Università degli Studi di Ferrara

A.I.B.G.

21 e 22 Settembre 2018

XVIII Congresso Nazionale AIBG

Università degli Studi di Ferrara

Associazione Italiana di Biologia e Genetica Generale e Molecolare

XVIII Congresso Nazionale AIBG

Associazione Italiana di Biologia e Genetica Generale e Molecolare

FERRARA, 21-22 Settembre 2018

Aule E2-E3
Nuovi Istituti Biologici
Via Luigi Borsari, 46

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PRESENTAZIONE

Care/i Colleghe/i Socie/i dell'AIBG,
siamo lieti di ospitarVi a Ferrara in occasione del XVIII Congresso Nazionale AIBG che si svolgerà nei giorni 21-22 settembre 2018 presso il Plesso Biologico della Università di Ferrara, situato in centro Città, dentro la cerchia delle mura storiche di Ferrara.

Sono previste comunicazioni di 15 minuti seguite da 5 minuti di discussione. I contributi verranno inseriti nel libro degli abstracts che sarà disponibile online e sarà consegnato agli iscritti all'atto della registrazione a Ferrara. Sono previste 20 borse congressuali per giovani non strutturati.

La Giunta AIBG ha deliberato in merito alla istituzione del Premio Guido Tarone, nostro illustre Collega scomparso prematuramente. L'Assemblea dei Soci AIBG si svolgerà presso la sede del Congresso. A seguire, la cena sociale presso il Castello Estense.

Certi che l'alto livello qualitativo dei vostri contributi determinerà il vero successo del Congresso, vi aspettiamo numerosi.

PROGRAMMA

VENERDÌ, 21 SETTEMBRE 2018

APERTURA CONGRESSO

12.00 – 13.30 Registrazioni

13.30 – 14.00 Cocktail di Benvenuto/Light Lunch

14.00 – 14.05 Benvenuto delle Autorità

14.05 – 14.10 Introduzione e presentazione, Mauro Tognon

14.10 – 15.00 Core Curriculum e strumenti didattici, Lucio Nitsch
Relazione sulla didattica nei Corsi di Laurea in Medicina e Chirurgia dal titolo: "La mente è un fuoco da accendere".

15.00 – 17.20 SESSIONE 1:
EPIGENETICA E REGOLAZIONE DELL'ESPRESSIONE GENICA
Moderatori: Olivieri C. (Pavia), Marmiroli N. (Parma)

15.00 – 15.20 BONONI I.
Circulating microRNAs found to be dysregulated in pleural mesothelioma patients as potential new biomarkers
Università di Ferrara

15.20 – 15.40 CHIANESE R.
Mammalian spermatozoa retain circular RNAs: a possible role of NAPEPLD
Università della Campania "Luigi Vanvitelli"

16.00 – 16.20 COTELLA D.
Interactome profiling of embedded SINE B2 RNAs reveals a role of the RNA-binding protein ILF3 in the nucleocytoplasmic shuttling of AS-UCHL1 lncRNA
Università del Piemonte Orientale e IRCAD, Novara

16.20 – 16.40 *DI EMIDIO G.
Exposure to anticancer drug cyclophosphamide results in a heritable modification of DNA methylation in mouse oocytes
Università dell'Aquila

16.40 – 17.00 DI GIORGIO E.
Class IIa HDACs revolution: from neglected epigenetic regulators to drivers of malignancy
Università di Udine

***COMUNICAZIONE CHE CONCORRE PER IL PREMIO TARONE**

17.00 – 17.30 Pausa caffè

17.30 – 19.30 SESSIONE 2:
RISPOSTE CELLULARI ALLO STRESS
Moderatori: Modesti A. (Firenze), Tripodi M. (Roma-La Sapienza)

17.30 – 17.50 FIOCCO D.
Small heat shock proteins characterization in a probiotic model
Università di Foggia

17.50 – 18.10 FIUME G.
Activation of NF-κB in B-cell receptor signaling through Bruton's tyrosine kinase dependent phosphorylation of IκB-α
Università di Catanzaro

18.10 – 18.30 FRANCESCHELLI S.
Modulation of the oxidative plasmatic state in gastroesophageal reflux disease with intake of electrolyzed reduced water (ERW)
Università di Chieti

18.30 – 18.50 SANTARSIERO A.
Essential role of mitochondrial citrate export in inflammatory response and oxidative stress
Università della Basilicata

19.10 – 19.30 CRISTOFANI R.
Chaperone mediated autophagy responds to macroautophagy inhibition in motor neuron diseases
Università di Milano

19.30-19.40 CONSEGNA PERGAMENE AI PROFESSORI SOCI EMERITI:
Sergio Barlati, UNIBS; Giovanni Delrio, SUN; Giovanni Battista Chieffi, SUN; Ranieri Cancedda, UNIGE; Franco Mangia, UNIROMA1; Giacomo De Leo, UNIPA; Enrico Ginelli, UNIMI

SERATA LIBERA

SABATO, 22 SETTEMBRE 2018

08.15 – 10.35 SESSIONE 3:
MECCANISMI E SEGNALI NEL CONTROLLO DI PROLIFERAZIONE, DIFFERENZIAMENTO E MORTE CELLULARE
Moderatori: Brunelli S. (Milano-Bicocca), Retta S.F (Torino)

08.15 – 08.35 DEIANA M.
Mesenchymal stem cells differentiation is associated to autophagy induction during physical activity
Università di Verona

08.35 – 08.55 *BARBAGALLO C.
LncRNA UCA1, upregulated in CRC biopsies and downregulated in serum exosomes, controls mRNA expression by RNA-RNA interactions
Università di Catania

08.55 – 09.15 BIANCHI E.
Role of MAF/SPP1 axis in primary myelofibrosis
Università di Modena e Reggio Emilia

09.15 – 09.35 FONTANA S.
Exosomes derived from metastatic colon cancer cells transfer malignant phenotypic traits to surrounding cells: their emerging role in tumor heterogeneity
Università di Palermo

09.35 – 09.55 CUSANELLI E.
Investigating the dynamics and function of the telomeric long noncoding RNA TERRA in living cancer cells
Università di Trento

09.55 – 10.15 PIVA F.
LncRNA co-expression network analysis reveals novel biomarkers for pancreatic cancer
Università Politecnica delle Marche

10.15 – 10.35 BRAGHETTA P.
EMILIN-3 deposition during bone development suggests a role in skeletal growth
Università di Padova

***COMUNICAZIONE CHE CONCORRE PER IL PREMIO TARONE**

10.35 – 10.50 Pausa caffè

10.50 – 13.10 SESSIONE 4:
GENOMICA STRUTTURALE E FUNZIONALE
Moderatori: Zavattari P. (Cagliari), Marini M. (Bologna)

10.50 – 11.10 *DI NISIO V.
Repeated gonadotropin treatments and protein expression in mouse fallopian tubes
Università dell'Aquila

11.10 – 11.30 *CARACAUSI M.
Plasma and urinary metabolomic profiles of down syndrome correlate with alteration of mitochondrial metabolism
Università di Bologna

11.30 – 11.50 DI PIETRO L.
Regulation of RUNX2 gene during evolution: functional implications in nonsyndromic craniosynostosis patients
Università Cattolica del Sacro Cuore, Roma

11.50 – 12.10 DONATO L.
New omics perspectives unveil an innovative scenario of retinitis pigmentosa etiopathogenesis: synaptic alterations from inner to outer retinal layers
Università di Messina e IEMEST, Palermo

12.10 – 12.30 FIORENZA M.T.
Pathogenetic mechanisms responsible for altered developmental trajectories in Niemann Pick C disease
Università La Sapienza, Roma

12.30 – 12.50 ROMANO R.
Alterations of the endocytic pathway in Charcot-Marie-Tooth type 2B
Università del Salento, Lecce

12.50 – 13.10 ZOPPI N.
Fibroblast-to-myofibroblast transition mediated by an αvβ3 integrin-ILK-SNAIL1 signaling in Hypermobile Ehlers Danlos syndrome
Università di Brescia

***COMUNICAZIONE CHE CONCORRE PER IL PREMIO TARONE**

13.10 – 14.00 COLAZIONE DI LAVORO PRESSO IL CHIOSTRO DEL '400 DI SANTA MARIA DELLE GRAZIE

14.00 – 15.40 SESSIONE 5:
MECCANISMI MOLECOLARI DI DIFESA CELLULARE
Moderatori: Talesa V.N. (Perugia), Bagni C. (Roma-Tor Vergata)

14.00 – 14.20 SALLUSTIO F.
Adult renal stem/progenitor cells can revert LPS-induced endothelial-to-mesenchymal transition of endothelial cells secreting BPIFA2, CXCL6, SAA2 and SAA4 antiseptic proteins
Università di Bari

14.20 – 14.40 SARNATARO D.
Regulation of subcompartmental targeting and folding properties of the prion-like protein Shadoo
Università Federico II, Napoli

14.40 – 15.00 TASSO R.
Mesenchymal stem cell-derived extracellular vehicles (EVs) as mediators of anti-inflammatory effects: endorsement of macrophage polarization
Università di Genova

15.00 – 15.20 MOLTENI M.
MIR-146a induced by cyanobacterial LPS antagonist (CyP) mediates inhibition of TNF-α in human monocytes
Università dell'Insubria

15.20 – 15.40 TORREGGIANI E.
Innovative protocol for long-term culture of human primary keratinocytes from the normal colorectal mucosa
Università di Ferrara

15.40 – 15.55 Discussione finale

15.55 – 16.05 Consegna Premio "Guido Tarone" al vincitore con pergamena dedicata Presidente di Giuria, Lucio Nitsch.

16.05 – 16.15 Conclusione lavori scientifici

16.15 – 16.25 Comunicazione dei vincitori e consegna delle borse congressuali

16.25 – 16.40 Pausa caffè

16.40 –18.40 Assemblea soci A.I.B.G.

20.30 Cena sociale presso la Sala dell'Imbarcadero del Castello Estense di Ferrara.

COMUNICAZIONI ORALI

CIRCULATING MICRORNAS FOUND TO BE DYSREGULATED IN PLEURAL MESOTHELIOMA PATIENTS AS POTENTIAL NEW BIOMARKERS

Ilaria Bononi, Francesca Frontini, Antonella Santoro, Elena Torreggiani, Lucia Oton-Gonzalez, John Charles Rotondo, Carmen Lanzillotti, Chiara Mazziotta, Maria Rosa Iaquinta, Fernanda Martini, Mauro Tognon

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Malignant pleural mesothelioma (MPM) is a very aggressive malignancy of the pleural surface due to the asbestos exposure. The MPM incidence increased over the past decade, with an estimated peak in 2025. MPM will continue to represent a significant health concern even after the peak incidence mentioned above [1]. As MPM is largely unresponsive to chemo- and radio-therapies and considering the long latency period of MPM onset, the identification of new and specific markers is of a paramount importance for an early diagnosis and treatment of MPM. In recent years, together with protein markers, microRNAs (miRNAs) from MPM cells or sera, have been proposed as new biomarkers [2]. Indeed, miRNAs expression was found dysregulated, both in cancer cells and sera, in patients affected by tumors of different histotypes, including MPM. Moreover, it has been reported that circulating miRNAs are stable in biological fluids and, consequently, they could be employed as potential MPM biomarkers. In this investigation, circulating miRNAs from serum samples of MPM patients and healthy subjects (HS) were comparatively analyzed by microarray and RT-qPCR technologies. Our results allowed (i) to select miR-3665, an endogenous stable miRNA, as the internal control to quantify circulating miRNAs in our analyses; (ii) to detect miR-197-3p, miR-1281 and miR-32-3p up-regulated in MPM compared to HS [3]. In conclusion, the three circulating up-regulated miRNAs, mentioned above, are proposed as potential new MPM biomarkers and targets for innovative therapeutic approaches.

References

[1] Park E.K. et al. Environ. Health Perspect.119:514-8, 2011.
[2] Balatti V. et al. J. Thorac. Oncol. 6:844-51, 2011.
[3] Bononi I. et al.Oncotarget.7:82700-11, 2016.

MAMMALIAN SPERMATOZOA RETAIN CIRCULAR RNAs: A POSSIBLE ROLE OF NAPEPLD

Rosanna Chianese[1], Marco Ragusa[2,4], Teresa Chioccarelli[1], Gilda Cobellis[1], Cinzia Di Pietro[2], Duilia Brex[2], Davide Barbagallo[2], Silvia Fasano[1], Bruno Ferraro[3], Carolina Sellitto[3], Luisa Perillo[3], Michele Purrello[2], Riccardo Pierantoni[1]

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Through a back-splicing reaction from linear RNAs, cells produce circular RNAs (circRNAs). As non-coding RNAs, circRNAs are able to sequester miRNAs through their sponge activity thus to inhibit their function; in doing so, circRNAs and miRNAs constitute a circRNA-associated-competitive endogenous RNA network (ceRNET).
Brain and testis are the main sites of circRNA production. Until today a possible circRNA function in spermatozoa (SPZ) physiology or a possible contribution of a SPZ-derived circRNA cargo in fertilization and embryo development in mammals is still unknown.
Using a computational approach we verified the ability to circularize of several linear transcripts, known to be involved in SPZ/testicular and epididymal physiology. Therefore, we identified two circRNA isoforms of NAPEPLD, an important component of the endocannabinoid system, able to synthesize anandamide (AEA), the main endocannabinoid. The expression of both isoforms - verified in both human ejaculated SPZ and mouse epididymal extracted SPZ - was higher than linear counterpart. Sponge activity of circNAPEPLD isoform 1 (iso1) and 2 (iso2) was demonstrated by computational analysis. Interestingly, 3 and 6 sponged miRNAs for circNAPEPLDiso1 and iso2, respectively, are expressed in human follicular fluid and oocytes, supporting our hypothesis concerning a possible ceRNET in reproduction, potentially regulated by SPZ circRNAs.
CircRNA translatability was also verified. Thus, circNAPEPLDiso1 showed two Internal Ribosome Entry Sites (IRESs) and an ORF different in length with respect to its linear counterpart.
These data suggest for the first time that SPZ-derived circNAPEPLD could represent a paternal contribution to zygote and embryo development.

INTERACTOME PROFILING OF EMBEDDED SINE B2 RNA REVEALS A ROLE OF THE RNA-BINDING PROTEIN ILF3 IN THE NUCLEOCYTOPLASMIC SHUTTLING OF AS-UCHL1 LNCRNA

Francesca Fasolo[1], Laura Patrucco[2], Massimiliano Volpe[3], Carlotta Bon[1], Olja Tarasiuk[2], Claudio Santoro[2], Silvia Zucchelli[1,2], Daniele Sblattero[4], Remo Sanges[3], Diego Cotella[2], Stefano Gustincich[1,5]

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Transposable Elements (TEs) compile about half of the mammalian genomes and, as embedded sequences, up to 40% of long non-coding RNAs (lncRNAs) transcripts. Embedded TEs may represent functional domains within lncRNAs, providing a structured RNA platform for protein interaction [1].
Here we show the interactome profile of the mouse inverted SINEB2 (invSINEB2) element alone and embedded in AS Uchl1, a lncRNA antisense to Uchl1 gene. AS Uchl1 is the representative member of a functional class of antisense lncRNAs, named SINEUPs, where the invSINEB2 element enhances translation of sense protein-coding mRNAs [2].
By using the RIDome technology [3], we identified the interleukin enhancer-binding factor 3 (ILF3) as a protein partner of AS Uchl1 RNA. By in vitro and in vivo experiments, we demonstrate that the interaction is mediated by the RNA Binding Motif 2 of ILF3 and the invSINEB2 element.
Bioinformatic analysis of ENCODE eCLIP data shows that ILF3 binds transcribed SINEs sequences at transcriptome-wide levels. Functionally, we prove that ILF3/invSINEB2 interaction regulates the nuclear localization of AS Uchl1 RNA together with an embedded partial Alu element.
This work unveils the existence of a specific interaction between an embedded TE and a protein partner regulating RNA localization strengthening the model of TEs as functional modules in lncRNAs.

References

[1] Johnson R. et. al. RNA 20(7):959-76. 2014.
[2] Zucchelli S. et al. Front Cell Neurosci. 9:174. 2015.
[3] Patrucco L. et al. RNA Biol. 12(12):1289-300. 2015.

EXPOSURE TO ANTICANCER DRUG CYCLOPHOSPHAMIDE RESULTS IN A HERITABLE MODIFICATION OF DNA METHYLATION IN MOUSE OOCYTES

***Giovanna Di Emidio[1], Marco D'aurora[2], Valentina Gatta[2], Carla Tatone[1]**

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Radio- and chemotherapy have increased survival rates but exert toxic effects in germline cells. Paternal exposure to the anticancer drug cyclophosphamide (CPM) induces mutations and transgenerational instability, but hereditary effects of maternal exposure have not been addressed. Many imprinted genes, including the maternally methylated *Igf2r* and *Peg3*, are erased when primordial germ cells arrive at genital ridges, and re-established during gametogenesis. Here, we investigated whether exposure to CPM can result in hereditable effects on DNA methylation of imprinted genes in the offspring's oocytes. Moreover, the effects of crocetin and AS101 known to protect the ovary from CPM injury were studied. Female CD1 mice received a single dose of CPM; crocetin or AS101 for 15days prior to CPM. To avoid CPM-related foetal malformations, mice were mated 12weeks after CPM. Female offspring (F1) derived from all-groups were housed and natural mated. Female offspring (F2) were reproduced from F1-female mice. DNA was isolated from pools of 250oocytes collected from 21day-aged F1 and F2-mice.

In CTRL-F1 and CPM-F1, methylation in *Igf2r* was 71% and 64 %; in *Peg3* was 68% and 4%, respectively. In CTRL-F2 and CPM-F2, methylation in *Igf2r* was 88% and 54%; in *Peg3* was 90% and 62%, respectively. In F1, crocetin or AS101 seemed to mitigate the effect of CPM only on *Peg3*, but in F2, they induced a reduction of *Peg3* methylation higher than CPM.

In conclusion, we showed that CPM influences epigenetic programming of imprinted genes in offspring and that molecules protecting ovarian reserve do not prevent these effects.

CLASS IIA HDACS REVOLUTION: FROM NEGLECTED EPIGENETIC REGULATORS TO DRIVERS OF MALIGNANCY

Eros Di Giorgio, Harikrishna Reddy Paluvai, Elisa Franforte, Valentina Cutano, Raffaella Picco, Emiliano Dalla and Claudio Brancolini

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Among the 18 mammalian Histone DeACetylases (HDACs), the relevance of the four class IIa HDACs (HDAC4, 5, 7, 9) as epigenetic regulators has been for long overlooked because of their lack of catalytic activity and their impotence in directly promoting the deacetylation of histone tails. Recently, we have demonstrated that the nuclear fractions of these HDACs own pro-oncogenic properties. This transforming potential is achieved through the binding to MEF2 transcription factors and their conversion into transcriptional repressors. Curiously, the repression exerted by these HDACs is locus specific and in a same cell-population some MEF2-target genes actively transcribed and others strongly repressed can coexist.

Sarcomas, melanomas and some types of breast cancer are addicted for the epigenetic reprogramming exerted by class IIa HDACs. In these tumors, the inhibition or the depletion of class IIa HDACs lead to a transient reacetylation of H3K27 that is followed by a stable methylation of H3K4 in the regulative regions of specific target loci. The selective expression of these genes causes senescence and/or apoptosis in the targeted cells and a profound modification of the extracellular environment; the slow kinetics of the mechanism suggests that the altered transcription rates of a limited number of genes could be at the basis of all the observed phenotypes.

The selective action of class IIa HDACs on this new class of bivalent poised promoters can be exploited for the treatment of HDACs addicted malignancies and offers a new interpretation of the epigenetic remodeling that drives oncogenesis.

SMALL HEAT SHOCK PROTEINS CHARACTERIZATION IN A PROBIOTIC MODEL

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Small heat shock proteins (sHSP) are ubiquitous, low molecular weight proteins with chaperon-like activity. sHSP protect cell under stress conditions and critically contribute to survival to heat shock. *Lactobacillus plantarum* (Lp), a member of the lactic acid bacteria (LAB), is a probiotic species with biomedical and biotechnological applications. Unlike most lactobacilli, which have single *shsp* genes, three sHSP-encoding genes were identified in Lp WCFS1. Such redundancy might endow Lp with the capacity to cope with a broad range of stresses, thus accounting for its extraordinary environmental adaptability.

To unravel the role of Lp sHSP, knock out (KO) mutants for *hsp1* and *hsp3* were generated and phenotypically characterized. Growth and survival rates under diverse stress conditions, which are typical for probiotics, revealed a different contribute of the two sHSP to thermotolerance induction and cryoprotection. However, neither *hsp* seemed essential to tackle such challenges. Accordingly, cellular protein aggregation in both mutants was not significantly different from the wild type. Comparative transcriptional patterns revealed that in the mutant genetic backgrounds there is an up-regulated basal expression of the un-mutated mate *hsp* and other stress-related genes, which might compensate for the loss of sHSP function, hence underlying the lack of a marked susceptibility to stress. *Hsp* KO affected biofilm adhesive capacity, altered cell surface physicochemical properties, and drastically modified membrane fluidity upon stress.

These findings indicate that Lp *hsp1* and *hsp3* have pleiotropic effects, fulfill overlapping activities in stress tolerance and housekeeping functions, and regulate membrane fluidity by a plausible direct association.

ACTIVATION OF NF-κB IN B-CELL RECEPTOR SIGNALLING THROUGH BRUTON'S TYROSINE KINASE DEPENDENT PHOSPHORYLATION OF IκB-α

Marilena Pontoriero, Eleonora Vecchio, Francesco Albano, Annamaria De Laurentiis, Enrico Iaccino, Selena Mimmi, Antonio Pisano, Gaetanina Golino, Valter Agosti, Giuseppe Scala, Ileana Quinto, Giuseppe Fiume

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The antigen-mediated triggering of B-cell receptor (BCR) activates the transcription factor NF-κB that regulates the expression of genes involved in B-cell differentiation, proliferation and survival [1]. The tyrosine kinase Btk is essentially required for the activation of NF-κB in BCR signalling through the canonical pathway of IKK-dependent phosphorylation and proteasomal degradation of IκB-α, the main repressor of NF-κB [1, 2]. Here, we provide the evidence of an additional mechanism of NF-κB activation in BCR signalling that is Btk-dependent and IKK-independent. In DeFew B-lymphoma cells, the anti-IgM stimulation of BCR activated Btk and NF-κB p50/p65 within 0.5 min in absence of IKK activation and IκB-α degradation. Within this short time, Btk associated and phosphorylated IκB-α at Y289 and Y305, and, concomitantly, p65 translocated from cytosol to nucleus. The mutant IκB-α Y289/305A inhibited the NF-κB activation after BCR triggering, suggesting that the phosphorylation of IκB-α at tyrosines 289 and 305 was required for NF-κB activation. In primary chronic lymphocytic leukemia cells, Btk was constitutively active and associated with IκB-α, which correlated with tyrosine phosphorylation of IκB-α and increased NF-κB activity compared to healthy B-cells. Altogether these results describe a novel mechanism of NF-κB activation in BCR signalling that could be relevant for Btk-targeted therapy in B-lymphoproliferative disorders.

References

[1] Herzog S., et al. Nat Rev Immunol. 9: 195-205, 2009.
[2] Hobeika E., et al. J Mol Med. 93: 143-158, 2015.

MODULATION OF THE OXIDATIVE PLASMATIC STATE IN GASTROESOPHAGEAL REFLUX DISEASE WITH INTAKE OF ELECTROLYZED REDUCED WATER (ERW)

Sara Franceschelli[1], Daniela Maria Pia Gatta[2], Mirko Pesce[2], Alessio Ferrone[2], Maria Anna De Lutiis[2], Ester Vitacolonna[2], Antonia Patruno[2], Alfredo Grilli[1], Mario Felaco[2] and Lorenza Speranza[2]

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Electrolyzed Reduced Water (ERW) produced near the cathode during water electrolysis exhibits high pH, high concentration of dissolved hydrogen and an extremely negative redox potential [1]. Proton pump inhibitors (PPIs) have been universally accepted as first-line therapy for management of GERD.Our previus study in U937 cell and other findings indicate that ERW had the ability of a scavenger free radical, which results from hydrogen molecules with a high reducing ability and may participate in the redox regulation of cellular function [2].The potential benefits of ERW, rich in molecular hydrogen, in improving symptoms and systemic oxidative stress associated with GERD was assessed. The study was performed on 84 GERD patients undergoing control treatment (PPI + tap water) or experimental treatment (PPI + ERW) for 3 months. These patients were subjected to the GERD-Health Related Quality of Life Questionnaire as well as derivatives reactive oxigen metabolites (d-ROMs) test, biological antioxidant potential (BAP) test, superoxide anion, nitric oxide and malondialdehyde assays, which were all performed as a proxy for the oxidative/nitrosative stress and the antioxidant potential status. Spearman's correlation coefficient was used to evaluate the correlation between scores and laboratory parameters. Overall results demonstrated that an optimal oxidative balance can be restored and GERD symptoms can be reduced rapidly.. The relative variation of heartburn and regurgitation score was significantly correlated with laboratory parameters. Thus,combination treatment with PPI and ERW improves the cellular redox state leading to the improvement of the quality of life as demonstrated by the correlation analysis between laboratory parameters and GERD symptoms [3].

References

[1] Franceschelli S, et al. Int J Mol Sci. 17(9). pii: E1461. 2016.
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ESSENTIAL ROLE OF MITOCHONDRIAL CITRATE EXPORT IN INFLAMMATORY RESPONSE AND OXIDATIVE STRESS

Anna Santarsiero [1], **Paolo Convertini** [1], **Vito Iacobazzi** [2], **Vittoria Infantino** [1]

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Recent evidence established that macrophages undergo metabolic rewiring in response to proinflammatory triggers to support the bioenergetic and biosynthetic demands of the cell. Changes in metabolite profiles are largely due to a broken Krebs cycle, one consequence of which is the accumulation of citrate. In these conditions most of citrate is diverted from Krebs cycle and channelled into the "citrate pathway", consisting in the increased export of citrate by the citrate carrier (CIC) from mitochondria to cytosol. Cytosolic citrate is cleaved by ATP citrate lyase (ACLY) into acetyl-CoA and oxaloacetate, precursors of inflammatory mediators. Acetyl-CoA is involved in PGE_2 biosynthesis while oxaloacetate is used to produce NADPH needed for NO and ROS. Inhibition of CIC or ACLY by different synthetic and natural molecules results in depletion of PGE_2, NO and ROS levels suggesting that the citrate pathway is a new target dealt with inflammation. The citrate pathway is upregulated in both Behçet's syndrome, a multisystemic inflammatory disorder, and in Down syndrome. Interestingly, citrate pathway inhibition induces beneficial effects on chronic inflammatory conditions as well as on oxidative stress which are relevant features of Down syndrome.

CHAPERONE MEDIATED AUTOPHAGY RESPONDS TO MACROAUTOPHAGY INHIBITION IN MOTOR NEURON DISEASES

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Protein aggregates in motoneurons are a common hallmark of motoneuron diseases (MNDs), including spinal and bulbar muscular atrophy (SBMA) and amyotrophic lateral sclerosis (ALS). In MNDs, the protein quality control system prevents protein aggregation.
We found that dynein mediated transport is an essential process for misfolded and aggregated protein delivery to degradative sites. Its blockage inhibits macroautophagy. In this condition the co-chaperone BCL2-Associated Athanogene 1 (BAG1) mRNA levels greatly increase to routes misfolded protein to proteasome. Indeed, exogenous BAG1 overexpression reduces misfolded protein accumulation via their proteasome degradation. However, dynein inhibition reduces misfolded insoluble species also when macroautophagy and proteasome are both inhibited. Interestingly, BAG1 can associate HSPA8/HSC70 routing misfolded proteins also to chaperone-mediated autophagy (CMA). After the recognition by HSPA8, proteins are translocated to the lysosomal receptor LAMP2A that allows their insertion into the lumen of competent lysosomes.
Dynein inhibition did not modify the mRNA levels of all autophagy markers tested, but significantly increased Lamp2A mRNA and protein levels, but it reduced Lamp1 protein levels suggesting that lysosomal number was reduced. To prove CMA involvement in mifolded protein clearance upon dynein inhibition we used alpha-synuclein (SNCA), a well-established CMA substrate and we found that dynein inhibition increased its clearance. These data show that when macroautophagy is impaired the accumulation of toxic misfolded protein could be prevented by proteasome and CMA degradation. Since CMA is a ubiquitous pathway, it is a conceivable that CMA can be used as a potential pharmacological target to increase the clearance of misfolded proteins.

MESENCHYMAL STEM CELLS DIFFERENTIATION IS ASSOCIATED WITH AUTOPHAGY INDUCTION DURING PHYSICAL ACTIVITY

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Physical activity is considered a favorable factor against obesity, diabetes, bone disease and hypertension. Therefore we have analyzed its effects on circulating progenitor cells harvested from runners before and after an half marathon performance. In particular, we investigated: i) progenitors differentiation profiles; ii) expression of telomerase-associated genes; iii) autophagy markers. Preliminary results showed that physical activity promotes Mesenchymal Stem Cells (MSCs) differentiation and affects the expression of telomerase associated genes. The expression of most genes involved in the osteogenic lineage commitment was upregulated after the run. Arrays data showed increased levels of BMP2 and BMP6 expression, respectively, along with BMP3, which induces MCSs proliferation. SOX9, COL2A1 and COMP genes expression was also enhanced, suggesting induction of chondrocitic differentiation as well. Post-run enhanced expression of both TERT and TERF1 in cMSCs was observed. Our findings indicate that physical activity affects progenitor cells by enhancing their proliferation as well as by inducing their commitment and differentiation. At the same time physical activity enhances autophagy, by upregulating the expression of ATG3, ATG5 and ULK1 genes; their expression correlates positively with MSCs differentiation.

LNCRNA UCA1, UPREGULATED IN CRC BIOPSIES AND DOWNREGULATED IN SERUM EXOSOMES, CONTROLS MRNA EXPRESSION BY RNA-RNA INTERACTIONS

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LncRNAs and circRNAs contribute to the onset of many neoplasias through RNA-RNA competitive interactions; in addition, they could be secreted by cancer cells into biological fluids, suggesting their potential diagnostic application. By analyzing the expression of 17 lncRNAs and 31 circRNAs in biopsies and serum exosomes from colorectal cancer (CRC) patients through qRT-PCR, we detected CCAT1, CCAT2, HOTAIR, UCA1 upregulation and CDR1AS, MALAT1, TUG1 downregulation in biopsies. In serum exosomes, UCA1 was downregulated, while circHIPK3 and TUG1 were upregulated. Combined ROC curves of TUG1:UCA1 and circHIPK3:UCA1 showed high values of sensitivity and specificity. Through in vitro (i.e., RNA silencing, MAPK inhibition) and in silico analyses (i.e., expression correlation, RNA-RNA binding prediction), we found that UCA1 could (1) be controlled by MAPKs through CEBPB, (2) sequester miR-135a, miR-143, miR-214, miR-1271, protecting ANLN, BIRC5, IPO7, KIF2A, KIF23 from miRNA-induced degradation; (3) interact with mRNA 3'-UTRs, preventing miRNA binding. UCA1 and its co-regulated antisense, LINC01764, could interact and reciprocally mask their own miRNA binding sites. Functional enrichment analysis of the RNA-RNA network controlled by UCA1 suggested its potential involvement in cellular migration. UCA1 regulatory axis would represent a promising target to develop innovative RNA-based therapeutics against CRC.

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ROLE OF MAF/SPP1 AXIS IN PRIMARY MYELOFIBROSIS

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Primary Myelofibrosis (PMF) is a myeloproliferative neoplasm characterized by hyperplastic megakaryopoiesis and deregulated cytokines production, which contribute to the development of bone marrow fibrosis, osteosclerosis and extramedullary hematopoiesis.
We recently showed that the transcription factor MAF is upregulated in PMF patients versus healthy donor-derived CD34+ hematopoietic progenitor cells (HPCs). In order to shed light into the role of MAF in PMF pathogenesis, we studied the effects of MAF overexpression and knockdown in CD34+ HPCs. We demonstrated that MAF favours the megakaryocyte and monocyte/macrophage commitment of HPCs. In addition, gene expression profiling of c-MAF-overexpressing CD34+ HPCs revealed that MAF enhances the expression of genes coding for proinflammatory and profibrotic secreted mediators, such as interleukin 8 (IL8), CCL2, MMP9, uPAR, LGALS3 and osteopontin (OPN). Among them, we focused on SPP1 and LGALS3, as their overproduction in PMF patients had never been reported before. Since SPP1 plasma levels resulted remarkably higher in PMF compared to ET and PV patients, we further investigated the effects of SPP1 on non-hematopoietic cells playing a key role in the bone marrow fibrosis, which is a hallmark of PMF. In vitro assays demonstrated that SPP1 promotes fibroblasts and mesenchymal stromal cells proliferation and collagen production. Strikingly, higher SPP1 plasma levels in PMF patients correlate with a more severe fibrosis degree and a shorter overall survival, further supporting the role of SPP1 in PMF progression.
Collectively our data unveil that MAF overexpression contributes to PMF pathogenesis by driving the deranged production of the profibrotic mediator SPP1.

EXOSOMES DERIVED FROM METASTATIC COLON CANCER CELLS TRANSFER MALIGNANT PHENOTYPIC TRAITS TO SURROUNDING CELLS: THEIR EMERGING ROLE IN TUMOR HETEROGENEITY

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Several studies have clearly demonstrated that within a heterogeneous tumor mass the inter-clonal cooperation between metastatic and non-metastatic cells can facilitate the tumor progression. Recent accumulating evidence has highlighted that tumor-derived exosomes (TDEs) play a relevant role as mediator of the inter-clonal collaborative cooperation affecting the properties of both tumor and non-tumor component.

In this context, our goal was to understand if exosomes derived from highly metastatic cells may influence the behaviour of less aggressive tumor cells and the properties of endothelium.

We found that metastatic SW620 cells transfer through exosomes (SW620Exos) their round/ameboid phenotype to elongated non-metastatic SW480 cells also inducing the increase of the motile and invasive activities. Moreover, SW620Exos caused endothelial hyperpermeability by altering the junctional complexes in HUVECs. The SWATH-based quantitative proteomic analysis highlighted that SW620Exos were significant enriched in several proteins related to the RhoA/ROCK sig¬naling, known to induce the amoeboid motility as well as the destabilization of endothelial junctional complexes. According to this data, we found that the treatment with ExoSW620 elicited in both SW480 cells and HUVECs the increase of RhoA activity, while the induced morphological and functional effects were reverted by co-treatment with a specific ROCK inhibitor. RacGap1 and thrombin were identified as putative key mediators of the effects induced by SW620Exos in target cells. Taken together our data indicates that within a heterogeneous tumor mass exosomes released by metastatic cells affect the features of both tumor and non-tumor cell components, thus contributing to accelerate the metastatic cascade.

INVESTIGATING THE DYNAMICS AND FUNCTION OF THE TELOMERIC LONG NONCODING RNA TERRA IN LIVING CANCER CELLS

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Telomeres are transcribed in a strand specific manner, giving rise to a telomeric-repeat containing long noncoding RNA called TERRA. TERRA plays key roles in telomere biology, including regulation of heterochromatin formation and DNA replication. TERRA also exerts extratelomeric functions by controlling gene expression through interaction with TERRA-binding sites within the genome. Furthermore, TERRA transcripts form RNA:DNA hybrids which can impact on genome stability and telomere biology. These findings indicate that TERRA molecules undergo complex dynamics within the nucleus. However, little is known about the dynamics and function of TERRA expressed from a single telomere in cancer cells.
We developed a live-cell imaging assay based on the MS2-GFP system in order to visualize single-telomere TERRA transcripts in human cancer cells [1]. To this aim, we used the CRISPR/Cas9 tool to generate clones containing MS2 sequences integrated at a single telomere. Co-expression of the MS2-GFP fusion protein which recognizes MS2 RNA sequences, enabled us to image the MS2-tagged TERRA transcripts in living cells. Confocal live-cell imaging analyses revealed the formation of two populations of TERRA-MS2-GFP foci: TERRA RNA single molecules, which freely diffuse within the nucleoplasm, and TERRA RNA clusters. Simultaneous time-lapse confocal imaging of TERRA particles and telomeres showed that TERRA clusters transiently localize with chromosome ends. Interestingly, depletion of MS2-tagged TERRA transcripts by antisense oligonucleotides resulted in the induction of DNA damage at telomeres and multiple extratelomeric sites. These findings suggest that single-telomere TERRA transcripts participate in the maintenance of genome integrity in cancer cells.

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LncRNA CO-EXPRESSION NETWORK ANALYSIS REVEALS NOVEL BIOMARKERS FOR PANCREATIC CANCER

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High mortality and low survival rates for pancreatic ductal adenocarcinoma (PDAC) mainly result from the delay in diagnosis and treatment. Therefore there is an urgent need to identify early PDAC biomarkers and new therapeutic targets. In this study, we applied a commonly used systems biology approach, the weighted gene co-expression network analysis (WGCNA), on lncRNA expression data. Eleven lncRNAs, namely A2M-AS1, DLEU2, LINC01133, LINC00675, MIR155HG, SLC25A25-AS1, LINC01857, LOC642852 (LINC00205), ITGB2-AS1, TSPOAP1-AS1 and PSMB8-AS1 have been identified and validated on an independent PDAC expression dataset. Furthermore, we characterised them by functional and pathway enrichment analysis and identified which lncRNAs showed differential expression, differential promoter methylation levels and copy number alterations between normal and PDAC samples. Finally, we also performed a survival analysis and identified A2M-AS1, LINC01133, LINC00205 and TSPOAP1-AS1 as prognostic biomarkers for PDAC. Interestingly, although only a few cancer-associated lncRNAs have been functionally characterized, LINC00675 and LINC01133 lncRNAs have been already demonstrated to be involved in PDAC development and progression. Therefore, our results provide new potential diagnostic/prognostic biomarkers and therapeutic targets for PDAC that deserve to be further investigated. Moreover, these lncRNAs may improve the understanding about molecular pathogenesis of PDAC.

EMILIN-3 DEPOSITION DURING BONE DEVELOPMENT SUGGESTS A ROLE IN SKELETAL GROWTH

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Elastin microfibril interface-located protein (EMILIN)-3 belongs to the EMI domain containing extracellular glycoprotein family composed of seven members in mammals and with unique functions in the extracellular space. Previous reports identified Emilin3 expression in mesenchymal and cartilaginous tissues of the developing mouse skeleton. However, the protein deposition and its biological relevance during mouse bone development and growth was never investigated before. In this study, EMILIN-3 skeletal distribution is reported and preliminary analyses are performed pointing at the in vivo characterization of the function of EMILIN-3 during osteogenesis and bone growth. At E12.5, EMILIN-3 is detected in the developing intervertebral disks, in the mesenchymal condensation of the limb buds and around the vertebral bodies primordia. At later stages, EMILIN-3 moves to the periphery of bone cartilaginous anlagens and becomes restricted to the perichondrium at E16.5, where is found also in the anulus fibrosus. In newborn mice, EMILIN-3 becomes progressively restricted to the periosteum and at P90 the anulus fibrosus labelling disappears. In vivo, Emilin3 deficiency leads to an overall increase of postnatal mice body size, with morphometric alterations in the hypertrophic zone of the growth plate that are not due to impairments in the columnar chondrocytes proliferation rate. Further, alterations are present in the growth plate gene expression outline. Finally, micro-CT analyses and mechanical tests did not reveal any alteration in morphometric values or in the elastic module of Emilin3-/- tibiae of P22 mice.

PLASMA AND URINARY METABOLOMIC PROFILES OF DOWN SYNDROME CORRELATE WITH ALTERATION OF MITOCHONDRIAL METABOLISM

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Down syndrome (DS) is caused by the presence of a supernumerary copy of the human chromosome 21 (Hsa21) and is the most frequent genetic cause of intellectual disability (ID). Key traits of DS are the distinctive facies and cognitive impairment. We conducted for the first time an analysis of the Nuclear Magnetic Resonance (NMR)-detectable part of the metabolome in plasma and urine samples, studying 67 subjects with DS and 29 normal subjects as controls selected among DS siblings. Multivariate analysis of the NMR metabolomic profiles showed a clear discrimination (up to of 80% accuracy) between the DS and the control groups. The univariate analysis of plasma and urine revealed a significant alteration for some interesting metabolites. Remarkably, most of the altered concentrations were consistent with the 3:2 gene dosage model, suggesting effects caused by the presence of three copies of Hsa21 rather than two: DS/normal ratio in plasma was 1.23 (pyruvate), 1.47 (succinate), 1.39 (fumarate), 1.33 (lactate), 1.4 (formate). Several significantly altered metabolites are produced at the beginning or during the Krebs cycle. Accounting for sex, age and fasting state did not significantly affect the main result of both multivariate and univariate analysis.

REPEATED GONADOTROPIN TREATMENTS AND PROTEIN EXPRESSION IN MOUSE FALLOPIAN TUBES

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Infertile women often undergo more than 1 cycle of ovarian stimulation with gonadotropins to get pregnant. However, repeating such stimulation protocols seems to increase the risk of developing different types of gynecological cancers, as ovarian cancer (OC). Moreover, OC can originate from metastatization of high-grade serous intraepithelial carcinoma of the Fallopian tubes (FT) [1]. In this study, by using the mouse as a model, we evaluated if increased rounds (R) of gonadotropin stimulation could affect 1. expression levels of proteins regulating cell cycle and DNA repair in FT, and 2. meiotic spindle morphology of ovulated oocytes. To this end, adult female mice were subjected or not (Ctr) to 6 or 8 R of gonadotropin stimulation, as described in a previous study [2,3]. Ovulated oocytes were immediately fixed and examined to evaluate spindle morphology. FT were analyzed to detect the following proteins: Cyclin D1, p-p53/p53, p-AKT/AKT, p-GSK3β /GSK3β, SOX-2, OCT-3/4 and p-β-catenin/β-catenin. After 6 R, the contents of Cyclin D1 and p53/p-p53 were higher than Ctr; after 8 R also the contents of p-AKT, p-GSK3β and OCT-3/4 were significantly increased in comparison with Ctr. Conversely, SOX-2 and β-catenin contents were unchanged after treatments. Oocytes number and percentage of normal meiotic spindles drastically decreased from 6 R onward. Our results demonstrated that, at least in mice, repeated cycles of gonadotropin stimulation can significantly impair reproductive performances by reducing oocyte quality at retrieval, and can alter the expression levels of proteins controlling cell proliferation and survival in FT.

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ACUTE ENDURANCE EXERCISE-INDUCED EARLY ACTIVATION AND TRANSLOCATION OF AB-CRYSTALLIN IN SKELETAL MUSCLE DEPENDS UPON FIBER TYPE AND OXIDATIVE STRESS LEVEL

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Numerous studies have shown the important function of αB-crystallin (HSPB5) in striated muscle during physiological or pathological changes. Here, we have chosen to analyse whether a non-damaging acute endurance exercise might modulate the response of HSPB5 protein and whether distinct changes might occur in relation of oxidative stress and to the fiber-type composition.
To this end, red and white *gastrocnemius*, as sources of slow-oxidative and fast-glycolitic/oxidative fibers, as well as the *soleus*, mainly composed of slow-oxidative type fibers, were obtained from BALB/c mice, before and at different times following exercise. Moreover, to better investigate at molecular level the response of muscle cells, we have exposed to oxidative environment C2C12 myotubes.
Although the total levels of HSPB5 were not changed, its phosphorylation form (p-HSPB5) was significantly increased specifically in skeletal muscle with a higher amount of type I and IIA/X myofibers, and it was correlated with both increased levels of lipid peroxidation and carbonylated proteins. Moreover, as a result of pro-oxidizing stimuli, there was a partial redistribution of HSPB5 from the cytosol to the cytoskeletal compartment. In particular, the phosphorylation of HSPB5 seems to be essential not only for its translocation, but it is necessary to interact with functional and structural proteins forming complexes essential for muscle integrity.
This study provides novel information that may improve understanding the protein function in skeletal muscle response to a non-damaging endurance exercise, suggesting a potential explanation of the process by which HSPB5 could protect from contraction-induced oxidative stress associated with physical exercise.

REGULATION OF RUNX2 GENE DURING EVOLUTION: FUNCTIONAL IMPLICATIONS IN NONSYNDROMIC CRANIOSYNOSTOSIS PATIENTS

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Runt-related transcription factor 2 (RUNX2) encodes a master bone transcription factor. RUNX2 mutations cause skull defects (cleidocranial dysplasia and craniosynostosis). RUNX2 affects indeed skull ossification and is thought to be involved in the different skull morphology of anatomically-modern humans (AMH) compared with ancient hominins (AH).

The aim of our study was to compare the genomic structure of RUNX2 in AMH and AH, to infer putative functional correlations for human skull morphogenesis, using nonsyndromic craniosynostosis (NCS) as a disease model.

RUNX2 counts 13 splice variants, alternatively transcribed from two promoters, and featuring two alternative 3'UTRs. In silico analysis allowed detecting nucleotide changes, between AMH and AH, in noncoding regulatory regions of RUNX2. The effect of changes at the 3'UTRs on the affinity of miRNA binding was analyzed by surface plasmon resonance (SPR). RUNX2 and miRNA expression were analysed by qPCR. Gene expression analysis of miRNAs and RUNX2 splice variants during in vitro osteogenic differentiation of suture-derived cells highlighted that miRNA levels were modulated during the ossification process and correlated with RUNX2 expression. Particularly the isoform containing the second 3'UTR was apparently stabilized by miRNAs binding. SPR validated the interaction between selected miRNAs and their RUNX2 mRNA binding sites.

Our data suggest that RUNX2 genomic evolution may have affected regulatory sequences of a specific splice isoform of the gene. The 3'UTR changes in AMH altered the binding of selected miRNAs which may have influenced the expression of bone-specific genes involved in the evolution of skull shape and volume.

NEW OMICS PERSPECTIVES UNVEIL AN INNOVATIVE SCENARIO OF RETINITIS PIGMENTOSA ETIOPATHOGENESIS: SYNAPTIC ALTERATIONS FROM INNER TO OUTER RETINAL LAYERS

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Retinitis Pigmentosa (RP) includes a varied group of inherited eye disorders characterized by progressive retinal degeneration. About 70 genes are already known to be causative of RP forms, but it is still difficult to determine a precise genotype-phenotype correlation. Many other genes are still unidentified, and the existence of multigenic inheritance patterns, along with modifier genes, makes molecular diagnoses difficult [1]. Analyzing big data coming from several NGS experiments, we found 82 genes still unassociated to RP carrying new mutations. Moreover, 51 genes, 22 Sense lncRNAs and 6 circRNAs showed expression alteration in a complex transcriptomic experiment of oxidative stress on RPE [2]. All previously analyzed genes and ncRNAs result involved in neurotransmission pathways, especially in synaptic regulation of inner retinal cells. Our working hypothesis is based on the bidirectional melanopsin-based signaling by photosensitive retinal ganglion cells influencing retinal visual function. This scenario suggests that the first biological event occurring in the retina during RP onset is not related to the photoreceptor outer layer but, rather, to inner retinal cells. Such data is corroborated by patients' clinical exams, showing an early impairment of optic nerve activity, before the other typical symptoms determined by photoreceptor death (here a consequence, not a cause). Additionally, genes related to retinal neurotransmission and their regulative ncRNAs showed expression changes during early stages of induced oxidative stress experiments before genes involved in other well-known RP causative pathways. In conclusion, the proposed scenario could provide new prognostic clues for genetic counseling, shedding light on the mechanisms of the disease.

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PATHOGENETIC MECHANISMS RESPONSIBLE FOR ALTERED DEVELOPMENTAL TRAJECTORIES IN NIEMANN PICK C DISEASE

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Niemann Pick type C (NPC) disease is an autosomal recessive, neurodegenerative lysosomal storage disorder caused by the abnormal function of NPC1 or NPC2 (95% and 5% of NPC patients, respectively), proteins involved in the intracellular trafficking of endocytosed cholesterol and other lipids. The accumulation/mislocalization of cholesterol, gangliosides, sphingolipids alters signaling pathways, likely causing developmental defects. As an example, the covalent cholesterol modification of Sonic hedgehog (Shh) and its downstream effector, Smoothened, is relevant for gradient formation and downstream signaling activation [1]. Hence, we have recently demonstrated that cholesterol dyshomeostasis in NPC1 affects Shh-mediated activities, at the primary cilium. This impairs the differentiation and functional maturation of neurons and glial cells, leading to abnormal cerebellar morphogenesis [2]. Downstream from Shh, the dysregulation of Brain-Derived Neurotrophic-Factor expression patterns is responsible for defective cell migration and synapse formation. In addition, our recent observations indicate that the reduced cholesterol availability at the plasma membrane affects the signaling of the endocannabinoid receptor CB1, in agreement with the influence that cholesterol content in lipid rafts exerts on the portioning and internalization of this receptor. Hydroxypropyl-β-cyclodextrin (HPβCD) represents the major treatment currently studied in both animal models and patients but it comes with several drawbacks. To overcome these limitations we have recently validated a novel polymer prodrug version of HPβCD, demonstrating that its enhanced pharmacokinetic/biodistribution profiles and longer terminal half-life leads to a significant rescue of cerebellar anomalies and neurobehavioral deficits of NPC1 mouse model, at a dose 5-fold lower than the efficacious HPβCD dose [3].

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ALTERATIONS OF THE ENDOCYTIC PATHWAY IN CHARCOT-MARIE-TOOTH TYPE 2B

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Charcot-Marie-Tooth type 2B (CMT2B) is a rare autosomal-dominant axonal disorder affecting the peripheral nervous system and characterized by distal weakness, muscle atrophy, prominent sensory loss, foot ulcerations and recurrent infections leading to toe amputations. CMT2B is caused by 5 mutations (L129F, K157N, N161T/I, V162M) of the *RAB7A* gene, encoding a small GTPase that regulates late endocytic trafficking and plays also important roles in neurons, regulating neurotrophin trafficking and signaling, neurite outgrowth and neuronal migration. Since several neurodegenerative diseases are caused by alterations of the endocytic pathway, we decided to investigate whether CMT2B-causing RAB7A mutations alter this process. Thus, we used healthy and CMT2B skin fibroblasts and we investigated expression of endocytic proteins, signaling receptor degradation and lysosomal enzyme activity. We found that CMT2B fibroblasts exhibited higher expression of late endocytic protein and of lysosomal enzymes, higher cathepsins activity and higher receptor degradation compared to control fibroblasts. In addition, we found in CMT2B cells an increased number of lysosomes. Therefore, our data demonstrate higher lysosomal activity in CMT2B cells. Thus, as hyperactivation of degradation within axons could induce a premature termination of signaling possibly contributing to axonal degeneration, our data suggest that higher lysosomal activity leads to neurodegeneration.
Experiments on healthy and CMT2B sensory neurons derived from induced pluripotent stem cells (iPSC) are in progress to confirm the data obtained in patient fibroblasts and to evaluate expression and organization of vimentin and peripherin, two intermediate filaments proteins involved in the endocytic pathway and in peripheral nervous system regeneration after injury.

FIBROBLAST-TO-MYOFIBROBLAST TRANSITION MEDIATED BY AN αvβ3 INTEGRIN-ILK-SNAIL1 SIGNALING IN HYPERMOBILE EHLERS-DANLOS SYNDROME

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During inflammation, myofibroblasts are involved in synthesis, remodeling, and reabsorption of tissues' extracellular matrix (ECM). The organization of the α-smooth muscle actin (α-SMA) cytoskeleton allows myofibroblasts' migration and ECM contraction. The persistent activity of myofibroblasts contributes to chronic inflammation.
Hypermobile Ehlers-Danlos syndrome (hEDS) is a dominantly inherited connective tissue disorder, orphan of a genetic etiology, defined by generalized joint hypermobility, joint instability complications, i.e., chronic pain, and mucocutaneous features. The hEDS phenotypic spectrum is broad and includes multiple signs and symptoms shared with chronic inflammatory systemic diseases.
To shed light into pathomechanisms underlying hEDS, we performed gene expression profiling and a detailed cellular characterization of patients' skin fibroblasts.
Transcriptome analysis revealed the altered expression of genes involved in maintenance of ECM organization/homeostasis and in immune, inflammatory, and pain mechanisms. Protein studies showed the disassembly of collagens-, fibrillins-, fibronectin (FN)-ECM and the organization of the αvβ3 integrin. The FN-ECM disarray and the presence of proteolytic FN-fragments in hEDS cells' media might be explained by enhanced levels of the metalloproteinase-9. These cells also organized specific myofibroblasts markers, such as the α-SMA-cytoskeleton and the adhesion molecule cadherin-11, showed an increased migratory capability compared with control fibroblasts, and an altered expression of the inflammation mediators CCN1 and CTGF. These findings are consistent with a fibroblast-to-myofibroblast transition, which is triggered by an ILK-mediated αvβ3 integrin signaling involving the transcription factor Snail1. This myofibroblast-like phenotype suggests an *in vitro* inflammatory-like condition, which correlates well with the systemic clinical manifestations of the patients.

ADULT RENAL STEM/PROGENITOR CELLS CAN REVERT LPS-INDUCED ENDOTHELIAL-TO-MESENCHYMAL TRANSITION OF ENDOTHELIAL CELLS SECRETING BPIFA2, CXCL6, SAA2 AND SAA4 ANTISEPTIC PROTEINS

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Acute Kidney Injury (AKI) is the major complication encountered in sepsis. Lipopolysaccharides (LPS) are frequently involved in the pathogenesis of AKI, that is mainly characterized by endothelial cell (EC) dysfunction [1]. EC acquire a myo-fibroblast phenotype, by endothelial-to-mesenchymal transition (EndMT), contributing to the renal fibrosis [2]. Resident adult renal stem/progenitor cells (ARPCs) enhance tubular regenerative mechanism during AKI [3], but little is known about their effects on endothelial compartment. The aim of this study is to investigate the effects of ARPCs on endothelial dysfunction.
Endothelial cells were stimulated in vitro with LPS for 48h and co-cultured with ARPCs for 24h. MTT cell viability assay was used to analyze the EC proliferation rate. FACS analysis was used to study the expression of fibrosis markers. Gene expression profiles of ARPCs and EC were generated using microarrays.
We observed a significant increase of EC proliferation after stimulation with LPS. ARPCs normalized EC proliferation rate, even in presence of LPS. Moreover, LPS induced a significant decrease of EC markers and a significant increase of EC dysfunction markers. ARPCs in co-culture with EC abrogated the LPS-induced EndMT by restoring the high expression of CD31 and VE-cadherin and limiting Collagen I and Vimentin expression.
In addition, LPS induced the modulation of specific genes in ARPCs. Pathway analysis identified 27 genes involved in prevention and recovery from infections caused by external agents.
In conclusion, our data demonstrate that ARPCs could preserve EC phenotype by regulating LPS-induced EndMT secreting BPIFA2, CXCL6, SAA2 AND SAA4 antiseptic proteins.

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REGULATION OF SUBCOMPARTMENTAL TARGETING AND FOLDING PROPERTIES OF THE PRION-LIKE PROTEIN SHADOO

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Shadoo (Sho), a member of prion protein family, has been shown to prevent embryonic lethality in *Prnp* $^{0/0}$ mice and to be reduced in the brains of rodents with terminal prion diseases [1]. Sho can also affect PrP structural dynamics and can increase the prion conversion into its misfolded isoform (PrP^{Sc})[2], which is amyloidogenic and strictly related to expression, intracellular localization and association of PrP^{C} to lipid rafts. We reasoned that if Sho possesses a natural tendency to convert to amyloid-like forms *in vitro*, it should be able to exhibit "prion-like" properties, such as PK-resistance and aggregation state [3], also in live cells. We tested this hypothesis, by different approaches in neuronal cells, finding that Sho shows folding properties partially dependent on lipid rafts integrity whose alteration, as well as proteasomal block, regulated generation of intermediate Sho isoforms and exacerbated its misfolding. Moreover, a 18 kDa isoform of Sho, likely bearing the signal peptide, was targeted to mitochondria by interacting with the molecular chaperone TRAP1 which, in turn controlled Sho dual targeting to ER or mitochondria. Our studies contribute to understand the role of molecular chaperones and of PrP-related folding intermediates in "prion-like" conversion.

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MESENCHYMAL STEM CELL-DERIVED EXTRACELLULAR VESICLES (EVS) AS MEDIATORS OF ANTI-INFLAMMATORY EFFECTS: ENDORSEMENT OF MACROPHAGE POLARIZATION

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Mesenchymal Stem Cells (MSCs) are effective therapeutic agents enhancing the repair of injured tissues. Preliminary results indicate that the paracrine activity of MSC promotes a functional switch of macrophages from a pro- (M1) to an anti-inflammatory (M2) state. Since extracellular vesicles (EVs) are relevant components of the MSC secretome, the aim of the present study was to carry out a detailed characterization of EVs released by human adipose derived-MSCs to investigate their involvement as modulators of MSC anti-inflammatory effects. The EV-isolation method was based on differential centrifugations of the medium conditioned by MSC exposed to either normoxic or hypoxic conditions (EV^{Normo} and EV^{Hypo}). Both types of EVs were efficiently internalized by responding bone marrow-derived macrophages, eliciting their switch from a M1 to a M2 phenotype. Observations that different macrophage subsets are associated with different stages of muscle regeneration led us to investigate whether EV treatment could influence macrophage polarization from M1 to M2 phenotype in vivo. Taking advantage of a cardiotoxin (CTX) injury mouse model, we observed, in injured and EV-treated muscles, a down-regulation of IL6 and Nos2, concurrent to a significant up-regulation of Arg1 and Ym1, late markers of alternative activation. These effects, accompanied by an accelerated expression of the myogenic markers Pax7, MyoD, and eMyhc, were even greater following EV^{Hypo} administration. Collectively, these data indicate that MSC-EVs possess effective anti-inflammatory properties, making them potential therapeutic agents more handy and safe than MSCs.

MIR-146a INDUCED BY CYANOBACTERIAL LPS ANTAGONIST (CyP) MEDIATES INHIBITION OF TNF-α IN HUMAN MONOCYT

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Lipopolysaccharide (LPS) is the main component of gram-negative bacteria able to elicit a strong activation of innate immune cells. LPS challenge induces a NF-κB-mediated release of pro-inflammatory cytokines, such as TNF-α and IL-1β. Differently from LPS, CyP, a LPS-like molecule isolated from the cyanobacterium Oscillatoria planktothrix FP1, does not induce any pro-inflammatory response. Intriguingly, when co-incubated with gram-negative LPS, CyP induced a dose-dependent reduction of pro-inflammatory cytokine production mainly by an antagonistic interaction with TLR4-MD2 receptor complex. Indeed, TNF-α production resulted to be post-transcriptionally regulated, thus suggesting a more complex mechanism of action exerted by CyP. Recently, it has been demonstrated that monocytes incubated with LPS induce micro-RNA (miR)-146a, known as anti-inflammatory miRNA, which has a key role in the negative regulation of TNF-α production observed after repeated challenges with LPS. MiR-146a targets are IRAK1 and TRAF6 adaptor proteins, involved in the MyD88-dependent intracellular signaling pathway. In this study, we incubated human monocytes with CyP to study whether the mechanism of regulation of TNF-α expression by CyP could be mediated by MiR-146a induction. Results showed that CyP specifically and time-dependently induced mi-R146a expression. Up-regulation of miR-146a by CyP significantly affected subsequent cell response, in term of TNF-α production, not only when monocytes were incubated with gram-negative LPS but also when cells were incubated with other TLR ligands, as lipotheicoic acid. These results indicate that CyP could modulate the pro-inflammatory response at two different levels, by blocking TLR4-MD2 receptor complex and by inducing miR-146a expression.

INNOVATIVE PROTOCOL FOR LONG-TERM CULTURE OF HUMAN PRIMARY KERATINOCYTES FROM THE NORMAL COLORECTAL MUCOSA

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Until now, procedures for in vitro culturing of human primary keratinocytes from normal colon mucosa specimens were not fully feasible. Currently, the main methods exploit to set up primary human colon epithelial cell cultures, foresee the use of replication-inactivated murine 3T3 embryonic fibroblasts as feeder cells, as well as the employment of digestive enzymes, e.g. collagenase, dispase, trypsin-EDTA or thermolysin, to dissociate colonic mucosal cells [1]. However, these methods are tricky and long-lasting. Moreover, some treatments are not effective in yielding a sufficient amount of cells and give rise to only short term cell cultures, which are often contaminated with fibroblasts [2]. Our approach, described herein, allows primary keratinocytes from small tissue fragments of colorectal mucosa biopsies to grow in vitro. The procedure was set up and developed in three steps: (i) the enzymatic digestion of the tissue biopsy; (ii) the use of cloning rings to purify primary keratinocyte colonies, (iii) a defined keratinocyte medium to grow these cells in long-term culture. Our cultural method enables normal primary keratinocytes to be obtained by simple and rapid techniques [3]. In our culture condition, primary keratinocytes express specific epithelial markers. Colorectal mucosa keratinocyte colonies require approximately two weeks to grow. Compared to previous approaches, our protocol provides a valuable model of study for human primary keratinocytes from colorectal mucosa both at the cellular and molecular levels. In addition, normal keratinocytes grown employing our protocol will be very useful to investigate comparatively different colorectal pathologies, including the colorectal carcinoma.

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ABSTRACTS

NEW INSIGHTS ON OXIDATIVE STRESS IN AUTISM SPECTRUM DISORDERS (ASD)

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairment in social communication and restricted and repetitive behavior and interests. The etiology of ASD is unknown, but evidences support the involvement of a complex interplay of genetic, epigenetic and environmental factors, where oxidative stress could be a key mediator in the pathogenesis of ASD. Recently, we found an increase in advanced glycation endproducts (AGEs), Nε-carboxymethyl-lysine (CML) and Nω-carboxymethylarginine (CMA), in plasma proteins of ASD children with respect to healthy age-matched subjects. These products, which we propone as new potential biomarkers for ASD diagnosis, mainly originate from lipid peroxidation, which we found to be increased in ASD children, suggesting that lipids, and consequently cell membranes, are particular affected by reactive oxygen species (ROS). In fact, ASD children show alterations in erythrocyte membrane fatty acid composition and fluidity, NKA enzymatic activity as well as erythrocyte morphology. Moreover, plasma of ASD children displayed high levels of dityrosine (DT), an oxidative damage marker, likely due to the increase in activity of Dual Oxidase (DUOX), an important enzyme involved in gut mucosal immunity. Gut microbiota, as well as oxidative stress and inflammation, may be involved in the complex epigenetic modifications supporting the establishment of the autistic phenotype. In conclusion, we identified several oxidative-stress related biological and biochemical alterations in the blood of ASD children, thus advancing the suggestion that antioxidant intervention might alleviate ASD symptoms.

DIFFERENTIAL CO-EXPRESSION: A DIFFERENT STRATEGY TO DISSECT TUMOR (UNKNOWN-)PLAYERS

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Cancer is a very complex disease that involves many different aspects from genetic background to genes' mutations, from lifestyle to environmental influence. All these factors impinge on a crucial point: genes' expression, often causing a change in expression and function of related proteins.
As a consequence of these effects, some specific re-wiring of transcriptional networks happen: some groups of genes, usually joint in pathways, fail their coordination and are not able to accomplish the right biological task and, potentially, some other genes, usually not correlated, acquire a novel co-expression level and, potentially, new functional relationships.
The principal aim of this work is to explore genes communities obtained from differential co-expression analysis characterizing their biological information and to highlight their relevance in cancer survival analysis.
Starting from public available gene expression data for seven cancer types and their normal counterpart, we have developed a strategy to identify pairs of genes that change their relative expression profiles, acquiring or loosing correlation amongst normal and tumor conditions.
Interestingly, groups of differentially co-expressed genes show peculiar biological and functional characterizations: not only flags related to general tumor disease but subtle implications in more specific biological processes. In addition, many communities cluster genes with promoter regions enriched for particular Transcription Factor Binding Sites and exhibit over-representation of specific miRNA targets. Another engaging aspect of this approach is the ability of some of these communities to detach patients differences in the survival analysis of independent tumor datasets.

GLYOXALASE 2 DRIVES TUMORIGENESIS IN HUMAN PROSTATE CELLS IN A MECHANISM INVOLVING ANDROGEN RECEPTOR AND p53-p21 AXIS: A NOVEL NON-ENZYMATIC ROLE FOR AN ANCIENT METABOLIC ENZYME

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Glyoxalase 2 (Glo2) is overexpressed in some human cancers [1], which suggests a role for this metabolic enzyme in human tumorigenesis [1,2]. In prostate cancer (PCa), the scanty information available is limited to the involvement of Glo2 in the progression of this malignancy [3]. Here we examined the immunohistochemical profile of Glo2 in human PCa and benign adjacent tissues and investigated Glo2 involvement in PCa development in human prostate cell lines. PCa and matched adjacent normal tissues were obtained from paraffin sections of primary PCa from 20 patients who had undergone radical prostatectomy. Histopathological diagnosis was confirmed for each sample. Glo2 expression analysis was performed by immunohistochemistry in prostate tissues, and by qRT-PCR and immunoblotting in prostate cell lines. The causative and mechanistic role of Glo2 in prostate tumorigenesis was demonstrated by Glo2 ectopic expression/silencing and employing specific activators/inhibitors. Our results showed that Glo2 was selectively expressed in PCa but not in the luminal compartment of the adjacent benign epithelium consistently in all the examined 20 cases. Glo2 expression in PCa was dependent on androgen receptor and was aimed at stimulating cell proliferation and eluding apoptosis through a mechanism involving the p53-p21 axis. Glo2 was also intensely expressed in the basal cells of benign glands but was not involved in PCa genesis. Our results demonstrate for the first time that Glo2 drives prostate tumorigenesis independently from its traditional role as a metabolic enzyme and suggest that it may represent a novel adjuvant marker in the pathological diagnosis of early PCa.

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NOTCH SIGNALLING IS INVOLVED IN THE PROTECTIVE EFFECTS OF IVABRADINE IN THE ENDOTHELIUM OF APOE-DEFICIENT MICE

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In dyslipidemic mice, ivabradine, a heart rate reducing agent, improves vascular function and reduces aortic plaques area by undefined mechanism. Since all the previous studies have tested the beneficial effects of ivabradine in the context of advanced stages of atherosclerosis [1], we sought to determine if ivabradine, started before plaque formation, could result in transcriptional changes leading to maintenance of normal endothelial function. To this aim, 6 week-old ApoE-/- mice were treated with ivabradine to determine the effect on early transcriptional changes (2- and 4- week treatment) and on lesions formation (19-week treatment) in the endothelium of the aortic arch. We found downregulation of pro-inflammatory genes, the majority of which are nuclear factor-κB (NF-κB)-and/or Angiotensin II-regulated genes, and upregulation of anti-inflammatory genes. Many shear stress-responsive genes were affected by the treatment and the Notch signaling, indispensable for the maintenance of endothelium integrity [2], was among the most significantly affected pathways. We observed that these effects of early treatment were linked with reduction of endothelial damage in aortic arch and reduction in plaque area in the aortic root of ApoE -/- mice, detected at a later stage, together with increased levels of Hes5, a marker of Notch signalling activation, required to repair the endothelium damaged by dyslipidemia [3]. Our results showed that an early treatment with ivabradine induces an atheroprotective gene expression profile. Activation of the Notch signalling through the modulation of shear-stress forces acting on the endothelium could be part of the mechanism underlying this protection.

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PROTEIN-PROTEIN INTERACTION BETWEEN GLYOXALASE II AND SPECIFIC REDOX DEPENDENT PROTEINS THROUGH S-GLUTATHIONYLATION MODIFICATION

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Glyoxalase II (Glo2) catalyzes the hydrolysis of S-D-lactoylglutathione (SLG) to form D-lactic acid and glutathione (GSH). GSH, the most important thiol reducing agent inside the cell, plays a key role in the cellular redox state and in various cellular processes, including S-glutathionylation which involves the reversible formation of a mix disulphide-bridge between cysteine and GSH. S-glutathionylation can be spontaneous or catalyzed, it is involved in protection of protein thiol groups from irreversible oxidation and plays key role in redox regulation.
During the hydrolysis of SLG in the active site of Glo2 there is unprotonated glutatione molecule (GS-) which can be transferred to protein target. To demonstrate the active involvement of Glo2 in glutathionylation the enzyme and SLG were incubated with different proteins that are known to be glutathionylated, like malate dehydrogenase, actin or cytochrome c purified proteins. In vitro studies demonstrated high propensity of Glo2 to aggregate with other proteins through its catalytic site. To better understand the role of Glo2 in the mechanism of S-glutathionylation, in silico analysis have been performed. This approach consists in protein–protein docking investigations followed by atomistic Molecular Dynamics (MD) simulations, and it is useful to predict molecular associations between human Glo2 and investigated proteins. Computational data confirmed the propensity of the enzyme to interact with the studied proteins through its catalytic site. These studies, revealed that Glo2 allows a rapid and specific protein-SSG formation using SLG substrate, leading to an enzymatic regulation of S-glutathionylation in proteins of different origin and cellular compartmentalization.

CircSMARCA5 INHIBITS MIGRATION OF GLIOBLASTOMA MULTIFORME CELLS BY REGULATING A MOLECULAR AXIS INVOLVING SPLICING FACTORS SRSF1 / SRSF3 / PTB

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Circular RNAs have recently emerged as a new class of RNAs, highly enriched in the brain and very stable within cells, exosomes, body fluids [1]. Through real-time PCR, we assayed the expression of twelve circRNAs (physiologically enriched in several regions of the brain) in fifty-six brain biopsies from GBM patients and seven normal brain parenchymas. We focused on hsa_circ_0001445 (circSMARCA5): it was significantly downregulated in GBM biopsies as compared to normal brain tissues (p-value < 0.00001, student's t-test), contrary to its linear isoform counterpart that did not show any differential expression (p-value = 0.694, student's t-test). Negative correlation between the expression of circSMARCA5 and glioma's histological grade suggests its potential negative role in the progression to malignancy. Overexpressing circSMARCA5 in U87MG cells significantly decreased their migration, but not their proliferation rate. CircSMARCA5 sequence revealed an enrichment in binding motifs for SRSF1, a splicing factor overexpressed in GBM and known to be a positive controller of cell migration. Direct interaction between circSMARCA5 and SRSF1 is supported by eCLIP data for SRSF1 in K562 cells from ENCODE. Consistently, U87MG overexpressing circSMARCA5 showed an increased expression of SRSF3 RNA isoform containing exon 4, normally skipped in a SRSF1-dependent manner, resulting in a non-productive non-sense mediated decay (NMD) substrate. Interestingly, SRSF3 is known to interact with two other splicing factors, PTBP1 and PTBP2, that positively regulate glioma cells migration. Collectively, our data show circSMARCA5 as a promising druggable tumour suppressor in GBM and suggest it may exert its function by tethering the RBP SRSF1[2].

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CHANGES IN BDNF RNA DENDRITIC TRAFFICKING ASSOCIATED WITH FUNCTIONAL AND MORPHOLOGICAL ALTERATIONS INDUCED BY CHRONIC STRESS AND KETAMINE

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Stress-based animal models of depression have shown dendritic atrophy and spine remodeling in the hippocampus (HPC) and prefrontal cortex (PFC), suggesting that stress-induced maladaptive changes have a primary role in psychopathology. Recent studies showed that a single sub-anaesthetic dose of ketamine (KET) exerts a fast and sustained antidepressant effect, accompanied by a rapid rescue of dendritic remodeling/loss of synapses in the PFC.
Several mechanisms have been proposed to unravel the molecular bases of the fast antidepressant action of KET, including a rapid increase of brain derived neurotrophic factor (BDNF) local translation at synapses. Dendritic trafficking of selected mRNAs was suggested to subserve local protein synthesis, and chronic treatment with antidepressants was shown to increase the trafficking of BDNF transcripts in distal dendrites.
Our study aims at analysing the changes in the expression levels and dendritic trafficking of selected BDNF mRNA splice variants in the HPC of rats subjected to CMS and acute treatment with KET. We found that CMS reduced BDNF expression levels in all animals (resilient, vulnerable and vulnerable treated with KET). However, total BDNF mRNA trafficking was reduced mostly in vulnerable animals, while acute KET partly rescued this alteration. Moreover, morphological analysis of CA3 pyramidal neurons showed a reduction in length and branching of apical dendrites after stress; KET restored these changes to control levels.
Our results suggest that alterations in BDNF trafficking may be involved both in mechanisms of stress resilience, and in the fast antidepressant effect of KET.

RNA-MEDIATED INTERCELLULAR MISCOMMUNICATION: ROLE OF EXTRACELLULAR VESICLE CARGOS IN AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic Lateral Sclerosis (ALS) is a rare neurodegenerative disorder that primarily targets upper and lower motor neurons. The progression of the disease is mediated by altered intercellular communication in the spinal cord between neurons and glial cells. Intercellular communication, mainly happening through extracellular vesicles (EVs) is responsible for the horizontal transfer of proteins and RNAs to recipient cells. Previously, we proved that EVs released from ALS mutant astrocytes selectively induced toxicity in wild type motor neuron, thus reinforcing the notion that astrocytes contribute to ALS disease propagation and suggesting EVs as mediators of toxicity [1]. Although, multiple factors can play a role in motor neuron degeneration, recent evidences point toward a fundamental role for RNA and RNA-binding protein dys-homeostasis as crucial players in ALS pathogenesis. Therefore, we used a model in which mutant TDP43 alters RNA homeostasis and it is not released in EVs at detectable levels. We isolated EVs from spinal and cortical astrocytes derived from transgenic mice with a novel and efficient methodology, called NBI. ALS astrocyte-derived EVs were significantly different in size and amount compared to controls, suggesting impairment in EV biogenesis. Accordingly, ALS EVs induced neuronal death of wild type motor and cortical neurons. Of note, alterations in EV profile was confirmed in plasma EVs derived from ALS patients at different stages of the disease, suggesting EVs not only as players of neuronal degeneration but also as source of biomarkers of neurodegenerative conditions.

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IDENTIFICATION OF EXTRACELLULAR VESICLES AND CHARACTERIZATION OF MIRNA EXPRESSION PROFILES IN HUMAN BLASTOCOEL FLUID

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During cavitation, at day 4 of human preimplantation development, embryo cells begin to differentiate into the Inner Cell Mass (ICM) and Trophectoderm Lineages (TE) and secrete fluid into the morula to create a fluid-filled cavity, the blastocoel. As the embryo further divides, the blastocoel expands and the ICM becomes positioned on one side of the trophoblast cells forming the mammalian blastula, called blastocyst, ready for implantation. In this study,we demonstrated, for the first time, the presence of microRNAs and extracellular vesicles in human blastocoel fluid (BF). The bioinformatic and comparative analyses identified the biological function of BF microRNAs and suggested a potential role inside the human blastocoel. We found 89 miRNAs, expressed at different levels, able to regulate critical signaling pathways controlling embryo development such as pluripotency, cell reprogramming, epigenetic modifications, intercellular communication, cell adhesion and cell fate. Blastocoel fluid microRNAs reflect the miRNome of embryonic cells and their presence, associated to the discovery of extracellular vesicles, inside blastocoel fluid, strongly suggests their important role in mediating cell communication among blastocyst cells. Their characterization is important to better understand the earliest stages of embryogenesis and the complex circuits regulating pluripotency. Moreover, blastocoel fluid microRNA profiles could be influenced by blastocyst quality, therefore, microRNAs might be used to assess embryo potential in IVF cycles.

PRODUCTION OF MEMBRANE AND SOLUBLE PD-L1 BY LUNG TUMOR CELLS

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Programmed Death-Ligand 1 (PD-L1) was first described as a membrane molecule expressed by antigen presenting cells to regulate T lymphocyte immune response through the engagement of its receptor PD-1 [1]. The observation that many tumors express PD-L1 to escape the immune surveillance augmented the interest in the immune checkpoint PD1:PD-L1 for its potential as tumor biomarker and molecular target of cancer immunotherapy [2]. Recently, PD-L1 secreted forms (soluble and vesicular) have been identified, too. They derive from alternative splicing or surface protein shedding and are able to potentiate the immunoregulatory message of membrane-bound PD-L1 in a remote, cell contact independent manner [3]. Our studies on the lung cancer human cell line H1975 confirm the positive modulation of membrane PD-L1 by IFN-gamma, enlightened the presence of PD-L1 secreted form in vesicles from H1975 culture supernatant and provided evidence for a possible reverse signaling triggered by a recombinant soluble PD1 on PD-L1 expressed by H1975 cells. Moreover, the analysis of a wide population of patients affected by NSCLC revealed the absence of correlation between the histological expression of PD-L1 in surgical tissue samples and the concentration of soluble PD-L1 measured in serum of the same individuals. This observation suggests that different cell mechanisms and/or different cell sources could contribute to the soluble form of the immune regulatory ligand PD-L1.

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NOVEL *KRIT1* MISSENSE MUTATIONS IN ITALIAN FAMILIES WITH CEREBRAL CAVERNOUS MALFORMATION LEADS TO ABERRANT SPLICING AND HIGHLY VARIABLE CLINICAL EXPRESSIVITY

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Cerebral Cavernous Malformation (CCM) is a cerebrovascular disease of genetic origin affecting about 0,5% of the world population, and characterized by abnormally enlarged and leaky capillaries that predispose to seizures, neurological deficits and intracerebral hemorrhage. It may arise sporadically or is inherited as an autosomal dominant condition with incomplete penetrance and variable expressivity, and has been associated with mutations of three genes, *CCM1* (*KRIT1*), *CCM2* and *CCM3*. Our previous studies in CCM patient cohorts identified genetic variants of modifier genes related to differences in vascular sensitivity to oxidative stress and inflammation, which contribute to inter-individual differences in CCM disease susceptibility and severity[1,2]. Consistently, we also demonstrated that CCM proteins modulate distinct redox-sensitive signaling pathways and mechanisms involved in cellular responses to oxidative stress and inflammation, suggesting a novel pathogenetic mechanism[3]. Here we report the identification of novel putative *KRIT1* missense mutations in Italian CCM patients, which were demonstrated to cause splicing defects leading to frameshifts and premature termination codons, resulting in a broad spectrum of clinical presentations, including wide differences in disease onset and severity in distinct family members carrying the same CCM mutation. Overall our findings underscore the need for systematic genetic testing approaches to better identify genetic causes of CCM disease and associated susceptibility factors, including accurate mRNA analysis of point mutations and the application of next-generation sequencing (NGS) technologies, such as whole exome sequencing (WES), which may improve our understanding of disease etiology, enable early diagnosis, risk stratification and long-term outcome prediction, and advance medical care.

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ROLE OF SPHINGOSINE 1-PHOSPHATE SIGNALING AXIS IN THE DIFFERENTIATION OF EPITHELIAL COCHLEAR PROGENITORS

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Age-related hearing loss is the second most common cause of disability in older people. The unique available treatment is cochlear implant, which has important use limitations due to neurons degeneration after the loss of neurotrophic support provided by epithelial hair cells. ERM (ezrin-radixin-moesin) proteins, cross-linkers that connect plasma membrane and the actin cytoskeleton, have critical roles in the auditory system since radixin deficiency causes deafness. ERM recently emerged as novel targets of sphingolipids. Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid that exerts its effects mainly through specific G-protein coupled receptor, $S1P_{1-5}$, after its export mediated by transporters. S1P signaling is essential for the maintenance of the auditory function: mice lacking $S1P_2$ and S1P specific transporter spinster homolog-2 (SPNS2) are deaf. In this work we unveiled the role of S1P on ERM activation and its involvement in inner ear progenitor differentiation employing the murine auditory epithelial progenitor cell line US/VOT-E36. We observed that these cells express the enzymes involved in S1P metabolism, its transporter SPNS2, $S1P_1$ and $S1P_2$. Interestingly, S1P induces ERM phosphorylation via $S1P_2$ engagement in US/VOT-E36. Moreover, ERK1/2 and PI3K/Akt pharmacological inhibition robustly reduces S1P-induced ERM phosphorylation. S1P biological effect in US/VOT-E36 was assessed by confocal immunofluorescence experiments: the bioactive sphingolipid provokes changes in actin filament organization in a $S1P_2$- and ERM-dependent manner. Moreover, patch-clamp experiments demonstrated that S1P induces changes of both passive and outward K^+ currents in a $S1P_2$- and ERM-dependent manner, suggesting a crucial role for the bioactive sphingolipid in the onset of a differentiated phenotype.

CARDIAC REGENERATIVE POTENTIAL OF THE HUMAN AMNIOTIC FLUID STEM CELL SECRETOME

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The mammalian heart retains residual regenerative capability via endogenous cardiac progenitor cell (CPC) activation and cardiomyocyte proliferation. We reported the paracrine cardioprotective action of human amniotic fluid-derived stem cells (hAFS) following ischemia or cardiotoxicity. Here we analyze hAFS secretome sub-fractions for cardiac regeneration for future clinical translation.
c-KIT+ hAFS were isolated from leftover samples of prenatal screening amniotic fluid. Cells were stimulated under 1% O_2 to enrich their conditioned medium (hAFS-CM). Anti-apoptotic, angiogenic and proliferative effects were evaluated on rodent neonatal ventricular cardiomyocytes (r/mNVCM), human endothelial colony forming cells (hECFC) and human CPC. Mice undergoing myocardial infarction (MI) were treated with hAFS-CM, hAFS-extracellular vesicles (hAFS-EV), or EV-depleted conditioned medium (hAFS-DM).
hAFS-CM improved survival of mNVCM under oxidative and hypoxic damage, induced Ca2+-dependent angiogenesis in hECFC and triggered CPC and rNVCM proliferation. hAFS-CM intra-myocardial delivery after MI was cardioprotective and curbed down inflammation within 24h. 4 weeks post-MI, hAFS-CM and hAFS-DM equally sustained angiogenesis. In contrast to hAFS-DM, hAFS-CM counteracted scarring, supported cardiac function and triggered cardiomyocyte cell cycle progression. hAFS-CM induced reactivation of endogenous WT1+ epicardium CPC (EPDC), similarly to hAFS-EV. Although no direct EPDC differentiation was detected, our data suggested a contribution to local angiogenesis by paracrine modulation and to resident cardiomyocyte cell-cycle progression via transfer of hAFS-EV microRNA to the myocardial tissue.
While hAFS-CM and hAFS-DM seem to equally support therapeutic angiogenesis, hAFS-EV sustained local progenitor reactivation and cardiomyocyte cell cycle re-entry. Therefore, the different hAFS secretome components may represent an interesting source for future cardiac regenerative medicine.

CIRCULAR RNAOME VARIATION IN NORMAL HEMATOPOIESIS AND MLL REARRANGED ACUTE LYMPHOBLASTIC LEUKEMIA

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Circular RNAs (circRNAs) regulate cellular processes, acting as microRNA sponges or binding proteins, and are translated into biologically active peptides. CircRNA abundant expression in the hematopoietic compartment (*Bonizzato et al. Blood Cancer J. 2016*), alterations in acute myeloid leukemia and oncogenic fusion-derived circRNAs in cells with chromosomal translocations have been reported. CirComPara (*Gaffo et al. ncRNA J. 2017*) was used to detect, quantify and annotate circular and linear RNAs from RNA-seq data of B-cells, T-cells, monocytes and CD34+ cells from healthy donors (N=4 per population), and Acute Lymphoblastic Leukemia with MLL rearrangements (MLLre ALL, N=3). Considering mature populations, over 36,000 circRNAs were identified, 34% novel. New circRNA-genes were uncovered. Alternative circularization was frequent: 67% of genes expressed multiple circular isoforms, and 33% 5 up to 78 circRNAs. CircRNA and linear gene expression were poorly correlated and 88% of the circRNAs differentially expressed between mature populations derived from genes not differentially expressed. Cell-type specific circRNA expression signatures were defined. Expression, circularity and backsplice junctions of 13 circRNAs, including newly uncovered ones and others expressed by the leukemia-associated genes, were experimentally validated. Further, a deep deregulation of the MLLre ALL circRNAome was observed. Comparing MLLre-ALL with B and CD34+ cells, 1175 and 464 circRNAs specific or significantly differentially expressed were identified, respectively. These circRNAs derived from leukemia-associated loci, non-coding RNAs and new circRNA genes. An unprecedented view of the circRNAome complexity in blood cell populations was provided and new circRNAs potentially involved in leukemogenesis were uncovered.

EXOME AND PANEL SEQUENCING POINT TO THE DNA DAMAGE RESPONSE GENE ATM AS A CANDIDATE FOR SUSCEPTIBILITY TO PANCREATIC CANCER AND MELANOMA NOT DRIVEN BY *CDKN2A*

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The *CDKN2A* melanoma (MM) predisposition gene also explains 20% of hereditary pancreatic cancer (PC) in our population. We hypothesized a role of the *ATM* gene as a candidate among the yet to be discovered genes involved in tumorigenesis of both PC and MM, prompted by our subset of data from a recent collaborative Whole Exome Sequencing (WES) study [1]. Indeed, WES and multigene panel testing in 37 PC and 100 MM families showed several loss of function (LOF) germline variants in multiple genes, including the DNA damage response (DDR) *ATM* gene. These data suggest that an altered DDR may be a partly UV exposure-independent mechanism of tumorigenesis shared by both PC and MM. Germline *ATM* LOF-variants were found in 3% of our families and interestingly were mutually exclusive with *CDKN2A* variants. As expected, the number of non-LOF ATM variants of unknown significance (VUS), limiting the analysis to the coding region, was elevated. *CDKN2A* analysis extended to the 5'UTR region in PC patients showed that a small fraction of them could be explained by VUS germline 5'UTR variants which were demonstrated to affect the post-transcriptional regulation of p16INK4a mRNA that is dependent on an IRES in the 5'UTR, and therefore reclassified as likely pathogenic[2]. Overall we have included selected genetic variants in a pipeline of population, computational and functional evaluation to clarify their functional role and hypothesize other non-*CDKN2A* genes in PC and MM susceptibility, as well as to identify actionable molecular targets.

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EVALUATION OF BIOLOGICAL EFFECTS OF *TERT* INHIBITION IN HUMAN THYROID CANCER CELLS

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The ribonucleoprotein polymerase telomerase reverse transcriptase (TERT) plays a key role in tumorigenesis of several cancers by permitting unlimited cellular proliferation. Mutations in the TERT promoter, associated both with higher levels of TERT expression and activities, have been detected in different human neoplasia, including thyroid cancer. We evaluated the biological effects of *hTERT* silencing in human thyroid cancer cells to verify the validity of targeting TERT as a potential strategy for the treatment of these tumors. We first observed increased expression levels of *hTERT* in subgroups of both papillary (PTC) and anaplastic (ATC) thyroid cancer, compared to normal thyroid tissues. *hTERT* silencing was able to reduce the growth, invasion and migratory ability of in human ATC and PTC cells. We also encapsulated an anti-*hTERT* oligonucleotide in biocompatible nanoparticles and tested the effect of this formulation both *in vitro* in human ATC cell lines and *in vivo* in a xenograft model of thyroid cancer. Anti-*hTERT* nanoparticles were able to reduce the viability and migration of human ATC cells and the same activity was confirmed *in vivo*. In fact, intravenous administration of these nanoparticles determined a significant lower expression of hTERT associated with a reduction in the growth (about 50%), invasion capacity, vascularization of the tumor with no sign of toxicity. In conclusion, our findings demonstrate that *hTERT* silencing determines an anti-proliferative effect in thyroid tumor cells, suggesting that it may represent a potential candidate to be targeted in the ATC treatment.

TRICYCLIC ANTIDEPRESSANTS INHIBIT *CANDIDA ALBICANS* GROWTH AND BIOFILM FORMATION

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Candida albicans is a commensal yeast of the human body, able to form biofilms on solid surfaces such as implanted medical devices, and contribute to nosocomial infections. Biofilms have the capacity to resist to higher levels of antifungals compared to planktonic cells and can develop tolerance to commonly used treatments. The necessity to overcome acquired drug resistance, and identify new active molecules with low toxicity is a significant problem. It has been reported that some antidepressants have antibacterial properties, but very little is known on the effect that these drugs have on fungi. The capacity of three tricyclic antidepressants to inhibit *Candida* growth and biofilm formation was demonstrated. The antimicrobial potential of the drugs was assessed by studying gene expression, hyphae formation, biofilm growth, and maturation. In this study, three tricyclic antidepressants, doxepin, imipramine, and nortriptyline, were considered. Their negative impact on the growth of *Candida albicans,* and other *Candida* species is shown *in vitro,* and with the hepatic S9 system. Indeed, it is demonstrated that the molecules considered can inhibit not only hyphae, and biofilm formation, but also killed cells in a mature biofilm. Moreover, cell lysis by the tricyclic antidepressant nortriptyline is reported, along with its synergistic activity with amphotericin B. These findings suggest that tricyclic antidepressants, and particularly nortriptyline, might be further studied in drug repositioning programs to fully assess their antimycotic capacity. Ongoing research is now trying to identify the targets of TCAs in *C.albicans*.

A MULTIDISCIPLINARY APPROACH TO THE CHARACTERIZATION OF CANCER STEM-LIKE CELLS OF PAPILLARY THYROID CARCINOMA: A PILOT STUDY

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Telomere shortening is a well known phenomenon leading to chromosome instability in cancer cells, including thyroid papillary carcinoma (PTC) [1]. Little is known on three-dimensional (3D) telomere dynamics in PTC cancer stem cells (CSCs) and no study has investigated a possible association of this feature with metabolomic profile of CSCs. We obtained and characterized thyrospheres (containing cancer stem-like cells, CSLCs) from 3 PTC-derived cell lines (B-CPAP, K1, TPC-1) with different molecular alterations and used immortalized normal human thyrocytes (Nthy-ori 3-1) for comparison. We analyzed telomere dysfunction by 3D Q-FISH in thyrospheres and parental cells of all cell lines, and metabolomic profile by gas chromatography-mass spectrometry in B-CPAP and Nthy-ori-3-1 cells. The 3D telomere profile showed different telomere architecture between thyrospheres and parental cells in all examined cell lines. Thyrospheres had longer telomeres compared to parental cells. In particular, B-CPAP thyrospheres had shorter telomeres then thyrospheres of the other cancer cell lines, as well as of nontumoral thyrospheres. Interestingly, differences in metabolomic profile were observed between B-CPAP CSLCs and non-CSLCs. The differences were mainly observed in metabolites of the glycolytic and Kreb's cycles, and in amino acids, polyols and lipids (cholesterol and fatty acids). This preliminary finding is in keeping with the pivotal role of TP53 gene (which is mutated in B-CPAP CSLCs) [2] in regulating the expression of genes involved in balancing glycolysis/OXPHOS and in maintaining genome integrity. The authors thank FUV (Fondazione Umberto Veronesi) fellowship (PC). Funding: PRIN N° 20122ZF7HE.

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18 kDa TSPO: DATA IN FAVOR OF A PRO LIFE ROLE AND ITS IMPORTANCE ON STEROIDOGENESIS REGULATION

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The mitochondrial Translocator Protein (TSPO, 18 kDa) has elicited considerable interest for medicine as it has been imputed in inflammation, HIV biosynthesis, cancer, neurodegenerative diseases, and psychiatric disorders [1]. TSPO, first detected in 1977 as an alternative peripheral binding site for the benzodiazepine diazepam (Valium®), has been proposed to be an important player in several fundamental biological processes [2]. Since its discovery, most of the information about TSPO functions derives from the use of synthetic ligands characterized by high binding affinity. However, this approach led to question the specificity of the observed effects as a poor correlation between the TSPO ligand binding affinity and in vitro efficacy has often been observed [3].

Our work framed on this subject with a dual perspective: i) on the one hand, we investigated whether the use of ligands characterized by a long Residence Time to TSPO can be a useful strategy to shed light on the functions ascribed to this protein. To this end, "competition kinetic association" assay to measure the Residence Time of TSPO ligands belonging to several chemical classes was set up; then ligands showing different Residence Time profiles were selected and tested for their in vitro and in vivo activities; ìì) on the other hand, we explored the role of a missense polymorphism in steroidogenesis/neurosteroidogenesis, one of the most debated function of this protein.

The obtained results were in accordance with a pro-life activity of TSPO and confirmed its prominent role in steroid/neurosteroid synthesis regulation.

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AUTOPHAGY AND APOPTOSIS IN HIPPOCAMPAL NEURONS OF TRIMETHYLTIN-TREATED RATS

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Trimethyltin (TMT) is an organotin compound known to produce significant and selective neuronal degeneration and reactive astrogliosis in the rodent CNS.
Autophagy is the main cellular mechanism for degrading and recycling protein aggregates and damaged organelles, which in different stress conditions, as starvation, generally improves cell survival. Autophagy is documented in several pathologic conditions, including neurodegenerative diseases.
This study aims to investigate the autophagy and apoptosis signaling pathway in hippocampal neurons of TMT-treated rats to explore molecular mechanisms involved in toxic-induced neuronal injury.
LC3 (autophagosome marker) and P62 (substrate of autophagy-mediated degradation) expression is examined by western blotting at different time points after treatment. Interestingly, the results demonstrate that the LC3 II/I ratio significantly increased 3 and 5 days after treatment, and the P62 levels significantly decreased after 7 and 14 days. Immunofluorescence images of LC3/NeuN show numerous positive-LC3 neurons in the hippocampus 3 and 5 days after treatment, in accordance with molecular data.
TUNEL assay demonstrates an increment of apoptotic cells starting from 5 days after treatment. In order to demonstrate activation of a caspase-dependent or independent apoptosis pathway, immunofluorescence images of AIF/NeuN show failure nuclear translocation of AIF in neurons. Increased expression of cleaved Caspase 3 is revealed after 5 days in all hippocampal regions by western blotting and immunohistochemistry analysis.
These data clearly demonstrate that TMT intoxication induces a marked increase both of autophagy and caspase-dependent apoptosis, and that autophagy occurring before apoptosis could have a potential role in neuronal loss in this experimental model of neurodegeneration.

EXPRESSION OF LEPTIN RECEPTOR AND EFFECTS OF LEPTIN ON PAPILLARY THYROID CARCINOMA CELLS

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Epidemiological studies indicate a strong association between obesity and thyroid cancer. It is debated whether obesity may promote tumor progression and exacerbate poor outcome in thyroid cancer. Increased expression of leptin and its receptor are well documented in papillary thyroid cancer (PTC). Aim of this study was to understand the molecular mechanisms underlying the link between leptin and aggressiveness of thyroid cancer. The expression levels of leptin receptor were analyzed in 44 PTC tissues well characterized in genotypic and clinical-biological features, divided in groups according to ATA risk (low and intermediate) and the presence or not of the BRAF V600E mutation. In addition, we evaluated the effects of leptin treatment on the growth and migration properties on two PTC cell lines (K1 and BCPAP) and the signal transduction pathways involved in its action. All PTCs expressed leptin receptor mRNA and protein. Slight differences were found between the groups of intermediate vs low risk PTCs, while no significant differences between BRAF mutated vs BRAF wild-type. In thyroid cancer cell lines, treatment with leptin (500 ng/ml for 96h) increased the proliferation and the migration of cancer cells (about 20% over control, $p<0.05$). Western blot analysis revealed no variation of the activation of AKT and ERK mediated pathways, but an increase of β-catenin expression levels. These results demonstrate the contribution of leptin to increase the aggressive phenotype of PTC cells, suggesting a potential role of targeting leptin receptor to reverse the progression of PTC in obese individuals.

THE CRH IN AUTOSOMAL DOMINANT NOCTURNAL FRONTAL LOBE EPILEPSY: FUNCTIONAL CHARACTERISAZION OF IDENTIFIED MUTATIONS AND *IN VITRO* ASSESSING OF A NEWLY DESIGNED CRISPR/CAS9-MEDIATED GENE EDITING

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Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is an autosomal dominant childhood-onset focal epilepsy characterized by the presence of clusters of nocturnal frontal lobe seizures. ADNFLE has been associated with mutations in genes coding for subunits of the neuronal nicotinic acetylcholine receptor (nAChR), in a potassium channel (*KCNT1*) and in the DEPDC5 gene.

However, these mutations account for a minority of disease cases. In a large cohort of ADNFLE Italian patients, we excluded the involvement of these genes, while we detected nucleotide variants in the *CRH* gene (coding for the corticotropin releasing hormone), suggesting its possible role in the disease pathogenesis.

Through in vitro studies, we already demonstrated that all mutations in the CRH cause the production/release of altered levels of the hormone and this recurrence suggests that individuals with such an altered hormone level could be more prone to develop the disease. However, a direct role of *CRH* mutations in the ADNFLE pathogenesis has still to be demonstrated, so further studies are necessary to verify this hypothesis, by generating in vivo transgenic mouse models.

We plan to substitute the murine *Crh* coding sequence with the human orthologous one, by CRISPR/Cas9-mediated genome editing. The possibility to target the murine *Crh* locus has been thus evalutated by designing all necessary molecular constructs and evaluating their efficacy by in vitro studies after transient transfections in Neuro2A cells. Our preliminary data demonstrated that this approach resulted successful.

ALDO-KETO REDUCTASES CONFER RESISTANCE TO FERROPTOSIS EXECUTION

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Cutaneous melanoma remains one of the most difficult to treat human cancer. More than 70% of all melanomas are characterized by oncogenic BRAF resulting in extremely aggressive and resistant to any treatment. In the past decade, treatment options have significantly increased with the introduction of BRAF/MEK inhibitors although the development of rapid resistance limits the success. Very recently, an immunotherapy approach has been introduced with combined treatments possibly improving efficacy but also carrying increased risk of adverse effects, such as autoimmune disorders. Therefore, novel therapeutic strategies are needed and under investigation. We therefore explored the possibility to induce ferroptosis to kill melanoma cells. This is a recently discovered cell death pathway in which the inhibition of the system Xc^- stimulates the iron-mediated production of lipid-ROS responsible for cell killing. We found that: ferroptotic stimuli initiate an ER stress-independent process resulting in LOX-mediated lipid-ROS generation, which are destroyed by the downstream Nrf2-mediated up-regulation of specific aldo-keto reductases (AKRs), thus conferring resistance to most melanoma cell lines. Importantly, we demonstrate that the abrogation of AKR activity resensitize melanoma cell to ferroptosis execution. Collectively our data indicate that combined treatment with pro-ferroptotic drugs and AKR inhibitors might represent a potential new valuable therapeutic strategy to treat human skin melanoma malignancy.

MELATONIN AND VITAMIN D ORCHESTRATE THE OSTEOGENIC FATE THROUGH EPIGENETIC MECHANISMS

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Fat tissue represent an important source of stem cells, the "Adipose derived stem cells" (ADSCs), which can differentiate towards different phenotype under external stimuli. Melatonin, a natural hormone that regulate many physiological processes, including circadian rhythms, is a molecule able to promote osteoblast maturation in vitro and to prevent bone loss in vivo and adipocyte metabolism [1]. In the present study, we aimed at evaluating the specific phenotype elicited by melatonin and vitamin based medium, considering also the involvement of epigenetic regulating genes. Histone deacetylases 1 and Sirtuins (Silent Information Regulator) 1 and 2, belongs to the same superfamily of enzyme able to remodel chromatin structure, seem particularly related to metabolic homeostasis, aging and embryonic stem cell differentiation [2-3].
ADSCs were cultured in a specific adipogenic conditioned media (ADM), in the presence of 0.01 M melatonin (Melatonin+DM) alone or in combination with 10-6 Vitamin D (Melatonin+VitaminD+DM). Our results show that Melatonin and Vitamin D are able to modulate ADSCs commitment towards osteogenic phenotype trough inhibition of adipogenesis and the upregulation of HDAC, Sirtuins 1 and 2 and the specific osteogenic related genes Bmp2, Osteocalcin and Stanniocalcin. The Alizarin Red shows the calcium accumulation in cells treated with melatonin together with Vitamin D, as compared to undifferentiated cells, despite the presence of the adipogenic medium.
Our findings unfold an epigenetic regulation of melatonin in stem cell differentiation inducing HDAC gene and protein expression and could open up novel strategies for future therapeutic balancing stem cell fate toward adipogenic or osteogenic phenotype.

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DETECTING CANCER BIOMARKERS ON POLYDISPERSE EXTRACELLULAR VESICLES ISOLATED FROM BLOOD

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Extracellular vesicles (EVs) are heterogeneous membranous particles intensively studied for their potential cargo of diagnostic markers. We developed a new rapid biochemical method to obtain polydisperse EVs in a physiological pH solution, preserving their morphology, dispersity, and stability. We challenged the reproducibility of this method by isolating EVs from different biological fluids. In plasma of healthy donors, the abundance of recovered EV populations, in the range of 10^9 per milliliter, positively correlated with the density of blood erythrocytes, platelets, and leukocytes. Quantitative analyses using specific haematopoietic and epithelial markers demonstrated the unbiased recovering of polydisperse EV lineages. Individual isolated vesicles were used in newly-designed homogeneous assays. We detected a picomolar concentration of PSMA on 10^5 EVs isolated from plasma of prostate cancer patients and *BRAF* V600E mutation-carrying mRNA in 10^3 EVs from plasma of colon cancer patients, reaching unprecedented matching with tissue biopsy results.

THE FRAGILE X MENTAL RETARDATION PROTEIN IS INVOLVED IN MEMBRANE PLASTICITY AT THE LEADING EDGES OF INTRAHEPATIC CHOLANGIOCARCINOMA CELLS

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Intrahepatic cholangiocarcinoma (iCCA) is a rare malignancy of the intrahepatic biliary tract with an outstanding poor prognosis. Although some clinicopathologic parameters have been shown to be prognostic factors for iCCA, the molecular prognostic markers and potential mechanisms of iCC have not been well elucidated. Here, we report that the Fragile X mental retardation protein (FMRP), a RNA binding protein lacking or mutated in patients with the Fragile X syndrome (FXS) and also involved in breast cancer [1] and melanoma [2] progression, is overexpressed in human intrahepatic cholagiocarcinoma and its silencing affects migration and invasion in iCCA cell lines. In particular, we show evidence that FMRP is localized in cytoplasmic RNA granules that accumulate in pseudopodia and invadopodia protrusions of iCCA cancer cells, where it is able to bind several mRNAs codifying key proteins for invadopodia formation and/or function. All together, our findings suggest that FMRP could promote cell invasiveness modulating membrane plasticity and invadopodia formation at the leading edges of invading iCCA cells.

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TRIO WHOLE EXOME SEQUENCING FOR DISEASE-GENE DISCOVERY: FROM GENES TO PATIENTS AND BACK

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Albeit one million Italian children are affected by a disorder with supposed genetic background, 40% of them remain without a genetic diagnosis. While whole exome sequencing (WES) is a common approach for attempting genetic diagnoses, WES results return several hundreds of sequence variants per patient, making the selection of the disease-causing gene difficult, unless the mutated gene is known for, or can be easily associated to, the clinical phenotype. Yet, such event occurs in a scant percentage of cases, thus limiting the power of WES for novel disease-gene discovery.

We performed trio-WES, i.e. the WES of the affected individual together with that of the healthy parents, in two families affected by a mitochondrial disorder for whom inconclusive results were obtained after the only WES of each patient. The trio-WES analysis led to a 10-to-100-fold decrease in the number of candidate sequence variants, compared to patient WES, for each class of mutations, i.e. *de novo*, homozygous and compound heterozygous mutations, pointing out biallelic mutations in two distinct genes, i.e. SLC25A10 (encoding a mitochondrial carrier protein) in the first family [1] and CRAT (encoding a carnitine acetyltransferase) in the second family.

The pathogenicity of detected mutations was assessed by functional analyses on patient cells and recombinant-purified proteins, demonstrating functional alterations of mutated proteins compared to control. Besides showing the power of trio-WES and proposing two novel disease-causing genes, our results go back from patients to genes, suggesting novel and unexplored functions of SLC25A10 and CRAT in the biology of human cells.

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AN INNOVATIVE TECHNOLOGY FOR THE DIRECT DETECTION OF MICRORNAS IN BIOFLUIDS

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Circulating microRNAs have been widely proposed as new promising non-invasive biomarkers for several diseases, including cancers. However, their clinical use is hampered by limitations of available technologies. Most assays for miRNAs quantitation still lack the sensitivity and specificity required for reliable clinical diagnostics. Here, we propose a new assay, that has single-base specificity, and no need for either RNA extraction or target pre-amplification and labelling. This novel compact and miniaturized platform is based on a Silicon Photomultiplier (SiPM)-based device manufactured by Optoi, integrated with DestiNA's abasic PNA probes and SMART chemistry, and a chemiluminescence-based assay. Calibration curves were made using DNA oligomers (synthetic mimic hsa-miR-21 and cel-miR-39) and measured by the combined technologies. The device showed a Limit-of-Detection (LoD) of 1.6 pM and was validated for the direct detection of hsa-miR-21 in plasma samples of lung cancer patients. We have evaluated the concentration of hsa-miR-21 in lung cancer plasma samples in the high picomolar range (100 – 800 pM), via calibration curves with gold-standard TaqMan RT-qPCR. We detected hsa-miR-21 from eight lung cancer plasma samples (Cq values from 21.7 to 24.4) directly adding magnetic beads coated with DestiNA probes in the plasma samples, obtaining a proof of concept for this innovative technology.

FUNCTIONAL ROLE OF MICRORNA-23b-3p and MICRORNA-193a-3p IN CELL MIGRATION OF CANCER CELLS

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In recent years we have shown that the microRNAs-23b-3p and -193a-3p exert a tumor suppressor function in hepatocellular carcinoma (HCC).Here we demonstrate that these miRs exert their biological role in inhibiting the migration ability of human HCC cell lines (SKHep1C3, HA22T/VGH).The ectopic expression of miR-23b significantly reduced migration of SKHep1C3 cells (by 45% at 50 nM).The single transfection of miR-23b and -193a impaired the migration ability of these cells and the cotransfection of both miRs led to an additive trend.Each miR also impaired cell proliferation, but the combined transfection did not induce an additive effect. Considering that these miRs target common and different pathways,it is plausible to suppose that for cell migration the final biological effect leads to an additive inhibiting effect.Thus we tested the combined treatment of HA22T/VGH cells with each miR and sorafenib (sf), the drug currently used for advanced HCC, to examine their additive trend in cell proliferation and apoptosis.Sf is a multikinase inhibitor that can act by both Ras/Raf/MEK/ERK-dependent and -independent mechanisms.The quantification of TUNEL-positive SKHep1C3 cells showed that each miR induced apoptosis of HCC cells and the cotreatment with Sf significantly increased apoptosis.The growth of the HA22T/VGH cells was significantly reduced upon the combined treatments of miR193a-3p and sf thus suggesting that miR193a 3p could sensitize HCC cells to sf.We developed a model of HA22T/VGH-SR resistant to Sf that displayed a downregulation of miR-193a and -23b compared with the sf sensitive cells. We will study whether the ectopic miR expression could counteract the sf resistance.

THE PARAPTOSIS INDUCED BY δ-TOCOTRIENOL IN PROSTATE CANCER CELLS IS ACCOMPANIED BY A PROFOUND ALTERATION OF MITOCHONDRIAL METABOLISM

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Castration resistant prostate cancer (CRPC) is an aggressive tumor with still limited therapeutic outcomes. Preliminary experiments performed in our laboratory demonstrated that vitamin E-derivative δ-tocotrienol (δ-TT) exerts a proapoptotic activity in CRPC (PC3 and DU145) cells. In the present study, we found that δ-TT can also trigger massive cytoplasmic vacuolization in both PC3 and DU145 cell lines, as seen in paraptosis. The vacuoles formation was prevented by the protein synthesis inhibitor cycloheximide and by the ER stress inhibitor salubrinal, indicating their ER origin. Moreover, by TEM analysis we confirmed the induction of mitochondrial swelling in these cells, further supporting the involvement of paraptosis in the antitumor activity of δ-TT. In order to clarify the molecular mechanisms underlying the cytotoxic effect of δ-TT, we then focused our attention on the study of mitochondrial metabolism and we observed that, in both PC3 and DU145 cells, δ-TT: decreases the expression levels of the protein complexes of the respiratory chain, specifically of complex I; decreases the mitochondrial activity as well as the mitochondrial activity/mass ratio; decreases both ATP production and oxygen consumption; induces ROS production. These data demonstrate that δ-TT exerts a significant proapoptotic and paraptotic activity in CRPC cells, rewiring the mitochondrial metabolism.

NOTCH1 IS REQUIRED FOR THE PROTECTIVE ACTION OF ESTROGENS AGAINST INFLAMMATION-INDUCED ENDOTHELIAL CELLS APOPTOSIS

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The risk of coronary artery disease (CAD) in women increases after menopause, suggesting cardiovascular benefits of estrogens, which act with molecular mechanism still poorly understood. It is known that both estrogens [1] and Notch [2] protect the endothelium against TNFα-induced apoptosis, one of the hallmarks of endothelial dysfunction leading to CAD. We have previously reported that in endothelial cells, treatment with 17β-estradiol (E2) activates Notch [3], which is, instead, inhibited by TNFα [2]. Based on these observations, the aim of this study was to establish whether Notch1 is involved in the estrogen-dependent protection of the endothelium against apoptosis induced by TNFα. Human umbilical vein endothelial cells were treated with E2, TNFα, DAPT, a gamma-secretase inhibitor that prevents the activation of Notch or siRNA against Notch1, in the presence or absence of Notch1 overexpression. The effects of each treatment on apoptosis were then assessed by flow cytometry. We found that TNFα treatment reduces the intracellular levels of Notch1 and increases the endothelial cells apoptosis and E2 counteracts these effects. When Notch1 is inhibited by DAPT or siRNA, E2 is not able to protect against TNFα-induced apoptosis but Notch1 ectopic overexpression restores E2-mediated protection. In our model, Notch1-dependent Akt phosphorylation contributes to the pro-survival action of E2 in the presence of TNFα. Taken together our data show that estrogen-mediated activation of Notch1 protects against endothelial apoptosis, which precedes atherosclerosis. Thus, in women with an impaired endothelial Notch1, estrogen therapy may be less efficient in preventing endothelial dysfunction and thus CAD.

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WORKERS EX-EXPOSED TO ASBESTOS FIBERS CARRY CIRCULATING DYSREGULATED MICRORNAS

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The WHO estimates that 125 million workers are at present exposed to asbestos fibers. The asbestos is a cancerogenic mineral responsible of a fatal cancer, the malignant pleural mesothelioma, (MPM), which causes more than 100,000 deaths/year [1]. MPM could arise in a long latency period (up to 50 years) after the asbestos exposure. Workers/subjects potentially at risk may benefit of an early diagnosis based on specific biomarkers. Cellular and circulating microRNAs (miRNAs) have been proposed as new biomarkers [2]. Indeed, miRNAs expression was found dysregulated in patients affected by MPM, suggesting their potential role as oncogenes or tumor suppressor genes. The use of circulating miRNAs as MPM biomarkers may simplify the surveillance procedure of subjects exposed to asbestos and facilitate the early detection of MPM, with a simple blood test. In this investigation, circulating miRNAs from serum samples of workers ex-exposed to asbestos (WEA) and healthy subjects (HS) were comparatively analyzed by microarray and RT-qPCR technologies. Our results allowed to select miR-3665, an endogenous stable miRNA, as the internal control to quantify in our analyses circulating miRNAs, and to detect miR-1281 up-regulated in WEA compared to HS [3]. As miR-1281 was found up-regulated also in MPM patients, this miRNA could be proposed as potential new predictive MPM biomarker in WEA, and in screening/preventive programs.

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IMPACT OF SNP AND ENHSNP OF MERTK ON HEPATOCELLULAR CARCINOMA DEVELOPMENT RISK

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GWAS identified the variant G (rs4374383 A/G) of MERTK, a TAM receptor mainly expressed in M2 macrophages, as associated with progression of liver fibrosis from HCV infection [1]. We demonstrated that AA genotype, associated with lower hepatic MERTK expression, is protective against fibrosis in Nonalcoholic Fatty Liver Disease [2], suggesting a pro-fibrogenic role of MERTK in the cross-talk between macrophages and Hepatic Stellate Cells. On the other hand, in patients with HCV liver cirrhosis treated with PEG-IFN/ribavirin (N=540), we found that rs4374383 AA genotype confers a significant risk of developing Hepatocellular Carcinoma (HCC). Remain unknown how this intronic SNP is able to conditioning the progression of liver disease in neoplastic direction. Recently was found that the intronic MERTK dubbed enhSNP (deSNP) rs6726639 A/C is in high linkage disequilibrium with rs4374383. DeSNP is located within the binding sites of Transcriptional Repressor Factors which are able to bind stronger the A allele [3], suggesting that the down regulation of MERTK, observed in patients with rs4374383 AA genotype, could be due to the association with rs6726639 AA genotype. In the same way, this genotype could influence in a negative manner the transcription of genes involved in fibrogenesis and in tumor suppression. Early results suggest that the AA rs4374383 and AA deSNP rs6726639 genotypes are associated with a higher risk of HCC occurrence in patients without SVR after IFN based therapy (N=403) and in cirrhotic patients who achieved SVR after Direct Antiviral Agents therapy (N=453). Furthermore, a major statistical significance for DeSNP was observed.

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ROLE OF RAB7A IN CHEMORESISTANCE

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Cisplatin (CDDP) is a widely used chemotherapeutic drug for the treatment of different types of cancer. The majority of the platinum treated patients develops resistance to CDDP with consequent therapeutic failure. Interestingly, several reports have shown a relationship between reduced CDDP accumulation in lysosomes, defects in the lysosomal compartment and reduced cytotoxicity, or even resistance. Resistance has also been associated with increased efflux of CDDP and reduced size of the lysosomal compartment. As vesicular trafficking and exosome production are regulated by Rab GTPases, we decided to investigate expression of endocytic Rab GTPases in chemosensitive and chemoresistant cells. We demonstrated that CDDP-resistant cells are characterized by a reduction of the number and size of acidic compartments and a significant downregulation of RAB7A. Notably, through modulation of Rab7 expression, we were able to alter CDDP response. In particular, RAB7A depletion in CDDP-sensitive cells and its overexpression in CDDP-resistant cells determined increased resistance and increased sensitization to the drug, respectively. ICP analysis on resistant and sensitive cells after CDDP treatment and subsequent evaluation of extracellular extracts allowed us to demonstrate that less CDDP is present inside chemoresistant cells. This decrease correlated with increased production of extracellular vesicles that was obtained also by depleting RAB7A. Thus, for the first time, we demonstrated that RAB7A regulates CDDP resistance determining alterations in late endocytic traffic and drug efflux through extracellular vesicles. This discovery contributes to shed light on molecular mechanisms underlying chemoresistance and may lead to the future design of new chemotherapeutic strategies.

THE eIF2α ROLE IN PROLIFERATING CELLS USING AS MODEL MELANOMA CELL LINES

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Tumor cells develop adaptive responses in order to cope particular conditions of the tumor microenvironment, characterized by stress conditions and by a dysregulated proliferation. These signals induce the activation of cellular response to stress. The most recent knowledge of melanoma biology focuses on the role of endoplasmic reticulum (ER) stress, autophagy and translational reprogramming. We analyzed the role of eIF2α translation factor in proliferating melanoma cells, wtBRAF and V600BRAF-mutated, extracted from primary or metastatic tumors, used as cellular model [1,2]. Our results showed higher levels of phosphorylated eIF2α (peIF2α in the metastatic V600BRAF-mutated melanoma cells as compared to the wtBRAF and primary V600BRAF-mutated melanoma cells [1]. The most striking result of our work is the finding of nuclear localization of peIF2α, both in the melanoma cell lines from the primary and metastatic lesions. Dogmatic molecular biology knowledge usually relates eIF2α activity into the cytoplasm. The role of eIF2α/peIF2α into the nucleus is not clear. However, a possible explanation is provided by studies suggesting the possible link between this translation factor and RNA polymerase (3). Therefore its nuclear localization would be crucial in ER stress response and in driving metastatic spread of melanoma. Furthermore, we found in all BRAF-mutated cells with respect to wtBRAF metastatic melanoma cells, higher LC3II/I ratios and TFEB levels, markers of increased autophagy [1] leading to support the activation of autophagy through the lysosomal pathway in melanoma cell lines.

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HUMAN ADIPOSE STEM CELLS INDUCED TO OSTEOGENIC DIFFERENTIATION BY AN INNOVATIVE HYDROXYAPATITE HYBRID SCAFFOLD

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In this study we investigated human adipose stem cells (hASCs) for biocompatibility, osteoconductivity and osteoinductivity effects of an innovative hydroxylapatite hybrid scaffold composed of granular hydroxyapatite and collagen (Coll/HA) materials [1-2]. In hASCs cultures, grown on this scaffold, showed normal cellular cytoskeleton organization, cellular morphology and cell viability when investigated by immunohistological staining (IHS), metabolic assay (Alamar blue) and scanning electron microscopy (SEM) [3]. Expression of the extra-cellular matrix (ECM), adhesion molecule, and osteogenic genes were evaluated by quantitative PCR (Q-PCR) array technologies. Osteocalcin, osteopontin, Alkaline phosphatase (ALP) and phosphorylated focal adhesion kinase p-FAK (Tyr397) proteins were detected in hASC by HIS and E.L.I.S.A.assays. It turned out that the cytoskeleton architecture of hASC seeded on biomaterial was well organized. hASC expression of CLEC3B, LAMB3, ITGAM, ITGA3, LAMA2, ITGB5, COL6A2, SELE, COL6A1, and SPP1 genes was up-regulated. In hASC culture mRNAs of 24 genes of the ossification pathway, i.e. CSF 2/3, SP7, SPP1, TNFSF11, BMPR1B, BMP1/2, BGLAP, IGF1, NOG, RUNX2, TGFB1, EGFR, FGFR1/2, VDR, TWIST1, SOX9, ALPL, IGF1R, COL1A1, EGF, ITGA2 were upregulated compared to the control. The hASC culture expressed the osteogenic proteins such as ALP and osteocalcin. In addition, the mineralized matrix was present in hASC culture. These results suggest that the hASC cultures are a reliable tool to evaluate the biocompatibility, osteoconductivity of the biomaterial. Our data demonstrate that the innovative scaffold provides the good microenvironment in which hASCs adhesion and proliferation are enhanced, while inducing the up-regulation of osteogenic genes with improvement in matrix mineralization.

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GENE EXPRESSION CHANGES DURING CANCER PROGRESSION IN CERVICAL NEOPLASTIC KERATINOCYTES

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Gene expression changes occurring in cervical intraepithelial neoplasia (CIN) progression are poorly understood. Using microarray analyses, large-scale gene expression profile was carried out in cervical neoplastic keratinocytes naturally infected by HPV-16, CIN2 and CIN3 keratinocytes, and normal cervical keratinocytes derived from normal cervical tissues belonging to the same CIN affected patients [1]. Comparative analyses of differentially expressed genes identified 37 candidate genes progressively up- or down-expressed from CIN2 to CIN3 keratinocytes. One of these genes, the phosphoglycerate dehydrogenase (PHGDH), was selected for further characterization. Quantitative reverse transcription-polymerase chain reaction and immunohistochemical analysis confirmed that expression of PHGDH consistently increases during progression of CIN toward cancer [2, 3]. In conclusion, this study revealed 37 downexpressed or overexpressed genes which may contribute to CIN progression. In addition, protein expression of PHGDH increased from CIN1 to cancer according to the degree of malignant transformation. Thus, PHGDH likely plays an important role in the initiation and progression of cervical tumorigenesis and may be a prognostic marker for progression of CIN to invasive cancer.

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IN VITRO CHARACTERIZATION OF THE CALVARIAL SUTURE OSTEOGENIC STEM CELL NICHE IN NONSYNDROMIC CRANIOSYNOSTOSIS

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Nonsyndromic craniosynostosis (NCS) is a congenital defect due to the premature fusion of skull sutures. The suture mesenchyme houses a skull-specific stem cell niche, which is plausibly impaired in NCS fused suture sites. To test this hypothesis, we characterized the stem cell niche of open and fused sutures of NCS patients.

The following lineage-specific markers were analyzed in suture tissues and in calvarial mesenchymal stem cells (CMSC), by qPCR and immunofluorescence: THY1 (skeletal stemness-marker), GLI1 (putative calvarial stemness-marker), AXIN2 (mesenchymal cell fate determinant), TEK and ENPEP (bone marrow stem cells differentiation markers).

AXIN2 resulted mainly expressed at the endosteal ossified side, while THY1 and GLI1 were primarily expressed within the trabeculae, enriched with proliferating cells.

NCS suture tissues and CMSC isolated thereof, expressed reduced levels of TEK and ENPEP compared with controls. AXIN2 levels were higher in open suture-derived CMSC than in fused suture cells and in controls. Upon in vitro osteogenic induction, the expression of THY1 and GLI1 decreased, whereas AXIN2 levels increased, in both open- and fused- suture derived CMSC.

CMSCs isolated from both fused and unfused sutures shared the same marker expression profile, indicating that explant cultures allowed selecting comparable cell populations, THY1+/GLI1+ representing the stem cell population within the human calvarial niche. Our data seem to suggest that in NCS the in vivo tissue microenvironment, rather than than the stem cell population itself, may cause the enhanced osteogenic differentiation of suture MSCs leading to premature suture closure.

INVESTIGATING THE COMPLEX ROLES OF DNA METHYLATION

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Aberrant DNA methylation of promoter CpG islands (CGI) and genome-wide hypomethylation are common and early events in cancer. Promoter CGI hypermethylation has often been associated with gene silencing. Recently, an increasing number of global methylation and gene expression data have revealed that CGI promoter hypermethylation frequently occurs in normally poorly expressed genes, questioning the role of DNA methylation in gene silencing [1-3]. Genome-wide methylation and targeted gene expression analyses of three types of cancer (colorectal cancer, pilocytic astrocytoma and chronic lymphocytic leukemia) conducted in our laboratory, have confirmed that DNA methylation targets CGI of genes already poorly expressed in the normal tissues that give rise to tumors. Nevertheless, we have observed that promoter CGI hypermethylation is significantly associated to a decreased gene expression in cancer. Moreover, our preliminary data have shown that gene-body CGI hypermethylation might have a functional role in the regulation of alternative transcript isoforms expression, probably by regulating cell-context-specific alternative promoters in gene bodies.
The loss of methylation in extended region of the genome, defined as partially methylated domains (PMDs), is another feature of cancer methylome. My placement project, carried out at Sproul Lab, had the aim to understand whether DNA transposons methylated *in vitro* represented a valid approach to elucidate the mechanism of DNA methylation maintenance in PMDs in cancer cells. Our preliminary results have indeed shown an integration of these methylated transposons in the genome of cancer cell lines and a loss of methylation of a subset of transposons over time.

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PROTEOME ANALYSIS IN DYSTROPHIC MDX MOUSE MUSCLE REVEALS A DRASTIC ALTERATION OF KEY METABOLIC AND CONTRACTILE PROTEINS AFTER CHRONIC EXERCISE

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Mdx mouse model of Duchenne muscular dystrophy shows a phenotype of the disorder milder than in human sufferers [1]. This phenotype can be worsened by a different protocols of forced exercise, in relation to the mechanical susceptibility of dystrophin deficient muscles. The standardized protocols on treadmill can mimic the muscle progressive damage observed in humans, and have been largely used to assess the ability of a drug to reduce the pathology related muscle damage [2]. In the present study, we describe, the pattern of differentially abundant proteins that is associated to the worsening of dystrophy phenotype induced by chronic exercise. Our proteomic analysis pointed out 34 protein spots with different abundance between sedentary and exercised mdx mice. These proteins belong mostly to glucose metabolism, energy production and sarcomere structure categories. Interestingly exercise induced an increase of typical fast twitch fiber proteins (Troponin T fast skeletal muscle, Troponin I fast skeletal muscle and Myozenin-1) combined with an increase of several glycolytic enzymes. Concerning energy transfer, Adenylate kinase, showed a marked decrease when compared with non-exercised mdx. The decline of this enzyme correlates with increased Creatin kinase enzyme, suggesting that a compensatory energy metabolism mechanism could be activated in mdx mouse skeletal muscle following exercise. Overall our data indicate that mdx exercised muscle are not able to carry out the metabolic changes associated to fast-to-slow transition typically observed in aerobically trained muscle.

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THE EFFECT OF CHILDHOOD TRAUMA ON BLOOD EXPRESSION OF *MED22* IN PATIENTS WITH MAJOR DEPRESSIVE DISORDER IS MEDIATED BY GENETIC VARIANTS REGULATING GENE EXPRESSION

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There is evidence that childhood trauma (CT) increase the risk of developing major depressive disorder (MDD) during adulthood. However, the biological mechanisms underlying this relationship remain largely undetermined. It is unclear how the physiological and psychological consequences of early life stress are influenced by an inherited vulnerability to stressful events. To clarify these issues, we studied differences in gene expression among 368 MDD patients experienced different type of CT. Moreover, for differentially expressed genes, the genetically regulated component of gene expression (GReX) and the environmental component (EReX) were calculated (applying PrediXcan method) and correlated with CT.

Expression analysis revealed a significant association between neglect CT and MED22 gene (FDR=0.016). The association was significant also for the GReX (p=2.6x10-4) and EReX (p= 6x10-3) components. Intriguingly, MED22 GReX component, but not EReX, resulted associated also to sexual (p=2.1x10-3) and emotional (p=2.2x10-5) abuses.

In a cohort of 177 controls, we observed a significant association between SNPs responsible for changes in MED22 expression and neuroticism (best p= 0.00848). Usually, people with high score on neuroticism dimension exhibit a decreased amount of resilience to stressful events.

In conclusion, our results suggest that neglect exert a strong impact on gene expression in MDD. In addition, they provide insights suggesting that the biological and psychological consequences of CT also depend on the effect of the genetic background that could induce vulnerability/resilient to stressful events by shaping people's personality traits and trauma memories.

DETECTION OF MERKEL CELL POLYOMAVIRUS DNA IN SERUM SAMPLES OF HEALTHY BLOOD DONORS

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Merkel cell polyomavirus (MCPyV) has been detected in 80% of Merkel cell carcinomas (MCC) [1]. Whereas Ig antibodies against MCPyV were revealed in approximately 80% of healthy subjects [2]. In the host, the MCPyV reservoir remains elusive. MCPyV DNA sequences were revealed in blood donor buffy coats [3]. In this study, MCPyV DNA sequences were investigated in the sera (n = 190) of healthy blood donors. This investigation was addressed to two viral LT gene sequences, which encode the MCPyV large T antigen (LT) oncoprotein. Two MCPyV DNA sequences, coding for the viral oncoprotein LT, were investigated using polymerase chain reaction (PCR) methods and DNA sequencing. Circulating MCPyV sequences were detected in sera with a prevalence of 2.6% (5/190), at low-DNA viral load, which is in the range of 1–4 and 1–5 copies/μl by real-time PCR and droplet digital PCR, respectively. DNA sequencing carried out in the five MCPyV-positive samples indicated that the two MCPyV LT sequences which were analyzed belong to the MKL-1 strain. Circulating MCPyV LT sequences are present in blood donor sera. MCPyV-positive samples from blood donors could represent a potential vehicle for MCPyV infection in receivers, whereas an increase in viral load may occur with multiple blood transfusions. In certain patient conditions, such as immune-depression/suppression, additional disease or old age, transfusion of MCPyV-positive samples could be an additional risk factor for MCC onset.

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HYPERMETHYLATION-INDUCED INACTIVATION OF IRF6 AND RARB GENES IN VULVAR SQUAMOUS CELL CARCINOMA ASSOCIATED WITH LICHEN SCLEROSUS

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Vulvar squamous cell carcinoma (VSCC) represents 5% of gynecological malignancies. About 80% of VSCCs arises from an inflammatory dermatosis, lichen sclerosus (LS) [1]. The molecular alterations involved in onset/progression of LS -associated VSCC are unknown. IRF6 and RARβ tumor-suppressor genes are downregulated by promoter methylation in cancer. In our investigation we aimed to evaluate the possible involvement of the IRF6 and RARβ in the development of VSCC from LS [2]. We analyzed the IRF6 and RARβ mRNA expressions by quantitative PCR and the promoter methylations by sequencing of PCR-amplified bisulfite-treated DNA [3], in VSCC (n=20) and the corresponding adjacent LS (n=20), cancer-free LS (cfLS, n=20) and normal skin (n=20). mRNA expression of p63 and c-jun, IRF6 and RARβ pathway-related genes, respectively, was also investigated. IRF6 was down-regulated in progression from cfLS, LS, VSCC, and p63 was over-expressed in progression from cfLS, LS, VSCC. IRF6 promoter was hypermethylated in 10% cfLS, 45% LS, and 80% VSCC. In VSCC,
RARβ was downregulated and c-jun overexpressed. RARβ promoter was hypermethylated in 90% VSCC, 55% cfLS, 50% LS and 25% normal skin. Our data indicate hypermethylation-induced IRF6 down-expression is involved in development of VSCC from LS, whereas hypermethylation-induced RARβ down-expression is a late event in LS-associated VSCC. IRF6 and RARβ promoter methylation may be used as prognostic biomarker in clinical management of LS and LS-associated VSCC patients.

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MIR-96-3P, THE NEGLECTED MIRNA "SISTER" OF THE DEAFNESS-ASSOCIATED MIR-96-5P, REGULATES C-KIT DURING MOUSE DEVELOPMENT

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MiR-96-5p is a microRNA with established role in vertebrate neurosensory organ development, and germline mutations in the MIR96 gene cause hereditary hearing loss in humans and mice. While the function of miR-96-5p has been extensively investigated, the biological role of its partner strand still remains unclear. To better define it, we compared miR-96-3p and miR-96-5p expression in several mouse tissues at different developmental stages. Real-time qPCR assays showed that, although the two miRNAs have a similar expression profile, miR-96-3p is upregulated compared to miR-96-5p in embryonic stem (ES) cells and at 10.5 days post coitum (dpc), suggesting a possible switch in the pre-miRNA dominant arm during early embryogenesis. These results were further corroborated by in-situ hybridizations on 10.5-17.5 dpc mouse embryos. Indeed, both miR-96-3p and miR-96-5p were co-expressed in several tissues with a specific spatio-temporal distribution.
We then sought to identify miR-96-3p targets by analyzing the transcriptome of murine ES cells upon miRNA inhibition. A total of 39 significantly differentially expressed genes were found (FDR adjusted p-value<0.05), the majority of which were upregulated. Among them, we confirmed Kit upregulation also at the protein level by western-blot analysis. Interestingly, immunohistochemical analysis of Kit expression during mouse embryonic development highlighted temporal co-expression with miR-96-3p but with a complementary spatial distribution in several tissues (e.g. neural tube, somites), which is suggestive of miR-96-3p regulation.
In conclusion, we report for the first time the highly-specific and regulated pattern of expression of miR-96-3p during mouse development, and suggest its role in regulating Kit expression.

ANTIBODIES AGAINST SIMIAN VIRUS 40 LARGE T ANTIGEN, THE VIRAL ONCOPROTEIN, IN SERA FROM OSTEOSARCOMA PATIENTS

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Human osteosarcoma (OS) is a rare human cancer, mostly occurring in children and adolescents [1]. Simian virus 40 (SV40) sequences have been detected in different human cancers, including osteosarcoma. SV40 is an oncogenic virus in vivo, whereas it transforms different kinds of mammalian cells, as well as distinct human cell types. SV40 injected in rodents induces tumors of different histotypes, such as brain and bone tumors. Herein, the association between OS and SV40 large T antigen (Tag) was studied by employing indirect ELISAs using synthetic peptides that mimic different epitopes of the SV40 Tag, the viral oncoprotein. Indirect ELISAs were used to detect serum IgG antibodies against this oncogenic virus in samples from OS patients [2,3]. Controls were sera from healthy subjects (HS) and oncological patients affect by breast cancer, a tumor which is not associated with SV40. It turned out that sera of OS patients had a higher prevalence of SV40 Tag antibodies, 35%, compared to HS, 20% and BC, 19%, respectively. The different prevalence of SV40 Tag antibodies revealed in OS vs HS and vs BC is statistically significant with $P<0.05$ and $P<0.01$, respectively [3]. Our immunological data suggest a significantly higher prevalence of IgG antibodies against SV40 Tag epitopes in serum samples from OS patients compared to HS and BC, the controls. These results suggest an association between OS and SV40 Tag, indicating that this oncogenic virus may be a cofactor in the OS onset/progression.

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CHRONIC LYMPHOCYTIC LEUKEMIA TESTED POSITIVE FOR THE ONCOGENIC MERKEL CELL POLYOMAVIRUS, MCC-350 STRAIN

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Merkel cell carcinoma (MCC) have been found to be associated with the oncogenic Merkel cell polyomavirus (MCPyV) [1]. However, MCPyV sequences have been detected at low prevalence in buffy coats [2] and sera of blood donors [3]. Chronic lymphocytic leukemia (CLL) were also found associated with MCPyV by some investigators, whereas other studies did not confirm this result. In our investigation, DNA sequences belonging to MCPyV were identified with a different prevalence in sera from CLL patients and blood donors. MCPyV sequences in sera of CLL patients and blood donors had a prevalence of 7% (16/224) and 5% (14/284), respectively. Specifically, MCPyV DNA sequences, coding for the viral oncoprotein large T antigen (LT), analyzed by the droplet digital polymerase chain reaction (ddPCR) method and DNA sequencing, showed the circulation of two different MCPyV strains, MCC350 and MKL-1. Indeed, DNA sequencing performed in MCPyV-positive sera indicated that MCPyV LT sequences belong to the ubiquitous MKL-1 [1-2] and oncogenic MCC350 strains. Interestingly, the more oncogenic MCC-350 strain was present at higher prevalence, 81% (13/16), in CLL samples, while the prevalence of MCC-350 was only 21% (3/14) in sera of blood donors ($P<0.05$). It is worth recalling that MCPyV MCC350 was the strain originally identified in MCC, a rare skin cancer. In our study, this oncogenic strain is found to be more prevalent in CLL patients, whereas the ubiquitous MKL-1 is more prevalent in blood donors. These data suggest that the MCC 350 strain could be responsible of the CLL onset in a fraction of these patients, whereas the MKL-1 strain seems to be the MCPyV circulating in normal subjects.

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NATRIURETIC PEPTIDES: NOVEL INSIGHTS INTO THE MOLECULAR MECHANISMS OF IL-1β AND NF-KB/ERK1/2/NA

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IL-1β and related cascade are being regarded as suitable targets for a large variety of diseases. Recently, we demonstrated a novel anti-inflammatory/immune-modulatory role for Atrial Natriuretic Peptide (ANP) and Brain Natriuretic Peptide (BNP), hormone/paracrine/autocrine factors exerting their biological functions by guanylyl-cyclase Natriuretic Peptide Receptor-1 (NPR-1). Indeed, we showed that they inhibit the NF-kB and ERK 1/2 pathways thus down-regulating NALP3-inflammasome cascade activation and related LPS/ATP-induced IL-1β secretion in THP-1 monocytes. The aim of this study was to further investigate the signaling mechanisms involved in ANP/BNP-dependent anti-inflammatory/immune-modulatory effect. cGMP cascade is the key event leading to ANP/BNP cellular and physiological responses mainly via cGMP-dependent protein kinase I (PKG-I) activation. We showed that 8-Br-cGMP, a permeable analogue of cGMP, mimic the ANP/BNP effects, by inhibiting LPS+ATP-induced IL-1β secretion in THP-1 monocytes and interfering on the molecular mechanisms that preside both pro-IL-1β synthesis and release. Likewise, by using KT-5823, a selective PKG-I inhibitor, we demonstrated the direct involvement of PKG-I in ANP/BNP/8-Br-cGMP-related IL-1β/NALP3-inflammasome inhibition via NF-kB pathway de-regulation. All together our data strongly suggest that ANP and BNP exert their anti-inflammatory/immune-modulatory molecular cascade via NPR-1/cGMP/PKG-I pathway activation. Pharmacological modulation of cGMP pathway is a potential target for a large variety of diseases. Therefore small molecules that can increase cGMP levels are regarded as compounds that can be used in several pathological conditions. Thus, we propose that ANP and BNP, already used in the treatment of heart failure, are of interest also in the treatment of IL-1β/NF-kB/ERK1/2/NALP3/ASC/caspase-1-associated diseases.

DELTA-TOCOTRIENOL INDUCES AUTOPHAGY AND APOPTOSIS IN HUMAN PROSTATE CANCER CELLS

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In men, prostate cancer (PC) is the second most common form of cancer and one of the leading causes of cancer death. PC is initially hormone-dependent and androgen deprivation therapy (ADT) is the preferred treatment. Unfortunately, many patients develop metastatic castration-resistant PC (CRPC) that present severe resistance towards conventional chemotherapeutic agents, so a new therapeutic approach is required.
Delta-Tocotrienol (δ-TT), a member of the vitamin E family, displays potent antiproliferative and cytotoxic effects in a variety of cancer cell type, but mechanisms underlying these actions are not clear. In this study we first demonstrated that δ-TT induces cytotoxicity in human CRPC cell lines (PC3 and DU145) affecting cellular viability (MTT assay), clonogenic growth and percentage of annexinV-PI positive cells. Then, we established that δ-TT triggered intrinsic apoptosis pathway inducing cytochrome *c* release from mitochondria and increasing cleaved caspase-3 and cleaved PARP levels. Autophagy is tightly regulated lysosomal self-digested process that can either promote cell survival or programmed cell death, but the role of autophagy in mediating δ-TT-induced cytotoxicity in prostate cancer is not yet completely understood.
The results obtained demonstrate that δ-TT causes an increased conversion of LC3, from its cytosolic form LC3-I to its lipidated form LC3-II, and enhances p62/SQSTM1 expression in PC cells. Moreover, the autophagy inhibitor 3-MA significantly inhibits δ-TT-induced apoptotic cell death.Taken together, the results indicate that δ-TT-induced autophagy triggers apoptosis in PC cells and inhibition of autophagy subsequently decreases cell death.

ROLE OF CIRCULATING miRNAs IN HEREDITARY HEMORRHAGIC TELANGIECTASIA

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Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant rare disease, with a worldwide prevalence of about 1:5000, characterised by vascular dysplasias, as telangiectases and arteriovenous malformations (AVMs). Mutations in either *ENG* or *ACVRL1* account for 85% of patients and lead to HHT1 and HHT2, respectively. Both genes belong to the TGF-β/BMPs pathway and play a role in the regulation of angiogenesis. HHT shows a heterogeneous phenotype, even within families, but the molecular underlying mechanism is not completely understood.
Circulating microRNAs (miRNAs) regulate several physiological and pathological processes and represent a class of non-invasive biomarkers for several disorders. We investigated the expression of circulating miRNAs in plasma samples of 15 subjects: 5 HHT1, 5 HHT2 patients and 5 age and gender matched controls. The aim was to define an HHT-related miRNAs signature, paying particular attention to the correlations with the disease genotypes and phenotypes. Both parametric and non-parametric tests were used for statistical analyses. Obtained data revealed the dysregulation of seven miRNAs; five of them are angiomiRs, angiogenesis regulating miRNAs, as members of the let-7 family, miR-16-5p and miR-20a-5p.
This study provides an "HHT signature" for circulating miRNAs and highlights a relevant involvement of angiomiRs. Furthermore, new correlations between circulating miRNAs and pulmonary AVMs and, for the first time, differences between HHT1 and HHT2 are identified.

HUMAN PAPILLOMAVIRUS DNA STATUS TOGETHER WITH THE DOWNREGULATION OF IRF6 AND RARB GENES CORRELATE WITH PROGNOSIS IN HEAD AND NECK CANCER PATIENTS

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Human papillomaviruses (HPVs) are detected in approximatively 40% of the head and neck squamous cell carcinomas (HNSCCs). The incidence of HPV-positive HNSCCs is rapidly increasing worldwide. The presence of HPV usually confers an improved overall- and progression-free survival, yet 30% of the HPV-positive HNSCC patients will have poor prognosis. In this study the viral DNA status, episomal, integrated or a mixture of both, was investigated in correlation to the patient's outcome, along with expression of IRF6 and RARβ, two tumor suppressor genes previously found hypermethylated in vulvar carcinomas [1-2]. DNA and RNA were extracted from frozen tumor biopsies. DNA was studied for the viral presence using the GP5+/GP6+ universal primers in real-time PCR (RT-QPCR) [3]; the melting temperature was used to determine the HPV genotype and the cycle threshold was used to calculate the viral load. Specific primers amplifying the E2 and E6 viral regions in RT-QPCR were used to determine the integration status. IRF6 and RARβ expression was assessed by RT-QPCR. Our preliminary data show a correlation between the viral status and the prognosis of the patients. Interestingly, HPV-positive HNSCCs tumors showed a greater IRF6 and RARβ downregulation compared to HPV-negative HNSCC and to other tumor tissues. Therefore, IRF6 and RARβ, along with the viral integration status may be used as potential prognostic factors in HNSCCs.

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RESCUING PROTEIN HOMEOSTASIS IN FRAGILE X SYNDROME

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Fragile X syndrome (FXS) is the most frequent form of monogenic intellectual disability and autism caused by the absence of the fragile X mental retardation protein (FMRP). This leads to an increase in mRNA translation at synapses and consequently, to deficits in synaptic architecture and plasticity. Here, we measured the rate of protein synthesis in fibroblasts derived from individuals with FXS as well as in *Fmr1* KO embryonic fibroblasts (MEFs) and primary neurons. We show that levels of protein synthesis are increased in fibroblasts of FXS individuals and in *Fmr1 KO* mice. However, protein synthesis levels are not increased in all individuals with fragile X and, of note, a proportion of fragile X individuals and *Fmr1 KO* mice have measures in the normal range. Furthermore, our findings show that increase of protein synthesis is sustained by the excessive production of soluble amyloid precursor protein α (sAPPα) due to the impaired processing of amyloid precursor protein (APP) during a critical developmental window in mice and in human patient cells. Treatment of FXS mice and human fibroblasts with a cell permeable peptide (TAT-Pro ADAM10$^{709-729}$) is able to modulate ADAM10 activity and therefore APP processing, restoring protein synthesis to wild type (WT) levels and rescuing behavioral deficits that constitute a hallmark of the disease. Importantly, we now recapitulate those findings in developing and differentiating FXS iPS cells and show that the TAT-Pro peptide might be a specific treatment to reduce sAPPα release and exaggerate protein synthesis rates in a subsets of human cells.

ALTERATION OF ENDOSOMAL TRAFFICKING IS ASSOCIATED WITH WITH EARLY-ONSET PARKINSONISM CAUSED BY SYNJ1 MUTATIONS

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The endosomal system, which is at crossroads of distinct intracellular pathways, plays crucial roles in various cell functions, including protein and lipid sorting. In the last years, several genes responsible for hereditary forms of Parkinson's disease are implicated in distinct steps of the endolysosomal pathway. Recently a new form of autosomic recessive early-onset parkinsonism (PARK20), due to a mutation in the phosphoinositide phosphatase Synaptojanin 1 (Synj1) has been reported. However, the nature and the degree of endocytic membrane trafficking impairment in early-onset parkinsonism remain elusive.

We show that depletion of Synj1 causes drastic alterations of early endosomes, which become enlarged and more numerous, while it does not affect the morphology of late endosomes both in non-neuronal and neuronal cells. Moreover, Synj1 loss impairs the recycling of transferrin, while it does not alter the trafficking of the epidermal growth factor receptor. Importantly, the same alterations of early endosomal compartments and trafficking defects occur in fibroblasts of PARK20 patients. All together, these data indicate that Synj1 plays a crucial role in regulating the homeostasis and functions of early endosomal compartments in different cell types, and highlight defective cellular pathways in PARK20.

Overall, our data strengthen the link between endosomal trafficking and Parkinson's disease.

KLF4 MEDIATES THE EFFECT OF 5-ASA ON THE β-CATENIN PATHWAY IN COLON CANCER CELLS

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Mesalazine (5-ASA) is an aminosalicylate anti-inflammatory drug capable of inducing μ-protocadherin, a protein expressed by colorectal epithelial cells that is down regulated upon malignant transformation. Treatment of colorectal cancer cell lines with 5-ASA restores μ-protocadherin expression and promotes the sequestration of β-catenin to the plasma membrane. Here, we show that 5-ASA–induced μ-protocadherin expression is directly regulated by the KLF4 transcription factor. In addition, this compound is capable of inducing KLF4 expression, therefore we suggest the existence of a dual mechanism whereby 5-ASA–mediated β-catenin inhibition is caused by μ-protocadherin–dependent sequestration of β-catenin to the plasma membrane and by the direct binding of KLF4 to β-catenin. Our experiments demonstrate that KLF4 is capable of directly sequestering β-catenin, this effect is accompanied by the release of TCF4 from β-catenin, which prevents its transcription activity. In addition, we found that 5-ASA treatment suppresses the expression of miR-130a and miR-135b, which target KLF4 mRNA, raising the possibility that this mechanism is involved in the increased expression of KLF4 induced by 5-ASA.

MODULATION OF CALCIUM (CA2+) SIGNALING AND INTRAPLEURAL PERFUSION OF COMBINATORY TREATMENTS AS NEW THERAPEUTIC APPROACHES FOR THE TREATMENT OF MALIGNANT PLEURAL MESOTHELIOMA

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The human malignant pleural mesothelioma (MPM) is a highly aggressive asbestos-related cancer that develops via mesothelial cell transformation [1]. After diagnosis MPM leads invariably to death patients in a few months/years. Recent estimations indicate that one-quarter million people will die of this neoplasm in Europe in the next three decades [1]. There is no standard curative therapy for MPM that is largely unresponsive to conventional chemotherapy and radiotherapy [1]. Modern targeted therapies that have shown benefit in other human tumors have so far failed in MPM. This has led to MPM being listed an "orphan disease" by the EU.As a consequence, effective therapeutic strategies are needed for this fatal disease, as well as new biomarkers for an early diagnosis. Calcium ions (Ca^{2+}) act as second messenger to regulate gene transcription, cell proliferation, migration and death. A growing body of evidences suggests that intracellular Ca^{2+} homeostasis is altered in cancer cells and the alteration is involved in tumor initiation, angiogenesis, progression and metastasis. Accordingly, we recent demonstrated that intracellular Ca^{2+} signaling is deregulated in MPM [2,3]. Hence, the reactivation of optimal intracellular Ca^{2+} levels may represent a new therapeutic option to counteract MPM progression. Our data confirm this possibility. Indeed, by increasing Ca2+ levels the efficacy of conventional chemodrugs used for MPM treatment is augmented. Most important, we propose an in vivo protocol aimed to recover the efficacy of chemodrugs by increasing Ca^{2+} levels, that would be transfer in clinic.

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ADAPTIVE REDOX HOMEOSTASIS MECHANISMS UNDERLIE CEREBRAL CAVERNOUS MALFORMATION DISEASE PATHOGENESIS

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KRIT1 is a disease gene responsible for Cerebral Cavernous Malformation (CCM), a major cerebrovascular disease of proven genetic origin affecting 0.3-0.5% of the population. Previously, we demonstrated that KRIT1 loss-of-function affects distinct redox-sensitive signaling pathways and mechanisms, including pro-oxidant and antioxidant pathways and autophagy, suggesting that it may impair molecular and cellular responses to oxidative stress and inflammatory insults [1]. Using both cellular and animal models of CCM disease, and surgical samples of human CCMs, we demonstrated that KRIT1 loss-of-function leads indeed to pleiotropic effects on major redox-sensitive pathways and enzymatic systems involved in cellular responses to oxidative stress and inflammation. In particular, we found that KRIT1 depletion increases NADPH oxidase signaling and endothelial ROS production, which directly contributes to the loss of barrier function in KRIT1 deficient animals and cells, and exacerbates vascular permeability triggered by inflammatory stimuli [2]. Furthermore, loss of KRIT1 leads to a sustained activation of the Nrf2 antioxidant defense pathway and its downstream target Glyoxalase 1 (Glo1), a pivotal stress-responsive defense enzyme involved in cellular protection against glycative and oxidative stress through the metabolism of methylglyoxal (MG). However, these effects are associated with a redox-sensitive downregulation of major apoptosis-protective proteins, including MG-modified Hsp70 and Hsp27 proteins, and a consequent increased susceptibility to oxidative DNA damage and apoptosis [3]. Overall, these findings demonstrate that KRIT1 loss-of-function leads to a sustained adaptive redox homeostasis (allostasis) that counteracts intrinsic oxidative stress but sensitizes cells to further oxidative challenges, thus providing novel options for the development of targeted therapeutic strategies.

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THE HISTONE H3 LYSINE 27 METHYLTRANSFERASE EZH2 EMERGES AS A NOVEL EPIGENETIC TARGET FOR MESOTHELIOMA THERAPY

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Mesothelioma is a type of cancer that forms on the thin protective lining that cover the lungs and other internal organs, often associated with exposure to asbestos. Newly diagnosed individuals with mesothelioma are often treated first with chemotherapy. However, for patients with relapsed or refractory mesothelioma, there are currently no approved treatment options.
Tazemetostat, a first-in-class EZH2 inhibitor, is currently being studied as a monotherapy in ongoing Phase 1 and 2 programs. The methyltransferase EZH2 (Enhancer of Zeste homology 2) trimethylates histone H3 lysine 27 (H3K27me3) on chromatin and this repressive mark is removed by the lysine demethylase KDM6B.
In accordance with published data [1], we observed that the mesothelioma derived MSTO-211H cells were resistant to Tazemetostat treatment when cultured in 2D as monolayer; while, we demonstrated that *SIRT1* silencing or SIRT1 catalytic inhibition, by EX527, sensitized cells [2]. We evidenced that SIRT1 inhibition resulted in increased EZH2 protein expression/stability and higher global H3K27me3 levels. Notably, MSTO-211H cells, when cultured in 2D, expressed negligible levels of KDM6B. Co-treatment with Tazemetostat and EX527, resulted in inhibition of AKT and ERK1/2 activity, $p16^{Ink4a}$ increased expression and G1 cell cycle arrest. Furthermore, we demonstrated that MSTO-211H cells were sensitive to Tazemetostat when cultured in 3D as multicellular spheroids. Treated spheroids were smaller and more densely packed, showed sustained $p16^{Ink4a}$ expression and accumulated DNA damages. Moreover, Tazemetostat treatment of cells cultured in 3D resulted in further increase in *EPAS1* and *KDM6B* expression that we previously demonstrated to be upregulated in the spheroids' hypoxic core [3].

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UNRAVELLING POSITION EFFECT AND PSEUDODOMINANCE IN NF1 MICRDELETION SYNDROME: NEW PERSPECTIVES FOR GENOTYPE-PHENOTYPE CORRELATIONS

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Neurofibromatosis type I microdeletion syndrome, accounting for 4–11% of NF1 patients, is associated with a severe phenotype as it is caused by the deletion of NF1 and other genes in the 17q11.2 region. The variable expressivity of the disease makes it challenging to establish genotype-phenotype correlations. The role of deletion events in promoting specific position effects on genes flanking the deletion has been poorly investigated. Furthermore, pseudo-dominance may contribute to specific phenotypic traits and may be involved in variable expressivity. We recently described an NF1 patient with a complex phenotype and an atypical deletion generating the RNF135-SUZ12 chimeric gene. The finding of high expression level of both the chimeric and a different flanking gene, together with the prediction of specific TADs' loss, lead us to hypothesize that deletion caused a position effect. We are going to carry out 4C experiments to identify the effects of 17q11.2 microdeletion on 3D chromatin organization. We previously sequenced a group of ten NF1-MD patients by a custom designed NGS panel including genes comprised in the deletion interval. A missense undescribed substitution was detected in RNF135, a gene associated with overgrowth, due to loss of function mutations. As bioinformatic predictors indicated a pathogenic effect of the substitution in a patient showing overgrowth, we will preformed in vitro studies to determine the functional effect of the mutation. The pseudo-dominance effect will be evaluated in more than 30 NF1-MD. We here propose a model addressing the molecular basis of other contiguous genes syndromes.

ENDOTHELIAL NOTCH1: THE ENDOTHELIUM HAS GOT A FRIEND

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The Notch pathway is a major determinant of stemness, differentiation, proliferation and cell survival during the development and in postnatal life. Given the crucial role of Notch in determining cell fate it is not surprising that in the last thirty years the dysregulation of Notch has been reported in the majority of solid tumors and leukemias. Clinical studies to investigate the efficacy of Notch inhibition at inhibiting tumor progression are ongoing. Growing evidence suggests a crucial role of Notch in the maintenance of the homeostasis of the endothelium. In this context, Notch1 seems to be required to mediate the protective effects of the tangential force exerted by the blood (laminar shear stress) on the endothelium, which is required for functional cell-cell junction, endothelial barrier function, elongated cell morphology, inhibition of the expression of mediator of inflammation [1]. Dyslipidemia-mediated endothelial dysfunction and formation of atherosclerotic plaque has been linked to inhibition of endothelial Notch1 [2]. Reduced levels of estrogens have also been linked to onset of atherosclerosis and we have shown the estrogens - mediated Notch1 activation is required to protect endothelial cell against apoptosis induced by TNF-□, the major mediator of inflammation [3]. All these data suggest that Notch1 could be a novel therapeutic target to prevent endothelial dysfunctions and therefore atherosclerosis. The challenge in the future is the identification of approaches to specifically activate Notch1 in the endothelium. To this aim the deep understanding of those factors that regulate Notch in this tissue will be required.

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AMNIOTIC FLUID STEM CELL DERIVED ESOVESICLES MODULATE PATHOGENIC IMMUNE RESPONSES IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Multiple sclerosis (MS) is a complex disease of immune dysfunction and neurodegeneration and shows a female preponderance, although late pregnancy is the one condition that most profoundly mitigates MS symptoms. Serum exosomes, released by specific cell types during pregnancy, modulate the immune and central nervous systems and contribute to pregnancy-associated suppression of experimental autoimmune encephalomyelitis (EAE), an induced model of MS.
The aim of our study was to control the inflammatory response and immune dysfunction in EAE, by administration of exovesicles (EVs) derived from Human Amniotic Fluid-derived Stem Cells (HASCs).
We found that vesicles from HASCs were able to prevent autoimmune responses in EAE, suppressing inflammatory cytokines and promoting immunoregulatory effects. Moreover, we found that treatment with EVs, significantly reduced disease severity in EAE, relative to controls. Specifically, treatment with EVs reduced neurological deficits and suppressed Rorc, IL-17 and IL-4 in brain lymphnodes (BLN), while increased the percentage of regulatory T (Treg-Foxp3+) cells. In addition, treatment with EVs during EAE, promoteed a significant increase of the immune regulatory indoleamine 2,3-dioxygenase 1 (IDO1) mRNA.
Taken together, the immunomodulatory effects observed from exosomes warrant further exploration into the active components mediating the proposed paracrine effects. The initial evidence strongly suggests that HASC-derived exosomes are the active therapeutic entities, and in this regard our study serves as an opportunity to develop biological therapeutic that harnesses the immunomodulatory and protective properties of stem cells.

METFORMIN INHIBITS MALIGNANT PLEURAL MESOTHELIOMA CELL PROLIFERATION AND INDUCES APOPTOSIS BY TARGETING NOTCH-1

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Malignant pleural mesothelioma (MPM) is an aggressive malignancy arising from the mesothelial cells lining the pleural cavity exposed to asbestos fibers [1-2]. Notch signaling is an evolutionarily conserved cell pathway involved in many cellular biology processes, such as cell proliferation and apoptosis [3]. Notch dysregulation has been reported in vitro, in primary MPM cells, indicating a role for Notch in mesothelial cell transformation [1]. In recent reports metformin (1,1-dimethylbiguanide hydrochloride), which is a common drug used to treat patients affected by type II diabetes, has been proposed as an adjuvant to treat solid tumors. Its indirect effects allow reducing the glucose and insulin blood levels, which results in the growth regression of insulin-dependent tumors [1]. In our study, the Notch1 signalling pathway, the anti-proliferative and pro-apoptotic effect of metformin in MPM cells were investigated. Specifically, protein levels of Notch-1 full length and its active form, represented by the intracellular domain (N1ICD), were investigated by western blot. Cell proliferation assay of MPM cells after metformin treatment it has been performed. Programmed cell death is part of a natural mechanism that regulates cell populations. Several therapeutic agents capable to modulate the apoptosis process are without effect in MPM. Here, the apoptosis was investigated after treatment with metformin in MPM cells. Taken together our preliminary data contribute to elucidate relevant issue of MPM, such as the identification of new therapeutic targets and biomarkers. In the mesothelioma model of study, metformin could act as an anti-proliferative and pro-apoptotic drug against MPM cells targeting Notch-1.

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MERKEL CELL CARCINOMA DEVELOPMENT IN PATIENTS AFFECTED BY AUTOIMMUNE DISEASES TREATED WITH BIOLOGICAL DRUGS

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Merkel cell carcinoma (MCC) is a rare but aggressive tumor with an incidence of 1/~3 million/yr in Europe and USA. The oncogenic Merkel cell polyomavirus (MCPyV) is its causative agent. In immunocompromised patients the anti-viral/cancer response is an adverse event, which may occur due to the therapies with biologics. In our investigation, three MCC arisen in patients (n=750) affected by autoimmune diseases and treated with biologics were characterized [1]. MCPyV DNA sequences were studied using PCR methods in MCCs and in peripheral blood mononuclear cells (PBMCs) [2] Sera from patients were analyzed for the presence and titer of antibodies against the oncogenic Merkel cell polyomavirus (MCPyV) antigens. IgG antibodies against the viral oncoproteins large-T (LT) and small-T (ST) antigens and the viral capsid protein-1 were analyzed by ELISA. Viral antigens were recombinant LT/ST and virus-like particles, respectively. Immunohistochemical (IHC) analyses were used in MCC tissues to reveal MCPyV-LT. MCPyV DNA sequences identified showed 100% homology with the European MKL-1 strain [3]. PBMCs tested MCPyV-negative. Viral DNA loads in the MCCs were in 0.1-30 copy/cell range. IgG antibodies against MCPyV LT/ST were detected in patients 1 and 3, whereas patient 2 was negative. Sera from the three patients contained IgG antibodies against MCPyV VP1. MCCs tested MCPyV LT-antigen-positive in IHC assays, with strong/nuclear LT expression. Normal tissues tested MCPyV LT-negative. We investigated three new MCCs in rheumatologic diseases-affected patients treated with biologics, including anti-TNF. A possible cause–effect relationship between pharmacologic immunosuppressive treatment and MCC onset is suggested.

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THE IMPACT OF PROTEIN CORONA ON CYTOTOXICITY OF ENGINEERED NANOPARTICLES: A LESSON FROM YEAST

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Engineered nanoparticles (ENPs) have found applications in different fields varying from medical science to electronics. The increasing interest in these materials, fuelled by the potential benefits of their use, has not as yet been matched by a concerted effort to gain a full understanding of potential negative effects they may have on the environment and on human health. In a biological environment, ENPs become coated by a so-called "protein corona", the structure of which defines their biological identity. The present research set out to characterize the interaction between cadmium sulphide quantum dots (QDs) and yeast cells, isolating and identifying the set of proteins which were adsorbed on the QD surface. Ring-shaped proteins were particularly prone to binding, and electrostatic and hydrophobic interactions were central for this interaction. QDs strongly increased the transcript levels of genes encoding the major hard corona proteins indicating a mechanism of genetic compensation in response to the "physical sequestration" of these proteins in the QD corona *in vivo*. The toxicological implications of the protein corona formation were explored using yeast mutant strains carrying deletions in genes encoding the corona proteins. Interestingly, these mutants were tolerant to doses of QDs that were lethal to wild type cells. These results reveal that the hard protein corona mediates the toxicity of QDs in yeast and a major resolution of this interaction at the molecular level is crucial for a better understanding of the *in vivo* response of ENPs.

Neuron-specific LSD1 controls plasticity-related transcription and provides a cue to deciphering intellectual disability

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One of the most intriguing questions in neurobiology is how the environment shapes our behavior and which molecular players are involved in this process. To appropriately interface with the environment, the mammalian brain relies on a set of epigenetic mechanisms that are just beginning to be elucidated. Interestingly, mutations in several chromatin modifiers cause different forms of intellectual disability (ID).

I will discuss the role of epigenetic enzyme lysine specific demethylase 1 (LSD1) as a mediator of neuronal plasticity that, by regulating stimuli-dependent gene transcription, impacts emotional and cognitive behavior in mice. LSD1 is ubiquitously expressed. We discovered that neurons carry a dominant-negative spliced isoform – neuroLSD1. LSD1 plays a further role in information processing: in mouse hippocampus, the ratio between LSD1 and neuroLSD1 is dynamically modulated by several paradigms of neuronal activation. Glutamatergic stimulation of hippocampal neurons will provide a possible perspective on LSD1/neuroLSD1 fine-tuning, leading to transient repression of plasticity-related target genes that might account for refinement of learning processes. The pivotal role of LSD1 as a transducer of environmental experiences is further evidenced by the identification of patients featuring a new form of ID caused by de novo LSD1/KDM1A point mutations. The three mutations cluster in the enzyme-active site and impair histone H3 Lys4 catalysis (cleft palate psychomotor retardation and distinctive facial features CPRF OMIM #616728), linking for the first time the activity of a specific epigenetic enzyme with both environmental adaptation and a neurodevelopmental disorder.

EXPLORING LRRK2 BIOLOGY IN GLIAL CELLS: IMPLICATION FOR PARKINSON'S DISEASE

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Mutations in *Leucine-rich repeat kinase 2* (*LRRK2*) gene are linked to familial Parkinson's disease (PD) and common variations in its locus increase the risk to develop the disease. LRRK2, a large and complex kinase/GTPase protein, has been for long time studied in neurons where it has been associated with different molecular pathways. However, how LRRK2 pathogenic mutations lead to neuronal degeneration and to pathology remains still unclear. The discovery that LRRK2 is highly expressed in immune cells, including microglia and astrocytes, has attracted much interest into the understanding as to whether LRRK2 biology in these cells impacts neuronal functions with implication for PD.

We recently demonstrated that LRRK2 is a positive modulator of microglial inflammation after LPS and α-synuclein fibrils insult. At the molecular level, we found that LRRK2 kinase activity negatively regulates PDE4-dependent cAMP degradation and content, thus leading to an enhancement of PKA- mediated NF-kB inhibitory signaling with consequent attenuation of the inflammatory response.

Additionally, LRRK2 appears to play an important role even in astrocytes. Here, we found that LRRK2 governs the endo-lysosome pathway *via* the extracellular chaperone clusterin, which binds misfolded/aggregated proteins to mediate their clearance. Overall, our findings indicate that LRRK2 controls key aspects of glial cells biology, which if dysregulated by pathological mutations might widely contribute to neurodegeneration and progression of the disease.

STUDY OF THE MOLECULAR MACHINERY CONTROLLING MIRNA SORTING IN EXOSOMES

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Exosomes are cell-secreted vesicles, important means of inter-cellular communication, carrying specific repertoires of proteins and RNA; notably their cargo is deregulated in pathological conditions.
Cellular mechanisms controlling specific cargo loading into exosomes are still poorly understood.
Our laboratory is committed to the investigation of the molecular machinery controlling specific miRNAS sorting into exosomes.
We recently identified and determined the functional role of both a specific miRNAs motif and a specific interactor , the RNA-binding protein SYNCRIP [1].
We showed that SYNCRIP directly binds to a specific miRNAs extra-seed sequence (hEXO motif), that was proven to have a pivotal role in the exosome miRNA loading, since its embedment into a poorly-exported miRNA enhances its sorting into exosomes [2].
Current studies are now focused on the characterization of the role of other proteins as further molecular regulator of miRNA loading in exosomes.

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CALRETICULIN INS5 AND DEL52 MUTATIONS IMPAIR UNFOLDED PROTEIN AND OXIDATIVE STRESS RESPONSES IN HEMATOPOIETIC CELLS

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Somatic mutations of calreticulin (CALR) have been described in approximately 30-40% of JAK2 and MPL-unmutated Essential Thrombocythemia and Primary Myelofibrosis patients. CALR is a chaperone that localizes in the endoplasmic reticulum (ER) where it is responsible for the control of proper protein folding and for calcium retention. Recent data have demonstrated that the TPO receptor (MPL) is essential for the development of CALR mutant-driven Myeloproliferative Neoplasms (MPNs). However, the precise mechanism of action of CALR mutants haven't been fully unraveled. In this study, we attempted to clarify whether CALR mutations affect the functions of CALR in the ER under homeostatic conditions. Our results showed that CALR mutants impair the ability to respond to the ER stress and reduce the activation of the pro-apoptotic pathway of the unfolded protein response (UPR), therefore allowing the accumulation of unfolded proteins and conferring resistance to ER-stress induced apoptosis. Moreover, our data demonstrated that CALR mutations induce increased sensitivity to oxidative stress, reducing the ability to counteract ROS accumulation and leading to increase DNA damage. We finally demonstrated that the downmodulation of OXR1 in CALR-mutated cells could be one of the molecular mechanisms responsible for the increased sensitivity to oxidative stress mediated by CALR mutations. Altogether, our data identify a novel MPL-independent mechanism involved in the development of MPNs mediated by CALR mutants. CALR mutations negatively impact on the capability of cells to respond to oxidative stress leading to genomic instability and on the ability to react to ER stress, causing resistance to UPR-induced apoptosis.

ASSOCIATION BETWEEN UVEAL MELANOMA AND THE ONCOGENIC POLYOMAVIRUS BK

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The uveal melanoma (UM) is the most common human intraocular tumor. BK polyomavirus (BKPyV) is a small DNA tumor virus, which footprints have been detected in different human cancers. In vitro BKPyV is (i) clastogenic (ii) mutagenic and (iii) transforms different animal and human cells of different histotypes, whereas in vivo induces tumors in animal models, such as mouse and hamster [1]. In this investigation, the association between UM and BKPyV was analysed investigating the presence and the titer of serum IgG antibodies against this DNA tumour virus. Serum samples were from UM affected patients and controls, represented by healthy subjects (HS) with the same mean age. Sera were analysed by indirect ELISAs employing two synthetic peptides as mimotopes/antigens belonging to the BKPyV viral capsid protein 1 (VP1) [2]. It turned out that serum samples from UM patients had a higher prevalence of BKPyV antibodies, 85% (51/60), compared to controls represented by two different groups of HS1, 62% (54/87) and HS2, 57% (68/120). The different prevalence of BKPyV antibodies detected in UM vs two control groups, HS1 and HS2, is statistically significant ($P<0.005$). Our immunologic data suggest a significant higher prevalence of antibodies against BKPyV VP1 epitopes in serum samples from UM patients compared to HS. These results indicate an association between UM and BKPyV, suggesting that this small DNA tumor virus may be a cofactor in the UM onset/progression [3].

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PRENATAL AND CHRONIC EXPOSURE TO A LOW DOSE OF BISPHENOL A IN DRINKING WATER AFFECTS SPERMATOGENESIS

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Bisphenol A (BPA) is an environmental endocrine disrupting chemical (EDC), diffused as monomer of epoxy resins and polycarbonate plastics. Itcan act as an estrogen agonist and androgen receptor antagonist, producing toxic effects on various organs and systems, such as liver, nervous, cardiovascular, immune and reproductive system. We investigated the possible effects of chronic exposure (*via* placenta first, lactation and drinking water later) to low BPA dose on heath. Pregnant Wistar rats received BPA (100µg/l) or vehicle (100µl ethanol) in drinking water during gestation and all over lactation; at weaning, newborn rats received the same treatments as dams until sex maturation. Male rats were sacrificed at 17, 45 and 60 post-natal die (pnd). Collected tissues were used to evaluate BPA levels (plasma and adipose tissue), testosterone levels, morphological analysis (liver and testis) and molecular markers (testis). Higher BPA levels than controlwere measured in plasma and adipose tissue of pups (17pnd) only. Liver morphology did not exhibit any statistically significant difference in inflammation/hypertrophy/steatosis, but body weight gain, testosterone levelsand testis morphology were affected in treated animals. Disorganization of blood testis barrier, oxidative stress damage and higher apoptosis rate were observed at 45pnd with partial rescue at sex maturation. Molecular mechanism involving the impairment of the NAD+-dependent deacetylaseSIRT1 has been demonstrated. The chronic exposure to a low BPA dose impacts the first round of spermatogenesis, probably as a consequence of BPA exposure during neonatal period.

CATALASE LEVELS REGULATE REDOX SENSITIVITY OF B CELL RECEPTOR SIGNALING AND DISTINGUISH LEUKEMIA PATIENTS WITH DIFFERENT CLINICAL COURSE

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B-cell receptor (BCR) signaling is a key determinant of variable clinical behavior and a target for therapeutic interventions in chronic lymphocytic leukemia (CLL). Endogenously produced H2O2 is thought to fine-tune the BCR signaling by reversibly inhibiting phosphatases. However, little is known about how CLL cells sense and respond to such redox cues and what impact they have on CLL. We characterized the response of BCR signaling proteins to exogenous H2O2 in cells from CLL patients using phospho-specific flow cytometry. Exogenous H2O2 in the absence of BCR engagement induced a signaling response of BCR proteins that was higher in CLL with favorable prognostic parameters and an indolent clinical course. We identified low catalase expression as a possible mechanism accounting for redox signaling hypersensitivity. Decreased catalase could cause an escalated accumulation of exogenous H2O2 in leukemic cells with a consequent greater inhibition of phosphatases and an increase of redox signaling sensitivity. Moreover, lower levels of catalase were significantly associated with a slower progression of the disease. In leukemic cells characterized by redox hypersensitivity, we also documented an elevated accumulation of ROS and an increased mitochondrial amount. Taken together, our data identified redox sensitivity and metabolic profiles that are linked to differential clinical behavior in CLL. This study advances our understanding of the redox and signaling heterogeneity of CLL and provides the rationale for the development of therapies targeting redox pathways in CLL.

ANALYSIS OF ENDOCANNABINOID SYSTEM IN RAT TESTIS DURING THE FIRST SPERMATOGENIC WAVE

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Endocannabinoids are lipid mediators, enzymatically synthesized and hydrolyzed, that bind cannabinoid receptors. Together with their receptors and metabolic enzymes, they form the "endocannabinoid system" (ECS). Anandamide (AEA) and 2-arachidonoylglyc-erol (2-AG) are the main endocannabinoids studied in testis. In this study, using the first wave of spermatogenesis as an in vivo model to verify the progressive appearance of germ cells in seminiferous tubules [i.e., spermatogonia, spermatocytes, and spermatids], we analyzed the expression of the main enzymes and receptors of ECS in rat testis. In particular, the expression profile of the main enzymes metabolizing AEA and 2-AG as well as the expression of cannabinoid receptors, CB1 and CB2, and specific markers of mitotic, meiotic, and post-meiotic germ cell appearance or activities have been analyzed by RT-PCR and appropriately correlated. Our aim was to envisage a relationship between expression of ECS components and temporal profile of germ cell appearance or activity as well as among ECS components. Results show that expres-sion of ECS components is related to germ cell progression. In particular, CB2 and 2-AG appear to be related to mitotic/meiotic stages, while CB1 and AEA appear to be related to spermatogonia stem cells activity and spermatids appearance, respectively. Our data also suggest that a functional interaction among ECS components occurs in the testis. In vitro-incubated testis show that AEA-CB2 activity affects negatively monoacylglycerol-lipase levels via upregulation of CB1 suggesting a CB1/CB2-mediated relationship between AEA and 2-AG. Finally, we provide the first evidence that CB1 is present in fetal gonocytes, during mitotic arrest.

ROLE OF SIRT1 IN THE OVARIAN ADAPTIVE RESPONSE TO METHYLGLYOXAL-INDUCED DICARBONYL STRESS

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Dicarbonyl stress is caused by an imbalance of the formation of dicarbonyl metabolites such as methylglyoxal (MG), a major precursor of advanced glycation end products (AGEs). In the ovary, dicarbonyl stress is associated with reproductive aging and polycystic ovarian syndrome (PCOS) [1]. In the present study we have investigated the possible role of the NAD(+)-dependent Class III-deacetylase SIRT1, a sensor of the redox state in oocytes and ovary [2], in the ovarian adaptive response to MG. We observed that MG-induced upregulation of glyoxalases 2 gene, a component of the MG detoxification enzymatic system, was prevented by oocyte exposure to the SIRT1 inhibitor EX527. Moreover, the inhibition of SIRT1 worsened the effects of MG on oocyte maturation rates. In order to study the ovarian response to MG in an *in vivo* model, female CD1 mice received 100 mg/kg MG by gastric administration for 28-days [3]. Western blotting analysis revealed that MG intake increased SIRT1 ovarian levels along with over-expression of catalase, superoxide dismutase 2, SIRT3, and PGC1α. Protein expression of glyoxalase 1, the main MG detoxifying enzyme, enhanced in MG mice whereas MG-AGEs remained unchanged. Although control and MG mice showed similar ovarian reserve and ovulation rate, oocytes ovulated by MG mice exhibited abnormal meiotic spindles, a condition predisposing to embryo aneuploidy. Our results suggest that MG triggers in oocytes and ovary an ovarian adaptive response involving a SIRT1 signalling and anti-glycation defence, which prevents MG-AGE accumulation, but negatively affects oocyte development.

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ANTHROPOMETRIC AND GLUCOMETABOLIC CHANGES IN AN AGED MOUSE MODEL OF LIPOCALIN-2 OVEREXPRESSION

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Lipocalin-2 (LCN2) is widely expressed in the organism with pleiotropic roles. In particular, its over-expression correlates with tissue stress conditions including inflammation, metabolic disorders, chronic diseases and cancer.
We show that LCN2-Tg mice, a transgenic mouse model with a systemic overexpression of LCN2, were smaller compared to controls but they ate and drank more and displayed a higher amount of visceral adipose tissue. Furthermore, LCN2-Tg mice with body weight ≥ 20g, showed adipocytes with a higher cell area, altered expression of genes involved in adipocyte differentiation and inflammation. In particular, mRNA levels of adipocyte-derived *Pparγ*, *Srbf1*, *Fbp4*, *Tnfα*, *Il6* and *Lep* were all increased. Furthermore, LCN2-Tg mice displayed a decreased amount of basal serum insulin and an impaired glucose tolerance and insulin sensitivity consistent with *Slc2a2* mRNA downregulated expression. On the other hand, *Insr* mRNA was upregulated and correlated with microPET analysis that demonstrated a trend in reduced whole body glucose consumption and MRGlu in muscles and a significantly reduced MRGlu in brown adipose tissue. Nevertheless, an almost nine-fold acceleration of hexokinase activity was observed in LCN2-Tg mice liver compared to controls. Moreover, AST and ALT were increased, which indicated a liver involvement also demonstrated by histological staining.
In conclusion we show that LCN2 profoundly impacts adipose tissue size and function and glucose metabolism suggesting that LCN2 should be considered as a risk factor in ageing for metabolic disorders leading to obesity.

IMMUNOLOGICAL EVIDENCE OF A STRONG ASSOCIATION BETWEEN NON-HODGKIN LYMPHOMA AND SIMIAN VIRUS 40 LARGE T ANTIGEN, THE VIRAL ONCOPROTEIN

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Association between non-Hodgkin lymphoma and Simian virus 40 (SV40) has been reported [1]. Herein, a new indirect ELISA was employed with two mimotopes from SV40 large T antigen (Tag), the viral oncoprotein, to analyse for specific reactions to antibodies in sera from non-Hodgkin lymphoma patients and controls, represented by healthy subjects (HS) and breast carcinoma (BC) patients [2]. This study allowed us to assay a new sera collection from non-Hodgkin lymphoma patients (NHL, n = 254). To verify the association between NHL and SV40 Tag, two totally independent cohorts were analysed: NHL1 n = 150 and NHL2 n = 104. The epidemiological survey included sera from HS1, n = 150; HS2, n = 104 and BC, n = 78. This new indirect ELISA revealed that antibodies against SV40 Tag mimotopes are detectable in NHL1 and NHL2 sera with a prevalence of 37 and 36%, respectively. The prevalence of SV40-antibodies detected in both NHL1 and NHL2 cohorts differs statistically from controls, at 19% for HS1 ($p < 0.01$), HS2 ($p < 0.05$) and BC patients ($p < 0.05$). This study, carried out with an immunological assay with specific Tag oncoprotein mimotopes of Simian virus 40 [2], reports the presence of IgG antibodies against the large Tumour antigen in non-Hodgkin lymphomas for the first time. Our immunological data with two independent NHL cohorts show a statistically significant association between Simian virus 40 Tag and non-Hodgkin lymphoma [3]. These results suggest that SV40-positive non-Hodgkin lymphomas could be treated differently from those tested SV40-negative.

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RARE RISK VARIANTS IDENTIFICATION BY IDENTITY-BY-DESCENT MAPPING AND WHOLE-EXOME SEQUENCING IMPLICATES NEURONAL DEVELOPMENT PATHWAYS IN SCHIZOPHRENIA AND BIPOLAR DISORDER

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Schizophrenia (SCZ) and Bipolar Disorder (BPD) are highly heritable disorders with an estimated co-heritability of 68%. Hundreds of common alleles have been implicated, but recently a role for rare, high-penetrant variants has been also suggested in both disorders. This study investigated a familial cohort of SCZ and BPD patients from a closed population sample, where the high recurrence of the disorders and the homogenous genetic background indicate a possible enrichment in rare risk alleles. A total of 230 subjects were investigated through a strategy that integrates identity-by-descent (IBD) mapping and whole-exome sequencing (WES). IBD analysis allowed to track high risk haplotypes (IBDrisk) shared exclusively by multiple patients from different families and possibly carrying the most penetrant alleles. A total of 444 non-synonymous sequence variants, of which 137 disruptive, were identified by WES in IBDrisk haplotypes. Interestingly, gene sets previously implicated in SCZ (i.e. post-synaptic density (PSD) proteins, voltage-gated calcium channels (VGCCs) and fragile X mental retardation protein (FMRP) targets) were found significantly enriched in genes carrying IBDrisk variants. Further, disruptive variants were preferentially affecting genes involved in the extracellular matrix (ECM) biology and axon guidance processes. Results thus confirm rare risk variants as key factors in SCZ and BPD pathogenesis and highlight a role for the development of neuronal connectivity in the aetiology of both disorders.

INVOLVEMENT OF DAAM1 IN ZINC PROTECTION AGAINST CADMIUM-INDUCED TOXICITY IN RAT TESTIS

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Cadmium (Cd) is a heavy metal which is a serious environmental hazard and if there is a continuous exposure, the concentration of this metal in the target tissues increases cumulatively. Testes are one of the major target organs for Cd toxicity due to their high sensitivity and the harmful effect of Cd on their function might result in lowering sperm production leading to reduce male fertility. On the other hand, several studies have reported that essential trace elements, such as Zinc (Zn) have a protective role against Cd toxicity. Here we report the protective role of Zn in testicular toxicity Cd-induced in male adult rats after gestational and lactational exposure and the effects of exposure to Cd and Zn on testicular DAAM1 gene and protein expression. DAAM1 is a protein belonging to the family formins, molecules involved in nucleation of unbranched actin filaments. Its role has been studied in cytoskeletal organization in juvenile, pre-pubertal, pubertal, and in adult rat testis. The results showed that exposure to Cd decreased the relative reproductive organ weight, altered the testicular histology, causing a significant reduction in the daily sperm production and altering the epididymal sperm quality. Furthermore, both mRNA and protein expression of DAAM1 were also inhibited in Cd-treated group. Zn supply has completely prevented most of these toxic effects. Our results imply that Zn could avoid Cd-induced testicular toxicity and sperm quality alteration, probably via the restoration of the testicular DAAM1 expression inhibited by Cd.

ROLE OF LONG NON-CODING RNAS HOTAIR, MALAT1 AND NEAT1 IN THE REGULATION OF CDK5R1 EXPRESSION AND POSSIBLE IMPLICATIONS FOR ALZHEIMER'S DISEASE PATHOGENESIS

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Cyclin-dependent kinase 5 regulatory subunit 1 (CDK5R1) gene encodes for p35, the main activator of Cyclin-dependent kinase 5 (CDK5). p35/CDK5 complex plays a key role in brain development and function, but its deregulation is associated to Alzheimer's disease (AD) onset and progression. We recently showed that miR-15/107 family can negatively regulate CDK5R1 expression through the binding to its 3'-UTR. Interestingly, miR-15/107 family is downregulated in AD brain while CDK5R1 is upregulated, suggesting a role in the pathogenesis of AD. The expression of CDK5R1 is also regulated at transcriptional level by the histone lysine specific demethylase LSD1. In particular, the expression of the neuronal isoform of LSD1, neuroLSD1 (nLSD1), causes CDK5R1 upregulation in cortical neurons.

An additional level of CDK5R1 regulation might be provided by long non-coding RNAs (lncRNAs). In this study, we focused on three lncRNAs: HOTAIR, MALAT1 and NEAT1. In order to assess their possible involvement in the regulation of CDK5R1 expression, we analyzed the effect of HOTAIR, MALAT1 and NEAT1 silencing on CDK5R1 and miR-15/107 levels, and, in the case of MALAT1, on LSD1:nLSD1 ratio in cell lines. We also measured HOTAIR, MALAT1 and NEAT1 expression levels and LSD1:nLSD1 ratio in human brain tissues of AD patients and control individuals, in order to verify whether they are deregulated in AD condition.

The elucidation of the role of HOTAIR, MALAT1 and NEAT1 in CDK5R1 regulation might add a new level of complexity to the control of CDK5R1 expression with possible implications for Alzheimer's disease pathogenesis.

NOTCH IMPAIRMENT IN KRIT1-DEFICIENT ENDOTHELIAL CELLS PROMOTES A PROATHEROGENIC PHENOTYPE

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Cerebral Cavernous Malformations (CCMs) are common cerebrovascular diseases with extremely variable clinical expressivity. CCM can cause symptoms of different severity including neurological deficits, seizures, and intracerebral hemorrhage. CCM is frequently a genetically inherited disease due to heterozygous mutations in one of three genes: CCM1 (KRIT1), CCM2, and CCM3 (PDCD10). These three proteins control endothelial cells junctions, proliferation, migration and, therefore, angiogenesis. Among the pathways controlled by CCM genes in the endothelium there is the Notch signaling system. Krit1 loss-of-function leads to downregulation of Notch activity in endothelial cells causing dysregulated angiogenesis and impaired EC-pericytes interaction, both hallmarks of CCMs [1]. Recently, it has been shown that Notch signaling is crucial in protecting endothelial cells against atherogenic factors such as dyslipidemia and inflammation [2], and it is required to mediate the atheroprotective effects of laminar shear stress on the endothelium [3]. The aim of this study was to investigate whether Notch impairment induced by Krit1 loss-of-function makes endothelial cells more susceptible to atherogenic stimuli. We show that Krit1 loss-of-function in HUVECs results in reduced levels of Notch1 and Notch4. HUVECs silenced for Krit1 are more prone to TNF-α induced apoptosis, a marker of EC dysfunction. Furthermore, treatment with a ROS scavenger reversed both the effect on Notch levels and on apoptosis. Taken together, our results suggest that Krit1 deficiency may increase susceptibility to atherosclerotic stimuli by disrupting the Notch pathway through a ROS-mediated mechanism.

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ROLE OF TGF-β1/miR-382-5p/SOD2 AXIS IN THE INDUCTION OF OXIDATIVE STRESS IN CD34+ CELLS FROM PRIMARY MYELOFIBROSIS

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Primary Myelofibrosis (PMF) is a myeloproliferative neoplasm characterized by an excessive production of pro-inflammatory cytokines resulting in chronic inflammation and genomic instability. Beside the driver mutations in JAK2, MPL and CALR genes, the deregulation of miRNA expression may also contribute to PMF pathogenesis. Recently, we reported the upregulation of miR-382-5p in PMF CD34+ cells. In order to unveil the role of miR-382-5p in PMF pathogenesis, we performed gene expression profiling of miR-382-5p-overexpressing CD34+ cells. Among the downregulated genes, we identified superoxide dismutase 2 (SOD2), which is a predicted target of miR-382-5p. Subsequently, we confirmed miR-382-5p/SOD2 interaction by luciferase assay and we showed that miR-382-5p overexpression in CD34+ cells causes the decrease of SOD2 activity leading to reactive oxygen species (ROS) accumulation and oxidative DNA damage. Furthermore, miR-382-5p inhibition in PMF CD34+ cells restores SOD2 function, induces ROS disposal and reduces DNA oxidation. Since the pro-inflammatory cytokine transforming growth factor-β1 (TGF-β1) is a key player in PMF pathogenesis, we further investigated the effect of TGF-β1 on ROS and miR-382-5p levels. We showed that TGF-β1 treatment enhances miR-382-5p expression and ROS accumulation by reducing SOD2 activity. Finally, inhibition of TGF-β1 signaling in PMF CD34+ cells by Galunisertib significantly reduced miR-382-5p expression and ROS accumulation and restored SOD2 activity.
Overall, we report that TGF-β1/miR-382-5p/SOD2 axis deregulation in PMF cells is linked to ROS overproduction that may contribute to enhanced oxidative stress and inflammation. Our results suggest that Galunisertib may represent an effective drug reducing abnormal oxidative stress induced by TGF-β1 in PMF patients.

IN ADDENDUM

RICTOR/mTORC2 DEFICIENCY ENHANCES KERATINOCYTE STRESS TOLERANCE VIA MITOHORMESIS

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How metabolic pathways required for epidermal tissue growth and remodeling influence the ability of keratinocytes to survive stressful conditions is still largely unknown. The mechanistic target of rapamycin complex 2 (mTORC2) regulates growth and metabolism of several tissues, but its functions in epidermal cells are poorly defined. Rictor is an adaptor protein essential for mTORC2 activity. To explore the roles of mTORC2 in the epidermis, we have conditionally deleted rictor in mice via K14-Cre mediated homologous recombination and found that its deficiency causes moderate tissue hypoplasia, reduced keratinocyte proliferation and attenuated hyperplastic response to TPA. Noteworthy, rictor-deficient keratinocytes displayed increased lifespan, protection from senescence, and enhanced tolerance to cellular stressors such as growth factors deprivation, epirubicin and X-ray in vitro and radioresistance in vivo. Rictor-deficient keratinocytes exhibited changes in global gene expression profiles consistent with metabolic alterations and enhanced stress tolerance, a shift in cell catabolic processes from glycids and lipids to glutamine consumption and increased production of mitochondrial reactive oxygen species (ROS). Mechanistically, the resiliency of rictor-deficient epidermal cells relies on these ROS increases, indicating stress resistance via mitohormesis. Thus, our findings reveal a new link between metabolic changes and stress adaptation of keratinocytes centered on mTORC2 activity, with potential implications in skin aging and therapeutic resistance of epithelial tumors.

NEW BIOLOGICAL ROLE OF THE PLASMATIC AND SALIVARY LEVELS OF IL-1β, IL-18 AND IL-6: ASSOCIATION TO EMOTIONAL DIFFERENCE DURING STRESS IN YOUNG MALE

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Saliva collection is considered a non-invasive method to detect inflammatory markers in response to emotional states within natural social contexts. Numerous studies have prompted an important role of cytokines in modulating distinct aspects of social and emotional behavior. The aim of this study was to investigate the reliability of plasma and saliva as investigative tools for measure some inflammatory marker levels (CRP, IL-1β, IL-18, and IL-6). At the same time, the relationships between these markers and emotional states in response to a socio-cognitive stress (Academic Exam, AE), were considered. It was demonstrated that the plasma and saliva concentrations of all immune-mediators analyzed were significantly related across the socio-cognitive stress. In addition, when there was a close correlation to AE, the anger state, the IL-1β, IL-18 salivary and plasmatic concentrations were significantly higher, while they decreased during the AE. On the other hand, the anxiety state and the IL-6 levels significantly increased throughout the AE. The IL-1β and IL-6 were positively associated to the anger and the anxiety state, respectively. In conclusion, our data highlight that different immune markers are similarly detectable in plasma and saliva during socio-cognitive stress. Also, they could be related to different emotional responses.

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CON IL CONTRIBUTO DI :

Università degli Studi di Ferrara

L'AIBG RINGRAZIA L'UNIVERSITA' DEGLI STUDI DI FERRARA PER AVER CONTRIBUITO ALLA REALIZZAZIONE DEL CONGRESSO