deletion mutants of the p165'UTRwere cloned as intervening sequence between Renilla and Firefly luciferase cDNAs. Results of reporters' relative activity coupled to control analyses of actual bicistronic mRNA transcription, indicated that the wild type p16 5'UTR could stimulate Firefly luciferase translation. The IRES-like activity was two-fold stronger compared to the cMYC 5'UTR, a known cellular IRES used as a control. Notably, hypoxic stress in particular, but also the treatment with mTOR inhibitors, enhanced the translation-stimulating property of the p16 5'UTR.RNA immune-precipitation (RIP) assays performed in a p16-positive MM-derived cell line suggest that the RNA-binding protein YB-1, known to actin translation control, can participate in p16 mRNA translation. Taken collectively our results suggest that the 5'UTR can modulate p16 mRNA translational fitness.

A disorder-to-order structural transition in the cytosolic tail of Fz4 is responsible for the misfolding of the L501fsX533 Fz4 mutant

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Frizzled 4 belongs to the superfamily of G protein coupled receptors. The unstructured cytosolic tail of the receptor is essential for its activity. The Mutation L501fsX533 in the *fz4* gene results in a new C-terminal tail of the receptor and causes a form of Familial exudative vitreoretinopathy. Here we show that the mutated tail is structured. Two amphipathic helices, displaying affinity for membranes and resembling the structure of Influenza Hemagglutinin fusion peptide, constitute the new fold. This tail induces the aggregation of the receptor in the Endoplasmic Reticulum and it is sufficient to block the export to the Golgi complex of a chimeric VSVG protein containing the mutated tail. Shifting the tail's fold to a more disordered conformation relocates the mutated Fz4 receptor to the Plasma Membrane. Such disorder-to-order structural transition was never described in GPCRs and opens a new scenario on the possible effect of mutations on unstructured region of proteins.

ABSTRACTS

MEF2 is a converging hub for HDAC4 and PI3K/Akt-induced transformation

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Gene transcription is under the influence of complex regulative networks integrating multiple signalling events that end up with the final decision of activating or repressing specific genetic programs. Histone deacetylases (HDACs) play important roles in the regulation of different genetic programs controlling differentiation, survival, tissue homeostasis and metabolism. The MEF2-class IIa HDACs axis operates in several differentiation pathways and in numerous adaptive responses. We show that nuclear active HDAC4 transforms NIH3T3 cells. This oncogenic potential depends on the repression of a limited set of genes, identified by DNA microarray experiments, most of which are MEF2-targets. Genes verified as targets of the MEF2-HDAC4 axis are also under the influence of the PI3K/Akt pathway that affects MEF2s protein stability. A signature of 25 MEF2-target genes identified by this study is recurrently repressed in soft tissue sarcomas. Correlation studies depicted two distinct groups of STSs: one in which MEF2 repression correlates with PTEN down-regulation and a second group, in which MEF2 repression correlates with HDAC4 levels. Finally, simultaneous pharmacological inhibition of the PI3K/Akt pathway and of the MEF2-HDAC interaction shows additive effects on transcription of MEF2-target genes and on sarcoma cells proliferation.

ABSTRACTS

Idiosyncratic roles of TSC22D4 and TSC22D1 proteins in the control of proliferation/ differentiation switch in developing cerebellar granule neurons and medulloblastoma cells

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The TSC22D (TGF ß1-stimulated clone 22 domain) protein family includes widely expressed members controlling multiple biological processes and having synergistic/antagonistic activities. To date, the functional activities of TSC22D proteins have mostly been gained on TSC22D1, a well-established tumor suppressor, encoding a long TSC22D1.1 (110 kDa) and two short TSC22D1.2 (11 and 15 kDa) splice variants. The antagonistic activities of TSC22D1 isoforms rely on their ability to compete with each other for heterodimerization with TSC22D4. TSC22D4 also displays high level of form complexity arising from alternative splicing (TSC22D4-42, TSC22D4-55) and post-translational modification of 42 kDa form (TSC22D4-67, TSC22D4-72). We reported that both TSC22D1.1/TSC22D1.2 and TSC22D4 forms are expressed in embryonal and postnatal mouse cerebellum and in *in vitro* cultured cerebellar granule neurons (CGNs), in a developmentally-regulated manner. By immunoprecipitation experiments we have found that, TSC22D4-42 heterodimerizes with TSC22D1.2 in CGNs at DIV1 (a large proportion of which is entering differentiation) but not at DIV7 (post-mitotic and morphologically differentiated). In agreement with this result, TSC22D4-42 - TSC22D1.1 heterodimer promote cell division/survival whereas TSC22D4 -TSC22D1.2 leads to cell senescence/death, raising the possibility that the heterodimerization of either TSC22D1 isoform with TSC22D4-42 is relevant to proliferation/differentiation switch. This is also suggested by the finding that TSC22D1.2 expression is up-regulated at the onset of CGN differentiation and consistently expressed thereafter. Moreover, proliferating DAOY medulloblastoma cells express high level of TSC22D1.1 compared to low level of TSC22D1.2 and TSC22D4-42. The findings described above suggest that idiosyncratic TSC22D4 and TSC22D1 localizations and heterodimerization abilities give rise to a repertoire of form-specific function(s) and pleiotropic activities.

Aquaporin 4 increased expression and blood brain barrier alteration occur in the rat hippocampus and cortex after trimethyltin intoxication

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Trimethyltin (TMT) is a neurotoxicant inducing in the rodent CNS neuronal degeneration, especially in the hippocampus and reactive astrogliosis. A previous magnetic resonance imaging investigation in TMT-treated rats has also evidenced dilation of lateral ventricles, possibly correlated to blood brain barrier (BBB) alterations and brain oedema. The Aquaporin 4 (AQP4), a glial water channel protein, expressed mainly in the brain, is known to play an important role in vascular permeability and in brain oedema conditions. We have studied both the AQP4 expression and BBB integrity in the hippocampus and cerebral cortex of TMT-treated rats to explore molecular mechanisms involved in the brain oedema. At first, we have tested AQP4 both by real-time PCR and western blotting analysis in hippocampus and cortex homogenates. The results have shown a significant up-regulation both of AQP4 mRNA and protein levels starting from 14 days of TMT treatment in the analyzed areas. These molecular data were confirmed by double immunolabelling experiments for AQP4 and GFAP (astrocytic marker). The immunofluorescence images have shown an intense astrogliosis and AQP4 immunoreactivity diffusely pronounced in the hippocampus and cortex at the same treatment times. Moreover, we have examined the effects of TMT on BBB breakdown, quantifying the IgG leakage via immunoblotting detection. Western blotting analysis indicated in the hippocampus an IgG significant increase ($p<0.05$) at 21 days of toxic administration. Double-label immunofluorescence for rat IgG and RECA-1 (vascular endothelium marker) has shown at 21 and 35 days, the presence of rat IgG in vascular endothelium and into the paravasal parenchyma both in the hippocampus and in cortex, indicating an IgG extravasation in TMT-treated rats. Furthermore, the extra-vascular IgG staining was coincident with up-regulation of neuronal vascular endothelial growth factor (VEGF) and phosphorylated VEGF receptor-2 (pVEGFR2), which are known mediate for BBB leakage.Taken together, our findings show that increased AQP4 expression and alteration of vascular permeability occurring in the brain of TMT-treated rats seem to be involved in BBB impairment and brain oedema conditions.

ABSTRACTS

L'asse ipotalamo-ipofisi: un target degli endocannabinoidi per inibire la steroidogenesi nel testicolo di rana

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Nel testicolo dei roditori gli endocannabinoidi - noti modulatori negativi della secrezione dell'ormone di rilascio delle gonadotropine (GnRH) - inibiscono la produzione di testosterone. Al fine di studiare il meccanismo molecolare (ipotalamo-dipendente o ipotalamo-indipendente) di tale processo, abbiamo scelto come modello sperimentale un riproduttore stagionale, l'anfibio anuro *Rana esculenta*, modello in cui il sistema del GnRH - costituito da due ligandi e tre recettori - è stato caratterizzato sia a livello ipotalamico che testicolare. A febbraio, all'inizio di una nuova ondata della spermatogenesi, abbiamo effettuato incubazioni *in vitro* sia di ipotalamo che di testicolo di rana con l'endocannabinoide anandamide (AEA). Nell'ipotalamo, l'AEA inibisce l'espressione di *GPR54* - il recettore che media gli effetti delle kisspeptine, noti modulatori positivi della secrezione del GnRH - e modula il sistema del GnRH. Nel testicolo, l'AEA non produce alcun effetto né sull'espressione di *GPR54* né su quella degli enzimi della steroidogenesi (*cyp17*, *3b-HSD*). Per comprendere se la funzionalità dell'asse ipotalamo-ipofisi possa supportare il ruolo degli endocannabinoidi nella steroidogenesi, abbiamo iniettato AEA nel sacco dorsale delle rane. Diversamente dall'esperimento *in vitro*, la somministrazione *in vivo* di AEA riduce l'espressione di *GPR54*, *cyp17*, *3b-HSD*. Inoltre, i livelli di testosterone - misurati nel testicolo di rana attraverso la metodica EIA - sono risultati anch'essi ridotti dal trattamento. Questi risultati indicano chiaramente che gli endocannabinoidi inibiscono la sintesi degli enzimi della steroidogenesi e la produzione di testosterone nel testicolo di rana spegnendo l'attività dell'asse ipotalamo-ipofisi, attraverso la riduzione di espressione di *GPR54*.

ABSTRACTS

Pituitary cells in juvenile individuals of *Ctenopharyngodon idella* (Cypriniformes Cyprinidae) studied by immunocytochemistry and electron microscopy

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The grass carp (*Ctenopharyngodon idella*, Cypriniformes Cyprinidae) was introduced in Italy for aquatic weed control since 1970 because thought unable to reproduce out of its original habitat: however, recent proofs of its reproduction in the wild were obtained in the Ferrara province. We studied by immunocytochemical and ultrastructural methods the pituitary cell types in juvenile *C. idella* individuals caught in irrigation canals. Nine secretory cell types were identified with antisera against fish and human hormones. In individuals of 51-69 mm of total body length (TL) with undifferentiated gonads, cells immunoreactive to prolactin were detected in rostral pars distalis (RPD), cells immunoreactive to adrenocorticotropin (ir-ACTH) and to somatotropin (ir-STH) respectively in RPD and proximal pars distalis (PPD) in close proximity to neurohypophysis (Nh). Cells immunoreactive to tireotropin were found intermingled with ir-ACTH in RPD and among ir-STH in PPD. Cells simultaneously immunoreactive to anti-follicle stimulating hormone and to anti-croaker gonadotropin were found in dorsal PPD. Cells immunoreactive to somatolactin (ir-SL) were detected in pars intermedia (PI) bordering the Nh. Unlike what observed in other teleosts, cells immunoreactive to melanotropin and to endorphin in PI did not cross react. A ninth cell type immunoreactive to gonadotropin II (ir-GtH II) was detected in PPD of individuals of 78-112 mm TL with a differentiated gonad: thus ir-SL cells could be involved in early gonad development and differentiation besides ir-GtH II. These results, supported by the ultrastructural observations, are the basis for further research on grass carp pituitary physiology.

Oleuropeina ed idrossitirisolo attivano pathways GPER-dipendenti inducendo apoptosi in cellule tumorali mammarie SKBR3 negative per i recettori estrogenici α e β

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Il recettore estrogenico α (ERα), quando è espresso, media la proliferazione di cellule tumorali mammarie attivando eventi molecolari sia genomici che rapidi. In assenza di ERα, un recettore accoppiato a proteine G denominato GPER permette alle cellule di rispondere agli estrogeni incrementando la crescita. E' stato dimostrato che fenoli di origine naturale con struttura chimica simile agli estrogeni possono interferire con la proliferazione delle cellule tumorali. Il nostro gruppo di ricerca ha precedentemente dimostrato che due fenoli di origine naturale oleuropeina (OL) ed idrossitirisolo (HT), riducono la proliferazione indotta da estradiolo in cellule tumorali mammarie MCF-7 ER-positive senza interferire con la classica azione genomica degli ER, ma attivando, invece, una via rapida che coinvolge le ERK1/2. Abbiamo, dunque, ipotizzato che tale inibizione potesse essere mediate da GPER. Per verificare tale ipotesi abbiamo investigato gli effetti di OL e HT sui pathways mediati da GPER e che coinvolgono le ERK1/2 utilizzando come modello sperimentale la linea cellulare di tumore mammario SKBR3 che è ER-negativa e GPER-positiva. Esperimenti di docking simulations e di ligand binding hanno evidenziato che OL e HT sono in grado di legare GPER. Inoltre, il trattamento con entrambi i fenoli riduce la crescita delle cellule SKBR3 e tale effetto viene abolito silenziando GPER con uno specifico siRNA, confermando l'interazione specifica tra OL, HT e GPER. L'approfondimento del meccanismo molecolare a valle ha evidenziato il coinvolgimento di una attivazione sostenuta delle ERK1/2 che determina l'attivazione di fenomeni apoptotici di tipo intrinseco. In conclusione, i risultati ottenuti indicano che OL e HT agiscono come agonisti inversi di GPER in cellule mammarie tumorali ER-negative e GPER-positive come le SKBR3, fornendo interessanti conoscenze sulle potenzialità di queste due molecole come nuovi tools farmacologici nella terapia di questi sottotipi di tumori mammari.

In vivo and in vitro characterization of human urethral epithelial stem cells

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Urethral strictures are the most common cause of obstructed micturition in men having a severe impact on the quality of patient's life. Standard treatments can be associated with complications and a high failure rate. We investigated the potential use of autologous urethral epithelial cells as a source of stem cells for the reconstruction of urethral epithelium. Since regenerative medicine, requires a deep knowledge of the biology of tissue-specific stem cells, we characterized urethral epithelial cells *in vivo* and *in vitro*. We showed the in vivo localization of stemness (p63, Bmi-1, p75), differentiation (Cytokeratin 4, 13, 8, 10), adhesion (laminin 5) markers specific of other stratified epithelia. We reported that urethral primary bulbar keratinocytes, isolated from human biopsies, could undergo an average of 100-120 cell divisions before senescence under selected culture condition. Moreover, molecular markers (p63a, Bmi1, p75), which characterized the stem cell of other stratified epithelium, are down modulated during clonal conversion in epithelial urethral culture. Human keratinocytes (limbal, skin, oral mucosa) when isolated in culture give rise to holoclones, meroclones, paraclones with different clonogenic properties. The holoclone is the stem cell of these stratified epithelia. We isolated and characterized several holoclones from bulbar urethral epithelium from different patients. We showed by clonal analysis that p63a is expressed by urethral holoclones but it is undetectable by paraclones. Our results suggest that urethral epithelial cells have regeneration ability comparable to that of other epithelial cells used for clinical application. In order to define preclinical protocols for the treatment of urethral strictures, we seeded under different conditions urethral epithelial cells on a biocompatible substrate suitable for urethral surgery. Preliminary results indicate the best condition for the preservation of urethral stem cells on the selected scaffold.

An evolutionarily conserved mode of modulation of a potassium channel by dynamic phosphorylation and dephosphorylation

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Voltage-gated K^+ channels of the *Shaw* family (also known as the KCNC or Kv3 family) play pivotal roles in the mammalian brain, and genetic or pharmacological disruption of their activities in mice results in a spectrum of behavioral defects. We have used the model system of *Caenorhabditis elegans* to elucidate conserved molecular mechanisms that regulate these channels. We found that the *C. elegans Shaw* channel KHT-1 and its mammalian homologue Kv3.1b are both modulated by acid phosphatases. Thus, the *C. elegans* phosphatase ACP-2 is stably associated with KHT-1, while its mammalian homologue, prostatic acid phosphatase (PAP; also known as ACPP-201) stably associates with murine Kv3.1b channels *in vitro* and *in vivo*. In biochemical experiments both phosphatases were able to reverse phosphorylation of their associated channel. The effect of phosphorylation on both channels is to produce a decrease in current amplitude and electrophysiological analyses demonstrated that dephosphorylation reversed the effects of phosphorylation on the magnitude of the macroscopic currents. ACP-2 and KHT-1 were colocalized in the nervous system of *C. elegans* and, in the mouse nervous system, PAP and Kv3.1b were colocalized in subsets of neurons, including the brain stem and the ventricular zone. Taken together, this body of evidence suggests that acid phosphatases are general regulatory partners of *Shaw*-like K^+ channels.

LASP-1 directly interacts with vimentin and its expression stratifies patients affected by human hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related mortality worldwide. We have previously reported that LASP-1 is a downstream protein of urokinase and its mediator during the HCC cellular migration.LASP-1 binds the actin microfilaments and this interaction is crucial for the cytoskeleton structure and cellular movement.LASP-1 is overexpressed in some malignant tumours, such as breast, ovarian, bladder and medulloblastoma cancers. To better understand the role of LASP-1 in HCC we have transfected HA22T/VGH cells with LASP-1-GFP expressing plasmid constructs and we have immunoprecipitated the fusion protein.The proteins differentially present in the controls and in the immunoprecipitated fractions were identified by mass spectrometer (MS). We have found that vimentin is a novel interactor of LASP-1.Vimentin is a member of the intermediate filament protein family and plays a central role in the cytoskeleton architecture and its overexpression was significantly associated with HCC metastasis. We have further assessed the expression levels of LASP-1 mRNA by qRT-PCR in tissues from biopsies of 44 patients and we have obtained a weak (average $R=RQ_{HCCn}/RQ_{PTn} = 1,27$) upregulation in HCC tissues compared to their correspondent adjacent PT (peritumoral) tissues. However, when we stratified the patients according to the background liver diseases we have found that the cirrhotic HCCs displayed an increased LASP-1 mRNA levels (R=1.424) while the non-cirrhotic HCCs showed unchanged expression levels (R=1.098). By stratifying the cases on the bases of hepatitis virus infections we observed higher levels of LASP-1 mRNA in the classes HBV/HCV (R=1.625; p=0.021) and HCV (R=1.400) and unvaried levels in the classes HBV (R=1.141) and HBV^-/HCV^- (R=1.120). Our results outline the proteomic identification of vimentin as novel LASP1 interactor;further they show a dysregulated expression in HCC,mainly an upregulation of Lasp1 expression in cirrhotic HCC.

Localizzazione della protimosina-α nei gameti maschili dei Mammiferi

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La protimosina-α (PTMA) è una delle proteine più acide conosciute ed è coinvolta, grazie alla sua struttura random coil, in diverse attività biologiche. Tra queste, quella inerente la gametogenesi maschile è ancora oggetto di discussione. Dal 2002 ad oggi il nostro gruppo ha riportato la conservazione dell'espressione testicolare di PTMA dai Vertebrati non-mammiferi (*Rana esculenta*) a quelli mammiferi (*Rattus norvegicus*): il trascritto è espresso nelle fasi meiotiche e post-meiotiche della spermatogenesi; l'espressione della proteina si estende anche agli spermatozoi (SPZ) (Aniello et al, 2002; Ferrara et al, 2009 e 2010). Più recentemente, abbiamo approfondito lo studio della localizzazione di PTMA nella testa degli SPZ di mammifero. I nostri risultati, ottenuti adoperando la microscopia confocale, mostrano che PTMA è localizzata specificamente nell'acrosoma degli SPZ epididimali di ratto (rSPZ) e provano, per la prima volta, la sua presenza negli SPZ umani (hSPZ) e la localizzazione acrosomale. L'analisi di Western blot su estratti proteici da regioni frazionate della testa degli hSPZ ha evidenziato che PTMA è specificamente associata alla frazione della membrana acrosomale interna (IAM). Una conferma fisiologica è stata ottenuta dalla reazione acrosomale *in vitro* degli rSPZ e hSPZ: anche dopo il rilascio del contenuto solubile dell'acrosoma, PTMA rimane associata alla IAM, esposta nella regione apicale degli SPZ. La conservazione del pattern di sub-localizzazione di PTMA nei gameti maschili dei Mammiferi supporta l'ipotesi di una sua funzione nella loro attività fisiologica, con un possibile ruolo nei fondamentali eventi della fecondazione (Ferrara et al, 2013).

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Development of a hemicornea from human primary ketatinocytes and stromal keratocytes on a biocompatible scaffold

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The aim of this project is the recostruction and characterization of an hemicornea, obtained by seeding primary human cells on a biocompatible scaffold.Scaffolds obtained from biological sources, were selected for transparency, biocompatibility and mechanical resistance. Selected materials have been sterilized with different methods, to guarantee cleaning and maintenance of biocompatibility, transparency and mechanical properties, by high pressure vapour or gamma rays. Limbal keratinocytes and stromal keratocytes were isolated from human corneas. Human limbal keratinocytes were cultured as preiously described by Pellegrini et al. Stromal keratocytes were cultured using different medium in presence or absence of serum to optimize their growth and differentiation in vitro.In order to define cell identity of the isolated cells and characterize cell impurities, we analized protein expression by immunofluorescence. Selected cell markers included CD34, specific for keratocytes, alpha-SMA, for myofibroblastic conversion and specific corneal epithelial cell markers such as cytokeratin 13, 3, 12. Stromal keratocytes and limbal stem cell- derived epithelial cells were seeded on the choosed scaffold, giving rise to hemi- corneas. These hemi-corneas, containing a well-differentiated epithelium and stroma, will be studied both histologically and ultrastructurally, characterizing the expression of specific markers.

Proliferative response induced by 50Hz magnetic field in neuroblastoma cells is accompanied by biphasic re-programming of energy balance, enhanced antioxidant defense and improved cellular protection against methylglyoxal

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Electricity-related extremely low frequency magnetic fields (ELF-MFs) are ubiquitous and epidemiologically linked to neurodegenerations and nervous system tumors. Pathways involved in such associations are still largely unknown, yet redox-sensitive responses may play a role. We previously showed that neuroblastoma cells exposed to ELF-MFs exhibit a proliferative response, together with novel protein expression associated with improved cellular defense against oxidative stress. So far, ELF-MF-induced alterations of glycolysis, the main energy source on which cancer cells mostly rely, are still unknown. Moreover, no previous work has studied ELF-MF-induced changes in cellular defense towards glycolytic toxic by-products, against which malignant cells are known to overexpress the glyoxalase system, to ensure a prompt removal of methylglyoxal (MG). MG is one of the major glycolytically-generated cytotoxic compounds, known to exert many cellular effects through reactive oxygen species (ROS) and oxidative stress. In order to fill this knowledge gap, we studied if the proliferative response induced by ELF-MFs is associated with changes in ROS- and MG-scavenging systems. SH-SY5Y cells underwent a prolonged exposure to ELF-MF (50Hz, 1mT), and the energy cellular balance, together with the activity of antioxidant and glyoxalase systems, was assessed. Our results indicated that the ELF-MF shifted the energy balance from glycolysis to mitochondrial catabolism, and enhanced cellular defense against oxidative stress and glycolysis-related toxic by-products. Interestingly, such adaptations were preceded by increased glycolytic flux, higher oxidative and MG-related damage and lower resistance against exogenous MG. In summary, here we reveal for the first time that the proliferative response induced by ELF-MFs in neuroblastoma cells is accompanied by a biphasic re-programming of energy balance and improved cellular defense against the cytotoxic compound methylglyoxal.

Proliferation of cerebellar granule neurons is impaired in Niemann-Pick C1 mice and is corrected by a single hydroxyproplyl-betacyclodextrin injection

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Niemann Pick type C (NPC) disease is an autosomal recessive, neurodegenerative lysosomal storage disorder due to the abnormal function of NPC1 or NPC2 (95% and 5% of NPC patients, respectively), proteins involved in the intracellular trafficking of cholesterol and other lipids. The deficiency of either protein leads to the accumulation of endocytosed unesterified cholesterol and other lipids, including sphingomyelin and gangliosides within lysosomes. The classic presentation of NPC disease is a child of either sex developing coordination problems, dysarthria and hepatosplenomegaly during early school-age years. A prominent feature of NPC1 disease in humans is the massive loss of cerebellar Purkinje cells (PCs). A similar feature is also observed in several mouse models of NPC1 disease, in which PC degeneration and loss initiates at PN28-PN40 and becomes very pronounced at PN60. Despite the huge interest for mechanisms underlying PC degeneration, the effect of *Npc1* loss of function on overall cerebellum cortex morphology and size has mostly been disregarded so far. Studying the early postnatal development of cerebellar cortex in two mouse models of NPC1 disease, the knock out, *Npc1*$^{-/-}$, and the hypomorphic, *Npc1nmf164*, we have observed that the cerebellum of these mice is significantly smaller compared to that of wild-type mice, because of a defective proliferation of granule neurons (GNs) apparently affecting all cerebellar lobules. A single injection of hydroxy-propyl-beta-cyclodextrin (routinely used to remove cholesterol from, or present cholesterol to, cells in culture) at PN7 doubles the number of GNs in the cerebellum of *Npc1*$^{-/-}$ and *Npc1 nmf164* re-establishing the normal size and shape of cerebellar lobules.

ABSTRACTS

Conventional treatment in acute congestive heart failure patients: modulation of TNRF1 signaling and endogenous NOS inhibitors system in monocytes

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There is poor understanding of the mechanisms of ordinary treatment in regulating inflammatory response in symptomatic acute congestive heart failure (ACHF). Blood monocytes were isolated from 40 hospitalized patients in Intensive Cardiology Care Unit (ICCU, Manfredonia, Italy) with ACHF before and after treatment with conventional drugs (RAAS, diuretics, and β-blockers). Microarray analysis (n=12) showed that pharmacological treatment modulates monocytes gene expression for 481 genes. The inflammatory response network constructed with Ingenuity Pathway Analysis (IPA) showed up-regulation of anti-inflammatory key molecules (NF-kBIA and phosphatase SHP1). IPA analysis also indicates the "TNFR1 signaling" as the most significantly pathway down-regulated after pharmacological treatment, these effects are associated to increased functional inactivation of IKKβ/NF-kB signaling by SHP1. Moreover we investigated the molecular mechanisms in a group of unresponsive patients. IKKβ/NF-kB signaling and SHP1 showed opposite trend between two patients groups. Since it was suggested that endogenous methylarginines could activate NF-kB in a concentration dependent manner, via increasing O_2^- production, we also explored the effects of treatment on levels of L-arginine, asymmetrical dimethylarginine (ADMA), symmetrical dimethylarginine (SDMA), endothelial nitric oxide synthase (eNOS) and cytochrome c oxidase relating to SHP1 expression. We reported that in unresponsive patients, the down-regulation of SHP1 and alteration of DDAH-1/CAT-1 system determine high ADMA and SDMA levels, reduction of eNOS expression and activity and increase of O_2^- production after therapy. In sum, our data demonstrate that treatment with conventional drugs of symptomatic ACHF affect TNFR1 signaling. In addition, we proposed that in unresponsive patients impaired recovery of cardiac function is associated with higher plasma levels of endogenous methylarginines downstream SHP-1 and nitrergic system function alteration.

Essential oils as tyrosinase inhibitors: a different effect on tyrosinase of different sources. A preliminary study

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Plant extracts are a rich source of bioactive compounds and have been traditionally used for both cosmetic and therapeutic purposes. In plants and fungi, the presence of a variety of phenolic compounds leads to the undesirable browning of fruits and vegetables (1). In humans, disorders in the amount and distribution of melanin pigments can be associated to disease states. Plant-derived chemicals have been shown to exert various biological activities and pharmacological effects (2). Because tyrosinase catalyzes the key steps of melanogenesis, most of the strategies for developing skin-lightening and/or anti-food-browning agents are based on the inhibition of tyrosinase activity (i.e. oxidation of L-DOPA) (3). In this study, the essential oils obtained by hydrodistillation from nine different plants were investigated for their potential inhibitory activity on mushroom tyrosinase (MT) and on B16 melanoma cell line tyrosinase (MCLT). The essential oils composition was assessed by GC-mass-spectrometry techniques, anyway we decided to investigate their possible anti-tyrosinase activity considering the oil in its entirety because the biological activity of plant extracts often derives from the combined effect of their different components (4,5). Our results indicate a different inhibitory efficacy on MT and MCLT activities. While extracts from *Cinnamonum zeylanicum*, *Syzigium aromaticum* and *Citrus aurantium amara* appear to be the best inhibitors of both MT and MCLT, with an oil dose-dependent effect, oils from O*riganum vulgaris* and *Rosmarinus officinalis* have major inhibitory effect on MT. Our findings indicate that cinnamon, clove and bitter orange essential oils have high potentials in applications as skin-whitening agents of natural source.

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ABSTRACTS

Rac1 inhibition affects polarity and modulates E-cadherin dynamics

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We are studying the role of Rac1 in the polarization process in the FRT thyroid epithelial cells. To this aim, we analyzed the effects on cell polarization of a specific inhibitor of Rac1, NSC23766, and of a tamoxifen-inducible, dominant-negative form of Rac1, ER-RacN17, which was stably expressed in FRT cells. Directional migration, transepithelial resistance acquisition by confluent monolayers grown on filters, cell aggregation and formation of polarized follicles in suspension cultures were found to be affected by inhibition of Rac1, indicating a critical role of Rac1 in the acquisition of the polarized phenotype. To understand by which molecular mechanisms Rac1 regulates this process, we investigated whether its inhibition affected E-cadherin dynamics and/or function since E-cadherin mediated cell-cell adhesion is the first necessary step of polarization process. We found that Rac1 inhibition determines a progressive loss of E-cadherin from the plasma membrane. Consistently upon Ca-switch assays Rac1 inhibition drastically reduced the amount of E-cadherin that re-localizes at cell-cell contacts from the cytosol where it is translocated following EGTA treatment. Thus, these data suggest that Rac1 regulates the physiological recycling of E-cadherin to the plasma membrane. Accordingly, we found that upon Rac1 inhibition E-cadherin is directed to intracellular degradation as demonstrated by its presence in endo-lysosomal compartments and by detecting its degradation products. In addition, both at the steady-state and upon Ca-switch, we found that the loss of Rac1 activity determines a significant shift of E-cadherin from the TX-100 insoluble pool to the soluble fraction, reflecting a loss of its binding to cytoskeleton. Taken together these data indicate that the crucial role of Rac1 in cell polarization is elicited by modulating the E-cadherin dynamics and membrane organization and, therefore, ensuring functional adherens junctions.

MafB is a downstream target of the IL-10 / STAT3 signaling pathway, involved in the regulation of macrophage de-activation

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In spite of the numerous reports implicating MafB transcription factor in the molecular control of monocyte - macrophage differentiation, the precise genetic program underlying this activity has been, to date, poorly understood. To clarify this issue, we planned a number of experiments that were mainly conducted on human primary macrophages. In this regard, a preliminary gene function study, based on MafB inactivation and over-expression, indicated the *MMP9* and *IL-7R* genes as possible targets of the investigated transcription factor. Bioinformatic analysis of their promoter regions disclosed the presence of several putative MARE elements and a combined approach of EMSA and luciferase assays, subsequently demonstrated that expression of both genes is indeed activated by MafB through a direct transcription mechanism. Additional investigation, performed with similar procedures to elucidate the biological relevance of our observation, revealed that MafB is a downstream target of the IL-10 / STAT3 signalling pathway, normally inducing the macrophage de-activation process. Taken together our data support the existence of a signalling cascade by which stimulation of macrophages with the IL-10 cytokine determines a sequential activation of the STAT3 and MafB transcription factors, in turn leading to an up-regulated expression of *MMP9* and *IL-7R* genes.

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Role of DHHC3 tyrosine phosphorylation in regulating NCAM palmitoylation

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S-palmitoylation is the postranslational addition of palmitate to cysteine residues of target proteins, which plays a role in their trafficking, subcellular localization, stability or function. Recently, zinc finger DHHC (Asp-His-His-Cys)-containing proteins have emerged as a large family of acyl transferases (PAT). Palmitoylation of specific neuro-substrates was shown to be important for synaptic function, plasticity, neuronal migration and maturation. In this context, a recent by study revealed that activation of fibroblast growth factor receptors (FGFRs) by FGF2 leads to palmitoylation of the neural cell adhesion molecule (NCAM) by DHHC3 and stimulation of neurite outgrowth. FGF:FGFR interaction induces the activation of signaling cascades operating through the sequential activation of tyrosine (Y) phosphorylation sites on target proteins. We thus questioned whether any role was played by tyrosine phosphorylation in the regulation of DHHC3 activity. We show that wild-type (wt) DHHC3 becomes highly tyrosine phosphorylated when co-transfected with Src or FGFR1 in neuroblastoma cells. Application of the selective Src inhibitor PP2 reduces the phosphorylation of DHHC3, which is further decreased by the FGFR inhibitor PD173074. DHHC3 has 5 tyrosines located in its cytoplasmic domains. By generating single and triple Y to F (phenylalanine) mutants we were able to dissect the ones responsible for src (Y295, Y297) or FGFR1 (Y18) mediated phosphorylation. Interestingly, the DHHC3 mutant lacking all 5 tyrosines is significantly more autopalmitoylated, as assed by the Click-IT protocol and shows higher palmitoylation activity towards NCAM compared to the wt. In sum, we describe for the first time a role for tyrosine phosphorylation in the regulation of the levels of DHHC3 autopalmitoylation, a postranslational event required for NCAM interaction and palmitoylation. Ongoing experiments aim to study how DHHC3 tyrosine phosphorylation modulates neuronal plasticity.

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

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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.

Human mesenchymal stromal cells exposed to an atmospheric pressure plasma source driven by different voltage waveforms

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Atmospheric pressure non-equilibrium plasma has recently raised much interest for its potential applications in many biomedical-related fields. We started to investigate the effects of exposure to atmospheric pressure non-equilibrium plasma of human mesenchymal cells, derived from fetal membranes (FM-hMSCs). FM-hMSCs have a fibroblast like morphology and easily proliferate in vitro; they express typical mesenchymal markers, comparable to those of bone marrow hMSCs. Moreover FM-hMSCs display some degree of pluripotency, as confirmed by the expression of some specific stem cells markers and may be induced to differentiate into different cell types. A dielectric barrier discharge (DBD) is used to generate a non-equilibrium plasma, driven by two different high-voltage pulse generators. In order to compare the effect of the treatments, we investigated cell survival and proliferation. Cells treated with plasma driven by nanosecond high-voltage pulses (peak voltage between 7 and 20 kV, a repetition rate between 50 and 1000 Hz, a pulse duration about 40 ns and rise time 3 ns) showed a very high mortality rate even at the lower exposure time (1 s) when the treatment was performed on cells deprived of the culture medium. Slightly higher survival and retention of proliferation were observed when cells were treated with the culture medium or the culture medium alone was treated and then added to the cells. Most cells surviving the treatment underwent senescence. The effect of plasma driven by microsecond high-voltage pulses (peak voltage between 15 and 25 kV, repetition rate between 50Hz and 3500 Hz, pulse duration between 1μs and 10 μs and rise time of about 5 μs) was less cytotoxic when administered in comparable conditions; rather, preliminary data suggest that the treatment could induce FM-hMSCs to differentiate. A wound-healing assay was also performed. Preliminary results suggest that treatment with microsecond high-voltage pulsed plasma enhances cell motility.

Insight into mechanism of in vitro insulin secretion increase induced by antipsychotic clozapine: role of FOXA1 and mitochondrial citrate carrier

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Adverse metabolic side effects, including diabetes mellitus, are associated to the use of clozapine and other antipsychotic drugs. These side effects could be, at least in part, the result of impaired islet cell function and abnormal insulin secretion, although the underlying mechanisms are unknown. We have identified a new target for antipsychotic drug clozapine that could be involved in abnormal insulin secretion. Clozapine induces a specific activation of the transcriptional factor FOXA1, but not FOXA2 and FOXA3, in HepG2 cells. In fact, clozapine enhances FOXA1 DNA-binding and its transcriptional activity, increasing mitochondrial citrate carrier gene expression, which contains a FOXA1 site in its promoter. Haloperidol, a conventional antipsychotic drug, does not determine any increase of FOXA1 gene expression. Moreover, clozapine upregulates FOXA1 and CIC gene expression in INS-1 cells only at basal glucose concentration. Interestingly, abnormal insulin secretion in basal glucose conditions can be completely abolished by FOXA1 silencing in INS-1 cells treated with clozapine. The identification of FOXA1 as a novel target for clozapine may shed more light to understand molecular mechanism of abnormal insulin secretion during clozapine treatment.

Molecular analyses in Hereditary Hemorrhagic Telangiectasia: our experience in a rare disease

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Hereditary Hemorrhagic Telangiectasia is an autosomal dominant vascular disorder, acknowledged by ISS as a Rare Disease (RG0100), causing mucocutaneous telangiectases and arterovenous malformations (AVMs). Telangiectases may lead to epistaxes and gastrointestinal bleeding, that may be severe enough to require transfusions. AVMs are mostly observed in liver (60%), lungs (18-70%) and brain (6%) and may cause severe life-threatening complications. The phenotype is highly variable, even among members of the same family, and penetrance is usually complete by the age of 40 years. In the late 90's we have introduced for the first time in Italy mutation analysis in HHT patients. The General Biology and Medical Genetic research group begun a tight collaboration with two clinical in Crema (dott.ssa Buscarini and collaborators) and the ENT Unit in Pavia (dott. Pagella and collaborators). The patients undergo to clinical routine HHT analyses in the clinical centers, then a blood sample is referred to the genetic lab for molecular analyses. A genetic counseling is offered to the patients before blood sample is collected and an informed written consent is requested. We have collected till now more than 1500 DNAs from HHT patients and relatives, belonging to more than 500 different families from all Italian regions. Moreover, we have samples of patients from other European countries. We have analyzed more than 350 Index Cases. We have found a disease causing mutation in more than 260 of them. We have observed a notable difference in mutation distribution within the two genes, with ACVRL1 (and so HHT2) being involved in more than 70% of the families. We have confirmed our previous observation on an high percentage of mutation involving ACVRL1 exon 3 in our population: about 40% of mutations in ACVRL1; about 30% of the sum of mutations considering both genes.

Pharmaceutically induced NDM29 ncRNA enhances chemotherapeutic efficacy in Neuroblastoma treatment

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We recently demonstrated both *in vitro* and *in vivo* that the stable over-expression of a specific non-coding RNA, NDM29, is sufficient to induce Neuroblastoma (NB) cancer cell differentiation restricting dramatically their malignancy [1]. The NDM29 over-expression drives malignant SKNBE2 cells toward a neuron-like phenotype characterized by differentiated traits such as (1) neuron-like morphology endowed with a well distinguishable network of neuritic processes, (2) very low proliferation rate, (3) excitatory properties associated to functional synapses, (4) inhibition of the synthesis of stemness markers, (5) anchorage-dependent growth, (6) expression of neuronal lineage-specific markers. The over-expression of this ncRNA also confers an unpredicted susceptibility to the effects of antiblastic drugs used in NB therapy on malignant NB cells, decreasing ABC transporters, responsible for resistance against anticancer drugs. These results suggest a possible novel strategy for cancer therapeutical purposes. We identified two promising drugs that modulate the expression of NMD29 screening a library of small molecules able to increase NDM29 expression. Next, we demonstrated in vitro the pharmaceutical induction of NDM29 synthesis, conferring to malignant NB cells a strongly increased susceptibility to the effects of antiblastic drugs used in NB therapy, such as cisplatin. We then confirmed these results *in vivo* showing that treated mice had significantly better progression-free survival rates and lower tumour growth compared with mice treated with the antiblast alone. Our preliminary results support the hypothesis that a possible anticancer treatment aimed to stimulate the expression of NDM29 in more aggressive cancer stem-like cells would constitute a possible therapy to cooperate with antiblastic therapies in those cases with a less propitious prognosis.

The Nerve Growth Effects (NGF) effects on the ocular surface

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The human corneal and conjunctival epithelia are constantly renewed during the life-time due to self-process depending on ocular epithelial stem cells (SCs). Several ocular diseases (autoimmune/trophic pathologies) may damage corneal and conjunctival SCs, leading respectively to Limbal Stem cells Deficiency (LSCD) and alteration in goblet cells numbers and mucin secretion. Sjögren's syndrome, a severe form of dry eye is ocular surface disease, which can be treated with NGF. Nerve Growth Factor (NGF) has a pleiotropic action on ocular surface, exerting trophic support and modulation of corneal and conjunctiva healing. The overall aim of this project is to investigate the potential role of NGF and TrkANGFR/p75 NTR for the cell fate of conjunctival and corneal SCs. In vitro studies were performed on both human conjunctival/corneal epithelial cell lines and primary cultures. TrkA and p75 expression are studied in correlation with stemness (ΔNp63α, Bmi1, C/EBPδ), differentiation (14-3-3σ, K3/K12, K19/MUC1 and MUC5AC) and proliferation (Ki67/Edu) markers after NGF treatment. Conjunctival and limbal epithelial cells are exposed to NGF, specific TrkA/p75 inhibitors and siRNAs. The effects on cell fate are evaluated by cellular (colony forming efficiency assay, clonal analysis), biochemical and immunohistochemical analysis. We demonstrated that NGF induces an increase of goblet cells and stimulates the synthesis of the specific "gel-forming" mucin 5AC, essential for the stability of the tear film. In limbal epithelial cultures, NGF induces an increase of clonogenic cells and an increased proliferation. We showed that at clonal level NGF maintains the stem cell compartment slowing down the shift from an activated stem cell to terminal differentiated cell. These results may have relevance for basic research, clinical knowledge and pharmaceutical industry, leading to potential therapeutic intervention.

Biological parameters determining the clinical outcome of autologous cultures of limbal stem cells

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Limbal cultures restore the corneal epithelium in patients with ocular burns. We investigate biological parameters instrumental for their clinical success.We report a long-term multicenter prospective study on 152 patients, carrying severe burn-dependent corneal destruction, treated with autologous limbal cells cultured on fibrin and clinical-grade 3T3-J2 feeder cells. Clinical results were statistically evaluated both by parametric and non-parametric methods. Clinical outcomes were scored as full success, partial success and failure in 66.05%, 19.14%, and 14.81% of eyes, respectively. Total number of clonogenic cells, colony size, growth rate and presence of conjunctival cells could not predict clinical results. Instead, clinical data provided conclusive evidence that graft quality and likelihood of a successful outcome rely on an accurate evaluation of the number of stem cells detected before transplantation as holoclones expressing high levels of the p63 transcription factor. No adverse effects related to the feeder-layer has been observed and the regenerated epithelium was completely devoid of any 3T3 contamination. Cultures of limbal stem cells can be safely used to successfully treat massive destruction of the human cornea. We emphasize the importance of a discipline for defining the suitability and the quality of cultured epithelial grafts, which are relevant to the future clinical use of any cultured cell type

Characterization of human gene *locus CYYR1*: a complex multi-transcript system

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Cysteine/tyrosine-rich 1 (*CYYR1*) is a gene we previously identified on human chromosome 21 (Hsa21). *CYYR1* was initially characterized as a four-exon gene that predicts a 154-amino acid product. We provide the first detailed description of the human *CYYR1 locus*. It is composed of a multigene system, which includes at least seven *CYYR1* alternative spliced isoforms and a new *CYYR1* antisense gene. In particular, we cloned the following isoforms: *CYYR1-1,2,3,4b* and *CYYR1-1,2,3b* present a different 3´ transcripted region; *CYYR1-1,2,4* lacks exon 3; *CYYR1-1,2,2bis,3,4* presents an additional exon between exon 2 and exon 3; *CYYR1-1b,2,3,4* presents a different 5´ untranslated region when compared to *CYYR1*. The meaningful differences in the protein isoforms of *CYYR1 locus* could indicate different functions and localizations of the predicted proteins. Moreover, we cloned a long transcript overlapping with *CYYR1* as an antisense RNA, probably a non-coding RNA. In order to verify the Hsa21 *locus* expression profile in altered conditions such as cancer and aneuploidy, expression analysis was performed in different tumour cell lines and in trisomy 21 (CCL54) and euploid fibroblasts (CCL110). The results obtained indicate a bare expression of the multi-transcript *CYYR1* in all the tumour cell lines studied, with the exception of U2OS, as well as in CCL110, while it is clearly detectable in CCL54. The characterization of the *CYYR1 locus* is a first step to clarify a possible role of the human *locus* related to tumorigenesis and Down syndrome disease. For this purpose, U2OS and CCL could be cell model systems useful to verify the real expression of the predicted proteins by studying the differences in the localization and/or the functional interaction and competition between each CYYR1 isoform. The results of the present study highlight the necessity to analyse thoroughly human gene *loci* that are still orphaned of role comprehension, but that could be related to complex diseases.

Universal tight correlation of codon bias and pool of RNA codons (codonome): the genome is optimized to allow any distribution of gene expression values in the transcriptome from bacteria to humans

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Codon bias is the well-known phenomenon, observed in species from bacteria to mammals, in which distinct synonymous codons are used with different frequencies. We define here the novel biological concept "codonome value" as the total number of codons present across all the expressed messenger RNAs (mRNAs) in a given biological condition. We have developed the innovative "CODONOME" software, which calculates the frequency of each codon in any reference mRNA sequence and, following integration with a gene expression profile, estimates the actual frequency of each codon in the mRNA pool derived from a specific tissue. Systematic analysis across different human tissues and multiple species shows a surprisingly tight correlation between the frequency of each codon at genome level (codon bias) and the proportion of that codon in the transcriptome (codonome bias). It seems that a global compensation exists between the codon bias of highly and of poorly expressed genes, even in tissues with an expression preponderance of a small number of proteins, as in human circulating blood erythrocytes. Moreover, an aneuploidy and cancer condition, such as that of Down Syndrome-related acute megakaryoblastic leukemia (DS-AMKL) trisomy 21 human cells, does not appear to alter this universal relationship. On the other hand, we found no correlation between specific aminoacyl-tRNA synthetase mRNA expression level and the abundance of their respective recognized codons in the codonome, so it would seem that cells do not use this process to optimize the translation. The universal law of correlation between codon bias and codonome at a genome scale emerges as a general property of the distribution and range of the number, sequence, and expression level of the genes included in a genome. This also implies the important conclusion that there is no constraint, in terms of codon bias, for the global distribution of gene expression values during transcription of a genome.

fas/fasl downregulation leads to zebrafish notochord defects including the expression of specific chordoma markers

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Chordoma is a malignant tumor characterized by chemoresistance and unforeseeable prognosis and originates from notochord remnants that do not correctly disappear during development of vertebral bodies. The apoptosis is necessary for the proper notochord development in zebrafish and xenopus and the apoptotic pathway mediated by Fas and Fasl is involved in the notochord cells regression of the intervertebral disks in the adult rat. We studied Fas and Fasl expression in 31 chordomas and in the U-CH1 cell line. The lack of FASL expression in most chordomas and the detection of FAS anti-apoptotic, besides the pro-apoptotic isoform, in both tumors and U-CH1 cells, are consistent with Fas/Fasl pathway inactivation, also supported by the detection of the Caspase 3 and Caspase 8 prevalent inactive forms. Moreover, we observed that apoptosis was induced in U-CH1 cells following treatments with soluble Fasl. We thus hypothesized that Fas/Fasl pathway deregulation may have a role in chordoma onset. To unravel this issue, we investigated the function of fas and fasl homologs in the zebrafish notochord development. We found that these genes were specifically expressed in zebrafish notochord cells. Morpholino-mediated knock-down of *fas* and *fasl* resulted in abnormal phenotypes mainly characterized by curved tail and altered motility. Histological and morphological analysis revealed notochord multi-cell-layer jumps, instead of the typical "stack-of-coins" organization, larger vacuolated notochord cells, defects in the peri-notochordal sheath structure and in vertebral mineralization. Interestingly we observed the persistent expression of *ntla* and *col2a1a*, the zebrafish homologs of the human T gene and COL2A1, which were found to be specifically upregulated in chordoma. These data demonstrate the role of *fas* and *fasl* in notochord development, differentiation and regression in zebrafish, suggesting the implication of this pathway in chordoma onset.

Supported by AIRC

ABSTRACTS

HSPB2 and HSPB8 prevent ARpolyQ aggregation in motoneuronal model of spinal and bulbar muscular atrophy

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Spinal and bulbar muscular atrophy (SBMA) is a motoneuronal disorder caused by an expansion of the polyglutamine (polyQ) tract in the androgen receptor (AR) protein. ARpolyQ misfolds and leads to the formation of intracellular aggregates in a testosterone-dependent manner. ARpolyQ impairs both Ubiquitin-Proteasome (UPP) and Autophagolysosome (APLP) Pathways leading to an accumulation of misfolded proteins and aggregates as well as protein degradation defects. Small heat shock proteins (HSPBs) are chaperones that prevent aggregation of misfolded proteins by assisting their refolding or facilitating their removal through the UPP or APLP. Among the HSPBs, we focused our attention on HSPB8 and HSPB2. HSPB8 is highly expressed in central nervous system and in skeletal muscle. In muscle, HSPB8 forms high molecular weight complexes by interacting with HSPB2. In SBMA motoneuronal cell model, we observed that HSPB8 counteracts ARpolyQ aggregation and facilitates the ARpolyQ clearance. In particular, HspB8 pro-degradative activity does not require a fully functional UPP because HspB8 enhances the mutant ARpolyQ clearance restoring a normal autophagic flux. Interestingly trehalose, an autophagy activator, is able to increase HSPB8 expression suggesting that HSPB8 might be one of the molecular effector of trehalose autophagic activation. We recently found that also HSPB2 is expressed in motoneuronal cell line and its expression, like HSPB8, is highly increased when the UPP is blocked. We analyzed the effects of ARQ(n) over-expression on HSPB2 expression and we found that in presence of testosterone-activated ARpolyQ, HSPB2 expression is highly increased, while HSPB8 expression is unchanged. Interestingly, when overexpressed HSPB2, like HSPB8, counteracts ARpolyQ aggregation. All these data suggest that HSPB2 and HSPB8 might play a relevant role in SBMA.

GRANTS
Fondazione AriSLA; AFM Telethon Foundation; Regione Lombardia multicentric project

ABSTRACTS

Antibody ondemand array

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The technology protein array allows the measurement of multiple proteins in parallel and in a miniaturized format. This high-throughput (HT) technology involves several steps including cloning, expression and purification of the proteins, spotting on microarray surface and downstream analyses. Even in a low throughput applications this approach is time- and cost-intensive. A new type of array, where the cDNA is spotted onto a slide with an anti-tag antibody that allow the capture of the protein produced with *in vitro* transcription/translation (IVTT), were produced in the last years. In this project we want exploit this idea producing protein array where the protein is capture onto the slide using a dsDNA fragment. This approach include the: i) DNA spotting on the array; ii) protein in situ production by IVTT; iii) proteins binding to the coding DNA and downstream assay. The protein array is obtained just when needed starting from spotted DNA. To allow the binding of proteins to their own template we have fused them to the *E. coli* protein TUS that can couple itself with very high affinity to the double stranded DNA sequence *ter*. As a first step we have find the best IVTT system that allow the production of all the proteins tested; secondary we have confirmed that all this proteins fused with TUS protein are able to bind efficiently to *ter* sequence on ELISA plate and on array; finally we have perform an antibody array based on these system. In conclusion we can state that our system is very promising.

Effects of miR-126 shuttled by chronic myelogenous leukemia exosomes on endothelial cells

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Exosomes are nanosized vesicles derived from endosomal compartment and released in extracellular space after fusion with the plasma membrane. Exosomes contain proteins, mRNA and microRNAs (miRNAs) and function as mediators in cell-to-cell communication. These vesicles can be transported within different cells thus affecting the phenotypes of the recipient cells. A number of studies have described exosomes as new players in modulating tumor microenvironment, promoting angiogenesis and tumor development. Our previous work showed that CML cells, LAMA84, release exosomes and that these vesicles stimulate *in vitro* and *in vivo* angiogenesis. Further investigation has showed thatthe treatment of endothelial cells with CML-exosomes downregulates CXCL12 gene expression and protein level in conditioned medium. We also observed a decrease of LAMA84 adhesion to endothelial cell monolayer when HUVEC were treated with CML exosomes for 24 hours. Because, a number of studies have shown that exosomes are involved in horizontal transfer of information through the export of miRNAs, we focused on the possible role of miRNAs on the crosstalk between CML cells and endothelium. We perfomed a miRNAs expression profiling and identified, in CML exosomes,124miRNAs;we focused our attention on miRNA 126 that targets CXCL12 and VCAM-1. We observed an increase in miRNA 126 expression in HUVEC treated with CML exosomes; we also showed that addition of CML exosomes to HUVEC transfected with 2'-O-methyl modified RNA oligonucleotides complementary to mature miR-126 (2'-O-Me-miR-126) reverts the decrease of CXCL12 protein level and restore the adhesion of LAMA84 cells on HUVEC monolayer. On the basis of these preliminary results, we hypothesized that the secretion of exosomes from CML cells affectsthe CXCL12-CXCR4 axis, probably through the shuttling of miRNA These results support the hypothesis that CML exosomes may facilitate the intravasation of leukemic blast and their diffusion in the bloodstream.

Antibodies reacting with Simian Virus 40, a small DNA tumor virus, in sera from malignant pleural mesothelioma affected patients

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Human malignant pleural mesothelioma (MPM) is considered a rare tumour, but recent estimations indicate that one-quarter million people will die of this neoplasm in Europe in the next three decades. The mineral asbestos is considered the main causative agent of this neoplasm. MPM is largely unresponsive to conventional chemotherapy/radiotherapy. In addition to asbestos exposure, genetic predisposition to asbestos carcinogenesis and to SV40 infection were also suggested. SV40 is a DNA tumor virus found in some studies to be associated at high prevalence with MPM. SV40 sequences have also been detected, although at lower prevalence than MPM, in blood specimens from healthy donors. However, some studies have failed to reveal SV40 footprints in MPM and its association with this neoplasm. These conflicting results indicate the need of further investigations with new approaches. We report on the presence of antibodies in serum samples from patients affected by MPM which specifically react with two different SV40 mimotopes. The two SV40 peptides employed in indirect ELISAs correspond to viral capsid proteins (VPs). ELISA with the two SV40 mimotopes gave overlapping results. Our data indicate that in serum samples from MPM affected patients (n = 97) the prevalence of antibodies against SV40 VPs antigens is statistically significant higher (26%, P=0.043) than in the control group (15%), represented by healthy subjects (n=168) with the same median age (66 years) and gender. Our results suggest that SV40 is associated with a subset of MPM and circulates in humans.

ABSTRACTS

Regulation of CDK5R1 expression by miR-15/107 family of microRNAs and its involvement in Alzheimer's disease

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CDK5R1 encodes p35, a protein that activates cyclin-dependent kinase 5 (CDK5). CDK5R1 is characterized by a large and evolutionary conserved 3'-UTR, which suggested a role in the control of its expression through miRNA binding. We previously demonstrated that miR-103 and miR-107 are able to regulate CDK5R1. These miRNAs belong to the miR-15/107 family, a group of highly conserved miRNAs, suggesting a role in the control of CDK5R1 expression for the whole miR-15/107 group. We observed a significant reduction of CDK5R1 mRNA and p35 levels in cells transfected with miRNA precursors of two other members of the family, miR-16 and miR-15a. In addition, antisense LNA molecules inhibiting the miR-15/107 group increased CDK5R1 transcript and p35 levels, supporting the action of the whole family on CDK5R1 expression. Alzheimer's disease (AD) is characterized by the presence of β-amyloid plaques and neurofibrillary tangles of hyperphosphorylated Tau. CDK5, activated by p35 or its proteolytic product, p25, has a key role in abnormal phosphorylation of Tau and β-amyloid Precursor Protein (APP). Since the miR-15/107 group was recently involved in AD pathogenesis, we hypothesize that reduced levels of miR-15/107 in AD can lead to Tau and APP hyperphosphorylation via upregulation of p35 levels and enhanced CDK5 activity. In order to test this hypothesis, we are studying CDK5R1 and miR-15/107 expression, as well as APP and Tau phosphorylation, in frozen brain tissues (hippocampus, temporal cortex and cerebellum) from AD patients and controls individuals. Future work will aim to verify the effect of miR-15/107 modulation on p25/p35 levels, CDK5 activity, APP and Tau phosphorylation and markers of neurodegeneration in primary neurons and differentiated cells. The involvement of miR-15/107-p35/CDK5 pathway in AD plaque formation and/or neurofibrillary degeneration can indicate these miRNAs as new therapeutic targets for the cure of Alzheimer's disease.

Project supported by FIRB2008 grant.

ABSTRACTS

The Fragile X Protein binds mRNAs involved in cancer progression and modulates metastasis formation

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The Fragile X Mental Retardation Protein (FMRP) is an RNA-binding protein involved in multiple steps of RNA metabolism in neurons, and is lacking or mutated in patients with the Fragile X Syndrome (FXS), the most frequent form of inherited mental retardation[1]. The role of FMRP is well established in brain but FMRP is almost ubiquitously expressed, suggesting that, in addition to its effects in brain, it may have fundamental roles in other organs. There is evidence that FMRP expression can be linked to cancer. *FMR1* mRNA, encoding FMRP, is overexpressed in hepatocellular carcinoma cells[2,3]. A decreased risk of cancer has been reported in patients with FXS[4,5], while a patient-case with FXS showed an unusual decrease of tumour brain invasiveness[6]. However, a role for FMRP in regulating cancer biology, if any, remains unknown. We showed that FMRP is deregulated in human carcinomas. In primary breast tumors, high FMRP and *FMR1* mRNA levels correlate with prognostic indicators of aggressive breast cancer, lung metastasis and Triple Negative Breast Cancer. Furthermore, reduction of FMRP in murine tumor cells decreases their ability to form lung metastases; as a result of a decreased cell intravasation while its overexpression increases metastatic potential. Finally, we identified specific FMRP target mRNAs involved in cell shaping and invasion, often a prerequisite of metastatization, and show that E-cadherin and Vimentin, hallmarks of EMT and cancer progression, are controlled by FMRP. The involvement of FMRP in the metastatic phenotype of breast cancer may open unexpected avenues for further research of therapeutic targets hindering metastatic dissemination.

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Characterization of oral mucosal epithelium

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The only cultured cell types widely used for tissue regeneration are the keratinocyte and the condrocyte. Cultured keratinocytes from biopsies of different epithelia have been used for many years to treat large wounds, but there have been many failures of engraftment due to absence of full characterization of cultures and an adequate number of stem cells. In this study we analyzed the proliferative and differentiation potential of human oral keratinocytes. Therefore oral mucosal biopsies were taken from patients to generate autologous oral cell cultures. Subconfluent cultures of oral cells were analyzed for expression of stem cells and differentiation markers. The expression of proliferative (p63), self-renewal (Bmi-1), differentiation markers (14-3-3σ) and cytokeratins (CK 4,13,14) was investigated. The analysis was performed by IHC in frozen tissue and Western Blot analysis in cell cultures obtained by different biopsies of oral mucosal epithelium. Holo-, mero-, and paraclones, previously identified in skin and ocular epithelia, were also found in the buccal epithelium. Holoclones are rich in p63α, meroclones have little and paraclones none. Therefore, p63α is likely to identify the stem cell population of human oral mucosal epithelium. The maintenance of 14-3-3σ in cultured cells revealed their ability to differenziate. We also assessed the expression of CK14 that was found in the basal compartment as seen in other stratified epithelia as well as expression of CK4 and 13, typical markers of suprabasal layers of non-cornified stratified epithelia. Here we demonstrated that in our culture conditions, oral keratinocytes, express stemness markers and retained their differentiation properties. However, since the possibility of transplanting reconstituted mucosal epithelia requires *in vitro* reconstruction on tridimensional scaffold, we analyzed cell cultures grown on different biocompatible supports in order to define the best conditions for future clinical application.

Regulatory mRNA/microRNA networks in CD34+ cells from primary myelofibrosis

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Primary myelofibrosis (PMF) is a clonal disorder of a hematopoietic stem cell included in the Philadelphia chromosome-negative chronic myeloproliferative neoplasms (MPNs), together with polycythemia vera and essential thrombocythemia. The molecular mechanisms of these diseases were partially unravelled in 2005 with the identification of somatic gain-of-function of Janus kinase 2 (JAK2) and Thrombopoietin Receptor (MPL), after which many other mutated genes were found. Moreover, aberrant microRNA (miRNA) expression seems to add up to the molecular complexity of MPNs, as specific miRNA signatures discriminates MPN cells from those of normal donors. In order to have a comprehensive picture of miRNA deregulation and its relationship with differential gene expression in PMF cells, we obtained mRNA and miRNA profiles in the same CD34+ cells from 31 healthy donors and 42 PMF patients by means of Affymetrix technology. Several miRNAs involved in hematological malignancies or known as oncomirs resulted upregulated in PMF samples (hsa-miR-155-5p, miRNAs belonging to the miR-17-92 cluster), whereas other aberrantly expressed miRNAs have never been described in the hematological context (hsa-miR-335). Next, we carried out an in silico integrative analysis (IA) with Ingenuity Pathway Analysis software, which combines the computational predicted targets with the gene expression data to construct regulatory networks of the functional miRNA-mRNA interactions. Of note, IA identified a network potentially involved in PMF pathogenesis, in which the upregulated oncomirs miR-155-5p and miR29a-3p could explain the downregulation of targets whose lower expression was already described in myeloproliferative phenotypes (NR4A3, CDC42, HMGB3), and of the chromatin remodeler JARID2, which is frequently deleted in leukemic transformation of MPNs. Finally, we demonstrated the JARID2 downregulation in CD34+ cells plays a role in the abnormal megakaryopoiesis, and contributes to PMF pathogenesis.

ABSTRACTS

In search of the *SLC2A10* gene role in the etiopathogenesis of arterial tortuosity syndrome by transcriptomic and biochemical approaches

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Arterial tortuosity syndrome (ATS) is an autosomal recessive connective tissue disorder, mainly characterized by arteries tortuosity, elongation, stenosis, and aneurysm formation. ATS is caused by loss-of-function mutations in *SLC2A10*, encoding for the facilitative glucose transporter 10 (GLUT10), also transporting vitamin C, a cofactor for collagen (COLL)/elastin (ELN) maturation. The GLUT10 role in the ATS pathogenesis is still controversial, although *SLC2A10* mutations lead to the extracellular matrix (ECM) disarray and TGFβ pathway activation. To identify gene expression patterns related to ATS, a gene transcription profile study was performed in ATS *vs* control fibroblasts. This approach revealed 217 differentially expressed genes (DEGs), 105 of which were down- and 112 up-regulated. DEGs functional analysis highlighted many dysregulated genes involved in cell-cell and cell-ECM interaction, cytoskeleton turn-over and TGFβ-signaling (*LTBP1, LOXL4, TGM2*), confirming the GLUT10 role in connective tissue biology. Among the up-regulated genes, emerged *ALDH1A1, FADS2, FAR2, PLA2G4A, PPARG,* involved in lipid metabolism and peroxisomal β-oxidation. ATS cells showed, compared to control ones, increased amounts of ALDH1A1 protein, a dehydrogenase scavenger of the aldehydes generating free radicals, and of PPARγ protein in the cytoplasm and nucleus. PPARγ is involved in the glucose/lipid homeostasis and anti-oxidant pathways and might be involved in the ECM disarray of ATS cells. The down-regulation of *LOXL4*, a lysyl-oxidase crucial in the COLL/ELN folding, also participates to the ECM disarray. Since in ATS cells the *LTBP1* gene expression is increased and that of *TGM2* down-regulated, both proteins should synergize in the TGFβ sequestration and TGFβ-receptor type I disorganization observed in these cells. These data confirm the TGFβ-signaling involvement in the ECM remodeling of ATS cells and provide new insights in unraveled biological pathways, *e.g.* the lipid metabolism and defense from oxidative stress, likely involved in the ATS pathogenesis.

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Design and layout

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