Libro degli Abstract

AIBGXII

Congresso Nazionale

ASSOCIAZIONE ITALIANA DI BIOLOGIA E GENETICA GENERALE E MOLECOLARE

Trento
8-9 Ottobre 2010

COMITATO DIRETTIVO:

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PROGRAMMA

Giovedì 7 Ottobre 2010

17.00 – 20.00 Registrazione

Venerdì 8 Ottobre 2010

8.00 – 14.00 Registrazione

11.00 – 12.00 Visita guidata al Castello del Buonconsiglio a Trento

13.00 – 14.00 Colazione di lavoro

14.00 – 14.10 Benvenuto

SESSIONE I ___RNA E REGOLAZIONE DELL'ESPRESSIONE GENICA

Chair: Giovanni Principato (Ancona) – Mario Felaco (Chieti)

14.10 – 14.30 Erica Diani (Università di Verona)- *Expression, localization and functional role of ribonucleoproteins in myoblasts differentiation*

14.30 – 14.50 Aldo Pagano (Università di Genova) - *The expression of a cytoplasmic Alu-like RNA promotes cell differentiation and reduces malignancy of human neuroblastoma cells*

14.50 – 15.10 Marco Ragusa (Università di Catania) - *Specific alterations of microRNA transcriptome and global network structure in colorectal carcinoma after cetuximab treatment*

15.10 – 15.30 Alessandro Barbon (Università di Brescia) - *Acute spinal cord injury persistently reduces R/G RNA editing of AMPA glutamate receptors*

15.30 – 15.50 Silvia Galardi (Università di Roma "Tor Vergata")- *Molecular dissection of the regulation of miR-221/222 in prostate carcinoma and in glioblastoma*

15.50 – 16.10 Pierluigi Strippoli (Università di Bologna) – *TRAM (Transcriptome Mapper): database-driven creation and analysis of transcriptome maps from multiple sources*

16.10 – 16.30 Gabriella Viero (Università di Trento) – *The shapes of native mammalian polysomes*
16.30 – 17.00 Coffee Break

SESSIONE II__CELLULE STAMINALI

Chair: Marco Tripodi (Roma Sapienza) – Silvia Fasano (Napoli II)

17.00 – 17.20 Mariana Lomiento (Università di Modena e Reggio Emilia)- *Dynamic correlation between differential gene expression and chromosome territories' topology during normal human myelopoiesis*

17.20 – 17.40 Letizia De Chiara (Università di Torino)- *Generation of Functional Hepatocytes from Mouse Germline Cell-derived Pluripotent Stem Cells in vitro*

17.40 – 18.00 Roberto Iorio (Università dell'Aquila)- *Extremely Low Frequency Electromagnetic Field (ELF-EMF) enhances the energy metabolism of human spermatozoa and does not interfere with sperm/oocyte interaction*

18.00 – 18.20 Marco Manfrini (Università di Ferrara)- *Mesenchymal Stem Cell Behavior in Biomaterials Assays*

18.20 – Serata libera

Sabato 9 Ottobre 2010

SESSIONE III _STRESS E MORTE CELLULARE

Chair: Paolo Remondelli (Salerno) – Marta Farnararo (Firenze)

8.30 – 8.50 Andrea Clocchiatti (Università di Udine)- *Cell death programs: one, two or many? Why discussing about programmed necrosis is no longer a heresy*

8.50 – 9.10 Stephanie François (Università di Milano "Bicocca")- *Necdin protects myoblast from programmed cell death by interfering with the mediator of apoptosis CCAR1/CARP1*

9.10 – 9.30 Cinzia Antognelli (Università di Perugia)- *Silencing of glyoxilase I defines a role in apoptosis in LNCaP and PC3 Human Prostate cancer cells*

9.30 – 9.50 Leonardo Rossi (Università di Pisa)- *Mitochondrial permeability transition: identification of new chemotherapy target and molecular diagnosis tools*

9.50 – 10.10 Sabrina Ceccariglia (Università di Roma "Cattolica del Sacro Cuore")- *Cathepsin D expression in the rat hippocampus after trimethyltin intoxication an in vivo and in vitro study*

10.10 – 10.30 Pasquale Bove (Università di Foggia)- *Novel hypotheses on the origin and regulation of a small heat shock gene in Lactobacillus plantarum WCFS1*

10.30 – 10.50 Coffee break

SESSIONE IV __PROLIFERAZIONE E DIFFERENZIAMENTO

Chair: Aldo Amato (Messina) – Arturo Bevilacqua (Roma Psicologia)

10.50 – 11.10 Gaetano Calì (Università di Napoli "Federico II")- *CDH16 gene expression in thyroid gland development and tumor progression*

11.10 – 11.30 Giulia Pinton (Università del Piemonte Orientale "A. Avogadro", Novara) - *Estrogen receptor beta exerts tumor repressive functions in human malignant pleural mesothelioma*

11.30 – 11.50 Giuseppe Fiume (Università di Catanzaro "Magna Graecia") - *HIV-1 Tat counteracts the post-activation turn-off of NF-κB through association with IκB-α repressor and p65 subunit of NF-Kb*

11.50 – 12.10 Alice Domenichini (Università di Padova) - *mother-of-snowwhite (msw): a maternal effect allele affecting behavior and the formation of the left-right body axis in zebrafish*

12.10 – 12.30 Fiorenza Magi (Università di Roma "Foro Italico") – *Cellular senescence in skeletal semitendiunous muscle: correlation between 4-HNE adducts, telomerase's length, stress proteins physical activity level*

12.30 – 12.50 Elisa Bianchi (Università di Modena e Reggio Emilia)- *c-Myb supports erythropoiesis by transactivating KLF1 e LMO2 expression*

12.50 – 13.40 Colazione di lavoro

SESSIONE V __BASI MOLECOLARI DELLE MALATTIE

Chair: Ranieri Cancedda (Genova) – Roberta Vanni (Cagliari)

13.40 – 14.00 Romina Combi (Università di Milano "Bicocca") - *Study of the genetic basis of Autosomal dominant nocturnal frontal lobe epilepsy*

14.00 – 14.20 Marco Venturin (Università di Milano) – *Identification of cis elements and trans factors involved in post-transcriptional regulation of the CDK5R1 gene*

14.20 – 14.40 Roberta Moretti (Università di Milano) - *Molecular mechanisms underlying the antitumor activity of Gonadotropin-Releasing Hormone and its receptors*

14.40 – 15.00 Paola Palanza (Università di Parma)- *Conditional knockout NPYYIR mice as an experimental model to study gene-environment interaction*

15.00 – 15.20 Massimo D'Agostino (Università di Napoli "Federico II")- *Molecular mechanism for the retention in the ER of the mutant Frizzled4 L501fsX533, which results in the dominant form of familial exudative vitreorethinopathy (FEVR)*

15.20 – 15.40 Laura Cogli (Università del Salento)- *Molecular basis of Charcot- Marie-Tooth type 2B disease*

15.40 – 16.00 Andrea Vettori (Università di Padova) - *Developmental defects e motor neuron alterations due to mitofusin 2 gene (MFN2) silencing in zebrafish: a new model for Charcot-Marie-Tooth type 2A neuropathy*

16.00 – 16.15 Conclusioni e chiusura lavori

16.15 – 19.00 Assemblea soci AIBG

19.00 – Cena Sociale

ABSTRACT PRESENTAZIONI ORALI

Expression, localization and functional role of ribonucleoproteins in myoblasts differentiation.

Diani Erica[1], Filippello Agnese[1], Morandi Carlo[1], Romanelli Maria Grazia[1].

[1]*Università degli Studi di Verona*

Large-scale expressed sequence tag and genome-wide analyses estimate that the majority of human genes undergo alternative splicing with a differential tissue distribution. More than 15% of human genetic diseases are associated to mutations in the consensus splice sites and disruption of splicing regulatory networks contributes to various diseases. Alternative splicing of pre-messenger RNA represents, consequently, an intensive post-transcriptional regulatory activity that involves several RNA binding proteins and splicing regulators. They belong principally to SR (serine/arginine rich proteins) and hnRNP (heterogeneous nuclear ribonucleoprotein particles) proteins. Both types can act as enhancers or repressors of alternative splicing helping in defining cis-regulatory elements positioned either in the exon or in the intron sequences. Dynamic antagonism between members of the SR and hnRNP protein families is demonstrated to be important for determining a number of alternative splicing patterns. Among the hnRNPs that have been implicated in regulating the alternative splicing events with tissue-specificity, polypyrimidine tract binding protein (PTB) and its paralog nPTB play a well established role as negative splicing regulators. We have previously characterized PTB, its paralog nPTB and Raver1 (a PTB co-repressor factor) gene expression in human tissues and cell lines (1-3). We showed that alternative spliced nPTB transcripts are distributed with a tissue specificity involving brain and skeletal muscle. During muscle development PTB regulates exons splicing in several specific transcribed genes. In the present study we are investigating the contribution of ribonucleoproteins to splicing events occurring during myoblasts differentiation. We are analyzing the expression, subcellular localization and functional role of PTB, Raver1 and Rbm20 (a SR protein which mutations are associated to familial dilated cardiomyopathy) during C2C12 myoblast differentiation. Preliminary results show that Raver1 expression is reduced both at transcriptional and translational levels during differentiation and that its overexpression can affect exon inclusion in calpain 3 (CAPN3) and myotubularin-related protein 1 (MTMR1). Confocal microscopy analyses show that Raver1 diffuses in the cytoplasm from the nucleus, localizing in polarized cytoplasmic area during myoblasts differentiation. These studies may shed new light on the role of ribonucleopreoteins in the post-transcriptional regulation events that occur during muscle development in both nuclear and cytoplasmic compartments. 1. Romanelli et al. Biochim Biophys Acta; 2001, 1520: 85 – 88. 2. Romanelli et al. Gene; 2005, 356:11-18. 3 Romanelli et al. Gene; 2007, 405: 79 – 87.

The expression of a cytoplasmic Alu-like RNA promotes cell differentiation and reduces malignancy of human neuroblastoma cells

Pagano Aldo[1].

[1]*University of Genoa & National Institute for Cancer Research*

SUMMARY: Neuroblastoma (NB) is a pediatric cancer characterized by remarkable cell heterogeneity within the tumor nodules. Here, we demonstrate that the synthesis of a pol III-transcribed non-coding (nc) RNA (NDM29) strongly restricts NB development by promoting cell differentiation, a drop of malignancy processes and a dramatic reduction of the tumor initiating cells (TICs) fraction in the NB cell population. Importantly, the overexpression of NDM29 also confers to malignant NB cells an unpredicted susceptibility to the effects of antiblastic drugs used in NB therapy. Altogether these results suggest the induction of NDM29 expression as possible treatment to increase cancer cells vulnerability to therapeutics and the measure of its synthesis in NB explants as prognostic factor of this cancer type. SIGNIFICANCE: Tumor masses are composed of cells at variable differentiation stages and with different malignant potentials whose relative abundance in the nodules affects the tumor fate. This work demonstrates that the synthesis of a small RNA, NDM29, strongly reduces the malignant capacity of NB cells promoting their differentiation. During the NDM29-driven maturation cells also acquire a marked susceptibility to low dosages of specific antiblastics used in NB therapy. Thus, NDM29 RNA is of particular interest as the determination of its amount in tumor nodules might provide prognostic indication whereas the pharmacological induction of NDM29 expression might constitute a possible treatment to be used in synergy with antiblastic therapy.

Specific alterations of microRNA transcriptome and global network structure in colorectal carcinoma after cetuximab treatment

Ragusa Marco[1], Majorana Alessandra[1], Statello Luisa[1] Salito Loredana[1], Barbagallo Davide[1], Guglielmino Maria Rosa[1], Duro Laura Rita[1], Angelica Rosario[1], Caltabiano Rosario[2], Biondi Antonio[3], Di Vita Maria[3], Privitera Giuseppe[4], Scalia Marina[1], Cappellani Alessandro[3], Vasquez Enrico[2], Lanzafame Salvatore[2], Basile Francesco[3], Di Pietro Cinzia[1], Purrello Michele[1]

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The relationship between therapeutic response and modifications of miRNA transcriptome in Colorectal Cancer (CRC) remains unknown. We investigated this issue by profiling the expression of 667 miRNAs in two human CRC cell lines, one sensitive and the other resistant to cetuximab (Caco-2 and HCT-116, respectively) through TaqMan RT-PCR. Caco-2 and HCT-116 expressed different sets of miRNAs after treatment: specifically, 21 and 22 miRNAs were differentially expressed (DE) in Caco-2 or HCT-116, respectively (t-test, $p<0.01$). By testing the expression of DE miRNAs in CRC patients, we found that miR-146b-3p and miR-486-5p are more abundant in KRAS mutated samples respect to wild-type ones (Wilcoxon test, $p<0.05$). 67% of DE miRNAs were involved in cancer, including CRC, while 19 miRNA targets had been previously reported to be involved in the cetuximab pathway and CRC. We identified 25 TFs putatively controlling these miRNAs, 11 of which already reported to be involved in CRC. Based on these data, we suggest that the down regulation of let-7b and let-7e (targeting KRAS) and the up regulation of miR-17* (a CRC marker) could be considered as candidate molecular markers of cetuximab resistance. Global network functional analysis (based on miRNA targets) showed a significant overrepresentation of cancer-related biological processes and networks centered on critical nodes involved in EGFR internalization and ubiquitin-mediated degradation. The identification of miRNAs, whose expression is linked to the efficacy of therapy, should allow to predict the response of patients to treatment and possibly lead to a better understanding of the molecular mechanisms of drug response.

REFERENCES

Di Pietro C, Ragusa M., Barbagallo D et al., The Apoptotic Machinery As A Biological Complex System: Analysis of its Omics and Evolution, Identification of Candidate Genes for Fourteen Major Types of Cancer and Experimental Validation in CML and Neuroblastoma. BMC MEDICAL GENOMICS, 2:20, ISSN: 1755-8794

Ragusa M., Majorana A, Statello L et al.,. Specific Alterations Of MicroRNA Transcriptome And Global Network Structure In Colorectal Carcinoma After Cetuximab Treatment. MOLECULAR CANCER THERAPEUTICS. Submitted For Publication.

Acute spinal cord injury persistently reduces R/G RNA editing of AMPA glutamate receptors

Barbon Alessandro[1], Fumagalli Fabio[2], Luca Caracciolo[3],. Laura Madaschi[3], Lesma Elena[3], Mora Cristina[1], Carelli Stephana[2], Slotkin Theodore[4], Racagni Giorgio[2], Di Giulio Anna Maria[5], Gorio Alfredo[5], Barlati Sergio[1]

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Spinal cord injury (SCI) triggers a complex ischemic and inflammatory reaction with cell death and alteration of neurotransmitter systems. The SCI mediated enhancement of excitatory glutamatergic neurotransmission activates a dramatic neuronal influx of Ca++ , which may results in a series of most detrimental events. In the current study, we hypothesized that SCI may lead to alteration of the editing of AMPA glutamatergic receptors thus producing functional and relevant changes in neuronal survival after SCI. We examined RNA editing of different AMPA receptor subtypes at different time points after lesioning and in different portions of the cord, i.e. the epicenter, the caudal site and the rostral site, in order to have a complete temporal and spatial profile of AMPA receptor changes triggered by SCI. Our study shows that SCI strongly reduced AMPA receptor R/G editing levels. Such regulation occurs at the different portions of the lesioned tissue, although with different magnitude and timing, and is long-lasting, being observed as long as 30 days after lesioning. These changes may correlate with a partial decrease in enzymatic activity of ADAR2 (adenosine deaminase acting on RNA 2), as deduced from the analysis of ADAR2 self editing. AMPA receptor containing an unedited (R) subunits show a slower recovery rate from desensitisation compared to the edited (G) forms. The reduced editing at the R/G site of the GluRs subunit might result in attenuated responses to glutamate and reduced activation of postsynaptic receptors, thus avoiding, or at least attenuating, calcium-induced activation of postsynaptic neurons, which has been shown to be pathologically relevant. Reduced editing at the R/G site of the GluRs subunit might represent a defensive strategy under extreme conditions such as following SCI. These observations provide the first and direct evidence of the possible contribution of RNA editing of AMPA receptors in some of the compensatory mechanisms triggered by acute SCI. RNA editing might represent an important determinant of long-term outcomes of SCI, and thereby a novel pharmacological target for treating SCI. Barbon et al., Journal of Neurochemistry 2010, 114, 397-407

Molecular dissection of the regulation of miR-221/222 in prostate carcinoma and in glioblastoma.

Galardi Silvia[1], Ciafrè Silvia Anna[1], Farace Maria Giulia[1].

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MicroRNAs (miRNAs) are small noncoding RNAs that suppress gene expression post-transcriptionally by base pairing to the 3' untranslated regions (3'UTR) of the target mRNAs. There is a large body of evidence that dysregulation of miRNAs is a hallmark of cancer (Bartel, 2004). We and others have shown that miR-221 and miR-222, encoded in tandem from a gene cluster located on chromosome X, are up-regulated in several human tumors, among which we have studied glioblastoma multiforme (GBM) and prostate carcinoma. In these two contexts, we have found that one important mechanism by which the two miRNAs affect proliferation and tumorigenicity is via the downregulation of p27, a key inhibitor of cell cycle progression (Ciafrè et al., 2005; Galardi et al., 2007). To date, the great scientific effort that has been made toward the understanding of microRNA role has been especially devoted to the discovery of their mechanism; on the contrary, we still know very little about the regulation of miRNA expression. Since the overexpression of miR-221/222 actively contributes to aberrant proliferation of GBM and prostate carcinoma cells, then the comprehension of miR-221/222 regulation is essential for understanding GBM and prostate carcinoma growth. In the present research, we have deepened our knowledge about miR-221/222, by defining how their transcription is regulated. In particular, we investigated the involvement of NFk-B, whose pathway is often deregulated in both GBM and prostate carcinoma (Palayoor ST et al., 1999; Nagai et al., 2002) . We performed a bioinformatic analysis of the genomic sequence (~ 12.000 bp) upstream of miR-221/222 transcriptional unit and identified predicted NFk-B binding sites that are embedded in two highly conserved regions in vertebrates. In vivo and in vitro experiments allowed us to show that NFk-B directly interacts with the predicted binding sites in the two regulatory regions and it is functionally involved in miR-221/222 modulation both in GBM and prostate tumors. Moreover, we identified in AP-1, a second regulatory factor that is involved in miR222/221 modulation and cooperates with NFk-B in activating mir222/221 expression. The data obtained from this research suggested a role of NFk-B and Ap-1 pathways in the transcription regulation of miR221/222 gene and can justify miR221/222 common overexpression in GBM and prostate tumor. We will transfer our knowledge to the in vivo reality of animal models, where treatments combining the use of specific NFk-B/AP-1 inhibitors and targeting of miR-221/222 can be proposed and assayed. References •Bartel DP. (2004). Cell, 116, 281-297 •Ciafrè, S.A., Galardi, S., Mangiola, A., Ferracin, M., Liu, C.-G., Sabatino, G., Negrini, M., Maira, G., Croce, C.M., Farace, M.G. (2005) Biochem Biophys Res Com.334(4), 1351-8. •Galardi S, Mercatelli N, Giorda E, Massalini S, Frajese GV, Ciafrè SA, Farace MG. (2007) J Biol Chem. 282(32), 23716-24. •Nagai S, Kurimoto M, Washiyama K, Hirashima Y, Kumanishi T, and Endo S. (2005). J. Neurooncol., 74, 105-111. •Palayoor ST, Youmell MY, Calderwood SK, Coleman CN, Price BD. (1999). Oncogene, 18, 7389-7394

TRAM (Transcriptome Mapper): database-driven creation and analysis of transcriptome maps from multiple sources

Strippoli Pierluigi[1], Lenzi Luca[1], Facchin Federica[1], Pelleri Maria Chiara[1], Vitale Lorenza[1], Casadei Raffaella[1], Canaider Silvia[1], Frabetti Flavia[1]

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Various tools have been developed to perform global gene expression profile data analysis, to search for specific chromosomal regions whose features meet defined criteria as well as to study neighbouring gene expression. However, most of these tools are tailored for a specific use in a particular context (e.g. they are species-specific, or limited to a particular data format) and they typically accept only gene lists as an input. TRAM (Transcriptome Mapper) is a new general software tool that allows the simple generation and analysis of quantitative transcriptome maps, starting from any source listing gene expression values for a given gene set (e.g. expression microarrays), implemented as a relational database. It includes a parser able to assign univocal and updated gene symbols to gene identifiers from different data sources, as well as to perform intra-sample and inter-sample data normalization methods, including an original variant of quantile normalization (scaled quantile) useful to normalize data from platforms with highly different numbers of investigated genes. When in 'Map' mode, the software generates a quantitative representation of the transcriptome of a sample (or of a pool of samples) and identifies if chromosomal segments of defined length are over/under-expressed compared to the desired threshold. When in 'Cluster' mode, the software searches the genome for a set of over/under-expressed consecutive genes. Statistical significance for all results is calculated with respect to genes localized on the same chromosome or to all genome genes. Transcriptome maps, showing differential expression between two sample groups, relative to two different biological conditions, may be easily generated. We present the results of a test biological model, based on a meta-analysis comparison between a human CD34+ hematopoietic progenitor cells sample pool and a megakaryocytic cells sample pool, identifying biologically relevant chromosomal segments and gene clusters with differential expression during the differentiation toward megakaryocyte. The large agreement with classical biological knowledge about megakaryocytopoiesis of TRAM results, obtained without any a priori specific assumption, shows that TRAM can perform integrated analysis of expression data from multiple platforms producing high confidence lists of over/under-expressed chromosomal segments and clustered genes. TRAM is the first complete software usable in a personal computer (Macintosh and Windows environments) designed to create, and statistically analyze, quantitative transcriptome maps, based on gene expression data from multiple sources. In conjunction with our previous implementation of a GenBank [1, 2] and UniGene [3] formats full parsing systems, TRAM may also contribute to the building of a novel, relational, multi-purpose, user-friendly and modular platform for the large-scale integrated analysis of genomic and post-genomic data. References 1. D'Addabbo P, Lenzi L, Facchin F, Casadei R, Canaider S, Vitale L, Frabetti F, Carinci P, Zannotti M, Strippoli P: GeneRecords: a relational database for GenBank flat file parsing and data manipulation in personal computers. Bioinformatics 2004, 20:2883-2885. 2. Frabetti F, Casadei R, Lenzi L, Canaider S, Vitale L, Facchin F, Carinci P, Zannotti M, Strippoli P. Systematic analysis of mRNA 5´ coding sequence incompleteness in Danio rerio: an automated EST-based approach. Biology direct, 2:34, 2007. 3. Lenzi L, Frabetti F, Facchin F, Casadei R, Vitale L, Canaider S, Carinci P, Zannotti M, Strippoli P: UniGene Tabulator: a full parser for the UniGene format. Bioinformatics 2006, 22:2570-2571. Acknowledgements This work has been conducted in collaboration with Francesco Piva, Matteo Giulietti and Giovanni Principato (Università Politecnica delle Marche), Stefania Bortoluzzi, Alessandro Coppe and Gian Antonio Danieli (Università di Padova) and Sergio Ferrari (Università di Modena e Reggio Emilia).

The shapes of native mammalian polyribosomes

Viero Gabriella[1], Lunelli Lorenzo[2], Gilbert Robert J. [3], Arseni Natalia[1], Provenzani Alessandro[1], Pederzolli Cecilia[2], Quattrone Alessandro[1]

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The precise assembly of native eukaryotic polysomes and the organization of ribosomes along the mRNA are still obscure. Despite different imaging approaches have been used to visualize bacterial and eukaryotic polysomes, human native polysomes have never been observed in physiological conditions. Nothing is known, moreover, about formation of the mRNA-ribosomes macroassembly. Atomic Force Microscopy (AFM) is a powerful technique to image biomolecules under near-native conditions, and without the addition of any fixing or external contrast agent. We employed an innovative approach to visualize polysomes from three mammalian cell lines (rat PC12, human MCF7 and HeLa cells) by AFM. After isolation by sucrose gradient centrifugation polysomal fractions have been characterized both in liquid and air, with a protocol aimed at maximally preserving their original assembly. Structural parameters of the ribosomes measured in these conditions (height, width and area) are compatible with those previously measured by AFM and electron microscopy (EM). For the first time to our knowledge, we could be able to observe ribosome aggregates associated to filaments compatible with single-stranded and double stranded RNA, providing therefore the imaging of intact polysome assemblies. The number of ribosomes for polysome ranges, depending on the density of the fraction analyzed, from 1-2 to more than 25 ribosomes. A common belief is that the more the polysome is packed the more constrained is expected to be the mRNA. This traditional model is not completely compatible with our images. Indeed, we have observed free RNA protruding from ribosomes, suggesting that the mRNA is not completely covered by translating ribosomes. This observation opens new questions concerning which and where proteins (most importantly components of the eIF4F complex) are in the polysome. In order to identify proteins in the polysomes, immunogold labelling was used. Golden beads associated to polysomes has been observed when using an antibody against the iIF4F components PABP or eIF4E, representing the first images of these proteins in ribosome associations.
Modulation of the conditions of deposition of polysomes on the mica allowed us also to visualize unpacked polysomes under the form of planar circular structures, therefore producing confirmation of the closed-loop model which has been indirectly demonstrated by twenty years of biochemical and genetic evidence.
Finally, we have used cryo-EM to explore the ribosome-ribosome structural interaction in native human polysomes. By using single particle reconstruction, the very first image of the native human ribosome assembly appears, suggesting the existence of a complex pattern of conformations but also the tendency to organize these conformations around a simple, modular structure. The systematic analysis of polysomal aggregates by this near-native methodology could reveal novel levels of polysomal organization, and provide structural testing of the current view about polysome physiology derived from biochemical data.

Dynamic correlation between differential gene expression and chromosome territories' topology during normal human myelopoiesis.

Lomiento Mariana[1], Orlandi Claudia[1], Ferrari Francesco[2], Ferrari Sergio[3]

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Human myelopoiesis is an interesting biological model to study cellular differentiation. It represents a plastic process during which multipotent stem cells gradually limit their differentiation potential, generating different precursor cells that finally evolve into distinct terminally differentiated cells. This process is regulated by ordered patterns of gene expression, where specific combinations of transcription factors or chromatin remodeling complexes result to be responsible for the different genetic programs underlying hematopoietic stem cell commitment and differentiation. The organization of a eukaryotic nucleus reflects its specific expression profile. On the genomic scale, this translates to preferred tissue-specific chromatin folding and chromosome positioning within interphase nuclei, including intermingling of looped segments within and between chromosomes. Currently, the development and utilization of high-resolution microscopy and high-throughput methods to detect gene interactions are vastly expanding the knowledge of chromatin dynamics modification in respect to the regulation of gene expression. The use of new bioinformatic methodologies has allowed us to define transcriptomal maps and to identify chromatin domains of constitutively, differentially expressed, coregulated or not expressed genes in myeloid cells. The possibility of confocal microscopy techniques has allowed us to characterize, in different cellular contests, the localization of chromosomes (which occupy distinct domains in the interphase nucleus, known as "chromosome territories" or CT) and the positional effect on expressed or silent genes by 3D-FISH. We have focused our attention in setting 3D-FISH analysis on human HSC (Hematopoietic Stem Cells) /progenitor as well as myeloid precursors interphase nuclei. Since there are no literature data in literature concerning the application of this technique on HSC interphase nuclei, it has been necessary to identify the best experimental conditions to preserve cell morphology and to perform the hybridization. The application of the 3D-FISH technique to the analysis of intranuclear localization of the chromatin domains already characterized in human myeloid cells, has allowed the evaluation of their possible role in the differentiation plasticity of HSCs and their potential modification throughout the differentiation process. According to the preliminary findings, it seems that highly expressed chromosome domains localize at the surface of the CT's volume, where they could be exposed to regulatory factors, thus contributing to proper gene function. On the contrary, silent clusters seem to localize at the interior of the CT volume and at the nuclear periphery, where transcription repressed heterochromatin domain are clustered. It has been postulated that the non-random, spatial organization of the genome within the cell nucleus contributes also to determine the outcome of chromosomal translocation. Comparative analysis of the spatial arrangement of translocations partners and their frequency of translocation suggests that translocations occur preferentially among proximally positioned genome regions. Early 3D-FISH experiment using WCPs (Whole Chromosomes Paintings) specific for a selection of human chromosomes involved in recurrent chromosome translocation in AML (Acute Myeloid Leukemia), has allowed us to examine the intranuclear localization of their respective CTs within the interphase nuclei. Preliminary findings obtained on HSCs and myeloblast cells, evidenced that all couples of chromosomes analyzed, have a strong tendency to associate within the interphase nuclei of precursor cells. These data strongly suggest a non-random spatial organization of the genome within the nucleus, also involving dynamic association of CTs' 3D positioning.

Generation of Functional Hepatocytes from Mouse Germline Cell-derived Pluripotent Stem Cells in vitro

Fagoonee Sharmila[1], Hobbs Robin[2], De Chiara Letizia[1], Tolosano Emanuela[1], Medico Enzo[3], Provero Paolo[1], Pandolfi Pier Paolo[2], Silengo Lorenzo[1], Altruda Fiorella[1].

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Germ line cell-derived pluripotent stem cells (GPSCs) are derived from spermatogonial stem cells and are similar to embryonic stem (ES) cells in that they can proliferate intensively and differentiate into a variety of cell types. Previous studies have revealed some inherent differences in gene expression between undifferentiated mouse ES cells and GPSCs. Our aims were to generate functional hepatocytes from mouse GPSCs in vitro and to investigate whether the differences in gene expression may impact on the hepatocyte differentiation capacity of the GPSCs compared with ES cells. Mouse GPSCs and ES cells were induced to differentiate into hepatocytes through embryoid body formation, with very high efficiency. These hepatocytes were characterized at cellular, molecular, and functional levels. The GPSC-derived hepatocytes expressed hepatic markers and were metabolically active as shown by albumin and haptoglobin secretion, urea synthesis, glycogen storage, and indocyanine green uptake. We also performed an unprecedented DNA microarray analysis comparing different stages of hepatocyte differentiation. Gene expression profiling demonstrated a strong similarity between GPSC and ES cells at different stages of induced hepatic differentiation. Moreover, Pearson correlation analysis of the microarray datasets suggested that, at late hepatic differentiation stages, the in vitro-derived cells were closer to fetal mouse primary hepatocytes than to those obtained from neonates. We have shown for the first time that adult GPSCs can be induced to differentiate into functional hepatocytes in vitro. Moreover, our ongoing in vivo work shows that GPSC-derived hepatocytes can colonize the liver of monocrotaline-treated, partially hepatectomised mice. These GPSC-derived hepatocytes thus offer great potential for cell replacement therapy for a wide variety of liver diseases.

Extremely Low Frequency Electromagnetic Field (ELF-EMF) enhances the energy metabolism of human spermatozoa and does not interfere with sperm/oocyte interaction

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The exposure of human spermatozoa to an ELF-EMF with a square waveform of 5 mT amplitude and frequency of 50 Hz improves sperm motility [1]. As the energy metabolism plays a central role in supporting flagellar movement, we studied the functional relationship between the energy metabolism and the enhancement of human sperm motility induced by ELF-EMF. Moreover, the effect(s) of ELF-EMF on human sperm functions involved in the fertilization process was investigated. RESULT AND DISCUSSION At 2 h and 3 h of exposure to ELF-EMF (5mT, 50 Hz) the stimulatory effect induced by ELF-EMF on sperm kinematic parameters (VAP and VSL) was associated with a progressive increase of mitochondrial membrane potential and levels of ATP, ADP and NAD+. No significant effects were detected on other parameters such as ATP/ADP ratio and energy charge. To examine which metabolic pathways was involved in the enhancement of sperm energy production, spermatozoa were exposed to ELF-EMF in medium containing different energy substrates (glucose or pyruvate and lactate) in the presence or in the absence of mitochondria or glycolisis inhibitor. When ClCCP (carbamoyl cyanide m-chlorophenylhydrazone) was applied to suppress the oxidative phosphorylation in sperm exposed to ELF-EMF with glucose as energy surge, the values of energy parameters and motility were maintained at the equivalent level to that of the control indicating that the glycolytic process was not involved in mediating the ELF-EMF stimulatory effect on sperm energy metabolism and motility. On the contrary, when pyruvate and lactate were provided instead of glucose, a significant increase of the energy metabolism and motility parameters was observed in ELF-EMF treated sperm, showing that the enhancement in the flagellar movement induced by ELF-EMF was supported by mitochondrial activity. However, in this experimental condition, it was not excluded the possibility that gluconeogenesis might be activated to produce glucose for use in glycolysis by the energy substrates obtained from respiration. To investigate this chance, spermatozoa exposed to ELF-EMF were incubated with mitochondrial substrates and with DOG (2-deoxy-D-glucose) to block glycolysis. Also in these experimental conditions, the values of energy parameters resulted significantly increased respect to the control, strengthening the idea that glygolysis is not involved in the enhancement of energy metabolism induced by ELF-EMF in human sperm. To investigate whether ELF-EMF could affect sperm functions implicated in the fertilization process, we evaluated the ability of human sperm exposed to ELF-EMF to fuse with oocytes by means of the hamster egg penetration test (HEPT). The effect on acrosome reactions (AR) was also evaluated. The exposure of sperm to ELF-EMF for 3 h did not interfere with sperm ability to fuse with oocytes either in the conventional version of the HEPT or in the version enhanced with progesterone (P). No interference with the stimulatory effect on AR exerted by P was revealed. Moreover, no effect on the DNA fragmentation (Tunel assay) was detected. In conclusion, the present study provides evidence that the key role in mediating the stimulatory effect exerted by ELF-EMF on energetic status and motility of human sperm is played by mitochondrial oxidative phosphorylation rather than by glycolysis. Moreover, these results indicate that the ELF-EMF does not interfere with sperm interaction with oocytes. This is noteworthy in the view of a possible clinical use of ELF-EMF as an "in vitro" stimulator of the sperm motility of asthenozoospermic patients for assisted reproduction techniques. BIBLIOGRAFIA [1] R. Iorio, R. Scrimaglio, E. Rantucci, S. Delle Monache, A. Di Gaetano, N. Finetti, F. Francavilla, R. Santucci, E. Tettamanti, and R. Colonna, "A preliminary study of oscillating electromagnetic field effects on human spermatozoon motility", Bioelectromagnetics, vol. 28, pp. 72-75, 2007

Mesenchymal Stem Cell Behavior in Biomaterials Assays

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Introduction: in the orthopedic perspective, tissue engineering is focused on the development of innovative materials, whose action consists in recruiting bone progenitor cells and in stimulating their proliferation. In this study, we investigated the interaction between human mesenchymal stem cells from adults and two types of biomaterials, which differ for chemical composition and format. To evaluate the effects of porosity on cell adhesion and proliferation comparative analyses were carried out on different forms of two bioceramic materials. Materials and Methods: Bone marrow aspirates from iliac crests were obtained from orthopedic patients who underwent bone marrow harvesting under general anesthesia. Mesenchymal stem cells (hMSCs) were isolated by Ficoll-mediated discontinuous density gradient centrifugation and polystyrene adherence capacity. Cell cultures at the second passage were characterized, by the flow cytometric analysis (FCA) of specific surface antigens. A surface marker-expression pattern, positive (Stro-1, CD29, CD44, CD71, CD73, CD90, CD105) and negative (CD45, CD34, CD235), was employed to characterize the cells obtained. hMSCs were expanded and duplication time evaluated. The AlamarBlue assay was used to evaluate the number of cells attached to the biomaterials and to the control in adhesion and proliferation assay. The quantification of the relative amount of Focal Adhesion Kinase was employed to evaluate the strength of the cell adhesion to the biomaterials after 36 hours from seeding. In order to verify the morphology of the hMSCs attached to bioceramics, stem cells grown on the biomaterials were analyzed by SEM. Moreover, to verify whether the cytoskeletal organization of hMSCs cultured on the analyzed biomaterials was altered, actin fibers were investigated using TRITC conjugated-Phalloidin. Results: the biomaterials assayed in our study provided a favorable environment for hMSCs adhesion and growth, without any evidence of cytotoxic effects. Indeed, hMSCs adhesion and growth assays indicate that shape and porosity influence the cytocompatibility of bioceramics. We may infer that intra- and iter- porosity of biomaterials investigated herein, are determinant factors influencing hMSCs behavior. Besides, the correlation between vitality and a greater amount of Focal Adhesion Kinase at molecular level show how cellular processes may be modulated by structured surface and three dimensional architecture of biomaterials. hMSC SEM analysis showed an elongated spindle-like morphology. The cells adhered to biomaterials with many dendritic projections with some differences in the morphology due to scaffolds structure. Moreover, hMSCs cytoskeletal architecture on biomaterials was indistinguishable from that of hMSCs grown on plastic petri dishes, used as a control. Conclusion: These assays confirm the feasibility to evaluate biomaterials biological features in pre-clinical environment with patients mesenchymal stem cells in order to obtain information about subject specific response. Moreover, the development of new matrices have to consider the possibility to employ bioactive surfaces able to modulate cell adhesion and differentiation processes. References Tampieri A, Celotti G, Landi E. From biomimetic apatites to biologically inspired composites. Analytical and Bioanalytical Chemistry 2005;381(3):568-576. Lennon DP, Caplan AI. Isolation of human marrow-derived mesenchymal stem cells. Exp Hematol 2006;34(11):1604-5. Morelli C, Barbanti-Brodano G, Ciannilli A, Campioni K, Boriani S, Tognon M. Cell morphology, markers, spreading, and proliferation on orthopaedic biomaterials. An innovative cellular model for the "in vitro" study. J Biomed Mater Res A 2007;83(1):178-83. Chou Y-F, Huang W, Dunn JCY, Miller TA, Wu BM. The effect of biomimetic apatite structure on osteoblast viability, proliferation, and gene expression. Biomaterials 2005;26(3):285-295.

Cell death programs: one, two or many? Why discussing about programmed necrosis is no longer a heresy

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Cells can efficiently eliminate themselves through a sophisticated genetic program known as apoptosis. Proteolytic processing of selected cellular proteins, as operated by a unique family of cystein-proteases called caspases, is necessary to induce apoptosis. Two main apoptotic pathways, the extrinsic and the intrinsic, keep in check caspase activation. The extrinsic pathway controls procaspase-8 maturation and it is triggered by the engagement of death receptors placed at the cell surface. By contrast, the intrinsic pathway, which is responsible for procaspase-9 maturation, is triggered by the release of killer mitochondrial proteins into the cytosol, after MOMP (mitochondrial outer membrane permeabilization). MOMP is emerging as the point of no return for many death pathways. In the presence of massive MOMP, the cellular demise could take place alternatively, as an effect of the mitochondrial metabolic failure or by means of some still mysterious caspase-independent deaths. MOMP and the release of the mitochondrial pro-apoptotic factors into the cytosol are under the antagonistic control of the master regulators of the apoptotic process: the members of the Bcl-2 family. We have recently identified a new caspase-independent death pathway, which is triggered, in cells resistant to apoptosis, by a new class of isopeptidase inhibitors known as NS-IIs (non-selective isopeptidase inhibitors). We will discuss of this new pathway and its role in triggering caspase-independent death in cells highly resistant to apoptosis such as glioblastoma.

Necdin protects myoblast from programmed cell death by interfering with the mediator of apoptosis CCAR1/CARP1

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Skeletal muscle tissue is characterized by a very slow turn over that increases however upon certain physiological stimuli or in pathological conditions, such as primary myopathies, leading to an extensive repair process aimed at preventing the loss of muscle mass. The initial phase of muscle repair is characterized by necrosis of the damaged tissue and activation of an inflammatory response . Local cues, produced by growth factors and inflammatory cytokines released by infiltrating cells, lead to the activation of quiescent myogenic cells, the satellite cells, located beneath the basal lamina of muscle fibers, that start to proliferate, differentiate and fuse, leading to new myofiber formation and reconstitution of a functional contractile apparatus. A delicate balance between cell proliferation and exit from cell cycle, differentiation and fusion is required for the correct muscle regeneration to occur, and many proteins have been found to play a crucial role in these processes. We have recently identified Necdin as a critical key player in muscle regeneration. Necdin is a member of the MAGE family (Barker and Salehi, 2002), a large family of proteins initially isolated from melanomas, carachterized by a large central region termed the MAGE homology domain MHD. We have shown that Necdin is express in satellite cells and is able to sustain efficient muscle differentiation and regeneration by acting on two different pathways: on myoblast differentiation, by direct transcriptional regulation of myogenin, in cooperation with MyoD and by protecting myoblasts from cell death (Deponti et al., 2007). We have shown that necdin inhibit the activation of both Caspase 3 and 9, probably by exerting its function via the intrinsic death pathway, but still the precise molecular mechanism is unknow. By using the Two Hybrid system in yeast we were able to identify new interactors of Necdin in myogenic cells. In our study, we identified for the first time an interesting protein-protein interaction between Necdin and CCAR1 in myogenic precursors. This protein also known as CARP1, cell cycle and apoptosis regulatory protein-1, is a perinuclear protein that mediates apoptosis signalling by diverse agents, leading to the activation of caspase 9 and 3, members of Jun N-terminal kinase (JNK) and p38 MAPK family of proteins. In this work we have shown that Necdin is able to counteract the apoptotic effect of CCAR1, as shown in C2C12 transfected with Necdin and CCAR1 and in satellite cells; we therefore postulate that Necdin can block the induction of cell death by blocking the action of CCAR1.

Silencing of glyooxalase I defines a role in apoptosis in LNCaP and PC3 human prostate cancer cells

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Glyoxalase I (GI) and Glyoxalase II (GII) constitute the glyoxalase system, a ubiquitous detoxification pathway protecting against cellular damage caused by potent cytotoxic metabolites such as methylglyoxal (MG). MG, a highly reactive alpha-oxoaldehyde, generated by oxidation of carbohydrate and glycolysis, binds to proteins and forms advanced glycation end products (AGE). Formation of AGE contributes to the development of pathological conditions such as diabetes and cancer. MG is converted to S-D-lactoylglutathione (SLG) by GI, with reduced glutathione as a cofactor, and GLS in turn is hydrolyzed by GII to D-lactate along with generation of reduced glutathione. MG is a cytotoxic metabolite, potent inhibitor of cellular growth and capable of inducing apoptosis (1). GI inhibitors have been proposed as potential anti-cancer agents in vivo, inducing intracellular increased of cytotoxic MG. Furthermore, most tumor cells displayed increased expression of GI, suggesting its involvement in cellular growth regulation. Furthermore, a possible involvement of GI in apoptosis mediated by MG-derived AGE, has been proposed (2). In order to further elucidate the biological function of GI in the regulation of cell proliferation, and or apoptosis, RNA silencing of GI gene by RNA interference has been performed in human prostate non aggressive and metastatic cell line, LNCaP and PC3, respectively. A comparative study of cell proliferation and apoptosis was performed, analyzing incorporation [3H]thymidine and flow cytometry, 72 h after GI silencing. AGE levels were further assessed by Western blot. The results show that GI silencing has not effect on cell growth of the two cell lines, but induces apoptosis, mediated by MG-derived AGE, in PC3 cells. On the contrary, neither apoptosis nor AGE significant modification were observed in LNCaP cells, after GI silencing. In order to evaluate if the AGE increased, observed in PC3 cell line, was effectively related to MG, the cells were treated for 72 h with a concentration of 0.8 and 1 mM MG in absence or presence of GI silencing. A comparative evaluation of the possible effect of MG on cell proliferation and apoptosis was performed, assessing the same assay on LNCaP cells. The evaluation of cell proliferation, apoptosis and AGE levels did not show any effect of MG on cell proliferation, but shows an induction of apoptosis in both cell lines, significantly increased in presence of GI silencing. However, the degree of the biological apoptotic effect induced by MG, and the dose related to the activation of the response, different in the two cell lines, could suggest different apoptotic mechanisms. The evaluation of protein levels of caspase3, Bax, BCl2 and BCl-XL, independent from AGE in PC3 and related to them in LNCaP cell lines, could seem to confirm this hypothesis. The apparent paradox in the obtained results could be explained on the basis of the different susceptibility of the two cell lines to MG. Its intracellular concentration, increased after GI knockdown or MG exposure or both, could be different and trigger different AGE levels. The present study pointed out, for the first time, the involvement of GI in the apoptosis process of adenocarcinoma cells and provides further evidence on the different susceptibility of cells to MG (2). 1. de Hemptinne V, Rondas D, Toepoel M, Vancompernolle K. Phosphorylation on Thr-106 and NO-modification of glyoxalase I suppress the TNF-induced transcriptional activity of NF-kappaB. Mol Cell Biochem. 2009,325(1-2):169-178. 2. Van Herreweghe F, Mao J, Chaplen FWR, Grooten J, Gevaert K, Vandekerckhove J, Vancompernolle K: Tumor necrosis factor induced modulation of glyoxalase I activities through phosphorylation by PKA results in cell death and is accompanied by the formation of a specific methylglyoxal-derived AGE. PNAS 99(2): 949–954, 2001.

Mitochondrial permeability transition: identification of new chemotherapy target and molecular diagnosis tools

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Mitochondrial membrane permeabilization is considered the point-of-no-return in numerous models of cell death. Mitochondrial permeability transition (MPT) occurs via multiple mechanisms, among which the opening of a multiprotein complex located at the contact sites between inner and outer mitochondrial membrane, the mitochondrial permeability transition pore (MPTP). The central role of mitochondria in mediating cell death has led to an interest in exploiting chemoterapeutic agents that, by acting on such organelles, may trigger cancer cell death. Among the different pharmacological targets, components of the MPTP such as the adenine nucleotide translocase (ANT) and the 18kDa translocator protein (TSPO, formerly known as the peripheral benzodiazepine receptor) are object of intense researches due to the promising antiproliferative effects of their ligands. TSPO and some ANT isoforms are robustly expressed in tumors and TSPO expression level has been positively correlated with tumor malignancy. In the last years we focused our research in the analysis of the effects elicited by classical and new-synthesized TSPO ligands on cancer cell death as well as in the development of new molecules targeting this protein as promising imaging tools. We demonstrated that TSPO ligands are able to induce apoptosis in different types of highly resistant cancer cells. We also investigated on the activity of some selected MPT-inducing drugs (Betulinic Acid, Lonidamine, CD437) thought to act through ANT and we demonstrated that these agents are able to produce concentration-dependent cytostatic and cytotoxic effects in parallel with MPT induction triggered through MPTP. Finally, our functional studies indicate that TSPO acts by facilitating cell proliferation, migration and invasiveness while the silencing of ANT isoform 1 strongly reduces tumor cell viability by inducing a non-apoptotic cell death process resembling paraptosis. We demonstrated that cell death induced by ANT1 depletion cannot be ascribed to the loss of the ATP⁄ADP exchange function of this protein. By contrast, our findings indicate that ANT1-silenced cells experience oxidative stress, thus allowing us to hypothesize that the effect of ANT1-silencing is mediated by the loss of the ANT1 uncoupling function. On the whole our studies suggest that the pharmacological or genetic targeting of MPTP components may be helpful as adjuvant treatment to combat tumor cell proliferation or invasiveness.

Cathepsin D expression in the rat hippocampus after trimethyltin intoxication: an in vivo and in vitro study

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Trimethyltin chloride (TMT) is know to produce in the rat hippocampus neuronal damage, especially in the CA1/CA3 subfields, and reactive astrogliosis. An our previous study indicates that in cultured rat hippocampal neurons the Ca2+ cytosolic increase induced by trimethyltin is correlated to apoptotic cell death (Piacentini et al., 2008), although some molecular aspects of the hippocampal neurodegeneration induced by the neurotoxicant remain still to be clarified. Cathepsin D (Cat D) is a lysosomal aspartic protease involved in several neurodegenerative processes that occur in ischemia and Alzheimer's disease and moreover seems to play an important role in the apoptotic regulation processes (Liaudet-Coopman et al., 2006). In this context we have investigated the specific activity and the cellular localization of Cat D in rat hippocampus (female Wistar) in vivo, after 1-21 days TMT intoxication (single intraperitoneal injection, 8mg/kg body weight) and in organotypic rat hippocampal slices cultured in vitro according to Stoppini (1991), exposed for 48 h to 10 µM TMT treatment. (Noraberg et al., 1998). Cat D activity was assayed in hippocampus homogenates of control and TMT-treated rats according to Barrett (1977), using haemoglobin as substrate. In order to visualize the distribution and cellular localization of Cat D immunoreactivity in the hippocampus, a double-label immunofluorescence for Cat D and NeuN, GFAP, OX42 was respectively carried out. The results obtained in vivo, show a progressive, significant increase of the total hippocampal Cat D activity up to 14 days of TMT treatment (60%) and then a decrease up to 21 days treatment (37%). Immunofluorescence analysis shows that after 5-7 days TMT treatment Cat D markedly labels several hippocampal neurons of CA3 region, but only few labelled glial cells were observed. Starting from the 7th day of TMT treatment, in all hippocampal areas, Cat D-immunolabelling was found mainly in glial cells (largely associated with reactive astrocytes and in a reduced extend with microglial cells) where a marked and progressive increase occurs. According to our findings in vivo, double immunolabelling experiments on rat hippocampal TMT treated cultures show a marked Cat D expression especially in neuronal cells of CA3 area. The present data taken together demonstrate that TMT treatment in rat hippocampus, induces height levels of Cat D activity both in vivo and in vitro, in glial cells and in CA3 neurons, where a marked TMT-induced neuronal loss occurs. In addition, in order to clarify the possible relationship between Cat D activity, neuronal calcium overload and neuronal death processes, organotypic hippocampal cultures were also treated with Ca2+ chelator (BAPTA–AM) 50 µM or Calpain inhibitor (Calpeptin) 100 µM or Cat D inhibitor (Pepstatin A) 10 µM in the presence of TMT. In treated cultures, both BAPTA-AM and Calpeptin, reduce significantly the immunoreactivity for neuronal Cat D, indicating that the TMT-induced increase of Cathepsin D is a Calcium-Calpain-dependent phenomenon. Furthermore, Pepstatin A, markedly reduces neuronal death in CA3 region of hippocampal organotypic cultures, supporting the hypothesis that Cat D was involved in neuronal cells death processes that occur in our experimental model of neurodegeneration.

Novel hypotheses on the origin and regulation of a small heat shock gene in Lactobacillus plantarum WCFS1

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Small heat shock proteins (sHsps) are ubiquitous molecular chaperones that prevent the unspecific aggregation of proteins, especially when cells are exposed to heat stress [1]. Three sHsp-encoding genes (hsp18.5, hsp 18.55, hsp19.3) of the lactic acid bacterium (LAB) Lactobacillus plantarum, have been recently characterized including their expression pattern in response to abiotic stress and the effects of their homologous over-expression on stress tolerance [2,3,4]. Sequence analysis of the three hsp promoters indicated potentially different regulation. The presence of CIRCE elements in the 5'non-coding regions of hsp18.5 and hsp19.3 points to a HrcA-dependent regulation. Moreover, the hsp 18.5 gene is under CtsR control [5]. However, the regulation of the third member of hsp genes family in L. plantarum is still a puzzle. In this work, the operon organization of L. plantarum lp_2669-hsp18.55 was analysed by 5' RACE. Sequence analysis of the promoter revealed a putative σβ cis-element, homologues to known σβ–dependent promoters of Bacillus subtilis - the genetic model organism of Gram-positive bacteria - suggesting that hsp18.55 might be transcriptionally controlled by the alternative sigma factor σβ under stress conditions, although this type of promoter has never been reported in L. plantarum. By contrast, a gene encoding the alternative sigma factor sigma H, analogous to the Gram negative sigma 32 factor, was identified in L. plantarum WCFS1 [6]. To gain insights into this aspect, hsp18.55 expression was examined in L. plantarum ΔctsR and ΔftsH mutant strains, being CtsR a transcriptional regulator of ftsH and FtsH a proteolytic regulator of the alternative σH factor. Based on the obtained results, we propose a potential regulation mechanism for the hsp 18.55 of L. plantarum via the alternative sigma H factor. In order to assess the phylogenetic relationship of sHsp homologs among LAB, currently available completely sequenced genomes were surveyed for the presence of sHsp genes. The unrooted neighbor-joining phylogenetic tree obtained through alignment of the sHsps α-crystallin domain and the comparative analysis of the corresponding genetic loci both suggested a horizontal gene transfer event between L. plantarum WCFS1 and Lactobacillus brevis ATCC 367. References 1) Narberhaus F. (2002) α-Crystallin-Type Heat Shock Proteins: Socializing Minichaperones in the Context of a Multichaperone Network. Microbiol. Mol. Biol. Rev.66, 64–93. 2) Spano G., Beneduce L., Perrotta C., Massa S. (2005) Cloning and characterization of the hsp 18.55 gene, a new member of the small heat shock genes family isolated from wine Lactobacillus plantarum. Research. Microbiol.156, 219-224. 3) Spano G., Capozzi V., Vernile A., Massa S. (2004) Cloning, molecular characterization and expression analysis of two small heat shock genes isolated from wine Lactobacillus plantarum. J. Appl. Microbiol.97, 774-782. 4) Fiocco D., Capozzi V., Goffin P., Hols P., Spano G. (2007) Improved adaptation to heat, cold, and solvent tolerance in Lactobacillus plantarum. Appl. Microbiol. Biotechnol.77, 909–915. 5) Fiocco D., Capozzi V., Collins M., Gallone A., Hols P., Guzzo J., Weidmann S., Rieu A., Msadek T., Spano G. (2010) Characterization of the CtsR stress response regulon in Lactobacillus plantarum. J. Bacteriol.192, 896-900. 6) Kleerebezem M., Boekhorst J., Kranenburg R., et al. (2003) Complete genome sequence of Lactobacillus plantarum WCFS1. Proc. Natl. Acad. Sci. USA.100, 1990–1995.

CDH16 gene expression in thyroid gland development and tumor progression

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Objectives: CDH16/Ksp-cadherin was characterized as a kidney-specific adhesion molecule belonging to the 7-D cadherin family. More recently, CRYAB/alpha B-crystallin was identified as a binding partner of its cytosolic domain and it was suggested to mediate the connection to the actin cytoskeleton. Following our preliminary expression data, we decided to investigate the expression of CDH16 in thyroid cells and its potential role in cell differentiation and transformation. Methods: The expression of CDH16 and CRYAB in thyroid gland and in thyroid cells was investigated by microarray analysis and by qRT-PCR. Protein localization in the thyroid gland was determined by immunofluorescence and confocal microscopy. TSH-dependent expression of CDH16 was investigated in rat thyroid cell cultures by western blot and immunofluorescence. CDH16, CDH1/E-cadherin and CRYAB expression in human thyroid tumors was determined by qRT-PCR and by TMA immunofluorescence and confocal microscopy analysis. Results: CDH16 was expressed in the follicular cells of human and mouse thyroid gland, where it co-localized with CDH1 at the basolateral plasma membrane. CRYAB was expressed as well in thyrocytes and its staining was more diffuse all over the cell. The expression of CDH16 in thyroid cell cultures was dependent upon TSH as other thyroid differentiation markers, such as Pax8, sodium/iodide symporter and thyroperoxidase. During embryonic development, CDH16 was expressed in the mouse thyroid at E10.5, 1-2 days after the main thyroid-specific transcription factors involved in thyroid cell differentiation, and its expression persisted throughout the adult life. In human thyroid tumors CDH16 and CDH1 expression was progressively lost as tumors progressed from benign adenomas to differentiated carcinomas (PTC, FTC) and anaplastic carcinomas (PDTC, ATC). CRYAB expression was also markedly decreased. By confocal microscopy, CDH1-negative tumor cells were negative for CDH16. However, CDH16-negative cells could be positive for CDH1 staining, indicating that the reduction in the expression of CDH16 occurred earlier and was more pronounced than that of CDH1. Conclusions: CDH16 is expressed in the thyroid gland during embryonic development and in the adult, and TSH regulates its expression in thyroid cell cultures. CDH16, as well as CDH1, is lost in thyroid tumors, but the former is the first to be lost during tumor progression. CRYAB is also down-regulated in thyroid tumors. Overall these results are suggestive of a role of CDH16 in thyroid gland development and in the process of tumor progression. CDH16 may turn out to be relevant as a diagnostic and/or prognostic marker in thyroid carcinomas.

Estrogen receptor beta exerts tumor repressive functions in human malignant pleural mesothelioma

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Background: The role of estrogen and estrogen receptors in oncogenesis has been investigated in various malignancies. Recently our group identified estrogen receptor beta (ERβ) expression as an independent prognostic factor in the progression of human Malignant Pleural Mesothelioma (MMe), but the underlying mechanism by which ERβ expression in tumors determines clinical outcome remains largely unknown. This study is aimed at investigating the molecular mechanisms of ERβ action in MMe cells and disclosing the potential translational implications of these results. Methods: We modulated ERβ expression in REN and MSTO-211H MMe cell lines and evaluated cell proliferation and EGF receptor (EGFR) activation. Results: Our data indicate that ERβ knockdown in ER positive cells confers a more invasive phenotype, increases anchorage independent proliferation and elevates the constitutive activation of EGFR-coupled signal transduction pathways. Conversely, reexpression of ERβ in ER negative cells confers a more epithelioid phenotype, decreases their capacity for anchorage independent growth and down-modulates proliferative signal transduction pathways. We identify a physical interaction between ERβ, EGFR and caveolin 1 that results in an altered internalization and in a selective reduced activation of EGFR-coupled signaling, when ERβ is over-expressed. We also demonstrate that differential expression of ERβ influences MMe tumor cell responsiveness to the therapeutic agent: Gefitinib. Conclusions: This study describes a role for ERβ in the modulation of cell proliferation and EGFR activation and provides a rationale to facilitate the targeting of a subgroup of MMe patients who would benefit most from therapy with Gefitinib alone or in combination with Akt inhibitors.

HIV-1 Tat counteracts the post-activation turn-off of NF-κB through association with IκB-α repressor and p65 subunit of NF-κB

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Background: Enhanced NF-κB activity is present in HIV-1-infected cells leading to up-regulation of NF-κB-dependent expression of pro-inflammatory genes. It is still unclear how HIV-1 counteracts the negative feed back of NF-κB operated by the NF-κB-dependent transcription and new synthesis of NF-κB inhibitors, including IκB-α. We previously reported that IκB-α directly associated the arginine-rich motif of the HIV-1-encoded Tat trans activator promoting the nuclear export of the viral protein (1). Here, we have investigated whether HIV-1 Tat sustains the NF-κB activity by interaction with IκB-α and the p65 transcriptional subunit of NF-κB. Results: Tat inhibited the post-activation turn-off of NF-κB in Jurkat cells upon single round infection with HXB2env-pseudotyped NL4-3 R-E- Luc virions as well as in HeLa cells stimulated with PMA. The NF-κB activity was analysed by western blotting of nuclear and cytosolic extracts to detect the nuclear translocation of p65, and by EMSA using the 32P-labelled-double-stranded NF-κB oligonucleotide. In PMA-stimulated HeLa cells, Tat was found associated with IκB-α by co-immunoprecipitation causing the release of p65 from the complex with IκB-α. By EMSA using in vitro translated proteins, Tat promoted the p65 binding to DNA in presence of IκB-α by interfering the inhibitory binding of IκB-α to p65. The Tat competition of the IκB-α/p65 complex was confirmed by co-immunoprecipitation assay. The mutant Tat R(49-57)A, lacking the arginine rich-domain required for the binding to IκB-α, did not counteract the IκB-α inhibition of p65. Accordingly with physical interaction, Tat interfered the IκB-α inhibition of the p65 transcriptional activity in transient expression assay using the κB-Luc reporter. As additional mechanism of NF-κB activation, Tat associated with p65 as measured by in vivo and in vitro co-immunoprecipitation. The Cys-rich region of Tat and Rel region of p65 (1-121 aa) were involved in physical interaction of the two proteins. By mobility shift assay, Tat and not Tat C(22, 25, 27)A increased the p65 binding affinity to DNA. By super-shift analysis, Tat was present in the p65 complex bound to the NF-κB enhancer. We determined the action of Tat on the expression of MIP-1α, a chemokine activated in neuronal glia cells in AIDS patients. A bioinformatic analysis by Jaspar software identified three putative NF-κB enhancers in the MIP-1α promoter. As measured by Real-Time PCR, Tat enhanced the expression of the MIP-1α gene in transfected Jurkat and HeLa cells, while TatC(22, 25, 27)A and Tat R(49-57)A did not have any effect. By chromatin immunoprecipitation, Tat increased the recruitment of p65 at the most distal NF-κB enhancer from the transcription initiation site of the MIP-1α gene. Moreover, Tat was present within the p65 complex bound to the NF-κB enhancer. Tat also promoted the removal of IκB-α from the same NF-κB enhancer while increasing the recruitment of Ser5-phosphorylated RNA polymerase II. Conclusions: This study describes a novel mechanism of NF-κB activation by HIV-1 Tat. We have shown that Tat hijacks the IκB-α inhibitor and associates with p65 at the NF-κB enhancer increasing the NF-κB DNA binding affinity and preventing the NF-κB removal by IκB-α. These findings suggest novel therapeutic strategies aimed to counteract the cross talk of Tat, IκB-α and p65 to interfere the abnormal inflammatory response in AIDS. Reference: 1. Puca A, Fiume G, Palmieri C, Trimboli F, Olimpico F, Scala G, Quinto I. IkappaB-alpha represses the transcriptional activity of the HIV-1 Tat transactivator by promoting its nuclear export. J Biol Chem. 2007 Dec 21;282(51):37146-57.

mother-of-snow-white (msw): a maternal effect allele affecting behavior and the formation of the left-right body axis in zebrafish.

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The vertebrate body plan displays distinct left-right asymmetries in the position of visceral organs. This asymmetrical organization extends to the vertebrate brain that is both anatomically and functionally asymmetric. The development of left-right patterning and cerebral lateralization are thought to be regulated by evolutionary conserved genes. Here we report the evidence of the maternal effect allele mother-of-snow-white (msw) controlling the establishment of LR body asymmetries in a vertebrate embryo suggesting conserved mechanisms in the evolution and establishment of this trait. In a recent study Facchin and colleagues [1] showed that the progeny of lines of zebrafish artificially selected for the right eye preference in scrutiny a mirror had a significant increase in the frequency of reversed left-right asymmetry in the epithalamus. In the present study it is proposed that Facchin's selection for behavioral lateralization could have lead to the isolation of a spontaneous maternal effect allele responsible for the disruption of normal left-right patterning in zebrafish neuroanatomical structures. We analyzed the genetic transmission of the msw allele and we identified three different classes of females according to the percentage of reversed brain asymmetries in their offspring. Females generating a frequency of 0-5% (class I), between 5 and 12.5% (class II) and females generating more than 12.5% (class III) of progeny with reversed asymmetries. Animals from the last group were considered as homozygous recessive females for the msw allele (HRF). We also investigated in the three classes the expression of members of signaling pathways responsible for the establishment of visceral and diencephalic left-right asymmetries and measured the size of Kupffer's vescicle (KV). We found that HRF offspring had smaller KV and, sometimes, no vesicle at all. We could observe a correlation between the frequency of reversed parapineal and the size of KV. The msw allele has shown to be semi-dominant as class II females showed an intermediate phenotype. Our hypothesis suggests that smaller size of KV can reduce the amount of morphogens accumulated by the leftward flow, thus leading to a randomization of the expression of genes of the Nodal pathway. Moreover we evidenced significant behavioral differences between fish with opposite parapineal position subjected to various laterality tests. We could also discuss a complex but relevant influence of neuroanatomical asymmetries on zebrafish personality [2]. Now using Paired-end Mapping and next-generation sequencing techniques (SOLiD approach) we are aiming at identifying the msw allele. 1. Facchin, L., F. Argenton, and A. Bisazza, Lines of Danio rerio selected for opposite behavioural lateralization show differences in anatomical left-right asymmetries. Behavioural Brain Research, 2009. 197 (1): p. 157-165. 2. Dadda, M., et al., Early differences in epithalamic left-right asymmetry influence lateralization and personality of adult zebrafish. Behavioural Brain Research. 206 (2): p. 208-215.

Cellular senescence in skeletal *semitendinosus* muscle: correlation between 4-HNE adducts, telomere's length, stress proteins and physical activity level.

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Telomeres shortening that occurs during normal cell division lead to cellular senescence and finally aging. Chronic oxidative stress is also involved in telomere shortening, increasing telomere loss, whereas antioxidants decelerate it (Saretzki and Von Zglinicki, 2002). 4-HNE, a major aldehydic end-product arising from lipid peroxidation in response to oxidative stress, is able to induce accumulation of modified proteins during aging, oxidative stress, and in a large number of human disease processes, often sustained by inflammatory reactions (Poli G. and Schaur RJ.). Besides, heavy exercise can increase mechanical damage to protein structure as well as ROS generation, but it is also well demonstrated that regular exercise can up-regulate antioxidant defences and stress protein response, providing adaptation towards related and non-related stressors, thus delaying cellular senescence (Ji, 2002). The main objective of this study was to investigate the relationship between telomere length, 4-HNE adducts, stress protein response and aging in human skeletal *semitendinosus* muscle, and their potential modulation by different degree of physical training. Semitendinosus muscle biopsies from 29 healthy male adults (16-56 yrs), with different sport activity levels, were collected during knee surgery. The samples were analyzed for telomere length (TRF analysis), HNE adducts, small HSPs (HSP27 and αB-crystallin) and catalase (CAT) activity content. Activity level (Sedentary, Recreational, Competitive) was classified according to Tegner scale, which mainly refers to the knee mechanical stress (Tegner and Lysholm, 1985). TRF analysis showed that telomere shortening is positively associated with aging ($p<0.05$), but no correlation was identified with respect to sport activity level. 4-HNE, HSPs levels and CAT activity showed individual variability not correlated neither with age nor with sport activity level, even if HNE damage seems to increase slightly with age. Moreover, HNE damage seems to be related to telomere shortening, given that subjects with higher telomere shortening showed a clear tendency to accumulate 4-HNE adducts, whereas subjects with lower telomere shortening showed an opposite correlation. Our study confirms the correlation between telomere length and aging in semitendinosus muscle of adult healthy subjects. Preliminary results indicates that the extent of HNE-adducts does not correlate with age, small HSPs expression, or physical activity level evaluated by Tegner scale, while a positive correlation was observed between HNE damage and telomere shortening. This data confirm that also oxidative stress, as well as physiological aging, is involved in telomere shortening, supporting the role of telomeres as a sensible target of oxidative DNA damage.

c-Myb supports erythropoiesis by transactivating KLF1 and LMO2 expression

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The c-Myb transcription factor is highly expressed in immature hematopoietic cells and down-regulated during differentiation. c-myb is essential for the hematopoietic development, as c-myb-/- mice die at E15 due to failure of fetal hepatic erythropoiesis. To gain further insights into the role of c-myb during the hematopoietic lineage commitment, we studied the effects of c-Myb silencing in human CD34+ hematopoietic stem/progenitor cells. c-Myb silencing in CD34+ cells was performed by transfection of siRNAs using the Amaxa Nucleofector® Technology. In order to keep c-Myb expression silenced for all the commitment phase of CD34+ cells, each sample was nucleofected 3 times, once a day. Moreover, to exclude non-specific effects of siRNA nucleofection, for each experiment, together with the sample transfected with the siRNAs targeting c-Myb, one sample electroporated without siRNAs and one transfected with a non-targeting siRNA were performed. c-Myb silencing effects on CD34+ cells differentiation ability were studied by methylcellulose and collagen-based clonogenic assays and by morphological and immunophenotypic analyses after liquid culture. Furthermore, we investigated by microarray analysis the changes in gene expression induced by c-Myb silencing. Methylcellulose assay revealed a remarkable increase of the percentage of monocyte (CFU-M) colonies and a decrease of the erythroid ones (BFU-E) in c-Myb-silenced CD34+ cells. Moreover, collagen-based clonogenic assay demonstrated that c-Myb silencing strongly enhances the megakaryocyte commitment of CD34+ cells. In agreement with these data, flow cytometric analysis showed an increase in mono-macrophage and megakaryocyte fractions in c-myb-silenced cells, while the erythroid population was strongly decreased. Morphological evaluation of May Grunwald-Giemsa stained cytospins further supported the conclusion that c-myb silencing forces the CD34+ cells commitment towards the macrophage and megakaryocyte lineages at the expense of the erythroid one. Gene expression profiling of c-Myb silenced CD34+ cells enabled us to identify new putative targets which can account for c-Myb knockdown effects. Indeed, Chromatin Immunoprecipitation and Luciferase reporter assay demonstrated that c-Myb binds to KLF1 and LMO2 promoters and transactivates their expression. Functional rescue experiments showed that the retroviral vector-mediated overexpression of KLF1 and LMO2 transcription factors in c-Myb silenced cells is able to rescue, at least in part, the impaired erythroid differentiation. Our data collectively demonstrate that c-Myb plays a pivotal role in human primary hematopoietic stem/progenitor cells lineage commitment, by enhancing erythropoiesis at the expense of megakaryocyte diffentiation. In particular, we identified c-Myb-driven KLF1 and LMO2 transactivation as the molecular mechanism through which c-Myb regulates erythroid versus megakaryocyte lineage fate decision.

Study of the genetic basis of Autosomal dominant nocturnal frontal lobe epilepsy

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Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is an idiopathic partial epilepsy characterized by a wide spectrum of stereotyped motor manifestations, mostly occurring during NREM sleep. ADNFLE is rare, but probably underdiagnosed since semeiological similarities make it difficult to distinguish this disorder from parasomnias. The main differentiating features characterizing ADNFLE are: a) several attacks per night at any time during the night; b) brief duration of the attacks; c) stereotyped motor pattern. Nocturnal video-polysomnography is crucial for the diagnosis. Since 1995, mutations causing a gain of function of the neuronal nicotinic acetylcholine receptors were reported in three different subunits (CHRNA4, CHRNB2 and CHRNA2). More recently, putative susceptibility nucleotide variations affecting the promoter of the CRH gene and altering the corticotropin releasing hormone levels were reported by our group in some patients. Altogether, mutations have been identified in only 10 to 15% of ADNFLE families and the causal gene remains unknown in the others. The limited number of families with a defined genetic cause so far published, makes phenotype-genotype correlations difficult. Moreover, the variability of expression of symptoms observed even among members of the same family with the same genetic defect emphasizes the need to understand the multiple influences responsible for the phenotype in each individual. CRH acts as a neurotransmitter or neuromodulator in extrahypothalamic circuits to integrate a multisystem response to stress that controls numerous behaviors such as locomotor activity, anxiety, food intake, sexual behavior, sleep, arousal and learning. A role of this hormone could explain specific ADNFLE aspects: in particular, the presence of at least one of the two identified nucleotide variations resulted to be associated with very high CAP rate, suggesting that an impaired regulation of the CRH level could led to the reported fragmentation of the NREM sleep and to an alteration of the sigma activity. Thalamocortical circuitry involved in the sleep spindles may be a factor in triggering seizures in ADNFLE. Even in families with mutant nicotinic receptors related to an increased sensitivity to acetylcholine, the exact pathophysiological mechanisms leading from abnormal nicotinic receptors to focal frontal lobe epileptic seizures remain poorly understood. The occurrence of ADNFLE seizures at arousal from non-REM sleep suggests the involvement of defective nAChRs in the cholinergic pathway ascending from mesencephalon to thalamus, at the time ACh is released during transitions from sleep to waking. Orexin is the other principal substance involved in arousal induction from sleep: the co-release of acetylcholine and orexin allows arousal from sleep with concomitant cortical activation and the presence of postural muscle tone. In contrast to acetylcholine, orexin is actively involved only in the transition from non-REM sleep to waking. Mutations in the orexin receptor and peptide have been found to induce narcolepsy in animal models, but only one mutation in the orexin gene has been identified in a patient with early onset narcolepsy. We postulated that some forms of ADNFLE could be caused by gain-of-function anomalies of the orexin system, and analysed the genes of the orexin system in 21 ADNFLE unrelated probands, to detect potentially causative genetic variants. We analysed the three genes of the orexin system: the single preproorexin gene (HCRT), which encodes orexin-A and orexin-B, and the HCRTR1 and HCRTR2 genes, which encode the two receptors. No potentially pathological variants were identified, in all three genes. The absence of mutations in the three tested genes in 21 patients makes improbable an involvement of the orexin system in the pathophysiology of ADNFLE. Orexin measures in the CSF of ADNFLE patients could further substantiate the absence of involvement of the orexin system in ADNFLE

Identificazione di elementi in cis e di fattori in trans implicati nella regolazione post-trascrizionale del gene CDK5R1

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Il gene CDK5R1 codifica per p35, una proteina richiesta per l'attivazione della chinasi CDK5, la cui attività gioca un ruolo chiave per il corretto sviluppo e funzionamento del SNC. L'iperattivazione di CDK5 mediata da p25, frammento proteolitico di p35, è inoltre implicata nella patogenesi di diverse malattie neurodegenerative, tra cui la malattia di Alzheimer. L'attività di CDK5 dipende unicamente dai livelli di p35, è quindi indispensabile che l'espressione di CDK5R1 sia strettamente regolata. CDK5R1 è caratterizzato da un 3'UTR di 2725 bp, fra i più lunghi finora annotati, che presenta un elevato grado di conservazione e diversi elementi regolatori predetti, in particolare elementi ARE; ciò sembra suggerire che questa regione sia importante nel controllo dell'espressione di CDK5R1 a livello post-trascrizionale. Abbiamo precedentemente dimostrato che il 3'UTR di CDK5R1 è implicato nel controllo della stabilità del trascritto, anche mediante il legame delle nELAV, fattori stabilizzanti neuronali che riconoscono sequenze ARE. Sempre nello stesso studio abbiamo individuato una regione del 3'UTR (C3.1) che ha un effetto fortemente destabilizzante sul trascritto di CDK5R1. Successivamente abbiamo focalizzato la nostra attenzione sulla regolazione post-trascrizionale mediata da un'altra importante classe di fattori in trans, i microRNA (miRNA). L'analisi bioinformatica del 3'UTR di CDK5R1 ha predetto la presenza di numerosi siti di legame per miRNA. L'analisi quantitativa dei miRNA con il maggior numero di siti target predetti in cinque linee cellulari ha mostrato una correlazione inversa tra l'espressione di miR-107 e miR-103 e i livelli di p35. È interessante far notare che questi due miRNA sono deregolati nel cervello di pazienti con Alzheimer e potrebbero quindi avere un ruolo nella sua patogenesi. Abbiamo quindi dimostrato che l'overespressione di miR-107 e miR-103 in cellule SK-N-BE porta ad una significativa diminuzione di p35, mentre la loro downregolazione porta ad un suo aumento. L'overespressione dei due miRNA causa inoltre una ridotta capacità di migrazione delle cellule. La co-trasfezione di un costrutto luciferasico che contiene il 3'UTR di CDK5R1 con il precursore dei due miRNA (pre-miR-103/107) porta ad un abbassamento dell'attività luciferasica rispetto allo stesso costrutto trasfettato da solo, a supporto di un'azione diretta dei due miRNA sul 3'UTR di CDK5R1. La generazione di costrutti luciferasici che contengono i diversi siti target predetti per miR-103/107, o privi dei siti stessi, e la loro co-trasfezione con il precursore dei miRNA ha permesso di individuare un sito sul quale i due miRNA sembrano agire in maniera specifica. Nel loro complesso, questi dati suggeriscono che miR-103 e miR-107 abbiano un'azione regolatoria sull'espressione di CDK5R1. Si sta ora analizzando la presenza dell'mRNA di CDK5R1 nella frazione polisomale, prima e dopo trasfezione con i due miRNA, per studiare il loro effetto sulla traduzione di p35. L'effetto destabilizzante causato dalla regione C3.1 non sembra tuttavia essere dovuto all'azione dei miRNA, in quanto la delezione del sito target all'interno di essa non causa un aumento dell'attività luciferasica rispetto al costrutto wild-type. Saggi di UV-crosslinking e pull-down di complessi RNA-proteine, finalizzati alla ricerca di fattori regolatori, evidenziano la presenza di bande specifiche per la regione C3.1: queste bande verranno analizzate mediante spettrometria di massa per l'identificazione dei fattori proteici che legano questa porzione di 3'UTR. Questo studio evidenzia come il 3'UTR sia coinvolto nella modulazione dell'espressione di CDK5R1 attraverso elementi in cis e fattori in trans, suggerendo che esso possa avere un ruolo chiave nel regolare i livelli d'espressione del gene durante lo sviluppo del SNC. La compromissione di questi meccanismi di regolazione potrebbe essere coinvolta nella patogenesi di disordini cognitivi e malattie neurodegenerative.

Molecular mechanisms underlying the antitumor activity of Gonadotropin-Releasing Hormone and its receptors

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The hypothalamic decapeptide GnRH (Gonadotropin-Releasing Hormone) is well known as the key regulator of the pituitary-gonadal axis, through the activation of specific receptors (GnRH-R) on pituitary gonadotropes. Both GnRH and GnRH-R are also expressed in tumors where they act as an autocrine loop, endowed with a strong antiproliferative (thorugh the arrest of the cell cycle) and antimetastatic activity. However, a possible link between the GnRH/GnRH-R system and the apoptotic machinery, in tumor cells, still remains to be defined. Recently, a second form of GnRH (called GnRH-II) has been identified in humans. GnRH-II, which is encoded by a different gene, is also associated with antitumor activity; in this context, efforts have been made to identify the receptor (called type II GnRH-R) which might specifically mediate the actions of this isoform of the decapeptide. However, to date, no transcripts that could be translated into a conventional, full length, functional receptor have been found in humans. Based on these observations, the present experiments have been performed to: 1) clarify the possible link between GnRH/GnRH-R and the apoptotic machinery; 2) identify the receptor which mediates the antitumor activity of GnRH-II. These studies have been carried out in androgen-independent prostate cancer cells, previously widely shown by our Laboratory to express the antitumor GnRH/GnRH-R system. 1) By genome wide transcriptomic analysis (Affymetrix Human Genome U133 Plus 2.0 Array) we observed that GnRH-R activation increases the expression of the pro-apoptotic protein Bax; these data were further confirmed by Western blot analysis. Moreover, GnRH-R activation significantly increased the expression of the dephosphorylated (active) form of the pro-apoptotic protein Bad, belonging to the BH3 only superfamily members. These data indicate that, in tumors in which it is expressed, in addition to its antiproliferative/antimetastatic effect, GnRH might also activate the apoptotic machinery. These data were further confirmed by the observation that GnRH-R activation sensitizes prostate tumor cells to the pro-apoptotic activity of chemotherapeutic drugs (i.e., Docetaxel), through the activation of the apoptotic effector caspase-3. 2) To identify the receptor which might mediate the antimitogenic activity of GnRH-II, the short interfering RNA (siRNA) technique was employed. Cancer cells were transfected with a GnRH-R siRNA (specifically targeting the classical form of the receptor) or with a type II GnRH-R siRNA; the efficiency of transfection was confirmed by RT-PCR, Western blotting and immunofluorescence. Silencing of GnRH-R was found to completely reverse the GnRH-II antiproliferative effect. On the other hand, silencing of type II GnRH-R did not affect the antimitogenic activity of GnRH-II. Moreover, this Laboratory has previously shown that, in cancer cells, the GnRH-R is coupled to the Galphai-cAMP signal transduction pathway. In the present experiments, we demonstrated that GnRH-II significantly counteracts the forskolin-induced cAMP accumulation; this effect was completely abrogated after silencing of GnRH-R. Taken together, these data demonstrate that, in prostate cancer cells: 1) the GnRH-R is linked to the pro-apoptotic machinery and its activation sensitizes tumor cells to the effects of pro-apoptotic agents; 2) the classical form of the GnRH-R mediates the antitumor activity of both GnRH and GnRH-II.
(Supported by MIUR/PRIN and by PUR)

Conditional knockout NPY-YIR mice as an experimental model to study gene-environment interaction

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The molecular mechanisms underlying psychiatric disorders are poorly understood. Among the genes most commonly associated with psychiatric disorders are those encoding for the neuropeptide Y (NPY) and its receptors. NPY exerts important functions as anxiolytic peptide modulating inter-individual variations in emotion and stress resilience in humans and animals. On the other hand, most psychiatric disorders arise from the interaction between genetic predispositions and environmental risk factors. In particular, stressful life events are known to play a prominent role in triggering the first episodes of anxiety and depressive disorder. As well, preclinical evidences highlight the prominent role of early life events, particularly of parental cares, in influencing the risk for multiple forms of adult psychopathologies, including anxiety, depression, drug addiction, schizophrenia and developmental disorders. Within this context, the purpose of the present study is to determine the role of the best characterized NPY receptor, i.e. Y1R, in emotional behaviour and vulnerability to pychopatologies by using a genetic and behavioural strategy. The animal model used is a conditional knockout mouse line (Y1Rfb-/-) in which Y1R function is inactivated postnatally in anterior forebrain and limbic brain structures, but not in hypothalamus. The role of maternal cares as the main environmental predisposing factor in early life will be investigated by fostering litters to dams of differing mouse strains and examining their emotional phenotype. Y1Rfb -/- conditional mutants showed increased anxiety in elevated plus maze and open field tests as compared to control littermates. In addition, starting at P38, forebrain-specific Y1R -deleted mice showed a rapid and permanent reduction of body weight, demonstrating direct evidence for a critical role of forebrain Y1R in the regulation of energy homeostasis. Behavioral phenotype of Y1Rfb-/- conditional mutants was associated with a significant decrease of CRH immunoreactive fibers in the central amygdala. However, variation in maternal care is a determinant factor to unmask the phenotype Y1Rfb -/- mice, suggesting a potential role of limbic Y1R in long lasting consequences of maternal care on offspring’s behavior.

Molecular mechanism for the retention in the ER of the mutant Frizzled4 L501fsX533, which results in the dominant form of familial exudative vitreoretinopathy (FEVR).

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Familial exudative vitreorethinopaty (FEVR) is an hereditary ocular disorder characterized by an abrupt cessation of the growth of peripheral capillaries leading to an avascular peripheral retina. This condition may lead to compensatory retinal vascularization which is thought to be induced by hypoxia from the initial avascular insult. New vessels easily break causing exudates and bleeding followed by scarring, retinal detachment and blindness. Mutations in Frizzled4 (Fz4), a member of the cell surface Wnt family receptors, were found in many FEVR patients. Fz receptors consist in seven transmembrane (TM) domains, a large extracellular domain which is the binding site for soluble ligands as Wnt, 3 extracellular and cytosolic loops that connect the TM domains, and a short cytosolic tail that plays an important role in signaling (1). In a autosomal dominant form of FEVR (the most frequent and henceforth referred as Fz4-FEVR), the deletion of two nucleotides in the coding sequence leads to a frameshift and to the synthesis of a mutant protein with a completely different truncated cytosolic tail (L501fsX533). The result of this mutation is the tight retention of the receptor in the endoplasmic reticulum (2). Furthermore, it has been proposed that Fz4-FEVR traps Fz4 in the ER by oligomerization and prevents its expression to the plasma membrane, thus performing its dominant effect (3). Our recent results showed that the transport to the plasma membrane of Fz4 requires the 2 last valine residues which are part of a C−terminal valine motif that binds PDZ domains-bearing proteins. We showed that two such proteins, GRASP65 and 55, mostly located in different Golgi compartments, binds Fz4 and are necessary for its transport to the cell surface. Fz4−dVV, a recombinant Fz4 deleted of the 2 terminal valine residues, reaches the plasma membrane much less efficiently than Fz4. In addition, Fz4−FEVR−VV, Fz4−FEVR extended with two terminal valine residues, rescues largely cell surface expression (4). But, how to explain the strong retention of Fz4-FEVR in the ER? Besides Fz4-dVV, the removal of a C−terminal valine motif does not prevents ER exit of several other proteins. In order to unravel the molecular mechanism of the retention of Fz4−FEVR in the ER, we have extensively mutagenized the cytosolic tail and intracellular loops of Fz4-FEVR. We found that the deletion of the last seventeen aminoacids rescues partially cell surface expression. This results suggested the presence of an ER retention motif in the distal portion of the cytosolic tail of the mutant receptor. To confirm this hypothesis, we replaced the cytosolic tail of the G glycoprotein coded by the ts-045 mutant strain of VSV (VSVG) with the tail of wilde-type and mutant receptor. We found that the chimeric VSVG-Fz4 FEVR tail was completely retained in the ER, whereas VSVG-Fz4 tail exited from the ER as well as the reporter VSVG protein. Intriguingly, also the substitution in the third intracellular loop of T425K426 with alanine residues partially rescued cell surface expression of Fz4-FEVR, suggesting that the third intracellular loop may be involved in ER retention of the mutant receptor. Current work is focussed on the identification of the ER retention motif and of the cellular protein(s) that should decode such motif. 1. Hsien-yu Wang et al. 2006 2. Robitaille J. et al. 2002 3. Kaykas A. et al. 2003 4. D'Angelo G. et al. 2009

Molecular basis of Charcot-Marie-Tooth type 2B disease

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The hereditary sensory neuropathies are a clinically and genetically heterogeneous group of disorders. Charcot-Marie-Tooth disease (CMT) is the most common inherited neuromuscular disorder, with a prevalence of 1 per 2500 individuals, and more than 30 genetic loci that are associated with different forms of the disorder have been identified. It is also known as hereditary motor and sensory neuropathy (HMSN) and is a degenerative nerve disorder that causes muscle weakness and atrophy in the feet, legs, hands and forearms. It is characterized by progressive loss of use and sensation in the limbs. Muscles fail to receive stimulation from the nerves, and then begin to waste away (atrophy). Atrophy in the small muscles in feet and hands causes the curl of fingers and toes. There are several forms of CMT. Charcot-Marie-Tooth hereditary neuropathy type 2 (CMT2) is an axonal (non-demyelinating) peripheral neuropathy characterized by distal muscle weakness and atrophy, mild sensory loss, and normal or near-normal nerve conduction velocities. Four missense mutations in Rab7 (a small GTPase evolutionarily conserved from yeast to human, expressed in all tissue, which controls transport to endocytic degradative compartments) cause the Charcot-Marie-Tooth 2B disease: Leu129Phe, Lys157Asn, Ans161Thr and Val162Met. These mutations target highly conserved amino acids. We have demonstrated that CMT2B-causative Rab7 mutant proteins have higher Koff for nucleotides compared to the wt protein (particularly high for GDP), and, as a consequence, lower GTPase activity. In addition, these mutant proteins are predominantly GTP-bound in the cells, and are able to rescue Rab7 function when expressed in Rab7-silenced cells. Since we previously demonstrated that a Rab7 dominant negative mutant (T22N) stimulates neurites outgrowth in PC12 cells, we decided to test if CMT2B mutants affected neurites outgrowth. Interestingly, expression of CMT2B-causing Rab7 mutant proteins in PC12 and in Neuro2A cells lines caused a strong inhibition of the outgrowth of neurites longer m. In addition, inhibition of neurite outgrowth by theseμthan 50 CMT2B-associated Rab7 mutants was confirmed by impaired up-regulation of the growth-associated protein 43 (GAP43) in PC12 cells and of the nuclear neuronal differentiation marker NeuN (Neuronal Nuclei) in Neuro2A cells. Expression of a constitutively active Rab7 mutant (Rab7 Q67L) had a similar effect to the expression of the CMT2B-associated mutants. These data, that have to be confirmed in neuronal cells, indicate that the CMT2B-causing Rab7 mutant proteins impair neurite outgrowth and neuronal differentiation. The onset of CMT2B is during the third decade of life and, therefore, development is not impaired in CMT2B patients. This suggests that the Rab7 mutant proteins selectively affect regeneration of neurons at later stages. These results indicate that strategies to control and lower Rab7 activity in neurons could be a targeted therapy for CMT2B.

Developmental defects and motor neuron alterations due to mitofusin 2 gene (MFN2) silencing in zebrafish: a new model for Charcot-Marie-Tooth type 2A neuropathy.

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The development of new animal models reveals to be a crucial step in determining the pathological mechanism underlying neurodegenerative diseases and is essential for the development of effective therapies. We have investigated the zebrafish (Danio rerio) as a new model to study Charcot Marie Tooth disease type 2, the most common form of peripheral axonal neuropathy characterized by the selective loss of motor neurons. CMT2A is caused by mutations of mitofusin 2 (MFN2) a dynamin-like GTPase located in the outer membrane of mitochondria required to promote the mitochondrial fusion processes and to control ER-mitochondria tethering. Using a knock down approach, we provide evidences that during embryonic development, mitofusin 2 loss of function is responsible of several morphological defects and motility impairment. Immunohistochemical investigations revealed severe alterations in motor neurons characterized by shortened and branched axons. Finally, we demonstrated the ability of human MFN2 to balance the downregulation of endogenous mfn2 in zebrafish, further supporting the conserved function of the MFN2 gene. These results highlight the essential role of mitofusin 2 in the motor axon development and demonstrate the potential of zebrafish as a suitable and complementary platform for dissecting pathogenetic mechanisms of MFN2 mutations in vivo.

ABSTRACT PARTECIPANTI

Oxidative stress in denervated muscle

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Muscle atrophy results from a wide range of conditions including cancer, neurodegenerative diseases, traumas and ageing, and partial or total lost of skeletal muscle innervation (denervation). Several evidences suggest that Reactive Oxygen Species (ROS) and mitochondrial dysfunction play an important role in muscle atrophy. In order to evaluate whether ROS may play an important role during denervation, rat Tibialis Anterior (TA) skeletal muscle was isolated after 15 days or 3 months from surgical sciatic nerve transection. Following denervation, a progressive increase of ROS production in TA muscle can be observed. We suggest that two vicious circles contribute to such increase. In fact the excess ROS lead to oxidative damage to the muscle membranes and to the contractile apparatus, as demonstrated by the increase in lipid peroxidation and by a progressive loss of ionic channel and pump activity, associated with an increase of cytosolic Ca2+. In a Ca2+ enriched environment, a calcium dependent Phospholipase A2 (cPLA2) may be activated, which hydrolyzes the membrane peroxidized lipids contributing to the increase in ROS amount. Furthermore, ROS may directly induce the expression of transcription factors such as PGC-1alpha, which enhances the biogenesis of the OXPHOS enzymes, leading to an unbalance in the expression of the different complexes of the mitochondrial respiratory chain. We identify in mitochondria the most important site of ROS production. Muscle cells react to the excess ROS by up-regulating the synthesis of cyto-protective and anti-oxidant enzymes. However, such up-regulation decreases with denervation time, probably owing to the progressive degeneration of the muscle cell. Since anti-oxidant defences appear inadequate to counterbalance increased ROS production with increased denervation time, an anti-oxidant therapeutic strategy seems to be advisable in the several medical conditions where the nerve-muscle connection is impaired.

Combined transcriptome and translatome profiling identifies HuR as a key mediator of estrogen-induced sequence-dependent translational control in MCF-7 cells.

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Estrogens are known to induce a well known transcriptional response in breast mammary cells, while less is known about their post-transcriptional influence. We report here evidence of a complex translational control exerted in the early phase of cellular response to 17β-estradiol (E2) in the estrogen-responsive MCF-7 cell line, involving dozens of genes and being strongly uncoupled from the transcriptional early program.
Indeed, a comparison of the transcriptome and the translatome (polysomally loaded mRNAs) after 1 hour of E2 treatment shows a surprisingly widespread translational repression activity, by which as much as 86% of transcriptome variations were buffered at the translatome level, while 66% of the translationally controlled mRNAs were not changed in their cell overall content.
Looking for determinants of this marked translational reshaping of a classical transcriptional program we firstly demonstrated activation of the mTOR pathway by E2 in MCF7 cells. But when we examined the structural mRNA feature expected to be influenced by this activation, folding complexity of the 5'UTR, we found that instead E2 selected for translational control transcripts with a complex 3'UTR, where it determined an enrichment of the widespread AU-rich elements (AREs), either in terms of upregulated and downregulated genes.
Since AREs are known to be 3'UTR docking sites for a number of RBPs behaving either as activators and repressors of translation, we focused on the ubiquitosly expressed member of the ELAV RBP family, HuR, the only ARE-binding protein demonstrated to act as a translational enhancer. Upon E2 treatment of MCF-7 cells, HuR rapidly shifted from the nucleus, where it is normally localized, to the cytoplasm, and bound en masse to ARE-containing, transcriptome-enriched and translatome-enriched mRNAs. This remarkable correlation of genome-wide transcriptional and post-transcriptional events following a mitogenic stimulus could be achieved by a coordinated interplay of the E2-induced stimuli, since we showed a combinatorial enrichment of experimentally validated promoter cis-acting estrogen response elements (EREs) and AREs in the commonly upregulated genes.
Notably, silencing of HuR rendered the cells less responsive to the mitogenic stimulus of E2, which functionally implicate HuR as the first known trans factor responsible to mediate translational regulation by E2.
This work revealed by a systems-level approach that a classically transcriptionally-targeted pathway, estrogen-induced stimulation of responsive mammary epithelial cells, is indeed markedly shaped in its gene expression regulation outcome by a concomitant, exquisitely sequence-specific and previously unappreciated translational control. This allowed also us to identify, originally on the basis of the study of mRNA population dynamics, a major factor involved in this control, this demonstrating the power of combined systems/mechanistic analysis of cell behaviour.

Isolamento, caratterizzazione proteomica ed analisi funzionale degli esosomi rilasciati dalle cellule di leucemia mieloide cronica

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La maggior parte delle cellule eucariotiche rilasciano nel mezzo extracellulare vescicole che differiscono per origine, dimensione, composizione e funzione. Le due popolazioni di vescicole maggiormente studiate sono gli esosomi e le vescicole di membrana. Gli esosomi hanno un diametro di 30-90 nm, vengono rilasciati per esocitosi dai corpi multivescicolari di molti tipi cellulari della linea emopoietica e da numerosi tumori solidi; essi sono ricchi di molecole MHC I e II e di tetraspannine, come CD63 e CD9 che formano complessi oligomerici ed interagiscono con le integrine e con i corecettori CD4 e CD8 dei linfociti T. Alcune proteine come l'HSC70, CD63 e l'acetilcolinesterasi, presenti negli esosomi rilasciati da tutti i citotipi, sono utilizzate come markers della popolazione esosomale. Gli esosomi sono coinvolti nei meccanismi di secrezione di molecole segnalatrici prive di sequenza segnale ed hanno un ruolo importante nella comunicazione cellula-cellula; essi sono stati evidenziati, in vivo, in asciti di pazienti affetti da carcinoma ovarico. In questo caso tali strutture appaiano avere un valore prognostico, infatti la quantità di vescicole presente nel fluido aumenta proporzionalmente allo stadio del tumore. Recentemente è stato ipotizzato un ruolo degli esosomi in alcuni meccanismi di scambio genetico, come "RNA shuttle". Dati di letteratura indicano che le cellule di leucemia mieloide cronica della linea K562 rilasciano esosomi con un meccanismo calcio-dipendente, ma nulla è descritto circa la loro composizione ed il loro ruolo biologico. Nel nostro laboratorio è stata effettuata una caratterizzazione proteomica, attraverso analisi MudPIT, delle vescicole rilasciate da cellule di leucemia mieloide cronica LAMA84 che ha confermato non solo la natura esosomiale di tali vescicole ma ha dato ulteriori indicazioni sulle molecole trasportate dalle microparticelle. Inoltre, dati sperimentali funzionali hanno permesso di evidenziare un loro potenziale ruolo nel processo angiogenetico. I saggi di motilità effettuati su cellule endoteliali trattate con gli esosomi delle LAMA84, mostrano che la capacità di migrare delle HUVEC aumenta in seguito al trattamento con gli esosomi; questo dato è confermato da esperimenti di "wound healing assay". Con esperimenti di Real Time PCR, in seguito a trattamento delle HUVEC con gli esosomi è stato osservato un incremento tempo e dose-dipendente dell'espressione di molecole di adesione come ICAM-1, VCAM-1 ed E-Selectina. Per valutare il significato funzionale dell'aumento di espressione di queste molecole sono stati effettuati dei saggi di adesione delle cellule di CML sul monostrato di HUVEC trattato per 6 ore con dosi crescenti di esosomi. I risultati indicano che il numero delle cellule adese al monostrato aumenta all'aumentare della dose di esosomi. Mediante esperimenti di microscopia confocale è stata analizzata la -catenina. Nelle HUVEC incubate per 6βdistribuzione della VE-Caderina e ore con esosomi si ha una delocalizzazione di queste molecole di adesione dalla membrana al citoplasma. Poiché in vivo il legame delle cellule leucemiche alle cellule endoteliali promuove la diffusione dei blasti leucemici nel circolo sanguigno, si ipotizza un ruolo degli esosomi nel meccanismo di intravasazione. Infine esperimenti effettuati in vivo in topi nudi hanno confermato il ruolo proangiogenetico degli esosomi rilasciati dalle cellule di Leucemia Mieloide Cronica. Resta da comprendere quali siano le molecole biologicamente attive presenti negli esosomi che potrebbero favorire le interazioni tra le cellule leucemiche e l'endotelio ed i pathways molecolari che si attivano in seguito all'interazione cellule endoteliali-esosomi. Individuare nuove vie di comunicazione intercellulare e nuovi target molecolari che favoriscono la diffusione delle cellule leucemiche potrebbe essere utile dal punto di vista clinico, per lo sviluppo di nuove terapie farmacologiche. Fondi AIRC Prof G. De Leo.

Mechanisms controlling liver stem cell fate

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We recently isolated and established in lines, from embryonal and newborn mouse livers, resident liver stem cells (RLSCs) with immunophenotype and differentiative potentiality distinct from other previously described liver precursor/stem cells. RLSCs showed i) a metastable phenotype typical of the stem cells, with a coexpression of epithelial and mesenchymal markers; ii) a multidifferentiative potentiality (hepatocytes/cholangiocytes and, if properly instructed, osteoblasts, condrocytes, glia and neurons) (Conigliaro A. et al. Cell Death And Diff. 2008).
A- We investigated on molecular mechanisms controlling the RLSC phenotype during self-renewal and differentiation. We clarified as Wnt/beta-catenin signalling integrates cellular program influencing both stemness maintenance and hepatocyte zone-specific differentiation. In particular, RLSCs were proven to spontaneous differentiate into periportal hepatocytes that, following Wnt pathway activation, switch into perivenular hepatocytes highliting a direct convergence of the canonical Wnt signaling on the HNF4α-driven transcription in the hepatocyte post-differentiative metabolic "zonation" (Colletti et al., Gastroenterology 2009).
B-The concept that terminal differentiation allowing cellular tissue-specific functions is stably maintained once development is complete is questioned by numerous observations showing that differentiated epithelium may undergoes an Epithelial-to-Mesenchymal Transition (EMT). Both the EMT and the reverse process Mesenchymal-to-Epithelial Transition (MET) are typical events of development, tissue repair and tumor progression. Our current efforts aim to the comprehension of the microenvironmental factors and the molecular mechanisms controlling the EMT/MET oscillations and hepatocyte differentiation.
In particular, we unveiled a direct reciprocal regulation between the EMT master gene Snail and the hepatocyte differentiation master gene HNF4α, which balance is crucial during liver stem/precursors cell maintenance, hepatocyte differentiation and HCC progression. Notably, we unveiled that in terminally differentiated hepatocyte, also in the context of the whole liver, the epithelial phenotype is strictly dependent from the HNF4α active repression of EMT master genes and of the entire mesenchymal program. In conclusion, both HNF4α activator and repressor functions are necessary for the identity of hepatocytes.
C- We are currently investigating the contribution of liver stem cells to the onset and progression of hepatic fibrosis. Our unpublished evidences suggest the capability of RLSCs to differentiate into mesenchymal cells reminiscent of activated Hepatic Stellate Cells, the major fibrogenetic cells in cirrhotic liver thus suggesting a yet uncharacterised pathogenetic mechanism of hepatic fibro-cirrhosis.

Ruolo dell'Unfolded Protein Response nel controllo della formazione del complesso di uscita dal Reticolo Endoplasmatico COPII

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Il Reticolo Endoplasmatico (RE) gioca un ruolo centrale nel controllo della sintesi, della qualità, dell'esporto e della degradazione di tutte le proteine della via secretoria. Nel lume del RE il macchinario molecolare del Controllo di Qualità (QC) trattiene le proteine che non hanno raggiunto il corretto ripiegamento e consente l'uscita delle sole proteine correttamente ripiegate, assicurando in tal modo il giusto funzionamento di tutti i compartimenti della via secretoria. Alterazioni del QC determinano un accumulo di proteine malconformate nel RE (stress del RE). All'aumento di proteine mal ripiegate il RE risponde attraverso un sistema integrato di segnali denominato Unfolded Protein Response (UPR), coordinato da tre trasduttori, IRE1, ATF6 e PERK, che hanno la funzione di ristabilire l'omeostasi proteica. In caso di stress prolungato, l'UPR stesso attiva almeno due differenti vie apoptotiche: la via IRE1/JNK e la via PERK/ATF4/CHOP. Abbiamo recentemente osservato che l'esposizione cellulare ad induttori dell'UPR perturba le dinamiche di traffico vescicolare fra RE e Golgi, riducendo sia il trasporto di proteine reporter che il numero di vescicole COPII. Questi risultati suggeriscono che l'UPR potrebbe influenzare la formazione e/o la stabilità del macchinario di uscita dal RE. L'esporto di proteine dal RE è mediato dalla formazione del rivestimento COPII che si costituisce a partire da sottodomini del RE noti come siti di uscita dal RE (ER Exit Sites, ERES). La componente prevalente degli ERES è la proteina Sec16 mentre il rivestimento COPII è costituito da due complessi eterodimerici il complesso interno Sec23-Sec24 ed il complesso esterno del rivestimento Sec13-31. L'attivazione della proteina Sar1 promuove il reclutamento sequenziale dei due complessi. Il turn over di COPII dipende dal ciclo di GTP su Sar1 che è sotto il controllo del fattore di scambio (GEF) Sec12 e della attività GTPasi di Sec23. Principale scopo del nostro lavoro sperimentale è stato quello di definire il ruolo dell'UPR nel controllo della funzione del complesso COPII studiando la rilevanza funzionale dei differenti trasduttori dell'UPR (ATF6, IRE1, PERK) sulla costituzione del macchinario di uscita dal RE e sul reclutamento del carico. A tale scopo, sono state esaminate in cellule esposte ad induttori dell'UPR: la localizzazione intracellulare e le dinamiche di interazione con la membrana del RE sia della componente proteica degli ERES, la proteina Sec16, che delle componenti proteiche di COPII vale a dire la proteina Sar1, la proteina Sec23 e la proteina Sec31. E' stata inoltre valutata la rilevanza funzionale delle differenti unità operative dell'UPR (ATF6, IRE1, PERK) sulla formazione e stabilità del macchinario di uscita dal RE e sulla efficienza di reclutamento del carico proteico. Le nostre evidenze sperimentali hanno dimostrato una stretta dipendenza fra la formazione delle ERES e la via IRE1/XBP1 dell'UPR.

Superoxide production detected in exhaled breath condensate of patients with chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is a relevant inflammatory pathology with high prevalence in the adult population. Many studies have emphasized the key role of reactive oxygen species (ROS) in the pathogenesis of several chronic inflammatory lung diseases, including COPD. An increased local production of oxygen radical species such as superoxide may unbalance lung redox equilibrium and trigger or exacerbate inflammatory events. The increased concentrations of ROS in lungs of COPD patients may be evaluated by analysis of oxidative biomarkers in airway fluids collected by bronchoalveolar lavage (BAL) through bronchoscopy. However, a non-invasive method of collecting airway fluids was recently developed, the exhaled breath condensate or EBC, obtained by cooling the exhaled air during tidal breathing through standardized procedures. Unlike BAL, EBC is non-invasive and well tolerated, allowing (when required) repeated sampling from the same patient without significant discomfort. EBC is composed of condensed water vapour and aerosolized airway fluid particles reflecting the molecular composition of lower respiratory fluids, especially in small soluble products related to oxidative stress. Since EBC can be analyzed in real time for oxidative stress biomarkers, it could be employed to obtain key information on redox balance and lung inflammatory status, with possible applications in screening and/or early diagnosis. After obtaining informed consent according to the guidelines established by the University of Ferrara and the Research Centre on Asthma and COPD, we analyzed for ROS content the EBC samples of 8 patients affected by COPD (7 males, 1 female, age range 65-70 years), together with EBC samples of 5 healthy non-smoking controls. To collect samples, each patient freely breathed for about 15 minutes in a sterile disposable plastic mouthpiece connected to a cooling apparatus (Transportable Unit for Research on Biomarkers obtained from Disposable Exhaled Condensate Collection System, Turbo Decs, Ital Chill, Parma, Italia), equipped with properly cooled, disposable sterile vials that could be sealed immediately after EBC collection to avoid contamination. For each sampling, about 2 ml of EBC were collected and freshly analyzed or stored at -20 °C for later tests. We evaluated the production of superoxide and other biologically relevant ROS by standard spectrophotometric tests (cytochrome c reduction kinetics, mannitol and catalase test). The results showed a significantly higher production of superoxide and other reactive oxygen species in EBC of patients affected by COPD, in comparison to healthy non-smoking controls. The identification of real-time profiles of oxidative stress biomarkers is relevant to evaluate the inflammatory status in respiratory diseases: although requiring further refining and standardization, non-invasive EBC appears a suitable tool for longitudinal studies and early assessment of COPD.

A molecular Systems Medicine approach to Diabetes Mellitus pinpoints the molecular bases of the higher resistance to cytokine-induced apoptosis of mammalian pancreas alpha cells respect to beta cells

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Background Diabetes Mellitus (DM) is a pathogenetically complex and heterogeneous systemic syndrome that afflicts millions of people worldwide. Seeking to gain a molecular systems view on its etiology, we exploited an in vitro model to investigate the global involvement of the Apoptotic Machinery (AM) in DM onset and progression. Methods Through high-throughput technology, we studied the alterations of AM transcriptome and AM critical protein nodes in two mouse cell lines from pancreas salpha- and beta-cells (alphaTC1 and betaTC1, respectively), after treating both with a cocktail of cytokines for a time course of 24 h, 48 h, 72 h: according to a general consensus, this model appropriately reproduces in vitro the inflammatory environment that through different mechanisms is involved in the pathogenesis of both T1DM and T2DM. AM protein- encoding genes, markedly over-expressed or down-regulated and conserved between the mouse and Homo sapiens, were ranked through functional (Fisher inverse chi-square test) and protein-protein interaction (Wilcoxon matched-pairs signed-ranks test) prioritization methods, as well as Genome Wide Association (GWA) and epidemiological data. MIR genes, targeting AM protein encoding genes, were ranked based on their dysregulation, that of their targets, and genome position. Results Overall, 31 of 92 AM protein-encoding genes significantly (more than threefold) varied their expression with respect to matched controls. In alphaTC1 cells, NOS2 was highly induced, whereas neither Ser 20-phosphorylated P53, Tyr 705-phosphorylated STAT3, nor the death receptor TNFRSF10B changed their levels. In betaTC1 cells, we detected the increase of proapoptotic proteins ATF3, BNIP3, NOS2, Ser 20-phosphorylated P53, TNFRSF10B, and the decrease of antiapoptoticTyr 705- phosphorylated STAT3. The AM molecular profile in alpha cells, including P53 and STAT3 pathways, was comparable to controls also after treatment with regulatory AM networks in cytokines. On the contrary, reconstruction of beta cells demonstrated the activation cytokine-treated of proapoptotic P53 and of both canonical and alternative NFkB pathways, coupled to inactivation of antiapoptotic STAT3 pathway. Conclusions Our molecular characterization of pancreas alpha cells, to date poorly studied, has allowed to pinpoint the molecular bases of their higher resistance to cytokine- induced apoptosis respect to beta cells. Based on our experimental data and computational analysis, we prioritized AM protein-encoding genes DDIT3, MAP3K14, NFKB1, NFKBIA, NFKBIB, NFKB2, RELA, STAT3 and AM MIR genes MIR16, MIR124, MIR199a, MIR497 as DM candidate genes: the previous identification of some of these through functional, GWA, epidemiologic studies strengthens our proposal. Bibliography Di Pietro C, Ragusa M, Barbagallo D et al: The apoptotic machinery as a biological complex system: analysis of its omics and evolution, identification of candidate genes for fourteen major types of cancer, and experimental validation in CML and neuroblastoma. BMC Med Genomics 2009, 2: 20. D Barbagallo, S Piro, M Ragusa et al: A molecular Systems Medicine approach to Diabetes Mellitus pinpoints the molecular bases of the higher resistance to cytokine-induced apoptosis of mammalian pancreas alpha cells respect to beta cells. BMC Genomics, inviato per la pubblicazione.

Molecular and functional analysis of the stem cell compartment of chronic myelogenous leukemia reveals the presence of a CD34negative cell population with intrinsic resistance to imatinib

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We show the molecular and functional characterization of a novel population of lineage-negative CD34-negative (Lin-CD34-) hematopoietic stem cells from chronic myelogenous leukemia (CML) patients at diagnosis. Molecular karyotyping and quantitative analysis of BCR-ABL transcript demonstrated that approximately one-third of CD34- cells are leukemic. CML Lin-CD34- cells showed kinetic quiescence and limited clonogenic capacity. However, stromadependent cultures induced CD34 expression on some cells and cell cycling, and increased clonogenic activity and expression of BCR-ABL transcript. Lin-CD34- cells showed hematopoietic cell engraftment rate in 2 immunodeficient mouse strains similar to Lin- CD34- cells, whereas endothelial cell engraftment was significantly higher. Gene expression profiling revealed the down-regulation of cell-cycle arrest genes and genes involved in antigen presentation and processing, while the expression of genes related to tumor progression, such as angiogenic factors, was strongly up-regulated compared with normal counterparts. Phenotypic analysis confirmed the significant down-regulation of HLA class I and II molecules in CML Lin-CD34- cells. Imatinib mesylate did not reduce fusion transcript levels, BCR-ABL kinase activity, and clonogenic efficiency of CML Lin-CD34- cells in vitro. Moreover, leukemic CD34- cells survived exposure to BCR-ABL inhibitors in vivo. Thus, we identified a novel CD34- leukemic stem cell subset in CML with peculiar molecular and functional characteristics.

Reconstruction and study of post-transcriptional regulatory networks governing tumour development and progression by integrated analysis of miRNAs and targets expression profiles

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MicroRNAs (miRNAs) regulate gene expression at post-transcriptional level by imperfect binding to target mRNA 3'UTR regions. To bypass limitations of computational predictions of miRNA–target relationships, we developed an original method for integrated analysis of miRNA and gene expression profiles in combination with target prediction, allowing the identification and study of post-transcriptional regulatory networks in specific biological contexts (Biasiolo et al., Pac. Symp. Biocomp. 2010). This method was applied to the definition of a miRNA/mRNA regulatory network in distinct molecular groups of multiple myeloma (Lionetti et al., BLOOD 2009). The combined analysis of miRNA and targets expression is complicated by different computational issues in managing multilayer sequence and expression data and is not straightforward for most experimental researchers. Thus, we designed a novel web tool (MAGIA, miRNAs and genes integrated analysis, http://gencomp.bio.unipd.it/magia; Sales et al., NAR Web Server Issue 2010). MAGIA query section allows the user to retrieve updated miRNA target predictions computed with different algorithms and Boolean combinations thereof. MAGIA analysis section comprises a multi-step procedure for (i) direct integration through different functional measures (parametric and non-parametric correlation indexes, a variational Bayesian model, mutual information and a meta-analysis approach based on P-values combination) of miRNA and genes/transcripts expression data, (ii) reconstruction of bipartite regulatory network of the best putative interactions and (iii) retrieval of genes, miRNAs and diseases information available in public databases and via scientific literature text-mining, thus facilitating the identification of cell activities under post-transcriptional control. The discovery power of MAGIA method was validated by analysis of an Acute Lymphoblastic Leukemia dataset, including expression profiles in T-lineage and B-lineage diseases, harbouring specific molecular lesions. Indeed, in a methodological study, we compared the results obtained from the analysis of expression profiles in 57 prostate cancer samples, using both gene and transcript based annotation, and showed that transcript-based annotations clearly improve the effectiveness of data integration for the reconstruction of post-transcriptional regulatory networks (Sales et al., NAR 2010). Currently, we are involved in the study of the role of miRNAs in clear cell renal cell carcinoma (ccRCC), the predominant form of kidney cancer, in which the status of the vonHippel-Lindau (VHL) tumor suppressor gene, regulating the hypoxia pathway, plays critical role. We investigated cancer development and progression by analysis of four cell lines (derived from normal kidney epithelium, metastatic and non-metastatic RCC, and with different VHL status) and identified miRNAs differentially expressed in metastatic cell lines and their targets. Functional enrichment analysis on reconstructed network showed that miRNA-based regulation in RCC may act in key signalling circuits (leukocyte extravasation, p53, ATM, and G2/M checkpoint). Among genes known as cancer biomarkers for diagnosis or prognosis and miRNAs known to be involved in diseases and tumours, we found some miRNAs (i.e. hsa-miR-1308, hsa-miR28-3p) not reported in literature as involved in cancer, which may be important for RCC pathology, and identified regulatory relationships involving differentially expressed miRNAs and genes of the hypoxia pathway. Functional validation and further characterization of specific results, undergoing completion, will further elucidate regulatory circuits acting in RCC also in relation to VHL and HIF1 alpha protein expression status.

Functional analysis of CDKN2A/p16INK4a 5'UTR variants predisposing to melanoma

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Assessing the pathogenicity of non-synonymous mutations in a high-penetrance melanoma susceptibility gene such as CDKN2A is critical to evaluate disease risk in carriers. While functional studies for determining the clinical significance of mutations in the coding regions are well described it is harder to draw solid conclusions and communicate information during genetic counselling for carriers of variants in the non-coding regions, with the exception of splicing variants whose relation to the disease may be clearly demonstrated. Currently, there are no systematic functional studies addressing the role of the 5'UTR variants identified during routine CDKN2A mutation screening in familial melanoma patients. To date only one proven mutation in the p16INK4a 5'UTR (c.-34G>T) has been described and its mechanism of action was related to the generation of an alternative AUG translation start site. In this study, we have evaluated in greater detail the frequency of occurrence of germline sequence variants in the p16INK4a 5'UTR and near-promoter region among 250 melanoma families and 782 hospital-based sporadic cases and their co-segregation with the disease. We identified melanoma patients that were heterozygous for non-coding germline variants in the 5'UTR of CDKN2A (c.-21C>T; c.-25C>T&c.-180G>A; c.-56G>T; c.-67G>C) and examined their impact on the p16INK4a 5'UTR activity using two luciferase-based reporter vectors that differ in basal transcription level and that were transfected into the melanoma-derived WM266-4 and in the breast cancer-derived MCF7 cells. The wild type 5'UTR sequence, containing a reported SNP (c.-33G>C) and the known melanoma-predisposing mutation (c.-34G>T), were included as controls. Results revealed that the variants at -21 and -34 severely reduced the reporter activity. The variants at -56 and at -25&-180 exhibited a milder impact, while results with c.-67G>C were dependent on the plasmid type. Quantification of the luciferase mRNA indicated that the effects of the variants were mainly post-transcriptional. Using a bicistronic dual-luciferase reporter plasmid, we confirmed that c.-21C>T and c.-34G>T had a severe negative impact in both cell lines. We also applied a polysomal profiling technique to samples heterozygous for the 5'UTR variants, including patient-derived lymphoblasts. Analysis of allelic imbalance indicated that in addition to the c.-21C>T variant, the c.-56T>G and c.-67G>C variants also reduced mRNA translation efficiency. Overall, our results provide tools to assess the functional significance of non-coding 5'UTR mutations and strongly suggest that the identified noncoding p16INK4a variants can be of clinical significance in melanoma-proneness due to their negative impact on the post-transcriptional dynamics of the CDKN2A/p16INK4a mRNA. Based on the results the c.-21C>T sequence variant could be classified as a melanoma-predisposing mutation. The c.-25C>T&c.-180G>A and particularly the c.-56G>T variants showed a range of intermediate functional defects in the different assays, were not observed in the control population and could be considered as potential mutations. We propose that the combined approach of reporter assays and polysomal profiling can be applied to studying the impact of other 5'UTR variants described in the literature for p16INK4a or other SNPs/variants in the 5'UTR of novel candidate genes/loci for susceptibility to melanoma and other diseases. We are currently investigating the pathogenicity of novel CDKN2A 5'UTR variants and their impact on a possible 5'UTR IRES activity. This study was funded by PRIN 2008

Stress tolerance of probiotic microorganisms in a simulated human gastro-intestinal system

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The market of functional foods is strongly growing, especially in the probiotics area. According to the guidelines established by FAO and WHO, probiotics are those "microorganisms which confer a health benefit to the host"[1] Most of the currently used probiotics belong to the genera of Lactobacillus and Bifidobacterium. Lactobacillus plantarum (Lp) is a lactic acid bacterium (LAB) which grows on various substrates including animal and vegetable foods (as local contamination or as added starter culture), soil and mammalian intestine. To be effective, orally-delivered probiotics need to reach the intestine in a viable state and at high titre. Therefore they must be able to tolerate stresses such as gastric acidity and high bile salts duodenal osmolarity. Another desirable feature is a strong adhesion capacity to the intestinal mucosa, so to ensure a stable colonization of the host which is fundamental to the competitive exclusion of pathogenic bacteria and to modulation of local immune response. The development of in vitro systems to study specific traits, such as stress tolerance, safety and immuno-modulatory properties, is important for preliminary screening of potential probiotic strains. In this work we designed a novel in vitro system that simulates the human gastro-intestinal (GI) tract. In such a model, the survival potential of Lp WCFS1 [2] and of the commercial probiotic strains Lactobacillus acidophilus (La) LA-5 and Bifidobacterium lactis (Bl) BB-12, was analyzed. The behaviour of Lp WCFS1 wilde type strain was compared with that of a set of Lp WCFS1 mutants obtained by knock-out of stress-related genes. ΔctsR strain was generated by deletion of the transcriptional repressor CtsR; ftsH mutant was obtained by insertional inactivation of the metalloprotease chaperon FtsH; and Δhsp2 strain was made through deletion of the small heat shock protein sHsp2 [3,4]. All the above-described genes allow bacteria to cope with abiotic stress conditions, e.g. temperature upshift, pH medium variation, starvation, etc. Because cell envelope is one of the main targets of chemical-physical stresses, these mutants might present distinctive cell surface characters determining a peculiar interaction with the host cells. Bacteria were mixed with a "milk medium" and stressed in the in vitro system which was prepared using specific saline solutions, supplied with the main enzymes characterizing the human digestive tract (lysozyme, pepsin and pancreatin) and with bile salts at the concentration found in the duodenum. The pH of the bacterial suspension was changed sequentially, reflecting the digestion process. Among the analyzed strains, Bl BB-12 showed the best resistance properties to the extreme GI conditions, La LA-5 reacted positively to saliva stress, whereas Lp WCFS1 revealed a significant reduction of cellular titre in the first steps of gastric stress, although it recovered viability in the intestinal conditions and survived the most drastic hydrogenionic concentrations. Unexpectedly the Lp WCFS1 mutant strains exhibited appreciable survival potentials, in some cases performing better than wilde type strain. This result prompts us to study the interaction of Lp wilde type and mutant strains with human intestinal epithelial cells. References 1.FAO/WHO (2002) ftp://ftp.fao.org/es/esn/food/wgreport2.pdf. 2.Kleerebezem M. et al. (2003) Proc. Natl. Acad. Sci. USA 100:1990–1995. 3.Fiocco D. et al. (2010) J. Bacteriol. 192(3) 896-900. 4.Spano G. et al. (2005) Res. Microbiol. 156(2): 219-224.

Cannabinoid receptor 1 influences chromatin remodeling in mouse spermatids by affecting content of transition protein 2 mRNA and histone displacement.

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Marijuana smokers and animals treated with Delta 9-tetrahydrocannabinol, THC, the principal component of marijuana, show alterations of sperm morphology suggesting a role for cannabinoids in sperm differentiation and/or maturation. Since the cannabinoid receptor 1 (CNR1) activation appears to play a pivotal role in spermiogenesis, the developmental stage where DNA is remodeled, we hypothesized that CNR1 receptors might also influence chromatin quality in sperm. We used Cnr1 null mutant (Cnr1-/-) mice to study the possible role of endocannabinoids on sperm chromatin during spermiogenesis. We demonstrated that CNR1 activation regulated chromatin remodeling of spermatids by either increasing Tnp2 levels or enhancing histone displacement. Comparative analysis of WT, Cnr1+/- and Cnr1-/- animals suggested the possible occurrence of haploinsufficiency for Tnp2 turnover control by CNR1, while histone displacement was disrupted to a lesser extent. Further, flow cytometry analysis demonstrated that the genetic loss of Cnr1 decreased sperm chromatin quality and was associated with sperm DNA fragmentation. This damage increased during epididymal transit, from caput to cauda. Collectively, our results show that the expression/activity of CNR1 controls the physiological alterations of DNA packaging during spermiogenic maturation and epididymal transit. Given the deleterious effects of sperm DNA damage on male fertility, we suggest that the reproductive function of marijuana users may also be impaired by deregulation of the endogenous endocannabinoid

How many roles for TSC22D4 protein in cerebellum granule neurons of the mouse: isoforms' subcellular localization and functional interactions

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During postnatal cerebellum development, the Tsc22d4 gene (belonging to the TGF-beta1-stimulated clone 22 domain,TSC22D, gene family) is consistently expressed in cerebellum granule neurons (CGNs) and the encoded 42 kDa TSC22D4 form is post-translationally modified giving origin to higher MW forms (42, 55, 67 and 72 KDa apparent MW, respectively). These forms vary in relative abundance during postnatal cerebellum development and CGN differentiation in vitro and arise from O-glycosylation and phosphorylation, but not alternative splicing (Canterini et al., 2009). The existence of multiple and developmentally-regulated TSC22D4 forms suggests that this protein is involved in function(s) mediated by a physical interaction with other protein(s). In fact, two-cell hybrids and in silico analyses have identified several putative TSC22D4 partners, including the Actin-binding double-zinc-finger protein ABLIM1 (Rual et al., 2005), the Prion Protein, the Apoptosis Inducing Factor (Lim et al., 2006) and the Kelch-like ECH-associated protein 1 (Sowa et al., 2009), a protein involved in the homeostasis of reactive oxygen species (Velichkova et al., 2005). Moreover, immunoprecipitation assays performed in our laboratory have recently shown that TSC22D4 interacts with the Slo1 subunit of large conductance calcium-activated potassium channels (BKCa) in the adult cerebellum. Studying TSC22D4 expression and subcellular localization in in vitro cultured CGNs, we observed that this factor is prominently localized in the cytoplasm and along neurites under normal conditions and that it rapidly and transiently translocates to nucleus when these cells are committed to apoptosis by experimental inhibition of membrane electrical activity (Canterini et al., 2009), suggesting a role in the interplay between CGN membrane electrical activity and survival/differentiation or apoptosis. We have recently addressed this issue by investigating the TSC22D4 intracellular localization during CGNs development in early postnatal cerebellum (PN6-40), when CGNs undergo a dramatic change in morphology and membrane excitability properties (Hatten and Heintz, 1995; Nakanishi and Okazawa, 2006). This study has shown that TSC22D4 is stably resident in both nuclear and somatodendritic compartments before PN12-18, namely the differentiation time at which CGN viability becomes dependent on membrane electrical activity (Gallo et al., 1987; Canterini et al., 2009). In mature CGNs, however, TSC22D4 apparently leaves the nucleus and accumulates at the level of synaptic connections with mossy fibers, namely cerebellum glomeruli. These features were also observed in cerebellum slices cultured in vitro under conditions favoring/inhibiting CGN differentiation by a variation in K+ concentration in the culture medium (Canterini et al., 2010). In light of these findings, it is tempting to hypothesize that major TSC22D4 functions take place (i) at the level of nucleus in differentiating CGNs; (ii) at the level of synapses in mature CGNs; and (iii) again at the level of nucleus, but with subnuclear targets different than those of immature cells, when CGNs are committed to apoptosis, as also suggested by the different fashions of TSC22D4 accumulation in the nucleus (Canterini et al., 2009; 2010). The characterization of TSC22D4 forms specific subcellular localizations and the identification of protein partners functionally interacting with TSC22D4 at the level of different subcellular compartments is currently under investigation in our laboratory. References Canterini, S. et al., Mol Cell Neurosci 40, 249-257 (2009). Rual, J.F. et al., Nature 437, 1173-1178 (2005). Lim, J., et al., Cell 125, 801-814 (2006). Sowa, M.E. et al., Cell 138, 389-403 (2009). Velichkova, M. & Hasson, T. Mol Cell Biol 25, 4501-451(2005). Hatten, M.E. & Heintz, N. Annu Rev Neurosci 18, 385-408 (1995). Nakanishi, S. & Okazawa, M. J Physiol 575, 389-395 (2006). Gallo, V., Kingsbury, A., Balazs, R. & Jorgensen, O.

ANALISYS OF GENE EXPRESSION PROFILE DURING MYOGENESIS EVIDENCES DIFFERENT MOLECULAR DEFECTS AT THE BASIS OF FSHD-1 AND FSHD-2 PATHOGENESIS

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disorder mainly associated with a contraction of the subtelomeric repeat D4Z4 on chromosome 4q (4q35.2). The D4Z4 copy number is highly polymorphic in normal individuals ranging between 11-150 copies, whereas almost all FSHD patients (FSHD-1) carry fewer than 11 repeats. Current hypothesis on FSHD-1 pathogenesis foresees the presence of an epigenetic molecular mechanism, but, how the contraction of D4Z4 array could determine alterations in chromatin structure and trigger transcriptional deregulation of target unknown genes is not clear. Interestingly, there is a small percentage of FSHD cases (< 5%) considered as patients with phenotypic FSHD (FSHD-2), presenting clinical signs of disease but showing no contractions of D4Z4 on chromosome 4q35. This subset of patients appears very heterogeneous and to date no disease locus has been identified. To derive the molecular perturbation of FSHD pathogenesis during muscle differentiation, we analyze the global gene expression profiles of three FSHD-1, two FSHD-2 and three controls proliferating myoblasts and of the corresponding myotubes, by using the Human GeneChip Exon 1.0 ST platform. FSHD-1 and FSHD-2 proliferating cells showed a total of 266 and 365 dysregulated probes, respectively, sharing only 17 genes. The same analysis performed on FSHD-1 and FSHD-2 myotubes evidenced a total of 129 and 1530 dysregulated probes, respectively. Also in this case the number of shared genes was very low (40 genes). The dysregulated probes were categorized in the DAVID program. The biological processes most affected in FSHD-1 myoblasts were mainly linked to cell cycle, organelle fission and DNA metabolic processes. More precisely, this classes, that represent the most significant ones, are essentially composed by down-regulated genes. The same analysis performed on FSHD-2 myoblasts highlighted that the most significant dysregulated functional classes were represented by multicellular organismal process and development, and response to external stimulus. The functional analysis performed on genes dysregulated in myotubes identified biological processes essentially involved in transport and biosynthetic processes for FSHD-1 and functional classes mainly involved in cellular metabolic processes, RNA metabolism and processing for FSHD-2. Interestingly, all the categories involved in cell cycle, identified as dysregulated in FSHD-1 myoblasts, were not evidenced in FSHD-1 myotubes. In general our results strongly indicate that gene dysregulation is mainly associated to proliferating cells in FSHD-1 and to differentiated cells in FSHD-2, thus suggesting that the molecular mechanisms at the basis of the two forms of disease are almost different. The gene expression analysis of FSHD-1 and FSHD-2 myoblasts and myotubes also evidenced several dysregulated miRNAs. Interestingly many of the predicted miRNA targets were effectively dysregulated in the chip analysis, suggesting a role of miRNAs in the regulatory network of FSHD. Functional studies are now necessary to better understand the biological significance of the data obtained and to experimentally verify the involvement of miRNA gene targets. In general, our approach provides new insights into the molecular mechanism of FSHD, allowing the identification of new candidate genes that may represent potential targets for clinical application.

Identification and characterization of a mitochondrial FLVCR isoform

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Feline Leukemia Virus subgroup C Receptor (FLVCR) was originally identified and cloned as a cell-surface protein receptor for feline leukaemic virus subgroup C, causing pure red blood cell aplasia in cats. Recent studies have demonstrated that FLVCR is a heme exporter essential for erythropoiesis. The heme efflux via FLVCR was shown to be essential for erythroid differentiation in K562 cells as well as in CD34+ precursors cells. Moreover, Keel and co-authors have reported that Flvcr-null mice die in utero due to the failure of fetal erythropoiesis1. We have identified an alternative transcription start site giving rise to a novel FLVCR isoform (FLVCRb). Flvcr-b transcript completely lacks the first exon of the canonical isoform (FLVCRa) and code for a putative 6 transmembrane domain containing protein ubiquitously expressed. In vitro over-expression of FLVCRa and FLVCRb showed that the two proteins display different subcellular localization. As expected FLVCRa localizes at the cell membrane, while FLVCRb localizes in the mitochondrial compartment. The mitochondrial localization of this novel isoform is further confirmed by the identification of a N-terminal mitochondrial sorting presequence. Because of FLVCRa is a heme exporter at the cell membrane, we hypothesized that FLVCRb could be the mitochondrial heme exporter. According to this hypothesis, FLVCRb expression increased following the stimulation of heme biosynthesis in vitro, in correlation with the increase in hemoglobin production. The ability of FLVCRb to bind and export heme out of the mitochondria is still under investigation. To gain insights into the specific roles of the two isoforms, we have generated Flvcr mutant mice different from those previously reported1. Keel and co-author generated a mouse model in which both FLVCRa and FLVCRb have been deleted. In our mouse model, FLVCRa has been specifically deleted and FLVCRb is still expressed (FLVCRa-null mice). Flvcr-a heterozygous mice were grossly normal, fertile and indistinguishable from their wild-type littermates. When Flvcr-a heterozygous mice were intercrossed, no Flvcr-a homozygous knock-out newborns were obtained, and the analysis of the embryos from timed Flvcr-a+/- intercrosses showed that the Flvcr-a-/- genotype was lethal between E14.5-E16.5. Flvcr-a-null embryos showed multifocal and extended hemorrhages, visible in the limbs, head and throughout the body wall, as well as subcutaneous edema. Alcian blue-alizarin red staining demonstrated skeletal abnormalities in limbs and head similar to that observed in Diamond-Blackfan anemia (DBA) patients. Interestingly, flow cytometric analyses of E14.5 fetal liver cells double-stained for Ter119 (erythroid-specific antigen) and CD71 (transferrin receptor) show normal erythropoiesis in Flvcr-a-null embryos, opposite to the previously reported Flvcr-null mice1. Taken together, these data demonstrated that FLVCRb is sufficient to support fetal erythropoiesis likely exporting heme from the mithocondrion for hemoglobin synthesis. The loss of FLVCRa leads to endothelial ruptures responsible for hemorrhages thus suggesting that FLVCRa is needed for detoxifying heme excess at these sites. 1.Keel SB et al. A heme export protein is required for red blood cell differentiation and iron homeostasis. Science 2008. Feb 8;319(5864):825-8.

Cloning and characterization of gpr54 in the testis of the anuran amphibian, Rana esculenta and possible crosstalk with GnRH and the endocannabinoid system.

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The reproductive axis, a dynamically regulated neuro-hormonal system, is arranged into three major levels of integration: hypothalamus, pituitary and gonads. The complicate network governing gonadotropin release - luteinizing hormone or LH and follicle-stimulating hormone or FSH - is essentially controlled by pulsatile secretion of GnRH, whose action is driven by the complex interaction of excitatory/inhibitory signals, of central and peripheral origin. Recent studies have contributed to the discovery of new central regulators of the reproductive axis: kisspeptins. They are a novel family of structurally related peptides encoded by kiss1 gene, able to bind and activate the G protein-coupled receptor, GPR54. In many species, KISS system expression in several peripheral tissues other than in discrete regions of the forebrain let to argue that it may be involved in numerous physiological mechanisms. Mutations in gpr54 gene exhibit hypogonadotropic hypogonadism, delayed sexual maturation and low levels of gonadal steroids. Despite GPR54 presence in the pituitary and kisspeptins release in the portal blood, these peptides directly send excitatory inputs to hypothalamic GnRH neurons, stimulating LH release. We cloned, in the past, gnrh1 and gnrh2 genes and related receptors in the anuran amphibian, Rana esculenta, a seasonal breeder with laminated encephalic structure - archetype of those more elaborated of the higher vertebrates - and with cystic organization of the germinal compartment. Although a functional crosstalk between endocannabinoid and GnRH systems in the frog forebrain and testis has been demonstrated, mechanisms remain unclear. In this paper, we evaluate the possible involvement of GPR54 in the progression of spermatogenesis and the interaction between the endocannabinoid and GnRH systems, both at central and testicular levels. We cloned a 503 bp gpr54 cDNA fragment, we analyzed its expression in a range of frog tissues (pituitary, brain, spinal cord, ovary, muscle, kidney, heart, liver, spleen), and in the testis during the annual reproductive cycle. In the testis, gpr54 is expressed at high levels in November and from February to April; this pattern correlates with testicular steroid-secreting activity and, in February-April, with the resumption of spermatogenesis and spermatogonial proliferations. By in situ hybridization, gpr54 is localized in the interstitial compartment and in mitotic stages of the germinal compartment. Possible autocrine-paracrine relationships among endocannabinoids, GnRH and kisspeptins were verified analyzing the localization of both cb1 cannabinoid receptor and gnrh system in testes, by in situ hybridization. Finally, the direct effect of the endocannabinoids on gpr54 expression was evaluated by in vitro treatments of testes and diencephalons with the endocannabinoid, anandamide (AEA, 10-9M). In diencephalons, this treatment inhibits gnrh1, gnrh2 and gpr54 expression. Conversely, in the testis - although a modulation of gnrh expression is observed - it does not affect gpr54. In conclusion, our data let to hypothesize an involvement of GPR54 activity in the regulation of testicular physiology, both in relation to steroid-secreting activity and progression of spermatogenesis. Whereas at testicular level endocannabinoids modulate gnrh expression, without affecting gpr54, at central level the AEA inhibitory effect on gnrh synthesis may be mediated by gpr54 down regulation.

Molecular approach to study the structure-function of a Pleurotus eryngii laccase isoform

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Laccases are biotechnologically interesting enzymes belonging to the polyphenol oxidases family. They are widely distributed throughout the phylogenetic scale from bacteria to mammals. In fungi the analysis of three-dimensional crystal structures of laccases indicate that ascomycete laccases are processed at their C-termini, at a conserved cleavage site, resulting in the proteolytic removal of C-terminal residues. We have isolated and cloned in expression vectors the cDNAs encoding two laccase isoforms (Ery3 and Ery4) from the basidiomycete Pleurotus eryngii. The Ery3 enzyme expressed in Saccharomyces cerevisiae, is functional, whereas the recombinant Ery4 protein does not show enzymatic activity. In order to explain this evidence, we investigated the relationship between the structure of the C-terminal extension and laccase enzymatic activity. The tasks of the present study were to determine the biological role of laccase C-terminal, and to validate a "molecular engineering" approach for the production of recombinant laccases with novel biochemical properties. Genetically engineered mutant genes were produced from Ery4 by: i) progressive 3'-terminal deletions, ii) point mutations, iii) Ery3/Ery4 chimeras. The mutant genes were expressed in S. cerevisiae and active recombinant laccase isoforms were produced, exhibiting each a different biological behaviour. The correlations between the structural information deriving from both biochemical and bioinformatic analyses shed light on the role of C-terminal region in determining laccase functions. The obtained data also indicated that our approach could represent an efficient method for laccase genetic engineering. To our knowledge, this study has produced the first evidences obtained by biotechnological approach of the involvement of the C-terminal tail in the inactivation/activation process of a basidiomycete laccase.

Rab7b controls trafficking from endosomes to the TGN

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Rab GTPases compose the largest family of small GTPases. These proteins function as molecular switches by cycling between two conformational states: an active GTP-bound ("ON" form) and an inactive GDP-bound form ("OFF" form). Nucleotide exchange factors (GEFs) promote GDP release and GTP binding, while GTPase activating proteins (GAPs) promote GTP hydrolysis. In mammalian cells, there are more than 60 members of the Rab GTPase family that are localized to distinct intracellular compartments and that are involved in the regulation of different steps of vesicular membrane transport. Rab7 is a small GTPase that regulates transport towards late endosomes and lysosomes. Recently, a Rab protein that shares about 50% identity and 65% similarity with Rab7 has been identified and named Rab7b. Sequences comparison of two proteins suggests that Rab7 and Rab7b are not isoforms. Therefore, we decided to study Rab7b expression, intracellular localization and function in comparison with Rab7. In order to establish the intracellular localization of Rab7b, we performed immunofluorescence analysis: we demonstrated that Rab7b colocalizes not only with late endosomal/lysosomal markers (as Lamp1 and CI-MPR) on late endosomes, but also with TGN and Golgi markers (as giantin, Golgin-97, TGN46 and Golgin-245). In addition, we demonstrated that the constitutively active Rab7b Q67L mutant is localized almost exclusively on TGN and Golgi. Interestingly, in cells transfected with the dominant negative mutant Rab7b T22N, several Golgi and TGN markers look dispersed and not concentrated in the perinuclear area. In contrast to Rab7, Rab7b is not involved in EGF and EGFR degradation suggesting that Rab7b has different function in intracellular membrane trafficking. In order to establish the step of transport controlled by Rab7b, we quantified hexosaminidase secretion in Rab7b depleted cells. Normally, this lysosomal enzyme is captured by mannose-6-phosphate receptors (M6PR) in the TGN for transport to lysosomes and only a small amount of it is secreted. In cells depleted for Rab7b (or transfected with the Rab7b dominant negative mutant, Rab7b T22N) we observed an increase of hexosaminidase secretion of approximately two fold. We excluded that this could be due to Rab7b involvement in the secretion pathway, as vesicular stomatitis virus G protein (VSV-G) secretion was not altered in cells depleted for Rab7b. We also discovered that in Rab7b-depleted cells the amount of M6PR and cathepsin D was increased compared to control cells. In addition, cathepsin D maturation was impaired as immature forms of cathepsin D accumulate upon Rab7b depletion, indicating that Rab7b function is essential for lysosomal enzymes trafficking. Importantly, Rab7b depletion prevented endocytosed cholera toxin B-subunit from reaching the Golgi. Altogether, these data indicate that Rab7b is required for normal lysosomal function, and, in particular, that Rab7b is an essential factor for retrograde transport from endosomes to the TGN.

Epigallo cathechin gallate inhibits estrogen receptor alfa gene expression in tamoxifen resistant breast cancer cells

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Acquired resistance to hormonal therapy for breast cancer commonly develops after an initial response to tamoxifen or aromatase inhibitors. It is well accepted that molecular mechanisms underlying hormone resistance development, involves the cross-talk of estrogen receptor alfa (ER alfa) with growth factor signalling pathways. Therefore agents to counteract ER alfa activity, which is involved in the adaptive change related to resistance, would substantially enhance the long-term benefits of hormonal therapy. We identified the inhibitory effects of epigallo cathechin gallate (EGCG), the main polyphenolic compound isolated from green tea, on tamoxifen resistant breast cancer cell proliferation. We found that under our control experimental conditions, basal cell proliferation of ER+ MCF-7 and Tamoxifen resistant breast cancer cells (MCF-7 TR) was inhibited by 2microM EGCG of 20% and 18% respectively. Increasing amounts (20 microM; 60 microM), reduced the MCF-7 cell number from 45% to 67% and MCF-7 TR1 from 42% to 70%, in a dose dependent manner. These effects are associated with increased expression of p21, decreased cyclin D1 and concomitant down-regulation of ER alfa both at mRNA and protein content. In order to evaluate the direct effects of EGCG on ER alfa gene transcription, ER alfa promoter region covered from -4100 bp to-212 bp was investigated and nuclear factor kappa B (NFjB), and activator protein 1 (AP1) sites were identified as within this region as potential target of EGCG action. Thus to identify the promoter regions involved in EGCG mediated down-regulation of ER alfa expression, five overlapping ERα promoter deletion constructs, −245 bp to +212 bp (A), −735 bp to +212 bp (B), −1000 bp to +212 bp (C), −2769 bp to +212 bp (D), and −4100 bp to +212 bp (E), all relative to the first transcriptional ATG start site were analyzed. The plasmids were transiently transfected into MCF-7 and MCF-7 TR cells then treated with increasing concentrations of EGCG. Our data show that EGCG significantly and reproducibly reduced the activity of fragment E and fragment D tested in both cell types indicating that the region between –4100bp to –1000 bp, which contains important regulatory elements necessary for ER alfa transcription, was involved in the EGCG mediated down-regulation of ER alfa activity. To evaluate the role of the NFjB and AP1 sites present within the above mentioned ER alfa gene promoter region, site-directed mutagenesis experiments to alter these sites are in progress.

Functional interaction between the G1 subunit of the V-ATPase and RILP

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The acidity of intracellular compartments and extracellular environment is crucial to various cellular processes. Proton transport is mediated by ATP-dependent proton pumps known as the vacuolar (V)-ATPases. V-ATPases are large multisubunit complexes composed of 14 different subunits that are organized into an ATP-hydrolytic domain (V1) and a proton-translocation domain (V0), that work together as a rotary machine. The V-ATPases have central roles, in normal physiology, to modulate pH homeostasis in a number of intracellular sites: (i) in sorting endosomes, to promote the uncoupling of internalized ligand–receptor complexes, following receptor-mediated endocytosis, (ii) in lysosomes and other digestive organelles to activate the degradative enzymes and (iii) in secretory vesicles where protons and membrane potential gradients are used to drive the uptake of small molecules, such as neurotransmitters. We have isolated, using the yeast two-hybrid screening, the G1 subunit of V-ATPase as a RILP (Rab Interacting Lysosomal Protein) interacting protein. RILP is required for biogenesis of MVBs (multivesicular bodies), is a Rab7 effector protein and, together with Rab7, regulates late endocytic traffic. The interaction data obtained with the two-hybrid system were confirmed, in mammalian cells, by co-immunoprecipitation. In addition, we demonstrated that RILP directly interacts with V1G1 using bacterially expressed and purified recombinant proteins. Confocal immunofluorescence analysis revealed co-localization of GFP-RILP and HA-V1G1. Further analysis suggests that RILP acts as a negative regulator of V1G1 because V1G1 protein levels are affected by RILP over-expression and RILP depletion. Indeed, in RILP-depleted cells the total levels of V1G1 strongly increase, while transient over-expression of RILP is accompanied by a strong reduction of the total V1G1 levels. The treatment with MG132, a proteasome inhibitor, shows that the decrease of V1G1 protein levels, after RILP-over-expression, is proteasome activity dependent. Reversible dissociation of V-ATPase complexes represents an important but yet incompletely understood process. Recently, some data suggest a possible ‘cross-talk’ between V-ATPase and small GTPases for control of disassembly/assembly of V-ATPase. Interestingly in cells expressing Rab7 dominant-negative mutants lysosome acidification was severely perturbed, presumably reflecting problems with the proton ATPase activity. To clarify if RILP, and possibly Rab7, may function to control assembly/disassembly of the V-ATPase modulating V1G1 stability, we are performing Western blot analysis on cellular fractions to analyze V-ATPase assembly on membranes after RILP over-expression and/or RILP silencing. Moreover, RILP abundance seems to modulate V-ATPase activity, as we detected changes in the extracellular pH in cells overexpressing RILP or in RILP silenced cells. Acidification, triggered by V-ATPase, in early endosomes of mammalian cells is required for the formation of MVBs, so our data support the idea that RILP might coordinate the biogenesis of MVBs through V1G1 activity regulation.

Molecular profiling of human oocytes after vitrification strongly suggests that they are biologically comparable to freshly isolated gametes

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Oocytes cryopreservation is a helpful fertility preservation technique for women at risk of loosing their ovarian functions following disease, surgery or chemotherapy. Moreover, avoiding embryo cryopreservation would solve religious, ethical and legal problems, connected to the laws that actually regulate medically assisted reproduction in various countries. There are two major techniques for cryopreservation: slow freezing and vitrification. Many published studies have compared frozen-thawed human oocytes, either after slow freezing, or vitrification with fresh collected ones and they have analyzed their biologic behaviour as well as more specific structural cellular features. However, there are no published data on the molecular profile of oocytes after cryopreservation. To assess the effects of vitrification on the biologic quality of oocytes, we compared the expression profile of mRNAs in single vitrificated-thawed oocytes with that of freshly collected oocytes without cryopreservation. We report the expression analysis of eight different genes: three perform housekeeping functions, since they encode proteins involved in the basic cellular functions and are constantly expressed in all human cells [HPRT, GAPDH, CYCLOPHILIN)]; the other five genes encode proteins essential for oocyte development and specific functions [BMP15, GDF9, FIGLA, OCT4, TAF4B]. The transcripts chosen for our analysis encode proteins that are essential for mature oocytes, due to their role in gamete viability and in follicular and embryo development: accordingly, they represent excellent molecular markers of oocyte quality. Human oocytes were collected from an IVF Centre (Servizio di PMA/ Azienda Ospedaliera Cannizzaro, Catania, Italy), after informed consent for the use of supernumerary ones. A total of 25 metaphase II (MII) oocytes were collected from 5 different women, whose primary infertility was due to a male factor: this excluded pathologies that could influence oocyte quality. For each patient, we collected 2 fresh and 3 vitrificated oocytes, choosing those with optimal morphology, for a total of 10 fresh and 15 vitrificated oocytes. Our data clearly show that the expression profile of the eight genes, chosen as biomarkers, did not change between fresh and vitrificated oocytes . To statistically validate our results, we compared the Ct value, normalized to HPRT (DeltaCt), in fresh and vitrificated oocytes by using the independent Student's t-test and demonstrated that there are no significant variations between fresh and vitrificated-thawed oocytes. Our molecular data, together with published results on oocyte survival, oocyte fertilization and pregnancy rates, confirm that vitrification might be very helpful for preserving women fertility. REFERENCES Purrello M, Di Pietro et al. Genes for human general transcription initiation factors TFIIIB, TFIIIB-associated proteins, TFIIIC2 and PTF/SNAPC: functional and positional candidates for tumour predisposition or inherited genetic diseases? ONCOGENE. 2001 Aug 9;20(35):4877-83. C Di Pietro et al Molecular profiling of human oocytes after vitrification strongly suggests that they are biologically comparable to freshly isolated gametes. FERTILITY AND STERILITY, (e-pub ahead of print on 9 June 2010).

S1P induces differentiation of human mesoangioblasts towards smooth muscle cells. A role for GATA6 and LMCD1 transcription factors.

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The sphingolipid metabolite sphingosine 1-phosphate (S1P) is a lipid mediator that regulates a wide number of fundamental biological processes. Complexity in S1P signalling is increased by the fact that endogenous S1P is mediator of the biological actions of a number of growth factors and cytokines. S1P has recently been shown to have important effects on vascular development and smooth muscle cells (SMC) growth and migration [1]. SMC control many fundamental functions such as arterial tone and airway resistance; alterations of vascular smooth muscle cells contribute to a number of major diseases in humans including atherosclerosis. Recent studies have provided evidence showing that circulating, SMC progenitor cells can contribute to neointima formation and repair following vascular injury [2]. Mesoangioblasts (Mb) are a new type of stem cells, isolated from explants of dorsal aorta, capable of differentiating into many mesoderm cell types [3]. We previously demonstrated that S1P acts as potent mitogen and antiapoptotic agent in murine and human Mb [4]. Supporting the important role of sphingolipid metabolism, we also recently showed that transforming growth factor beta (TGFbeta) exerts a marked antiapoptotic action in Mb with a mechanism involving regulation of sphingosine kinase (SphK)-1 [5]. In order to completely utilize the therapeutic potential of these cells, with the aim of fully individuating the pleiotropic biological action of S1P, we performed a microarray study to establish transcriptional profiles of human Mb treated with 1µM S1P for 6 h and 24 h. Obtained results, validated by Real time PCR, Western blot and immunofluorescence analysis, demonstrate for the first time that S1P promotes differentiation of human Mb towards SMC by enhancing the expression of myogenic marker proteins, such as calponin-1 (CNN-1), tropomyosin-1 (TPM-1), transgelin etc. Addition of 1µM S1P augmented Mb morphogenesis in 3D matrigel culture, while pharmacological inhibition of SphK impaired the process. Interestingly, GATA6, LMCD1 and TRPS1 transcription factors were identified involved in the molecular mechanism of S1P-induced increase of CNN1 and TPM1 in Mb. The S1P-induced increase of GATA6 and LMCD1 was also demonstrated to be mutually regulated. Moreover, we present evidence that TGFbeta-induced differentiation of Mb into SMC relies on SphK regulation, since pharmacological inhibition of the enzyme impaired the ability of the cytokine of increasing the expression levels of myogenic markers and on GATA6 and LMCD1 expression. Interestingly, TGFbeta-induced increase of GATA6 and LMCD1 depends on SphK regulation. This study individuates an important role of S1P as effector of TGFbeta-induced smooth muscle differentiation in Mb through the activation of specific transcription factors which can be exploited to favour vascular regeneration. [1] Pyne, S., Pyne, N. J. (2000) Sphingosine 1-phosphate signalling in mammalian cells. Biochem. J. 349, 385-402 [2] Owens GK, Kumar MS, Wamhoff BR. (2004)"Molecular regulation of vascular smooth muscle cell differentiation in development and disease" Physiol Rev. 84:767-801. [3] Cossu G, Bianco P. (2003) "Mesoangioblasts-vascular progenitors for extravascular mesodermal tissues" Curr. Opin. Genet. Dev. 13, 537-542 [4] Donati, C., Cencetti, F., Nincheri, P., Bernacchioni, C., Brunelli, S., Clementi, E., Cossu, G., Bruni, P. (2007) Sphingosine 1-phosphate mediates proliferation and survival of mesoangioblasts. Stem Cells 25, 1713-1719 [5] Donati C, Cencetti F, De Palma C, Rapizzi E, Brunelli S, Cossu G, Clementi E, Bruni P. (2009) "TGFbeta protects mesoangioblasts from apoptosis via sphingosine kinase-1 regulation" Cell Signal. 21, 228-236

The ADAR editing enzymes are novel HIV-1 proviral factors

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One of the best-characterized mechanisms of RNA editing is the conversion of adenosine into inosine (A-to-I) mediated by the Adenosine DeAminase enzymes or ADARs that act on double-stranded RNA. In mammals, three different ADAR enzymes have been identified: ADAR1, ADAR2, and ADAR3 (1). Inosine acts as guanosine during both splicing and translation events, therefore A-to-I editing within pre-mRNA can alter both splicing patterns and amino acid sequence with important consequences in the final function of the coded protein. Recent evidence demonstrated that most of the A-to-I substitutions occur within non-coding sequences of pre-mRNAs enriched in inverted repeated Alu elements, such as introns and untranslated regions (UTRs) (1). RNA editing of non-coding sequence can alter the fate of pre-mRNAs by affecting their splicing, localization, stability or translation (1). ADARs can target viruses, as suggested by numerous reports showing A-to-I changes identified in viral genomes or transcripts that are consistent with editing mediated by these enzymes (1). So far little effort has been dedicated to testing the involvement of ADARs in the life cycle of the human immunodeficiency virus type 1 (HIV-1). HIV-1 gene expression is tightly regulated at the level of transcription and maturation of an unspliced primary transcript (9 kb RNA) in distinct classes of partially and completely spliced RNA molecules (4 kb and 2 kb RNAs) and is accomplished by a coordinated interaction between viral and cellular factors (2). In addition, HIV-1 RNAs contain several double stranded regions, some of them critical for the different steps of the viral life cycle such as the Rev responsive element (RRE), trans-activation responsive element (TAR), and dimerization domain (DIS) (2) that could be possible ADARs substrates. Recently we provided evidence of a role for ADAR1 and ADAR2 in the regulation of some critical steps of the HIV-1 life cycle (3,4). We demonstrated that over-expressed ADAR1 and ADAR2 strongly increase the overall accumulation of HIV-1 proteins in producer cells independently of their editing activity. Through their RNA editing activity, both enzymes enhance the release of progeny virions from the producer cells. Of note, only the ADAR1-mediated editing increases the infectious potential of the virus. Finally, we demonstrated that both enzymes edit adenosines within the 5' UTR of viral transcripts, but only ADAR1 edits specific adenosines within the Rev and Tat coding sequence. Therefore, ADAR2 and ADAR1 share some but not all the functions that positively regulate HIV-1 replication. Future investigations are necessary to understand the impact of ADARs in the HIV-1 life cycle. This study, by providing further insight into the role of the ADAR enzymes in stimulating the sequence diversification and replication of HIV-1, will possibly help the disclosure of novel targets for antiviral intervention. REFERENCES: 1. Bass, B.L. (2002). Annu. Rev. Biochem., 71, 817-846. 2. Lama, J. and Planelles, V. (2007). Retrovirology, 4, 52. 3. Doria, M., Neri, F., Gallo, A., Farace, M.G., and A. Michienzi. (2009.) Nucleic Acids Res. 37:5848-58. 4. Margherita Doria, Angela Gallo, Francesca Neri, Sara Tomaselli, Silvia Anna Ciafrè, Maria Giulia Farace, and Alessandro Michienzi. Manuscript submitted

Mushroom tyrosinase inhibition by essential oils from lavender and mint.

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The screening and characterization of novel tyrosinase inhibitors are useful for potential applications in food technology and in cosmetic and medical treatment of human pigmentation disorders. In this study the essential oils obtained by hydrodistillation from the lavender Lavendula spica L. and the mint Mentha x piperita L. were investigated for their potential tyrosinase inhibitory activity. To gain insights into the potential active compounds of the oil mixtures, the essential oils composition was assessed by GC-mass-spectrometry techniques. Both extracts significantly inhibited the mushroom tyrosinase activity (i.e. oxidation of L-DOPA). Inhibition was oil dose-dependent and oil extracts concentrations inhibiting 50% tyrosinase activity (IC50) were determined. The inhibition kinetics analysed by Lineweaver-Burk plots, showed a non-competitive and mixed type inhibition for lavender and mint oils, respectively. Ki values were determined. Our results indicate that lavender and mint essential oils have high potentials in applications as skin-whitening agents of natural source. Therefore, they may be promising herbal ingredients for the development of depigmenting agents in clinical, beauty and industrial prospects.

16p13.3 and 17q21.3 chromosome breakpoints: are they randomly or non-randomly affected in Deep Fibrous Histiocytoma?

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Introduction. Fibrous histiocytoma (FH) encompasses a wide spectrum of common soft-tissue tumors exhibiting both fibroblastic and histiocytic differentiation. FH has a predominant dermal location, however a rare sub-type, occurring in deep soft tissues, has been recently formally characterized as a diagnostically-distinguishable variant (1). Due to the small number of cases published, information on its clinical behavior, including propensity for local recurrence and metastasis, is quite limited, and no molecular genetic or cytogenetic data are available. Aim. To add knowledge to the biology of this type of lesion, we reconsider from our cytogenetic file a case of deep fibrous histiocytoma with 46,XY,t(16;17)(p13.3;q21.3) karyotype. The tumor occurred in a subject with normal male karytype and was the first description of deep FH showing a clonal chromosome abnormality. Moreover the change was not found in any of the tumors reported in Mitelman's online database (2). To ascertain whether the breakpoints at 16p13.3 and 17q21.3 were non-randomly involved in changes in this rare tumor subtype we: a) searched for BAC clones spanning the translocation breakpoints in order to characterized them at the molecular cytogenetic level; b) identified BAC clones mapping proximally and distally to the breakpoints; c) used them to set up dual-color break-apart probes able to disclose the breakpoints by interphase fluorescent in situ hybridization (I-FISH); d) tested the probes in six archival cases. Results. FISH using BAC clones refined the translocation breakpoints within 119.9 kb at 16p13.3 and 214 kb at 17q21.3. BAC clones proximal and distal (about 100-200 kb) to the breakpoints were identified and a dual color break-apart probe for each breakpoint was set up. None of the two probes gave break-apart signals in the six archival cases studied. Comments. We previously reported that superficial FH (dermal location) has clonal non-recurrent chromosome changes (2). On the contrary, no data are available for the recently formally characterized deep variant. Since at the moment the t(16;17) is unique and never described in any solid tumor and these lesions rare and the diagnosis sometime controversial, we planned to ascertain whether the t(16;17) breakpoints were nonrandomly involved in changes in additional archival cases. After refining the breakpoint within 119.9 kb at 16p13.3 and 214 kb at 17q21.3 in the index case, we set up a dual-color breakapart probe and tested it by I-FISH in the additional cases. Our results indicate that, as with cutaneous lesions, deep FH may have clonal chromosome changes and that, at present, they seem to be rare and non-recurrent. Supported by Fondo per gli Investimenti della Ricerca di Base (FIRB) project No. RBIP0695BB and Regione Autonoma Sardegna (RAS) by L.R. 7/2007 (fondo ricerca fondamentale). References 1) B.C. Gleason, C.D. Fletcher, Deep "benign" fibrous histiocytoma: clinicopathologic analysis of 69 cases of a rare tumor indicating occasional metastatic potential, Am. J. Surg. Pathol. 32 (2008) 354-62 2) Mitelman database of chromosome aberrations in cancer 2010. Mitelman F, Johansson B, Mertens F, editors. Available from: http://cgap.nci.nih.gov/ Chromosomes/Mitelman updated on February 18, 2010. 3) R. Vanni, C.D. Fletcher, R. Sciot, P. Dal Cin, I. De Wever, N. Mandahl, F. Mertens, F. Mitelman, J. Rosai, A. Rydholm, G. Tallini, H. Van Den Berghe, H. Willén, Cytogenetic evidence of clonality in cutaneous benign fibrous histiocytomas: a report of the CHAMP study group, Histopathology 37 (2000) 212-7.

Genotoxicity of amorphous silica on murine alveolar macrophages (RAW 264.7). Effects of particles micro-morphology.

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Silicosis, one of the more ancient occupational diseases, has been associated to the prolonged exposure to crystalline silica dusts (quartz). In addition, lung cancer and autoimmune pathologies have also been related to the exposure to some crystalline forms of silica. Numerous in vitro and in vivo studies have compared the effects of crystalline and amorphous silica and demonstrated the last form being much less active or almost biologically inert with respect to the crystalline ones. However, the paradigm of crystallinity, as a prerequisite to make a silica particle toxic is contradicted by the evidence that vitreous (amorphous) silica powdered by grinding share similar surface reactivity and in vitro toxicity with quartz dust. In spite of an enormous amount of experimental studies, the physical-chemical features imparting toxicity to silica particles are not yet fully understood. In the present study the genotoxic potential of amorphous silica particles with different superficial micro-morphology was investigated. In particular, mesoporous silica particles (MCM-41) and dense silica spheres (diameter, 250 and 500 nm) were compared in term of DNA damaging effects on cultured murine alveolar macrophages (Raw 264.7). Mesoporous silica particles were characterized by sharp edges, while dense spheres had a smooth surface. Genotoxicity was evaluated by alkaline Single Cell Gel Elecrophoresis (SCGE) or Comet Assay (detecting DNA strand breaks) and Micronucleus Test (detecting chromosomal damage). Cytotoxicity was also assessed by the Trypan Blue exclusion assay. Particle suspensions (five increasing concentrations) were prepared in complete MEM solution and sonicated for 30' (35 KHz) to reduce aggregation. Methyl methanesulphonate (MMS) and Mytomicin C were selected as positive controls for Comet assay and Micronucleus test respectively. Comet assay results showed that both amorphous silica particles induced DNA fragmentation at the highest doses tested (40 – 80 µg/cm2); however, mesoporous silica was significantly more genotoxic than dense silica spheres. Neither exposure time (4 and 24 h) nor particle dimensions showed any significant effect on DNA integrity. Although preliminary, our results are in line with the idea that physical-chemical features and micro-morphology of particle surface affect cell response and that the inertness/toxicity of amorphous silica deserves to be further investigated.

IDENTIFICATION OF NOVEL MAFB PRIMARY RESPONSE GENES IN PHORBOL 12 - MYRISTATE 13 - ACETATE (PMA) DEPENDENT MACROPHAGE DIFFERENTIATION.

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Monocyte–macrophage differentiation of hematopoietic cells can be achieved in vitro upon treatment with Vitamin D3 (VD) or phorbol 12-myristate 13-acetate (PMA). Although the capacity of VD to induce monocyte differentiation is well known, the outcome of PMA stimulation is, on the contrary, quite controversial. To better characterize the effect exerted by PMA on monoblastic cells, we carried out an immunophenotype analysis and morphological examination of PMA stimulated U937 cells. The results of these experiments suggested that PMA is able to promote a combination of macrophage and dendritic cell maturation program. To clearly define the phenotype promoted by the considered compound, a genome-wide meta-analysis comparing the transcriptome of PMA treated U937 cells with publicly available gene expression profiles of human monocytes, macrophages and dendritic cells was performed. Transcriptional signatures unequivocally demonstrated that PMA stimulation of U937 cells give rise to macrophage differentiation. In addition, gene expression data also evidenced that MafB is one of the most up-regulated transcription factors (TF) by PMA. Subsequent silencing experiments, followed by EMSA and luciferase assays, allowed us to characterize MafB specific MARE binding sites in IL-7R and MMP9 gene promoter regions. On the bases of these data we can state that: (i) PMA induces a macrophage differentiation in monoblastic cells; (ii) MafB TF supports the PMA maturation pathway; (iii) MMP9 and IL-7R, up-regulated by PMA, are MafB primary response genes. Finally, these experiments allow us to define a component of the PMA molecular pathway, through the characterization of PMA-MAFB-MMP9/IL-7R transactivation cascade.

STATINS, FIBRATES AND RETINOIC ACID UPREGULATE MITOCHONDRIAL ACYLCARNITINE CARRIER GENE EXPRESSION

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The carnitine/acylcarnitine carrier (CAC) is an integral protein of the inner mitochondrial membrane that transports acylcarnitine esters (in exchange for free carnitine) into mitochondria where the acyl groups are oxidized. It therefore plays an essential role in fatty acid β-oxidation, which is the major source of energy for heart and skeletal muscles during fasting and physical exercise. The human CAC is encoded by the SLC25A20 gene that maps to chromosome 3p21.31. Since the first reported mutation in the SLC25A20 gene, others have been identified and associated to CAC deficiency, a severe autosomal recessive, nonpopulation-specific disorder presenting an equal male-to-female ratio. CAC deficiency provokes a disorder of fatty acid β-oxidation (OMIM 212138) that presents two phenotypes: the first and more severe form with early onset in the neonatal period and the second milder form with onset in infancy. Patients usually display a good correlation between phenotype and reduction of CAC activity. Early recognition is crucial in CAC-deficient patients and the only long-term treatment consists of fasting prevention with frequent meals, a diet rich in carbohydrates, low in lipids, and supplemented with essential polyunsaturated fatty acids. We find that CAC mRNA level in HepG2 cells is increased by both statins and fibrates in a dose-dependent manner. Fluvastatin and GW7647 also cause a CAC mRNA increase on rat hepatocytes and myocytes and, when used in combination, a synergistic effect is observed. Because statins and fibrates are known to act on gene transcription by activating PPARa we checked for the presence of PPRE in the CAC gene promoter and we find a PPRE sequence from -99 to -80 bp, which shares 80.9% identity with the canonical PPRE. The effects of both statins and fibrates on gene reporter activity are abolished by mutations in the PPRE site clearly indicating that CAC transcriptional activation by these drugs is mediated by the PPRE regulatory element present in the human CAC promoter. Moreover, the activation of CAC gene transcription by forskolin provides evidence that the PKA pathway regulates CAC gene expression by phosphorylation of PPARa. Finally, 9-cis-retinoic acid, the ligand of RXRa which heterodimerizes with PPARa for binding to PPRE, enhances CAC gene expression. The results of the present investigation on the regulation of CAC gene expression extend our knowledge of the molecular mechanisms by which statins, fibrates and retinoic acid exert hypolipidemic action. Furthermore, we propose that patients affected by CAC deficiency, who present a mild phenotype with some residual activity and for whom pharmacological strategies are at present very limited, might benefit from treatment with statins and fibrates acting via stimulation of CAC gene expression. Indeed, this approach has been used successfully for other disorders of fatty acid b-oxidation.

Proteins and carbonylation pattern in trained and untrained rat muscles

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The mode, the intensity, the duration of the training and the intrinsic characteristics of the different skeletal muscles influence the way physical exercise affects the muscle. We investigated how high intensity aerobic training (10 wks treadmill at ~ 80% VO2max) impinges on the formation of protein carbonyl adducts, a consequence of excess ROS production, in two opposite kinds of skeletal muscle, Anterior Tibialis (mostly fast glycolytic) and Soleus (mostly slow oxidative). In fact, during intense activity, the high rate of O2 consumption in skeletal muscles can cause incomplete oxygen reduction, leading to the generation of ROS. ROS production may be quantitatively limited or counteracted by anti-oxidants, so that oxidative stress does not necessarily become established. These considerations promped us to examine the products of ROS damage. In particular, protein carbonylation is an irreversible reaction, whose amount reflects the intensity of the oxidative stress . The formation of carbonyl groups occurs either directly by reaction with ROS or indirectly by reaction with peroxidated lipids. The pattern of carbonylated proteins was studied in both control and exercised muscles by 2D-GE followed by Western Blot with anti dNTP antibodies. In order to minimize the biological variability between different individuals, the images of Western Blots were used to compose four synthetic gels in which only matched spots were present. This study enabled us to obtain the basal pattern of carbonylation characterizing the two types of skeletal muscles and to compare them with the exercise-induced variations in the carbonylation level of specific proteins.

Application of SNP-Arrays, FISH and GenotypeColour in Molecular Diagnosis of Mental Retardation.

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Mental retardation (MR) occurs in 2-3% of the general population worldwide. Previously published reports of chromosomal aberrations in cohorts of MR patients have shown that many small aberrations are overlooked by conventional cytogenetics methods. Instead, the higher resolution level of microarray based technologies can reveal novel submicroscopic deletions or duplications. We report on the results of the SNP array screening of 39 subjects with apparent normal karyotype and idiopathic mental retardation. When available, also the parents were analysed. Copy Number Variations (CNVs) of potential clinical significance were found in 13 patients (33%). The analysis of parent's SNPs array data of 9 patients, using the GenotypeColour™ (GenCol) software (Barlati, Chiesa and Magri. BMC Bioinformatics. 2009), revealed that 5 patients (55%) were carrier of a de novo CNV. All the patients with relevant CNV alteration and their parents were also analysed by FISH using specific BAC probes. FISH validated all the potential pathological CNVs and was fundamental to determine position, orientation and exact number of duplicated fragments. The combined used of SNP arrays, FISH and GenCol has proved, therefore, an important tool for the definition and characterization of several cryptic chromosome aberrations. In addition to the 13 patients, carriers of a submicroscopic aberration, 4 samples (10.3%) with large Runs of Homozygosis (ROHs) were highlighted by the GenCol analysis of the SNPs data. The trio comparison revealed that ROHs were the consequence of endogamy and not of segmental uniparental disomies (UPDs). This result is relevant in the light of the fact that cases of MR due to recessive syndromes are reported in literature. Even though we don't known if the ROHs detected in our patients are related with the clinical phenotype, this result demonstrates that SNP arrays combined with visualisation tools like GenCol gives useful information for the genetic interpretation of the clinical phenotype. In conclusion, with a detection rate of 33%, SNP arrays turned out to be a powerful tool in clinical diagnostic of developmental disorders. Allele specific information from SNP arrays provides, actually, valuable information for interpreting copy number variation (CNV), allelic imbalance including ROH that goes beyond that obtained from the total DNA signal available from aCGH platforms. Genotyping platforms such as SNP arrays, combined with FISH and GenCol analysis should be therefore considered as a primary platform for genetic clinical testing of MR.

New Copy Number Variations in Schizophrenia Italian Patients

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Schizophrenia is a severe mental disorder characterized not only by the positive, negative, and disorganized domains of psychopathology but also by a progressive deficit of neurocognitive functioning. The disorder affects 1% of the world population and has a heritability estimated to be up to 80%. The inheritance patterns are complex and the number and type of genetic variants involved are not understood. The technical advancements since 2008 have led to a turning point in schizophrenia genetics with the publication of several large-scale, whole-genome schizophrenia association studies, which collectively point to a model of schizophrenia pathogenesis whereby submicroscopic deletions and duplications of the genome might account for a portion of the genetic variability of schizophrenia. Since the majority of these events are relatively rare, many studies are required to provide statistical confirmation. With this in mind, we screened 172 patients with schizophrenia and 160 healthy controls of Italian origin for detection of structural variations, using Affymetrix 6.0 arrays. The results of the study provided support for the CNVs most convincingly implicated as risk factors for schizophrenia such as the NRXN1 gene, and the 15q11.2, 15q13.3 and 16p13.1 loci. Furthermore, CNVs previously reported but never replicated, such as those at the 2q12.2, 3q29 and 17p12 loci have been found. Some new candidate schizophrenia susceptibility genes deleted by large CNVs (>900 kb) have been proposed. In conclusion, the identification of new, rare, large CNVs and the replication of others previously reported, further supports a model of schizophrenia that includes the effect of multiple, rare, highly penetrant genomic variants.

Translational control mechanisms in the progression of cutaneous malignant melanoma: the role of eIF2alpha

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Malignant transformation and tumorigenesis include a complex series of cellular and biomecular events which are not yet completely known. Emerging evidence supports the fundamental role of translational control mechanisms in the modulation of cell functions. Cell growth and proliferation depend on protein synthesis that is regulated, in part, by translational initiation factors. These factors transiently increase in normal cells in response to growth factors and are constitutively elevated in transformed cells. In particular, the eIF2 translation factor seems to be involved in the abnormal regulation of protein synthesis that leads to a tumorigenic phenotype. Recent works suggest the translation factor eIF2 to be involved in the pathogenesis and/or tumour progression in some kind of tumours such as bronchioloaveolar carcinoma, non-Hodgkin lymphomas, breast tumour, brain tumour, gastrointestinal carcinomas, melanoma. Moreover other data indicate that the pERK levels could influence the RAS/RAF/MAPK pathway modifying the cell proliferation parameters. In this study we have analysed the phosphorylated eIF2-alpha and ERK levels in different melanoma cell lines derived from metastatic or primary cutaneous melanoma lesions, as well as in a line from the primary skin lesion of a patient with metastatic melanoma. Our results show higher levels of phosphorylated eIF2alpha and ERK in the metastatic cell lines (including the line from the primary lesion in the patient with distant metastases) as compared to the primary melanoma cell lines. In such cell lines, the gene expression of proteins involved in cell cycle regulation (i.e., cyclin D1 and p53) was also examined. The results of our study suggest the relationship between p-eIF2 alpha and tumoral progression in the malignant melanoma. Further analyses could allow to identify phosphorylated eIF2 as a possible prognostic marker.

Genetic biodiversity of enological Saccharomyces isolated from grapes must natural fermentations

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Spontaneous grapes must fermentation, induced by the indigenous micro flora, is believed to be associated with a specific vineyard and to give a distinctive style and quality to that wine. The alcohol-tolerant Saccharomyces cerevisiae strains invariably dominate the latter stage of natural wine fermentation. The S. cerevisiae population and other specific yeasts present in the vineyard niche habitats are considered autochthonous and their involvement in natural fermentation allows the production of wines with particular features in each microclimatic area. The present study was aimed to the individuation of autochthonous yeast strains useful in the improvement of oenological production of Salento, which is a very important wine-producing area of Southern Italy. Grapes were sampled from the most representative areas of Salento region (Ugento e Guagnano for “Negroamaro” and Gioia del Colle for “Primitivo”) and separately subjected to natural fermentation in an experimental scale. The identification of micro biota present during the last step of wine fermentation (>1 °Bé) of grapes, was carried out to select autochthonous yeast strains for industrial wine production. Aliquots of must samples of several dilutions were isolated after spreading on YPD agar medium supplemented. As preliminary screening, averages of 1500 colonies for each of the three fermentations were assayed for H2S production on BIGGY agar. The colonies appearing white or light brown (about the 10% of initial 1500 individual clones) were selected and recognized as S. cerevisiae by a microbiological screening based on the implementation of the taxonomical keys for identification of yeasts belonging to Saccharomyces genus. Identification at strain level of S. cerevisiae isolates is a fundamental step to investigate the biodiversity of this yeast and to examine population dynamics during the fermentative process. The criterion for strain differentiation was provided by the amplification of genomic sequence blocks flanked by delta elements of retrotrasposon origin. Interdelta (ID) typing has proved to be a very convenient method that can advantageously replace the other methods for molecular characterization of S. cerevisiae strains. For the first time, a recently developed method has been applied for the ID by the use of fluorescent primers and automatic sequencers. When compared with gel-based analytical methods, the use of capillarity system and automated analysis increase data throughput, scoring and reliability, decreasing the overall experimental error. The results of the genetic characterization of S. cerevisiae strains associated to natural fermentations of must from “Primitivo” and “Negroamaro” grapes and their implications for the selection of autochthonous industrial starters will be discussed.

CEREBRAL DNA DAMAGE AND HSP70 EXPRESSION IN MICE EXPOSED TO EXTREMELY LOW FREQUENCY MAGNETIC FIELDS

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The biological effects of extremely low frequency magnetic fields (ELF-MF) and their potential adverse effects on health is still an open and unresolved question. Some epidemiological studies indicate an association between residential/occupational exposure to ELF-MF and an increased incidence in cancer and neurodegenerative diseases, whereas other studies do not support these associations. Experimental studies, focused on clarifying the role of ELF-MF in cancer promotion and neurodegenerative disorders, report inconclusive and often contradictory results. However, some laboratory investigations, both in vivo and in vitro, show that ELF-MF produce a variety of biological effects which might have a role in the etiology of cancer and neurodegeneration. These effects include tumor promotion and copromotion, lowering of endogenous cytoprotection, induction of DNA strand breaks and oxidative stress. Regarding genotoxic effects, it has been suggested that ELF-MF might harm DNA through alteration of free radical production, lifetime and/or activity. Our recent study shows that chronic exposure to 1mT ELF-MF induces transient cerebral DNA damage in mice, while it does not elicit Hsp70-mediated stress response (Mariucci G et al. 2010). These results, together with the literature, suggest that high intensities of or prolonged exposure to ELF-MF are needed to induce DNA damage. The present study was aimed at confirming these results and clarifying the relationship between exposure intensity and genotoxic effect of ELF-MF in mouse brain. Moreover, we investigated the effect of ELF-MF on Hsp70 expression which is thought to be cytoprotective and is commonly used as a marker of cellular stress. We also evaluated, as general stress markers, food intake, body weight increase and spontaneous motor activity. Young male CD1 mice, 30-40 days old, were exposed to a 50 Hz MF of 1 or 2 mT for a period of 7 days (15 h/day) and sacrificed either at the end of exposure or 24 h after exposure. Sham-exposed mice were used as controls. Food intake and weight gain over the 7 days of exposure and motor activity before sacrifice were evaluated. At sacrifice, mouse brains were collected and dissected into cerebral cortex-striatum, hippocampus and cerebellum. Primary DNA damage was evaluated by alkaline single-cell microgel-electrophoresis assay (Comet assay). Western blot and real time RT-PCR were used to determine the expression of Hsp70. The Comet assay revealed an increase in DNA fragmentation of up to four times in brain cells from MF-exposed animals sacrificed at the end of exposure. DNA damage was directly correlated with the intensity of the MF ($r>0.80$ for all the cerebral areas, $p<0.001$). Interestingly, after a 24 h recovery period, DNA strand-breaks were not evident in the group exposed to a 1mT MF, while mice exposed to a 2mT MF showed complete DNA repair only in the cerebellum. No changes in Hsp70 mRNA and protein expression were found in cerebral areas of all the exposed groups. Similarly, behavioural and metabolic parameters were unchanged. These data confirm that in vivo ELF-MF induce primary DNA damage. The effect on DNA integrity seems to indicate a dose-response relationship between MF intensity and the degree of damage. Moreover, the DNA repair capability seems to be reduced after exposure to the highest intensity (2mT) and dependent on the brain area. The lack of the Hsp70 stress response could be interpreted as an inhibitory effect of high-intensity ELF-MF on this cytoprotective system, as has already been suggested by results obtained with chicken embryos (Di Carlo A. et al. 2002).

A Dlx-Wnt5a regulation modulates GABAergic differentiation of progenitor cells, in vitro and in vivo

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In the basal forebrain, neural progenitor (NP) cells occupy the wall of the lateral ventricle, and are committed towards the GABA+ interneuron lineage. As these cells exit the cell cycle, they migrate either towards the cortex, the hippocampus or the olfactory regions, and differentiate in various GABA+ subtypes. The homeodomain transcription factors of the Dlx family are essential for migration and differentiation of these GABA progenitors, as revealed by the brain phenotypes of mice null for individual Dlx genes or combinations of them. For instance, in the absence of Dlx5 the number of TH+, CR+ and GABA+ olfactory interneurons is reduced, although the Dlx5+ cell lineage is not depleted. Since the Dlx proteins regulate expression of the GABA-synthesizing enzyme gene GAD1, a role of Dlx proteins to drive GABA differentiation cell-autonomously seems established. However, previous results from our team indicate that the Dlx genes also exert a non-cell autonomous function. The balance between proliferation and differentiation of NP cells is the result of a complex interplay between genetically determined cellular programs and local environmental cues. Among these, the Wnt class of ligands participate in several key developmental processes. Wnt/beta-catenin signalling maintains the proliferation potential of hippocampal, cortical and midbrain NP cells in vivo. Similarly, in the subpallium Wnt/beta-catenin signalling is required for NP cells proliferation, while does not influence their migration or differentiation. Wnt signalling also promotes NP cells differentiation, in fact Wnt3 and Wnt7a favour neuronal differentiation of late cortical NP cells, and Wnt5a stimulates differentiation of dopaminergic neurons. As Wnt ligands elicit such a variety of responses, their functions need to be determined in a cell type- and context-dependent manner. We have therefore investigated a possible link between the Dlx transcription regulation and Wnt signalling, and their effect on the proliferation and GABA+ differentiation of NP cells. Here we provide evidence that Dlx2 and Dlx5 promote differentiation of olfactory interneurons, non cell-autonomously, by regulating the expression of Wnt5a. We also show that Wnt5a favours GABA+ differentiation both in dissociated OB neurons and in slice cultures. Wnt ligands use several transduction pathways: the best known one (canonical) depends on stabilization/nuclear translocation of beta-catenin, and generally promotes self-renewal and proliferation of progenitor cells. Conversely, beta-catenin independent pathways, known as “non canonical”, either use JnK phosphorylation or Ca-dependent activation of PKC. In general, activation of non-canonical Wnt pathways promote neuronal maturation/differentiation, but the biochemical pathway by which Wnt5a modulates differentiation of NP cells is poorly known. To examine this, we derive adherent cultures of NP cells from the embryonic brain, a cell system prone to GABA+ differentiation in vitro. In these cells we observe that canonical Wnt3a specifically activates beta-catenin, while non-canonical Wnt5a promotes phosphorylation of JnK. Furthermore, JnK phosphorylation is reduced in the forebrain of Dlx5 null mice. Wnt5a interacts with the tyrosine-kinase receptor Ryk, and Ryk activation/cleavage promotes the differentiation of cortical NP. The application of a Ryk neutralizing antibody on basal NP alters their differentiation properties. Defining the pathway that links Wnt5-Ryk interaction with JnK during NP cell differentiation may open the way to activate/inhibit key steps of this regulation and thereby control GABA+ differentiation of neuronal progenitors.

New insights into molecular and functional properties of the LSD1/KDM1 neuronal mini-exon

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A variety of chromatin remodeling complexes are thought to orchestrate transcriptional programs that lead neuronal precursors from earliest commitment to terminal differentiation. We showed that mammalian neurons have a specialized chromatin remodeling complex arising from a neurospecific splice variant of LSD1/KDM1 - Histone Lysine Specific Demethylase 1- whose demethylase activity on Lys4 of histone H3 has been related to gene repression (Zibetti et al. 2010). We found that alternatively spliced LSD1 mammalian transcripts generate four isoforms. Two isoforms retain E8a, an unusually short exon four amino acids-long, internal to the amine oxidase domain, whose inclusion within transcripts is restricted to the nervous system. Remarkably, their expression is dynamically regulated throughout brain development, particularly during perinatal stages. While inclusion of exon E8a within LSD1 proteins does not affect the in vitro biochemical properties nor the interaction with known co-repressor partners, in vivo the mini-exon E8a enables LSD1 to pace-make early neurite morphogenesis. Our goal is now to understand the molecular mechanisms by which the mini-exon E8a confers a neurospecific function to LSD1 epigenetic activity. Structural studies show that the mini-exon E8a residues form a sort of protrusion from the amino oxidase domain that emerges from the main body of the protein without causing any local conformational change into LSD1. Such a feature indicates that these residues could easily form a docking site for other protein partners as well as they can be targeted by post-translational modifications (PTM). Functional assays based on these two possibilities might provide a strategy to uncover in vivo biochemical differences. We will present data relative to the physiological PTM pattern of immuno-isolated LSD1 from rat brain (analyzed by mass spectrometry) and to the functional role of such PTM in the acquisition and maintenance of normal neuronal phenotype.

Extremely low frequency Electromagnetic fields (ELF-EMF) effect on morphology and genetic expression profile of a human keratinocyte line (HaCaT).

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Keratinocytes represent the majority of the cell population in the epidermis. Cutaneous homeostasis is depending, among the other factors, from a correct proliferation and differentiation mechanism. Usefulness of electromagnetic fields have been demonstrated in several clinical application for the enhancement of tissue restoration (eg. bone tissue), however little is still known about the exact mechanism of action of ELF-EMF. Moreover concerning skin tissue restoration clinical results are still debated. The aim of our study was to evaluate the effect of ELF-EMF (frequency 50Hz, intensity 1 mT) on a keratinocyte cell line at different time points (1-24-48 h). We assessed gene profiling and morphological modulation by means of gene microarray and electron microscopy technique. ELF-EMF exposed cells presented, compared to control sham exposed cells, a modulation of cellular morphology with a better organization of citoplasmatic and nuclear compartments. Citoplasmatic organelles were better developed and nuclear arrangement was primarily eucromatinic in ELF-EMF exposed cells. Microarray analysis has evidenced the variation of 124 gene expression in ELF-EMF exposed cells. Most of the genes were involved in mechanisms such as: growth and proliferation, apoptosis and glutathion metabolism. These results have also been confirmed with Real Time PCR technique. Mammalian target of Rapamycin (mTOR) has been identified as a kinase with a pivotal role in cellular proliferation and survival in mammals. We performed Western blot assays on lysates from ELF-EMF exposed cells and we demonstrated that these cells presenta n activation of the PI3K/AKT/mTOR pathway. Moreover in exposed cells both mTOR and its effector protein Ribosomal Protein S6 Kinase, 70 kDa (p70 S6K) were also found to be highly phosphorilated compared to control cells. While eIF4E-binding proteins (4E-BP1) was found to be downregulated thus confirming a role of ELF-EMF in the translational increment.

EFFICIENCY OF ANTISENSE-MEDIATED EXON SKIPPING IN NORMAL AND MUTATED DMD GENES

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Duchenne and Becker muscular dystrophy (DMD, BMD) are caused by mutations in the dystrophin gene. In general, DMD mutations disrupt the reading frame and lead to prematurely aborted dystrophin synthesis; conversely, mutations that leave the reading frame intact lead to the production of low levels of internally truncated protein, which are sufficient to generate the milder BMD phenotype. This observation suggested that a therapeutic approach for DMD could be based on active intervention on primary RNA processing ("exon-skipping"), aimed at restoring a viable reading frame. The most commonly used exon-skipping approach relies on the use of sequence-specific antisense oligonucleotides (AO), which binds to intron-exon boundaries and/or to splice enhancer elements. So far, virtually all AO designing procedures have been tested on intact dystrophin genes. However, there are clear indications that the genomic re-arrangements (deletion, duplications, point mutations, etc.) present in patients can affect the efficiency of the desired skipping processes. In this work we hence decided to compare the efficiency of a set of AO in control and in DMD myoblasts, obtained from patients carrying different types of mutations. Our data indicated that not only AO skipping efficiency varied greatly between intact and mutated DMD genes, but also that in some instances patients' mRNA exhibited splicing patterns that could not be directly correlated to their specific genomic mutation.

Analisi citogenetica di cellule staminali mesenchimali a differenti passaggi di espansione

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Le cellule staminali mesenchimali (MSC) sono cellule multipotenti che rappresentano un prodotto per terapia cellulare molto promettente per applicazioni cliniche, nell'ambito dei trapianti e della medicina rigenerativa. In considerazione del fatto che per poter infondere numeri adeguati di MSC sono necessarie procedure estensive di espansione, studi recenti hanno riportato un'aumentata instabilità cromosomica delle cellule in coltura con il conseguente potenziale rischio di trasformazione neoplastica. Diventa pertanto indispensabile introdurre dei controlli citogenetici in grado di monitorare la presenza di possibili riarrangiamenti cromosomici nelle cellule utilizzate per il trapianto. Abbiamo studiato MSC espanse in vitro da sangue midollare (BM=7) e tessuto adiposo (AT=3). Le cellule sono state seminate (5 x 10^5/cm^2) in fiasca in IMDM addizionato di lisato piastrinico al 5%; dopo 2-3 giorni le cellule non aderenti sono state rimosse e la coltura proseguita con cambio bisettimanale del terreno. A crescita confluente le cellule aderenti sono state tripsinizzate e ripiastrate a bassa concentrazione per 4 o 5 passaggi successivi. Ad ogni passaggio sono stati valutati l'immunofenotipo (CD45, CD34, CD90, CD105, CD73), la capacità differenziativa (in senso osteocitario, adipocitario e condrocitario) e il cariotipo utilizzando tecniche standard di bandeggio Q. L'analisi citogenetica e la descrizione del cariotipo sono stati eseguiti secondo le indicazioni dell'International System for Human Cytogenetic Nomenclature (2009) Tutti i campioni analizzati hanno ottenuto un elevato indice di crescita, hanno mostrato fenotipo mesenchimale (negatività per CD45 e CD34, triplice positività>90% per CD90, CD105, CD73) e capacità differenziativa. Il grado di espansione medio al terzo passaggio stato di 1389 volte (da 46 a 5900) per BM e di 660 volte (da 504 a 969) per AT. Nella coltura primaria e dopo quattro passaggi, l'analisi citogenetica effettuata su i dieci campioni ha mostrato cariotipo normale diploide, cioè 46, XX o 46, XY. Anomalie strutturali (delezioni), a basso livello di mosaicismo con la linea normale, sono state osservate solo in due colture (BM15 e BM18 al primo passaggio). L'espansione di MSC può comportare la comparsa di anomalie cromosomiche, ma l'alterazione genetica non è associata ad un vantaggio proliferativo selettivo e pertanto il clone anomalo viene spontaneamente eliminato durante i passaggi successivi di espansione cellulare. Tuttavia, in considerazione della continua e progressiva estensione dei potenziali campi di applicazione clinica delle MSC, sono necessari ulteriori studi per confermare la conformità biologica e genetica di tali prodotti per terapia cellulare.

VARIATION OF FURIN AND TMPRSS6 EXPRESSION CAN CONTRIBUTE TO THE INADEQUATE HEPCIDIN PRODUCTION IN THE LIVER OF HFE-HEMOCHROMATOSIS PATIENTS

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Various studies have recently demonstrated the ability of different molecules to inhibit the production of hepcidin by hepatocytes. They include the protease TMPRSS6, which inhibits hepcidin induction by degrading membrane hemojuvelin, a potent activator of hepcidin transcription, and soluble hemojuvelin, which acts as decoy on the same pathway and is generated by furin cleavage. Currently there are no data on the expression of these genes in the liver of patients with different iron overload conditions. We previously reported a decreased hepcidin expression, compared to the iron burden, in liver biopsies of patients with HFE hemochromatosis and dismetabolic hepatic iron overload syndrome (DHIOS) [1]. We thus analyzed liver mRNA levels of furin and TMPRSS6 in patients with iron overload of different ethiologies to detect possible variation in expression and to correlate them with hepcidin expression, iron indexes and other clinical and histological data. Liver biopsies were obtained from patients with DHIOS (no. 45), HFE hemochromatosis (no. 14) and secondary iron overload due to beta-thalassemia (no. 6). Patients with non alcoholic fatty liver disease (NAFLD) without iron overload or inflammation served as controls (no. 14). mRNA levels were determined by quantitative PCR using Taqman probes and normalized over HPRT expression. Differences between groups were compared by ANOVA. Furin mRNA expression was downregulated in HFE hemochromatotic patients as compared to the NAFLD group, and a reduction in expression, although to a less extent, was observed also in DHIOS and thalassemic subjects. On the contrary TMPRSS6 mRNA expression was increased in the HFE patients. In the HFE group, furin expression was positively correlated to hepcidin mRNA levels ($p<0.001$), whereas no correlation in DHIOS and NAFLD groups was observed. No significant correlation was observed for TMPRSS6 expression for the analyzed iron-related parameters, includin hepcidin mRNA levels. Furin affects iron metabolism via the generation of soluble hemojuvelin, that should block the BMP pathway which increases hepcidin expression [2]. On the other hand, furin cleaves prohepcidin generating the mature hepcidin peptide, activity shared by other proprotein convertases [3]. Furin expression has been reported to increase in iron deficiency [2], and the data here presented suggest that, in iron overload due to HFE hemochromatosis, furin mRNA expression is downregulated by iron burden. In addition, an increased expression of TMPRSS6 has been detected in HFE hemochromatotic patients, fact that can further reduce the activation of the BMP pathway, and thus partially explain the blunted hepcidin response observed in these subjects. 1) Barisani D et al. J Hepatol. 2008;49(1):123-133. 2) Silvestri L et al. Blood 2008;57(11):1573-82. 3) Scamuffa N et al. Gut 2008;57(11):1573-82.

SpliceAid 2: An enhanced version to perform more accurate human splicing predictions

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Nowadays to foresee the splicing outcome starting from pre-mRNA sequence is an unsolved task. The difficulties are due to the fact that splicing language is overlapped to aminoacidic one, moreover the splicing regulatory elements are scattered in the entire pre-mRNA sequence and all nucleotide positions are potentially involved in the generation of the specific splicing pattern through the specific interaction with trans-acting RNA-binding proteins. Cell/tissue type-, development stage-, and/or growth condition-specificity constitute a further complexity layer. Previously we designed and realized SpliceAid [1], a tool, based on experimental RNA target motifs bound by splicing proteins in humans, able to reveal the presence of splicing regulatory element binding sites in pre-mRNA sequences. Therefore it is useful to predict the effect of mutations on the splicing process outcome. In this year we have developed an improved version: SpliceAid 2. This new version is able to perform tissue-specific predictions of splicing factor binding sites, it shows the splice site presence and scores, it is costantly updated and at present it collects 1067 experimental binding sites. To assess the performances of SpliceAid 2, we have compared the experimental results of some studies from the literature versus our predicted results. Since the comparison have pointed out the good accuracy of the predictions, we have dealt with the problem of the polymorphism preselection in association studies. In particular, we have selected polymorphisms regarding serotonin related genes because we have well studied them in collaboration with the Department of Neurosciences of the Polytechnic University of Marche. This application is important since the huge number of polymorphisms to evaluate and the large number of experimental data necessary to obtain a statistically significant assessment, make association studies expensive and time consuming, so only few polymorphisms are usually selected for genotyping in association studies. We have shown that SpliceAid 2 results can be useful to indicate the potentially phenotype-affecting polymorphisms and so worth considering in association studies [2]. SpliceAid 2, that may also help to explain an observed splicing pattern, is freely available at www.introni.it/spliceaid.html References 1. Piva F, Nocchi L, Giulietti M, Principato G SpliceAid: a web tool that joins a database of strictly experimentally assessed RNA target sequences with the RNA secondary structure prediction in humans. Bioinformatics 2009; 25: 1211-1213 2. Piva F, Giulietti M, Nardi B, Bellantuono C, Principato G An improved in silico selection of phenotype affecting polymorphisms in SLC6A4, HTR1A and HTR2A genes. Human Psychopharmacology 2010; 25:153-161

POTENTIAL ROLE OF AMPK IN THE CONTROL OF MIGRATION OF GnRH NEURONS

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The 5'-AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase that plays a fundamental role in regulating energy homeostasis in the periphery and it is also involved in the central regulation of feeding and metabolism. AMPK is activated by stress conditions (i.e. heat shock, ischemia/hypoxia and exercise) causing ATP depletion, which in turn leads to elevation of the AMP:ATP ratio, and also by different metabolic molecules, such as the adipocyte-derived hormones adiponectin and leptin, and pharmacological drugs, such as metformin and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR). Once activated, AMPK switches on catabolic pathways, leading to ATP generation, and inactivates ATP-consuming processes. Cell migration is an energy consuming function that is highly dependent on both ATP and GTP levels. Previous reports have shown that AMPK modulation, in addition to its role in metabolism, may affect the migration of different cell lines in response to various stimuli. The potential role of AMPK as an inhibitory signal affecting cell migration prompted us to explore the modulatory effect of AMPK activation on the migration of immature GnRH neurons, which, during fetal development, migrate from the olfactory placodes to the hypothalamus undergoing a complex developmental program which allows the acquisition of the reproductive competence in the adult age. To this aim, we took advantage of the immature and migrating GN11 cells, a cell line obtained by genetically targeted tumorigenesis of GnRH neurons in mice, which have been shown to retain many characteristics of GnRH neurons and represent a good model for the in vitro study of the biology of GnRH neurons. Therefore, GN11 cells were used as a model system to verify whether AMPK activation by adiponectin, selected according to its emerging functions as AMPK activator and modulator of reproductive function, or by the pharmacological compound 5-amino-imidazole-4-carboxamide riboside (AICAR), can affect cell migration, and whether the ERK1/2 and Akt pathways are involved in this process interacting with the AMPK pathway. The most relevant results of this study can be summarized as follows: 1. in GN11 cells, which express both adiponectin receptors (adipoR1 and adipoR2), adiponectin and the pharmacological activator AICAR were capable to induce AMPK, as well as ERK1/2 and Akt phosphorylation; 2. AMPK activation, induced by AICAR, but not by adiponectin, inhibited serum-induced GN11 migration; 3. differently from AICAR, which activated AMPK directly, adiponectin promoted a more complex signalling pattern whereby AMPK is indirectly activated via ERK1/2 and Akt pathways, which were further positively related to each other; 4. rapid activation of AMPK by AICAR, but not by adiponectin, caused the inhibition of pERK1/2, but not of pAkt levels. We conclude that the direct activation of AMPK, which in turn promoted the down-regulation of pERK1/2, may be an important signalling mechanism involved in stopping GnRH neuron migration. Thus, the identification of factors able to directly activate AMPK within the hypothalamus may lead to the discovery of potential signals able to affect GnRH neuron migration, further clarifying the biology of these neurons.

Pharmacological and molecular approaches to induce mutant protein clearance in cellular model of Spinal and Bulbar Muscular Atrophy.

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Protein misfolding and aggregation are typical hallmarks of neurodegenerative disorders, such as those of the family of polyglutamine (polyQ) diseases, which includes Huntington's disease (HD), Spinocerebellar Ataxias (SCAs), dentatorubropallidoluysian atrophy (DRPLA) and Spinal and Bulbar Muscular Atrophy (SBMA). SBMA is a motor neuronal disease caused by polyQ expansion in the androgen receptor (AR) protein, which affects motor neurons in the spinal cord and in the bulbar region. These motor neurons express very high level of AR. The AR is a nuclear receptor that in the inactive state is localized into the cytoplasm. The binding with testosterone induces AR activation, its dimerization and nuclear translocation were it activates AR-dependent transcritpion. In SBMA, testosterone induces ARpolyQ aggregation into the cytoplasm and in the nucleus. The aggregates contain ubiquitin, proteasome pathway (UPS) components and Heat shock proteins (Hsps), suggesting a possibile involvement of UPS in SBMA. Using an immortalized motorneuronal cell line (NSC34) expressing ARpolyQ, we observed that the inactive and soluble ARpolyQ impairs the UPS activity; testosterone-activated ARpolyQ induced both the nuclear translocation and cytoplasmic protein aggregation resulting in UPS desaturation. This is possibly due to the removal of misfolded protein from the soluble compartment confining the mutant and misfolded ARpolyQ into physically defined intracellular compartments (the aggregates) waiting for an alternative clearance, such as autophagy. To analyse autophagy in SBMA cell model, we studied the autophagic marker LC3, a protein associated to autophagosome. We observed that in presence of the mutant ARpolyQ, LC3 showed a intense punctate staining, suggesting an involvement of autophagy in the clearance of the mutant protein. To increase the capability of these two intracellular proteolytic systems involved in the clearance of the ARpolyQ, we used different approaches. (i) The first was a molecular appoach. We have studied the effects of the small Heat shock Protein, such as HspB8. Inhibition of the proteasomal function did not block the HspB8 activity suggesting that HspB8 did not require a functional proteasome to remove the misfolded protein. HspB8 overexpression increased ARpolyQ clearance acting through the macroautophagy; in fact HspB8 stimulated the formation of LC3-II, and increased the number of autophagosomes. It appears that HspB8 might act in an heterocomplex with other chaperones, as Bag3, Hsc70 and CHIP. (ii) The second was a pharmacological approach. We used 17-Allylamino-17-demethoxygeldanamycin (17-AAG), an Hsp90 inhibitor, which reduces ARpolyQ toxicity in SBMA transgenic mice. 17-AAG treatment reduced ARpolyQ aggregates, increasing ARpolyQ clearance, without altering the UPS activity. 17-AAG induced LC3 expression, increased LC3 turnover punctate distribution both in absence and in presence of T, suggesting a relevant role in the induction of autophagy. All these data suggest that the identification of compounds or molecole capable to potentiate ARpolyQ clearance through the main degradative systems may represent an important step for SBMA therapies. GRANTS: Telethon - Italy (GGP06063 and GGP07063); Italian Ministry of Health (2007-36 and 2008-15) Convenzione Fondazione Mondino/CEND-UNIMI; Universita' degli Studi di Milano; Fondazione CARIPLO (2008-2307); Thierry Latran's Foundation.

Hematopoeitic stem cells in coculture with BM stromal cells: molecular and functional characterization of the hematopoietic stem cell niche

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Hematopoietic stem cells (HSCs) are located in the bone marrow (BM) in a specific microenvironment referred as the hematopoietic stem cell niche, which plays a pivotal role in regulating their survival, self-renewal and differentiation. In this specific microenvironment, HSCs interact with a variety of stromal cells including fibroblasts, endothelial cells, reticular cells, osteoblasts and adipocytes. According to several recent studies, osteoblasts are able to support HSCs maintenance in vitro through the secretion of soluble factors and cell-cell contact, whereas adipocytes have been shown to act as negative regulators of the hematopoietic microenvironment. Though several components of the stem cell niche have been identified, the regulatory mechanisms through which such components regulate the stem cell fate are still unknown. In order to address this issue, we investigated how osteoblasts and adipocytes can affect the molecular and functional phenotype of HSCs and vice versa in a coculture system. We set up 2 different coculture systems: CD34+ cells purified from the Cord Blood (CB) in coculture with human osteoblasts isolated from the trabecular bone or with adipocytes differentiated from human CD146+ bone marrow stromal cells. After coculture, CD34+ cells and the hematopoietic cell fraction were separated from stromal cells and analyzed by gene expression profiling and clonogenic assay to assess how coculture with osteoblasts or adipocytes could affect the self-renewal and differentiation capacity of HSCs. On the other hand, we also assessed how the contact with CD34+ cells influence the molecular and functional phenotype of the stromal cell compartment. Our results show that osteoblasts induce a strong increase in the clonogenic capacity of CD34+ cells after coculture. Moreover, clonogenic assay results show an increase of the macrophage colonies and a decrease of the erythorid ones in CD34+ cells after coculture with osteoblasts. Gene expression profiling analysis will allow us to understand which signalling pathways are activated in the hematopoietic cell fraction and in the stromal compartment, therefore enabling us to identify the molecular mechanisms responsible for such biological effects. Our data suggest that osteoblasts induce an increase in the clonogenic ability of HSCs and are able to affect the differentiation capacity of CD34+ cells by favouring macrophage commitment at the expense of the erythroid lineage. Gene expression profiling and analysis of the cell culture medium by means of ELISA are necessary to understand the complex mechanisms underlying the effect exerted by stromal cells on HSCs and vice versa. An understanding of how the niche participates in the maintenance of hematopoiesis offers new opportunities for the development of novel HSCs ex-vivo expansion protocols that allow stem cells expansion without loss of ‘stemness’.

HEPATOCYTE IDENTITY IMPLIES STABLE REPRESSION OF THE MESENCHYMAL PROGRAM: A NOVEL ROLE FOR HEPATOCYTE NUCLEAR FACTOR 4alpha

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Epithelial-to-mesenchymal transition (EMT) is the process by which the highly organized, polarized cells of the epithelium lose cell-cell connections and polarization and acquire the mesenchymal characteristics of motility and invasiveness. The reverse process, mesenchymal-to-epithelial transition (MET), often occurs at a site secondary to the original EMT population. The dynamic EMT/MET processes are essential for embryonic development and wound repair and also initiate the pathological states of fibrosis and metastatic cancer. A number of master regulators of EMT have been identified. The transcriptional repressors of the Snail family, Snail (Snai1 gene) and Slug (Snai2 gene), induce the EMT partly through direct inhibition of E-cadherin gene transcription. Snail also acts as a point of signal integration for many other inducers of EMT , Notch, and estrogens. It is generally assumed thatβincluding Wnt, TGF completion of MET involve reverse sequential modulation of the mechanisms that led to EMT, thereby permitting to reacquire the epithelial phenotype; however, the molecular mechanisms driving MET are largely unknown. In the liver, EMT/METs have been postulated for several terminally differentiated cell types including parenchymal cells. Concerning hepatocytes, while there are solid experimental data supporting EMT occurrence in culture and also in vivo (where hepatocyte-derived fibroblasts could contribute to liver fibrosis), evidences for the reverse process MET are still elusive. It was previously shown that the orphan nuclear receptor hepatocyte nuclear factor 4-α (HNF4α) orchestrates the expression of several epithelial markers in hepatocytes. HNF4α can confer to fibroblasts an epithelial-like morphology and re-establishes a differentiated phenotype to invasive hepatocellular carcinoma. These findings together with our previous observations showing that HNF4α counteracts Snail-dependent down-regulation of epithelial markers, may act as a MET-inducing factor in hepatocytes. suggests that HNF4α In this study, we provide evidence on the molecular mechanisms by which HNF4α induces MET and maintains the differentiated phenotype. This transcriptional factor, in cooperation with its target HNF1α, directly inhibits transcription of Snail, Slug and HMGA2 (previously demonstrated to act as EMT master genes) and mesenchymal markers. Notably, we provided evidence that the HNF4α−mediated repression of EMT genes, induces MET in hepatomas and is required for the maintenance of the epithelial phenotype. The relevance of HNF4α in the active repression of mesenchymal program was confirmed in the in vivo model of mice with hepatocyte-specific deletion of Hnf4α alpha. In conclusion, the HNF4α pivotal role in the induction and maintenance of hepatocyte differentiation should be also ascribed to its capacity to continuously repress the mesenchymal program thus, both HNF4α activator and repressor functions are necessary for the identity of hepatocytes.

PKC-mediated phosphorylation of IBtkγ regulates the Btk signalling in human and mice B cells.

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Bruton tyrosine kinase (Btk) is a member of the Tec family of non-receptor protein tyrosine kinases and is expressed in B cells, macrophages and neutrophils (1). Btk sustains the developmental program of pre-B cells by limiting the pre-B cell expansion and by promoting B cell differentiation (1, 2). Consistently, mutations of BTK cause the human X-linked agammaglobulinemia (XLA) and the murine X-linked immunodeficiency (xid) syndromes, which are characterized by increased susceptibility to recurrent bacterial infections as a consequence of the impaired generation of mature B cells and low production of immunoglobulins (3, 4). Accordingly, DT40 Btk-/- chicken B cells show a drastic decrease in Ca2+ signalling and NF-κB activation upon antigen stimulation (5), and Btk-/- mice have a significant reduction of B cells (6, 7). We have previously identified the Inhibitor of Btk (IBtk) as a ≈25 kDa protein that bound to the PH domain of Btk and repressed the Btk-mediated Ca+2 mobilization and NF-κB-activation upon BCR triggering (8). We have recently reported the physical and functional analysis of the IBTK locus, which encodes for the IBtk isoforms α, β, and γ (9). IBtkα (150.53 kDa) and IBtkβ (133.87 kDa) are ubiquitous and might exert regulatory functions beyond the Btk regulation by associating with other proteins (9). IBtkγ (26.31 kDa), which was the first identified isoform (8), is mainly expressed in haematopoietic cells (8, 9), and might play a major role in Btk-mediated regulation of B cells by competing for the binding of Btk-PH domain to other signaling molecules, such as PIP3 and PI5K. In this study, we have addressed the role of IBtkγ in the regulation of Btk activity in response to the BCR triggering. The IBtkγ amino acidic sequence includes several PKC consensus sites, suggesting that IBtkγ may be regulated by serine-phosphorylation in response to B cell signaling. To address this possibility, we evaluated the PKC-mediated phosphorylation of IBtkγ and the outcome on the stability of both the Btk/IBtkγ complex and Btk activity. We show that IBtkγ is phosphorylated at serine 90 by PKC upon BCR engagement; this phosphorylation causes the dissociation of the Btk/IBtkγ complex and allows Btk to translocate to the plasma membrane. These findings underscore a novel signaling pathway where IBtkγ regulates the amplification of BCR signalling mediated by Btk. References 1. J. M. Lindvall et al., Immunol Rev 203, 200 (Feb, 2005). 2. A. Maas, R. W. Hendriks, Dev Immunol 8, 171 (2001). 3. K. E. Blomberg, C. I. Smith, J. M. Lindvall, Curr Mol Med 7, 555 (Sep, 2007). 4. S. Tsukada, D. J. Rawlings, O. N. Witte, Curr Opin Immunol. 6, 623 (1994). 5. M. Takata, T. Kurosaki, J Exp Med 184, 31 (Jul 1, 1996). 6. W. Ellmeier et al., J Exp Med 192, 1611 (Dec 4, 2000). 7. J. D. Kerner et al., Immunity 3, 301 (Sep, 1995). 8. W. Liu et al., Nat Immunol. 2, 939 (2001). 9. C. Spatuzza et al., Nucleic Acids Res 36, 4402 (Aug, 2008).

Modelling of ALK1 ectodomain and ALK1-ActRIIA-BMP9 receptor complex: hints from Type 2 Hereditary Haemorrhagic Telengiectasia

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Background Activin A receptor, type II-like kinase 1 (also called ALK1), is a serine-threonine kinase predominantly expressed on endothelial cells surface. Mutations in its ACVRL1 encoding gene (12q11-14) cause type 2 Hereditary Haemorrhagic Telangiectasia (HHT2), one of the two forms of HHT. The type 1 of this autosomal dominant multisystem vascular dysplasia depends on mutations of the ENG gene (9q34), which codes for Endoglin (CD105), a homodimeric membrane glycoprotein involved in Transforming Growth Factor-β (TGF-β) signalling. While a preliminary structural model of Endoglin extracellular domain is already available, structure determination of the ALK1 ectodomain (ALK1EC) has been elusive so far. We here describe the building of a homology model for ALK1EC to study the potential structural effect of HHT2-associated mutations. ALK1EC potential interaction mode with other partner proteins was then predicted combining modelling and docking data. Results The calculated model of the ALK1EC allowed the rating of HHT2 associated mutations according to their thermodynamic effect. The predicted interaction mode of wild-type ALK1EC with the structure of its BMP9 ligand and of the type II receptor ActRIIA shows an unexpected orientation of the type I receptor strongly supported by the localisation and thermodynamic effects of the HHT2 associated mutations. A potential interaction mode of the holocomplex with an ab initio model of Endoglin is consistent with previous electron microscopy and molecular biology data and might give an initial idea of its structural role as a coreceptor. Variants of BMP9 with a higher affinity for the mutant forms of ALK1EC were designed as molecules with a potentially patient-tailored pharmacological application. Conclusions This study gives a preliminary insight into the potential structure of ALK1EC and of the ALK1EC-BMP9-ActRIIA signalling complex and into the structural effects of HHT2 associated mutations, also suggesting a potential model of interaction for Endoglin and for higher affinity BMP9 mutants. From these models, the potential effect of each single mutation can be predicted, and used in the formulation of new biological experiments and in the interpretation of the biological significance of new mutations, private mutations, or non-synonymous polymorphisms.

Interactions between ERα, GPR30, EGFR and IGF1R and their role in controlling adrenocortical tumor cell proliferation.

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Adrenocortical cancer (ACC) is a rare tumor with a very poor prognosis. The unavailability of specific treatment come from limited knowledge of molecular mechanisms underlying ACC development. Chromosomal alterations can be involved in ACC genesis. The most consistent and dominant genetic changes in ACC is the perturbation of the insulin-like growth factor II (IGF-II) locus (11p15) that is imprinted. IGF-II is over-expressed in 90% of ACCs determining IGF1R activation inducing mitogenic effects through PI3K/Akt and/or ERK pathways. Moreover several growth factor receptors (i.e. IGF1R and EGFR) are overexpressed in most ACC and inhibitors for these growth factors are currently in clinical trials. However, ACC is a disease extremely heterogeneous and this new pharmacological approach could not be enough for the therapy of all forms of ACC, since several molecular mechanisms trigger ACC development. Epidemiological and experimental studies suggest that estrogens could also be involved in genesis and progression of ACC. Our prior works demonstrated that ACC are characterized by Estrogen Receptor (ER) α up-regulation and aromatase (the enzyme involved in the production of estrogens using androgens as substrate) over-expression and that estradiol enhances proliferation of the human adrenocortical carcinoma cell line H295R. In addition to transcriptional regulation, through cytoplasmic ER and membrane-mediated signaling (MIS), estrogen can also induce rapid cellular signals including the generation of second messengers as well as activation of receptor tyrosine kinases, such as EGFR and IGF1R. Recently, a seven-transmembrane G-protein-coupled receptor (GPCR), named GPR30, has been identified as a transmembrane estrogen receptor that can mediate rapid estrogen signaling in different cell types. Aim of this project is to elucidate the role of estrogen signaling and its cross-talk with IGF1R and EGFR system in adrenocortical tumor cell proliferation using H295R cells as model. Here we show that IGF-II induces aromatase mRNA and protein expression suggesting the ability of IGF-II to control in situ estrogen production. Moreover, IGF-II induces both ERα and GPR30 expression. On the other hand, E2, PPT (a selective ERα agonist) and G1(a selective GPR30 agonist) stimulate phosphorylation of IGF1R and EGFR and determine ERK1/2 and AKT activation. The presence of AG1024, a specific IGF1R antagonist, decreased IGF1R, ERK1/2 and AKT phosphorylation without affecting EGFR activation. The presence of AG1478, a specific EGFR antagonist decreased ERK1/2 and AKT phosphorylation, without changing IGF1R activation. These data suggest that IGF1R and EGFR do not work in a linear sequence downstream of estrogen receptors but can independently from each other mediate estrogen signaling. In the E2-dependent IGF1R and EGFR transactivation we found the involvement of the scaffold protein PELP1/MNAR. Ten minutes treatment with E2 determined the recruitment of PELP1/MNAR at the cell membrane, where it acted as an adaptor protein for connecting ERα-IGF1R-EGFR-GPR30-Src. These data suggest the ability for estrogens to activate IGF1R and EGFR even in the absence of their specific ligands. We found that treatments for 24 hours with E2, G1and PPT increased cyclin D1expression. However, despite estrogen's ability to transactivate IGF1R and EGFR, E2-dependent cyclin D1 expression was not mediated by the activation of these receptors. In fact, AG1024 and AG1478 did not block E2-dependent cyclin D1 expression. This was due to the ability of E2 to increase cyclin D1 transcription through direct binding of ERα to cyclin D1 promoter. All together these data highlight an involvement of estrogen receptors and GPR30 in controlling ACC tumorigenesis through both genomic and rapid pathways, giving indications for new promising therapies (antiestrogens and/or antiPELP1) of ACC.

Integrated analysis of microRNA and mRNA expression profiles in physiological myelopoieis: role of hsa-miR-299-5p in CD34+ progenitor cells commitment

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Cell fate decisions in the hematopoietic system appear to be directed by an antagonistic or synergistic interplay of transcription factors that pivot immature blood progenitors for cell specification. Multipotent progenitors initially trigger a promiscuous transcriptional program and, as soon as they commit to a restricted fate, they reinforce unilineage gene expression and withdraw transcripts affiliated with alternative blood cell types. MicroRNAs appear to be especially pertinent in driving this particular behavior representing a new component of the hematopoietic gene regulatory network. In fact, the archetypal microRNA can potentially regulate hundreds of genes even if most targets contain isolated microRNA recognition sites that may be inadequate for complete gene silencing. According to Bartel's theory, microRNAs mediated post-transcriptional control offers a more flexible and rapid way of tuning genes compared to transcriptional control (Bartel DP and Chen CZ, Nat Rev Genet 2004). These issues encouraged some investigators to explore the association of microRNAs and genes expression profiles obtained from the same cell type and advocated that microRNAs evolved to regulate gene expression programs and remove gene products unnecessary or potentially dangerous more rapidly than might occur by natural decay. Although many studies addressed the role of microRNAs during the normal myeloid differentiation process, only Georgantas and co-workers focused onto the impact of microRNAs on mRNA expression levels but limited the analyses to data obtained from human CD34+ stem/progenitor cells (Georgantas RW 3rd et al, PNAS 2007). In order to shed light onto the interplay of mRNAs and microRNAs during the normal myeloid commitment and verify that increased expression of a microRNA is skillful to modulate the levels of corresponding target mRNAs, we obtained microRNAs profiles from CD34+ hematopoietic progenitor cells (CD34 HPCs) and in-vitro differentiated precursors: erythroblasts, megakaryoblasts, monoblasts and myeloblasts (ERY, MKC, MONO and MYELO). We therefore analyzed these microRNA expression profiles together with the gene expression profiles of the same populations and observed that for the most part of the microRNAs specifically up-regulated in one single progeny an inverse correlation between microRNAs and down-regulated putative targets expression levels occurs, i.e. down-regulated genes showed an enrichment for the conserved putative targets of up-regulated microRNA. Among these microRNAs, hsa-miR-299-5p emerged as an interesting candidate to demonstrate how the integrated analysis of microRNA and mRNA expression data can help shedding light on the regulatory mechanisms governing cell differentiation. In particular, we used hsa-miR-299-5p to prove that the forced expression of a single lineage-specific microRNA is able to control the cell fate of CD34 HPCs grown in multilineage culture conditions. Clonogenic and liquid culture differentiation assays after gain- and loss-of-function experiments revealed that indeed hsa-miR-299-5p regulates hematopoietic progenitors fate modulating megakaryocytic-granulocytic versus erythroid-monocytic development.

ZFP36L1 negatively regulates erythroid differentiation of CD34+ hematopoietic stem cells by interfering with Stat5b pathway

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ZFP36L1 is a member of a family of CCCH tandem zinc finger proteins (TTP family) able to bind to AU-rich elements in the 3'-untranslated region of mRNAs, thereby triggering their degradation. The present study suggests that such mechanism is used during hematopoiesis to regulate differentiation by post-transcriptionally modulating the expression of specific target genes. In particular, it demonstrates that ZFP36L1 negatively regulates erythroid differentiation by directly binding the 3' untranslated region of Stat5b encoding mRNA. Stat5b down regulation obtained by ZFP36L1 over-expression results, in human hematopoietic progenitors, in a drastic decrease of erythroid colonies formation. These observations have been confirmed by silencing experiments targeting Stat5b and by treating hematopoietic stem/progenitor cells with drugs able to induce ZFP36L1 expression. Moreover, this study shows that different members of ZFP36L1 family act redundantly, since co-overexpression of ZFP36L1 and family member ZFP36 determines a cumulative effect on Stat5b downregulation. This work describes a mechanism underlying ZFP36L1 capability to regulate hematopoietic differentiation and suggests a new target for the therapy of hematopoietic diseases involving Stat5b/JAK2 pathway, such as chronic myeloproliferative disorders.

HDAC inhibitor valproic acid interferes with myeloip differentiation of human hematopoietic stem/progenitor cells

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Chromatin modifications such as histone acetylation and DNA methylation are important for gene expression regulation. Compounds like as histone deacetylases (HDAC) inhibitors are used in AML and MDS therapy because modify chromatin conformation by mean the deacetylation of core histone proteins, modulating gene expression patterns involved in cell growth, differentiation, and/or apoptotic cell death. Recently, the HDAC inhibitors have been shown to maintain the self renewal ability on hematopoietic stem/progenitor cells (HSPCs). However, the effects of HDAC inhibitors in changing the differentiation behaviour of normal human myeloid progenitors are poorly described. The aim of this study is clarify the interference of HDAC inhibitors on myeloid commitment and investigate molecular basis underlying biological effects on HSPCs following HDAC inhibitors treatment. HSPCs were treated with HDAC inhibitor valproic acid (VPA) and proliferation and differentiation capacities of VPA treated cells was studied. Moreover, to investigate changes in gene expression induced by VPA treatment on HSPCs, we performed microarray analysis of mRNA expression profile in VPA treated and untreated (CTR) CD34+ cells. In this study we demonstrated that VPA treated CD34+ cells showed increased number of cells in G0-G1 phase of cell cycle compared to CTR cells. Immunophenotype of CD34 cells at day 6 of treatment showed a physiological decrease of CD34 expression in CTR cells; on the contrary, CD34 expression is maintained for a longer time in the VPA treated cells. Moreover, VPA CD34+ cells showed higher expression of erythroid marker GPA, as well as a significant increase in the megakaryocyte marker CD41; on the contrary, granulocyte (CD66b, CD15, and MPO) and monocyte-macrophage (CD14, and CD163) markers resulted decrease in VPA CD34+ cells compared with CTR cells. According to this data, the clonogenic assay showed a significant increase in the percentage of BFU-E and CFU-E in VPA CD34+ cells, coupled to a decrease in CFU-G. Furthermore, a collagen-based culture system that supports megakaryopoietic progenitor growth in vitro, demonstrated VPA treatment induced a remarkable increase in CFU-MK. Gene expression profile analysis showed that among transcripts increased in VPA CD34+ cells, there are cell cycle and self renewal related genes. Moreover, VPA treatment up-regulated key genes involved in erythrocyte and megakaryocyte differentiation; on the contrary monocyte and granulocyte genes are down-regulated. These results indicate that the VPA treatment enhances erythrocyte and megakaryocyte differentiation at the expense of granulocyte and mono-macrophage differentiation. Moreover, microarray data provide for the first time a detailed molecular support for the biological effect promoted by VPA treatment on the proliferation, self-renewal and differentiation capacities of HSPCs.

Modulation of the facilitative glucose transporters (SLC2A) gene family expression in arterial tortuosity syndrome skin fibroblasts and aortic vascular smooth muscle cells

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In mammalian cells glucose and exoses homeostasis is manly mediated by the facilitative glucose transporters (GLUTs), encoded by the 14 members of the SLC2A gene family. Structurally, GLUTs are transmembrane proteins showing 12 hydrophobic domains and both the N- and C-termini located on the endofacial side of the membrane. GLUTs show tissue and cell-type-specific expression pattern. GLUT1 mediates glucose transport across blood tissue barriers; GLUT2 is the glucose sensor of the insulin-secreting β-cells. GLUT10 is expressed in liver, pancreas, adipose tissue, skin fibroblasts and aortic vascular smooth muscle cells (AVSMCs). Loss-of-function mutations in SLC2A10 gene, encoding for GLUT10, cause the arterial tortuosity syndrome (ATS) (OMIM#208050), a rare autosomal recessive connective tissue disorder characterized by tortuosity and elongation of the large and medium-sized arteries, pulmonary arteries stenosis, aneurysm formation, dysmorphic features and several connective tissue manifestations. We investigated the expression pattern of SLC2A gene family and organization of glucose transporter proteins in skin fibroblasts and AVSMCs strains derived from an unaffected subject and from an ATS patient, homozygous for the SLC2A10 frameshift c.1334delG (G445fsX484) mutation, leading to a premature termination codon (PTC), nonsense mediated mRNA decay (NMD) and lack of the protein (Coucke et al., 2006). Quantitative PCR showed that several GLUTs mRNAs were expressed to a different level in both cultured skin fibroblasts and AVSMCs from the control and the ATS patient. GLUT2 and GLUT7 mRNAs were the only GLUTs almost undetectable in both cell strains. GLUT1, 9 and 12 mRNAs were expressed at significantly higher levels in ATS than in control fibroblasts. GLUT6 and GLUT9 transcripts were detected at higher levels in ATS AVSMCs, compared to control cells. In the ATS AVSMCs, GLUT5, GLUT11 and GLUT12 mRNAs showed a significant reduction compared to the control cells. In both ATS cell strains GLUT10 mRNA was expressed at low levels, as expected by the NMD pathway activation. GLUTs protein organization was examined by immunofluorescence analysis when specific antibodies were available. Both control strains organized GLUT1, 3, 5, 10 and 11 on the cell membrane or nuclear and perinuclear regions, and did not organize GLUT6 and GLUT12. GLUT9 was detected at low levels only in skin fibroblasts. In both ATS cell strains GLUT1, GLUT3 and GLUT5 were organized as in their control counterparts. GLUT10 and GLUT11, abundantly organized in control cells, were not detectable and reduced, respectively, in ATS cells. GLUT6, GLUT9 and GLUT12 proteins, undetectable in control cells, were expressed at high levels in ATS strains. In particular, GLUT6 and GLUT9 were distributed in a spot-like pattern on the plasma membrane, whereas GLUT12 localized in the nuclear region. This study report for the first time the expression pattern profile of GLUTs in control and ATS skin fibroblasts and AVSMCs. In the ATS mutant GLUT10 defective cell strains, the expression and organization of the different GLUTs can be modulated at transcriptional, translational and post-translational level in order to maintain the exoses homeostasis by a co-regulated expression pattern, the molecular mechanisms of which are largely unknown. Coucke PJ, Willaert A, Wessels MW, Callewaert B, Zoppi N, De Backer J, Fox JE, Mancini GM, Kambouris M, Gardella R, Facchetti F, Willems PJ, Forsyth R, Dietz HC, Barlati S, Colombi M, Loeys B, De Paepe A. Mutations in the facilitative glucose transporter GLUT10 alter angiogenesis and cause arterial tortuosity syndrome. 2006, Nat. Genet. 38:452-457

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