Meeting annuale dei Gruppi Membrane, Nutrizione e Biologia computazionale e dei sistemi della SIB

25-26 Giugno
Sala Ulisse dell'Accademia delle Scienze
via Zamboni, 31, Bologna

Bio-energetics, Metabolism and Nutrition: from molecules to systems

Program and Abstract book

Comitato Scientifico: Vito De Pinto (CT), Silvana Hrelia (BO), Pier Luigi Martelli (BO), Alessandra Baracca (BO), Rita Casadio (BO), Angela Messina (CT), Giancarlo Solaini (BO)

Comitato Organizzatore: Vito De Pinto (CT), Silvana Hrelia (BO), Pier Luigi Martelli (BO), Alessandra Baracca (BO), Andrea Magrì (CT), Salvatore Nesci (BO), Castrese Savoiardo (BO), Giancarlo Solaini (BO)

Program

Monday 25 June 2018

9,30 Registration

10,30 Opening Address

10,45 Plenary Lecture

Chair: Rita Casadio

Lilia Alberghina (Milano Bicocca)
Mitochondria and metabolism: their time has come again!

Session 1: Structure, function, system biology of membrane proteins

Chair: Pier Luigi Martelli

11,30 Daniela Gaglio (CNR-Roma)
Building systems metabolomics 1: Biological challenges which will advance by using GC/LC mass spectrometry metabolome analysis

11,45 Chiara Damiani (Milano Bicocca)
Building system metabolomics 2: constraint-based modeling to identify patterns and rules of metabolics wirings

12,00 Federica Zinghirino (Catania)
Analysis of NRF1 and HIFS role in trascriptional regulation of VDAC isoforms under metabolic stress conditions

12,15 Salvatore Nesci (Bologna)
The ATP Synthase membrane-embedded domain: structural implications in health and disease

12,30 Michele Galluccio (Cosenza)
Structure modeling of the OCTN1 transporter: validation by site-direct mutagenesis

12,45 Maria Gaetana G. Pittalà (Catania)
Characterization of post-translational modifications of VDAC isoforms from rat-liver mitochondria by high-resolution mass spectrometry

13,00 Lunch break

14,30 Plenary Lecture

Chair: Silvana Hrelia

Paola Antonia Corsetto (Milano)
Nutrition and fasting in the prevention and treatment of cancer

Session 2: Natural substances and nutrition: from biochemistry to pathology

Chair: Silvana Hrelia

15,15 Eleonora Da Pozzo (Pisa)
Anti-oxidant and anti-senescence effects of Bergamot juice

15,30 Sofia Pugnaloni (Ancona)
Nutritional quality of whole grains: polyphenol content and glycemic load of Senatore Cappelli durum weath pasta

15,45 Enea Felrizza (Bologna)
Biochemical characterization of Arthrospira spp used as nutritional supplement

16,00 Coffee break

16,30 Laura Giusti (Pisa)
A proteomic approach to study the neuroprotective effect of oleocanthal in SH-SY5Y cells

16,45 Simona Daniele (Pisa)
Tumor Necrosis Factor ✔ regulates GRK2 turnover through the E3 ubiquitin ligase Mdm2 and supports osteogenesis

17,00 Tatiana Carrozzini (Milano-Bicocca)
To investigate the protective effect of phyto-complexes against oxidative strees in a cellular model of stroke

17,15 Deborah Pietrobono (Pisa)
Human glioblastoma cell apoptosis is induced by Rosemary officinalis through the p53 functional reactivation

17,30 Marco Necci (Padova)
PhytoTypeDB, a database for plant protein function and variability

Tuesday 26 June 2018

10,00 Plenary Lecture

Chair: Vito De Pinto

Cesare Indiveri (Calabria)
The intriguing amino acid transporter, LAT1: relevance to human health and drug discovery

Session 3: Membrane proteins in action

Chair: Giancarlo Solaini

10,45 Anna Costanzini (Bologna)
Role of the F1F0-ATPase Inhibitor IF1 in osteosarcoma cells under anoxic conditions

11,00 Cecilia Prata (Bologna)
Sulforaphane influences AQP8-linked redox signaling in a leukemic cell line

11,15 Coffee break

11,30 Lorena Pochini (Cosenza)
Regulatory aspects of the human organic cation transporter OCTN1 (SLC22A4)

12,00 Maria Tolomeo (Bari)
Altered expression of SLC52A members in human cancer

12,15 Maura Samarani (Milano)
A Lyposome-plasma membrane-sphingolipid axix linking lysosomal storage to cell growth arrest

12,30 Stefano Conti Nibali (Catania)
Protein purification's method affects the electrophysiological features of yeast VDAC2

12,45 Lunch Break

14,15 Plenary Lecture

Chair: Alessandra Baracca

Sabina Passamonti (Trieste)
Membrane transport of bilirubin and flavonoids: from kinetics to diet

Session 4: Nutrition and pathologies

Chair: Cristina Angeloni

15,00 Livia Cabitta (Milano)
Identification of the antigen recognized by RHIGM22, a remyelination-ptomoting human monoclonal antibody and his effect on glia cells

15,15 Chiara Giacomelli (Pisa)
Human gingival mesenchymal stem cell trophism is modulated by inflammatory microenvironment: effects of Ribes nigrum bud extract

15,30 Sonila Alia (Marche)
Taste sensitivity and body weigth: is there a link?

15,45 Elisa Boschetti (Bologna)
Intestinal epithelial barrier abnormalities in patients with chronic intestinal pseudo-obstruction

16,00 Giulia Frisco (Bologna)
Lactobacillus crispatus interferes with Chlamydia trachomatis infectivity through modulation of integrin exposure in cervical cells

16,15 Natalia Calonghi (Bologna)
Effect of 9-Hydroxy-stearic acid on glucose metabolism in a human colon cancer cell line

16,30 Antonina Orlando (Milano-Bicocca)
Evaluation of endothelial dysfunction markers in children with cardiovascular risk factors: obesity and/or Hypertension

16,45 Cristiana Caliceti (Bologna)
Is NOTCH involved in proteome effects of estrogen on endothelial function and angiogenesis?

End of the meeting

Meeting annuale dei Gruppi Membrane, Nutrizione e Biologia computazionale e dei sistemi della SIB

Bologna 25-26 Giugno, Accademia delle Scienze

Bio-energetics, Metabolism and Nutrition: from molecules to systems

ABSTRACT BOOK

PLENARY LECTURE

Mitochondria and metabolism: their time has come again!

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Session 1: Structure, function, system biology of membrane proteins

Building systems metabolomics 1: Biological challenges which will advance by using GC/LC mass spectrometry metabolome analysis

Daniela Gaglio[1,2], Marcella Bonanomi[1,3], Gloria Campioni[1,3], Giuseppina Votta[1,2], Marco Vanoni[1,3], Lilia Albergina[1,3]

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Metabolomics has been established as a robust tool describing complex biological networks and accurately the functional and physiological states of an organism. The metabolome is the set of all small molecules, such as amino acids, sugars and lipids, in a biological system. It is considered to be an endpoint of biological processes and carries an imprint of all genetic, epigenetic and environmental factors. Aiming at in-depth characterization of complex metabolite mixtures, the recent technological developments in the field of metabolomics have opened up a wide range of research fields: in biological, biomedical, environmental and nutritional research. In biomedical research, metabolomics is a key technique for systems biology, disease diagnostics and biomarkers especially for cancer staging, prediction of recurrence, prognosis and treatment selection. In fact is a powerful tool able to identify the main cancer metabolic alterations in tumors, aiming at targeting the cancer metabolic rewiring [1] as novel and personalized therapeutic approaches as opposite to current standard treatments. Moreover, metabolomics dataset can be used in silico linking the metabolic fluxes [2] and predictive metabolic models [3] able to investigate metabolic alterations of complex human diseases.

[1] Gaglio et al, Oncotarget. 2016 Aug 9;7(32):52017-52031;
[2] Gaglio et al, Mol Syst Biol. 2011 Aug 16;7:523;
[3] Damiani et al, PLoS Comput Biol. 2017 Sep 28;13(9):e1005758.

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Building system metabolomics 2: constraint-based modeling to identify patterns and rules of metabolic wirings

Chiara Damiani[1,2], Davide Maspero[3], Marzia Di Filippo[1,3], Dario Pescini[1,4], Giancarlo Mauri[1,3], Hans V. Westerhoff[5,6], Marco Vanoni[1,3], Lilia Alberghina[1,3]

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A focus on metabolism, which provides a functional readout of cellular biochemistry, is anticipated to clarify the mechanisms of complex diseases, leading to better diagnosis and treatment than genomics or proteomics alone. Regrettably, current metabolomics technologies often portray the average behavior of intermixed heterogeneous subpopulations, overlooking the internal interactions and differences within a cell population, taken for example from cancer biopsies or tumors-on-chips, that may be crucial for facilitating the disease progression. Along with heterogeneous genetic and epigenetic factors, variations in the tumor microenvironment contribute indeed to intra-tumor heterogeneity, leading to a complex cancer population architecture in which differently specialized cells exchange nutrients and cooperate to mass growth, by activating complex networks of interactions. Despite major advances in single-cell sequencing, single-cell metabolic analyses are lagging behind. To bridge this gap, we present a computational framework to characterize metabolism at the single cell level, by integrating bulk metabolomics and single-cell transcriptomics data, representative of different levels of a cell population. We exploit constraint-based modeling to simulate a set of replicates of a metabolic network of human central carbon metabolism, corresponding to different cells, which may interact by exchanging metabolites, given nutritional constraints on the total population. By simulating scenarios in which lactate cannot be fully released in plasma but can be exchanged in the tumor micro-environment, our methodology proved able to reproduce the existence of a phenomenon known as “reverse Warburg effect”, according to which tumor stromal cells aerobically produce lactate, which is used as carbon source by adjacent high proliferative cancer cells. Other scenarios may be simulated with our approach, by exploiting single-cell experimental information to specifically constrain the boundaries for the fluxes of the single cells in the population, instead of letting them free to adjust their consumption rates according to linear programming optimization. Single-cell fluxes are computed as a function of the transcriptome at the cell level, while assuring biomass formation at the population level. We integrated the gene expression profiles of dozens of individual tumor cells isolated from mouse xenografts derived from lung adenocarcinoma patients. We were able to characterize the existence of a more aggressive subpopulation in terms of metabolic growth rate.

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Analysis of NRF1 and HIFs role in transcriptional regulation of VDAC isoforms under metabolic stress conditions

Federica Zinghirino, Vito De Pinto, Lia Mela, Francesca Guarino
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Voltage-dependent Anion Selective Channels (VDAC) are a family of pore-forming proteins that play a crucial role in transport of ions and small metabolites through the mitochondrial outer membrane. In mammals there are three isoforms of VDAC (VDAC1, VDAC2 and VDAC3), encoded by three different genes [1]. To investigate on the role of VDAC isoforms in mitochondrial dysfunction and adaptation during metabolic stress, we analyzed their expression and regulation by inducing hypoxia and nutrient deprivation that are common features in pathologies such as cancer and neurodegenerative diseases. Interestingly, VDAC1 and VDAC2 expression levels increased in a time related manner while VDAC3 transcript levels remained unchanged or were slightly down-regulated. Using a bioinformatic approach, we performed a prediction analysis of transcription factor binding sites on VDAC promoters. We focused our attention on binding sites specifically recognized by NRF1 and HIFs factors, respectively involved in mitochondrial biogenesis induced by nutrient depletion [2] and in cell response to low level of O2 (hypoxia) [3]. We found that on all three promoters analyzed there are NRF1 putative binding sites but with a major numerical distribution and statistical significance on VDAC3 promoter sequence. Instead, the HIFs factor binding sites have been found only on VDAC1 and VDAC2 promoters. There are also several regulative modules formed by other factors such as CREB, ETS, SP1 that are involved in mitochondrial biogenesis and in response to oxidative stress. Furthermore, we found that on VDAC3 promoter there are binding sites for transcriptional repressors adjacent to NRF1 binding sites. Thus, we hypothesized a different transcriptional regulation of VDAC3 probably correlated with other factors that could repress the NRF1 activation or compete with its overlapping binding sites identified on the sequence. In conclusion, with this predictive analysis we identified, on VDAC promoters, the binding sites for NRF1 and HIFs that could be the regulators of VDACs expression in response to metabolic stress conditions. This suggests that VDAC proteins could have an important role in mitochondrial dysfunction and adaptation.

[1] Messina et al, Biochim Biophys Acta (2012) 1818, 1466-1476;
[2] Scarpulla, Biochim Biophys Acta (2012) 1819, 1088-97;
[3] Iommarini et al, Front Oncol (2017) 7:286.

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The ATP Synthase membrane-embedded domain: structural implication in health and disease

Salvatore Nesci, Fabiana Trombetti, Vittoria Ventrella, Cristina Algieri, Alessandra Pagliarani
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The mitochondrial F1FO-ATP synthase/ATPase is a bifunctional enzyme complex arranged in two main domains: the hydrophilic F1, protruding in the matrix and capable of ATP synthesis/hydrolysis, and the membrane-embedded FO which translocates H+ through the inner mitochondrial membrane (IMM). The H+ translocation coupled to ATP catalysis involves two asymmetric and discontinuous half-channels, located on horizontal α-helices of a subunit [1] and opening on either IMM sides. The equally spaced arrangement of crucial aminoacids involved in the H+ uptake/release in a subunit [2] guarantees the H+ flow across the IMM, whose direction is driven by the c-ring rotation [3] and allows the accommodation of differently-sized c-rings. The holoenzyme forms FO dimers, which bend the IMM to yield its characteristic curvature at the apex of cristae [4] and ensure mitochondrial bioenergetics. Conversely, the dimer dissociation impairs oxidative phosphorylation and forms the mitochondrial permeability transition pore between the detached monomers [5], thus initiating regulated cell death. FO is targeted by endogenous and exogenous modulators [6], which through post-translational modifications of crucial aminoacids [7], change the enzyme features. Indeed, any structural change which affects the H+ translocation mechanism and/or the dimer assembly impacts on mitochondrial efficiency and constitute a risk factor not only for mitochondrial diseases but also for a variety of pathologies in which mitochondrial dysfunctions are involved. So, FO emerges as molecular link between the (micro)environment and mitochondrial bioenergetics and a promising target for innovative drugs [6].

[1] Allegretti et al, 2015, Nature 521(7551):237–240;
[2] Srivastava et al, 2018, Science 360(6389);
[3] Nesci et al, 2015, J Membr Biol 248(2):163–169;
[4] Guo et al, 2017, Science 358(6365):936–940;
[5] Nesci, 2018, Trends Biochem Sci 43(5):311–313;
[6] Pagliarani et al, 2016, Mini Rev Med Chem 16(10):815–824.
[7] Nesci et a, 2017, Biochim Biophys Acta 1861(11 Pt A):2902–2912.

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Structure modeling of the OCTN1 transporter: validation by site-direct mutagenesis

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The Organic Cation Transporter Novel 1 (OCTN1) is a member of the SLC22 family involved in cation transport at plasma membrane level. Even though its physiological substrate is still under debate, its involvement in inflammatory pathways related to non-neuronal acetylcholine system is acknowledged [1]. To date, using different approaches and templates, homology models have been built by different research groups to gain insights in the structure/function relationships. However, the presence in the OCTN1 sequence of a large extracellular hydrophilic loop between the first and the second transmembrane domains, impairs the modeling due to the absence of a corresponding loop in any of the possible templates present in the PDB database. Modeller 9.19 software, I-TASSER and Phyre2 server have been used to model OCTN1 using as different template the glycerol-3-phosphate transporter from E. coli (1PW4), the high-affinity phosphate transporter (PiPT) from Piriformospora indica (4J05) or the human GLUT3 transporter (4ZW9). To validate the models, site-directed mutagenesis studies have been performed by exploiting the seven Cys residues of the protein and their reactivity toward hydrophobic or hydrophilic thiol specific reagents. Indeed four of the Cysteines are present in the large hydrophilic loop and three in the transmembrane domains. cDNA encoding for the OCTN1 protein has been codon optimized according to E. coli genome and cloned under the control of the tac promoter into the pH6EX3 plasmid. E. coli Lemo21(DE3) strain has been cultured at 28 °C for 6 hours in presence of 0.4 mM IPTG in order to over-express the protein of interest that has been purified by affinity chromatography. Site-directed mutagenesis has been performed substituting each of the seven Cys residues to Ala. The effect of –SH reagents, such as MTSEA, have been tested for the reactivity towards OCTN1 wt and Cys/Ala mutants. The data obtained are consistent with the homology model obtained by Phyre2 server.

[1] Pochini et al, Biochim Biophys Acta, 1818 (2012) 559-565.

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Characterization of post-translational modifications of VDAC isoforms from rat liver mitochondria by high-resolution mass spectrometry

Maria Gaetana G. Pittalà[1], Rosaria Saletti[2], Pierpaolo Risiglione[1], Salvatore Antonio M. Cubisino[1], Salvatore Foti[2], Vito De Pinto[1]

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Voltage-dependent anion selective channels (VDAC1, VDAC2, VDAC3) are integral membrane proteins found in the mitochondrial outer membrane whose primary function is to permit the communication and exchange of molecules related to the mitochondrial functions. The outer membrane can collect signals from and to mitochondria related to bioenergetics metabolism, to the dynamics of the organelle with its fusion/fission processes, to the quality control of the organelle itself. However there are only rare and limited information about the molecules involved in this role of switch between the in and out of mitochondria. The present work is part of a research line concerning the structural characterization of the VDAC proteins. We have recently reported about the peculiar over-oxidation of cysteines from VDAC3 rat liver mitochondria [1] and now we have extended the analysis to the other two isoforms VDAC1 and VDAC2 [2]. In particular, we have focused on the sequence analysis and some of their post-translational modifications (PTM). We have found also in these proteins, as for VDAC3, over-oxidation of cysteines. In fact, since the intermembrane space is a strongly oxidizing area of the cell, we wanted to see if this could modify the redox state of these proteins. The attention was therefore focused on the study of the oxidation state of the methionine and cysteine residues which, among all the amino acids, are the most easily susceptible to oxidation. Furthermore, serine, threonine and tyrosine phosphorylation and the cysteine succinations were studied. Interestingly, cysteine over-oxidation appears to be an exclusive feature of VDACs, since it is not present in other transmembrane mitochondrial proteins eluted by hydroxyapatite [2]. The analyses were performed by tryptic and chymotryptic proteolysis and high-resolution nano UHPLC/nano ESI-MS/MS and the results were submitted to bioinformatics research. We speculate that such modifications could have a signaling meaning, in the sense that they could show a modified interface between the external surface of the organelle and cytosol resident systems [3]. The assignment of a functional role to these modifications of VDACs will be a further step towards the full understanding of the roles of these proteins in the cell.

[1] Saletti et al, Biochim Biophys Acta. 2016, 1859, 301-311.
[2] Saletti et al, Biochim Biophys Acta. 2018, in press.
[3] Reina et al, Oncotarget. 2016, Jan 19;7(3):2249-68.

PLENARY LECTURE

Nutrition and fasting in the prevention and treatment of cancer

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Genetic and environmental factors, but also dietary habits, play fundamental role in cancer onset, progression and prognosis. Nutrient starvation is a promising approach to reduce metabolite availability to tumours cells, impairing the rapid synthesis of new intracellular components, such as lipid membranes and enzymatic or structural proteins. Several studies have suggested that cycles of prolonged fasting or of fasting-mimicking diets (FMDs) enhance the activity and the tolerance of chemo- and radio- therapy in preclinical cancer models. Nutrient starvation strongly increases the antitumor activity of tyrosine kinase inhibitors in mice carrying human tumour xenografts to block signalling via the pro-tumorigenic mitogen-activated protein kinase cascade. Fasting or short-term starvation decreases circulating insulin-like growth factor 1 (IGF-1), that is crucial for the establishment of the differential stress sensitization, a condition that makes cancer cells, but not normal cells, sensible to cytotoxic drugs. Moreover, fasting also down-regulates the mechanistic target of rapamycin (mTOR). mTOR is a metabolic regulator on which signalling pathways dependent on IGF-1, glucose, and amino acids converge to regulate growth and autophagy and which has a central role in cancer proliferation FMDs may enhance the chemotherapy efficacy by tumour immunogenicity increase, in part by an HO-1-dependent mechanism, which induces the recruitment of cytotoxic CD8+ T cells to the tumour, and reduces tumour-associated Tregs. Breast cancer is very intricate disease due to its heterogeneous nature and there is a positive association between obesity and breast cancer mortality. One subtype is Triple Negative Breast Cancer (TNBC), which is deficient in the estrogen receptor α, progesterone receptor, and human epidermal growth factor receptor expression. TNBC has an aggressive clinical behavior and is extremely metastatic. Since there is a complex network between exogenous nutrients and cancer abnormal metabolism, we have evaluated, as preclinical data, the effects of nutrient deprivation on cell migration and lipid metabolism in MDA-MB-231 cell line derived from TNBC; we assessed the consequences of medium glucose, glutamine and serum reductions on cell viability, migration and Epithelial Mesenchymal Transition. Moreover, we evaluated the effect on lipid phenotype by lipidomic approach. The results obtained indicate that nutrient restrictions reduce cell viability and tumor cell migration greatly influencing the lipid pattern of MDA-MB-231cells. We measured total, phospholipid and neutral lipid fatty acids, especially arachidonic (AA) and eicosapentaenoic (EPA) acids that are precursors of eicosanoids involved in the cross-talk between cancer cells and immune cells. The data suggest significant changes in lipid composition, especially in omega-6/omega-3. Moreover, we have observed alterations of triglyceride and sterol content. In conclusion, nutrient deprivation influences lipid phenotype and invasiveness of TNBC cells suggesting a possible future clinical approach for the prevention and treatment of breast adenocarcinoma, although further studies and clinical trials are needed to demonstrate its efficacy.

Session 2: Natural substances and nutrition: from biochemistry to pathology

Anti-oxidant and anti-senescence effects of Bergamot juice

Eleonora Da Pozzo[1,2], Marinella De Leo[1,2], Immacolata Faraone[3], Luigi Milella[3], Chiara Cavallini[1,2], Eugenia Piragine[1,2], Lara Testai[1,2], Vincenzo Calderone[1,2], Luisa Pistelli[1,2], Alessandra Braca[1,2], and Claudia Martini[1,2]

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Aging is one of the main risk factor for the onset of cardiovascular diseases; one of the possible explanations could be linked to the age-associated over-production of free radicals. This increase of oxidative stress can be overcome with a high intake of food antioxidants. In this context, a number of studies have been addressed to assess the anti-aging potential of natural antioxidant compounds. Recently, it has been shown that the juice of bergamot (Citrus bergamia Risso et Poiteau), a fruit mostly produced in the Ionian coastal areas of Southern Italy (Calabria), is a valuable source of health promoting constituents with, among other, antioxidant properties. In order to investigate the potential anti-aging effects of this Mediterranean natural antioxidant source, bergamot juices of three different cultivars ('Fantastico', 'Femminello' and 'Castagnaro') were herein characterized by the mean of high performance liquid chromatography-photodiode array-electrospray ionization-tandem mass spectrometry. Then, juices were investigated for the evaluation of total polyphenolic and flavonoid contents, cell free model antioxidant activities and in vitro anti-aging properties on two different cellular models of induced myocardial senescence. The best performing juice was also assessed in vivo. The phytochemical profiles confirmed that juices were rich in flavonoids, both flavone and flavanone glycosides. In addition, two limonoid glycosides were also identified in all cultivars. Each cultivar showed different phenolic and flavonoid contents. In tube results showed the juice robust antioxidant activities that correlate with their phenolic and flavonoid contents. Moreover, for the first time, the ability of juice to counteract the chemical-induced senescence was here demonstrated in both cellular models. Lastly, the in vivo data obtained from mice hearts evidenced an increase in transcription of genes involved in anti-aging and anti-oxidant responses. The overall results suggest that Bergamot juice exerts anti-oxidant and anti-senescence effects, making it useful for nutraceutical purposes.

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Nutritional quality of the whole grains: polyphenol content and glycemic load of Senatore Cappelli durum wheat pasta

Sofia Pugnaloni[1], Sonila Alia[1], Arianna Vignini[1], Tiziana Bacchetti[2], Marcello Gabrielli[3], Eleonora Salvolini[1], Gianna Ferretti[1], Laura Mazzanti[1]

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Pasta is one of the best parts of the Mediterranean diet. UNESCO recognized the Mediterranean diet as an Intangible Cultural Heritage of Humanity in November 2010. As a result, research regarding its potential health benefits has received considerable attention in the last decades. Pasta and bread, while often present in the dietary Italian habits, are consumed mainly in their refined forms. Several studies encouraged to consume whole grain because of its high levels of vitamins, dietary fiber and bioactive compounds (polyphenols) with antioxidant and anti-inflammatory properties. Evidences from different studies show a positive association between refined carbohydrates and insulin resistance. The Glycaemic Index (GI) has been extensively studied as an indicator of the physiological effects of a carbohydrate meal with applications in the management and prevention of diabetes and obesity. The aim of the present study was to evaluate the properties (such as glycemic index and polyphenol content) of a pasta obtained using Senatore Cappelli Durum Wheat. It is an Autumnal cultivar of durum wheat (Triticum durum), obtained at the beginning of the last century by the geneticist Nazareno Strampelli. We obtained the following information related to the total polyphenol and flavonoids contents in the studied pasta sample using the spectrophotometric method: total polyphenol=113.5 mg/100 g, flavonoids=52.96 mg/100 g. A standard assay was performed to measure the GI of two significant sources of carbohydrates following the World Health Organization (WHO) recommended methodology, determining the incremental area under the blood glucose response curve of a 50 g carbohydrate portion of the test food compared to the same amount of carbohydrate from a glucose solution by the same subject measured in capillary whole blood before and 15, 30, 45, 60, 90 and 120 minutes after ingestion in a total of 14 healthy adult volunteers (6 males and 8 females). Two formats of the same pasta (providing 50 g available carbohydrate) were tested in order to enable comparison of the resulting GI values, and to determine whether the different formats gave the same result. The following results were obtained: long format pasta (spaghetti)=47.9, short format pasta=68.5. Our study confirms the low glycemic index of pasta made from durum wheat, and it has also highlighted one of the multiple factors affecting the GI of a food, its physical form, which illustrates the importance of measuring the GI values of foods rather than applying values from foods of similar description. Such information is useful to researchers interested in calculating the GI in dietary surveys to study diet-disease relationships, and in the planning of dietary intervention studies, in order to have a clear idea of the GI of the intervention diets. It also provides valuable data for practitioners who have responsibility for advising individuals on their diet.

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Biochemical characterization of Arthrospira spp used as nutritional supplement

Enea Ferlizza[1], Giulia Andreani[2], Martina Bertocchi[2], Giorgio Fedrizzi[1], Gloria Isani[2]
1 Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna; 2 Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Bologna, Italy

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For centuries, native populations from Mexico used the cyanobacteria Arthrospira as food. Nowadays, this microorganism is globally recognized as a source of chemical constituents with wide applications in different fields, including human and animal nutrition as well as nutraceutical, cosmeceutical, and pharmaceutical industries. In fact, Arthrospira shows high concentrations of proteins, vitamins and minerals, in particular essential trace elements. In the last decade, the interest towards cyanobacteria as possible nutraceuticals and functional food grew exponentially, leading to massive commercialization of Arthrospira-based food supplements under the name of Spirulina. In consideration of the increasing popularity of these products and the associated quality problems, the purpose of this preliminary research was to analyse trace metal concentrations and to isolate proteins and pigments in commercial samples of Arthrospira spp used for human and animal nutritional supplementation. Samples of Arthrospira maxima and Arthrospira platensis were obtained from the market. Nineteen essential and non-essential trace elements (Mn, Fe, Co, Cu, Zn, Se, Mo, Cr, V, Pb, Cd, Hg, As, Al, Ag, Ni, TI, U, Sb) were analysed by ICP-MS. Cytosolic proteins were extracted and a molecular exclusion chromatography on Sephadex G-75 was subsequently performed to separate different protein fractions. In the resulting fractions total proteins and pigments were determined, as well as Fe, Zn and Cu. The analyses by ICP-MS revealed high concentrations of essential trace elements in the examined samples; in particular, Fe concentrations ranged from 432 to 576 μg/g dry weight resulting one order of magnitude higher than those generally found in foods of animal and plant origin. Hg concentrations were lower than 0.05 μg/g dry weight, while Al concentrations showed wide variations (4-175 μg/g dry weight) and raised concern due to high values found in some samples. Regarding cytosolic proteins, all the samples showed a peak containing the blue protein phycocyanin; among the different application of this protein, the possible use in medicine and biology has recently attracted increasing attention due to its antioxidant [1] and anti-inflammatory [2] properties. A second peak was present at the low molecular weights fractions, related to the presence of small peptides and free amino acids, including the so-called mycosporin-like aminoacids [3]. In conclusion, due to the abundance of interesting bioactive molecules and essential trace elements, Arthrospira-based supplements deserve more attention in future studies.

[1] Fernández-Rojas et al, 2014, J Funct. Food 11: 375-392;
[2] Qian et al, 2016, E-CAM, ID 7803846;
[3] Chrapusta et al, 2017, Mar. Drugs 15: 1-29.

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A proteomic approach to study the neuroprotective effect of oleocanthal in SH-SY5Y cells

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Olive leaves and virgin olive oil contain many phenolics effective against aging and several lifestyle-related diseases, including neurodegeneration, both in animal models and in humans. Oleocanthal is a secoiridoid, one of the most represented class of phenols in olive oil, and it is responsible of the stinging effect at pharynx level perceived after extra virgin olive oil ingestion. Recently, different studies demonstrated that oleocanthal possesses anti-aggregation activities on tau protein and Ibuprofen-like activity thanks to its ability to inhibits COX-1 and COX-2. The aim of this work is to investigate the neuroprotective effect of oleocanthal in neuron-like SH-SY5Y cells before and after oxidative stress induced by H2O2. Using 2DE coupled to mass spectrometry the protein maps for different conditions of treatment have been obtained and analyzed by Same spots (TotalLab). PCR analyses were performed to validate proteomic results. Seventeen spots resulted significantly differentially expressed with respect to control after treatment with hydrogen peroxide, twenty-seven after treatment with oleocanthal (10 μM) followed by hydrogen peroxide, while two spots for direct effect of oleocanthal. Spots of interest were excised and identified by LC/MS/MS. Oleocanthal significantly reverted the down-regulation induced by hydrogen peroxide of 26S proteasome non-ATPase regulatory subunit 1, proteasome subunit beta type-4, Ubiquitin carboxyl-terminal hydrolase and Pyruvate kinase, moreover it increased the expression of Heat shock protein HSP 90-beta and Protein DJ1. Moreover, 10 μM oleocanthal was able to counteract oxidative stress induced by H2O2 in SH-SY5Y as measured by MTT viability assay and to increase reduced-GSH level both in the absence and in the presence of H2O2 as measured by monochlorobimane assay. Our findings suggest that oleocanthal may have beneficial health effect in counteracting neurodegeneration.

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Tumor Necrosis Factor α regulates GRK2 turnover through the E3 ubiquitin ligase Mdm2 and supports osteogenesis

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Tumor Necrosis Factor alpha (TNF-α) is involved in bone healing by affecting mesenchymal stem cell (MSC) proliferation and differentiation in a dose- and time-dependent manner [1,2]. In the bone cell, TNF-α affects the expression and functionality of different G protein-coupled receptors (GPCRs), expressed on MSC membranes, and of their intracellular regulatory proteins, GPCR-regulated kinases (GRKs) [3], thus dictating their final biological outcome in controlling bone anabolic processes. Among these GPCRs, a primary role in osteogenesis has been emerging for the A2B adenosine receptor (A2BAR), a Gs-coupled receptor that triggers MSC differentiation into osteoblasts [4,5]. Herein, the effects of TNF-α were investigated in particular on the expression/responsiveness of the A2BAR, in order to investigate the functional consequences of the receptor modulation on MSC differentiation. To this purpose, MSCs were incubated with TNF-α and cellular differentiation was monitored by both Real-time PCR and fluorescence analyses of osteogenic markers. Low TNF-α concentrations showed a pro-differentiating effect on MSCs, promoting the osteoblast phenotype. In particular, the cytokine reduced A2BAR desensitization, forcing the receptor-mediated osteoblast differentiation, through to a regulation in GRK2 turnover and expression. These effects on GRK2 levels did not involve a transcriptional mechanism, but they were mediated, at least partially, by the proteasome system. In particular, the cytokine induced a significant GRK2 association with the E3 ubiquitin ligase Mdm2 and promoted the kinase ubiquitination. Low levels of the kinases GRK2 reduced A2BAR desensitization causing an increase of functional receptors. Overall, these data indicate that the release of cytokines in the inflammatory environment directs MSC differentiation and represents a useful target to enhance bone formation. Moreover, the pivotal role of A2BAR availability/functionality in osteogenesis appears to be strictly linked to a decrease of receptor desensitization mediated by GRK2, whose levels are controlled by the ubiquitin-ligase Mdm2 binding.

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To investigate the protective effects of phyto-complexes against oxidative stress in a cellular model of stroke

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The oxidative stress, a pathological condition caused by the breakdown of the physiological equilibrium between the production and the elimination of reactive oxygen species (ROS) is involved in the pathogenesis of different human disease. Indeed the presence of ROS can easily activate innate immune responses, neuroinflammation, microglial activation, cerebrovascular dysfunction, and alterations in the blood-brain barrier contributing to CNS pathology such as Alzheimer's and other neurodevelopmental disorders. Epidemiological and observational studies have focused the attention on the research of anti-oxidant substances able to reduce the effect of ROS on the nervous system. A healthy diet with adequate intake of essential micronutrients may be crucial to prevent the development of chronic diseases. Increased intake of antioxidants, as well as other anti-inflammatory nutrients, may attenuate the oxidative stress and inflammation, thereby providing a useful addition to current disease management strategies. Furthermore, the identification of natural phyto-compounds with antioxidant activity, could be used to enrich food, strengthening protective properties. Moreover, the potential health and economic benefits of establishing non-pharmacological approaches (e.g., dietary supplementation) to disease management could be enormous. For the reasons above mentioned, our research aims to identify new natural substances with antioxidant properties. The natural phyto-extracts deriving from the processing of the coffee bean waste, were used on liver cells to examine their toxicity since the liver is the organ responsible of detoxification. After the results indicated that the substances were not toxic, the antioxidant properties of these phyto-extracts were evaluated in presence of Tert-butyl hydroperoxide as pro-oxidant. The results obtained show that our phyto-extracts have a protective effect against oxidative stress. Considering that the reoxygenation of tissue after stroke determines the formation of ROS, we are testing the antioxidant effect of the coffee bean waste on the neuronal cells and the hematoencephalic barrier cells, where the oxidative stress was induced with OGD, an in vitro model of stroke (oxygen glucose deprivation and reoxygenation). In parallel, we are also carrying studies with coffee-derived substance modified by intestinal microbiome on the same cellular model.

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Human glioblastoma cell apoptosis is induced by Rosemary officinalis through the p53 functional reactivation

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In the last two decades, natural products have gained popularity as effective and low toxicity co-adjuvants to canonical therapy in reducing cancer cell growth (Oncotarget. 2018 Apr 24; 9(31): 22194–22219). Among these products, Carnosol (CAR) has attracted a great interest for its anti-proliferative effects on Glioblastoma Multiforme (GBM) cells. GBM is a high aggressive glial tumor with a high proliferation rate and resistance to chemotherapy. A dysregulation of p53 signaling pathway has been implicated in GBM cell resistance to standard therapy, highlighting the need of new agents able to reactivate this pathway and triggering apoptotic processes in these cancer cells. Herein, the ability of a Rosemary officinalis extract (RMO) to modulate the proliferation of different human GBM cell lines was demonstrated. Furthermore, its ability to promote the p53 reactivation was measured, in comparison to the single molecule CAR, evaluating the p53 protein levels and the expression of p53 target genes (e.g. p21, MDM2, PUMA and BAX). RMO caused a significant anti-proliferative effect on GBM, especially in cells expressing wild-type p53. This effect appeared to be significant also in GBM staminal cells (CSCs), which are the most resistant cellular component in the tumor bulk and are responsible for tumor resistance and recurrence. RMO anti-proliferative effects were mediated by the functional reactivation of p53, as demonstrated by the increase of apoptosis and by the active transcription of p53 target genes. The RMO activity was demonstrated to be higher respect to CAR alone demonstrating that the overall composition of the extracts potentiates the antiproliferative effects with respect to a single component. In conclusion, these data highlighted the ability of RMO to restore and reactivate the p53 functionality in glioblastoma cells. Furthermore, the augmented activity of RMO respect to CAR shed light on the possibility to develop combinatory therapy to potentiate the conventional GBM treatment.

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PhytoTypeDB, a database for plant protein function and variability

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Many important plant species are poorly studied at the point that more than half A. thaliana proteins completely lack functional annotation by Gene Ontlogy. With a focus on cultivated crops we started our work from Malus domestica. Malus domestica (Domesticated Apple) it's the most intensively grown fruit crop in the world, from which its importance. Despite its domestication (a process that usually shrinks down diversity), it retained a large amount of genetic diversity throughout evolution, originating many different cultivars that display different phenotypes, like fruit features or resitance to pathogens. Despite the great importance, M. domestica is poorly studied. To study M. domestica gene functions and variability among different cultivars we started from an experiment where more than 500,000 high quality SNPs were identified from the resequencing of 78 M. domestica cultivars. An a ccurate gene prediction identified around 46,000 genes for the reference genome, whose coding sequencing were then analyzed in order to identify their function. We developed and gathered a set of tools ad resources for the analysis of protein sequences, including widely used bioinformatics tools like BLAST or InterProScan. Annotations produced by standard tools are complemented by a function prediction from an improved version of INGA (among the winners of CAFA 2), namely INGA2. It scored among the best in CAFA 3 and excelled in prediction of plant protein localization, representing the perfect tool to achieve a finer prediction of protein function. Furthermore, mobiDB-lite, a tool we developed for protein disorder annotation and was later included in InterProScan 5, extends the coverage of domain annotation to regions of protein disorder, which host the most variability. This set of tool was organized in a pipeline that is used to annotate proteomes for function. To broaden our scope we are going to annotate 'orphan genomes', results of whole genome sequencing experiments that were never further analyzed (e.g. Theobroma cacao). The annotations were organized in a database (NOSQL MongoDB database) and a web app was developed with cutting edge technologies to query the database and visualize and filter the annotations.

PLENARY LECTURE

The intriguing amino acid transporter, LAT1: relevance to human health and drug discovery

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LAT1 (SLC7A5) is an Heterodimeric Amino Acid Transporter interacting with the glycoprotein CD98 (SLC3A2) through a conserved disulfide. LAT1 mediates an antiport of branched and aromatic neutral amino acids. Conversely, it was recently shown that His is a preferred substrate. LAT1 is over-expressed in many tumors being a potential pharmacological target. It has been studied by bioinformatics, intact cell and proteoliposomes using the native or recombinant proteins. CD98 is not required for transport since [3H]His transport was detected either in proteoliposomes harboring the LAT1/CD98 heterodimer or LAT1 alone. The homology model of LAT1 predicted four crucial residues for substrate binding, then confirmed by site-directed mutagenesis: F252 which has a gate function; S342 and C335 which are responsible for substrate docking; C407 which plays a minor role in the intracellular side. The presence of Cys has been exploited for designing inhibitors, based on dithiazole ring, able to react with thiols. Among 50 compounds, 8 have been identified as best inhibitors that interact with C407. Two of the compounds are the most potent inhibitors of LAT1 so far identified, and were able to induce cancer cells death. An intriguing aspect of LAT1 biology is its expression in Blood Brain Barrier. Interestingly, some inherited mutations of LAT1 are responsible of Autism Spectrum Disorders due to function impairment.

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Session 3: Membrane proteins in action

Role of the F1F0-ATPase inhibitor IF1 in osteosarcoma cells under anoxic conditions

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Mitochondria provide most of the ATP necessary to the cell via oxidative phosphorylation (OXPHOS), process in which the F1F0-ATPase catalyzes the ATP synthesis driven by the electrochemical gradient (ΔμH+). As shown in heart and liver, ischemia leads to a ΔμH+ collapse that induces the F1F0-ATPase to reverse its activity and to hydrolyze ATP. In this condition, the endogenous ATPase inhibitory factor 1 (IF1), can inhibit hydrolysis, preventing ATP dissipation to ensure cells viability. In normoxia, IF1 cooperates in the mitochondrial structure determination and promotes OXPHOS [1,2]. The overexpression of IF1 in several human cancer suggested its involvement in tumors development and growth. For its features, IF1 is proposed as a target for cancer therapy, but its mechanisms of action are still unclear. We recently investigated the bioenergetics of cancer cells in oxygen deprivation, proving that the ATP hydrolysis occurs only in anoxia conditions, but not in hypoxia. In anoxia-mimicking conditions, we also demonstrated that IF1 expression favors cancer cells growth and cellular ATP levels preservation, by inhibiting the ATPase [3]. As a further investigation, here we explored in cancer cells upon anoxia-mimicking conditions, the role of IF1 expression on the mitochondria content and composition. For this purpose, we exposed IF1-expressing and IF1-silenced osteosarcoma cells to the uncoupler FCCP and we evaluated the mitochondrial mass. Surprisingly, we found that the mitochondria mass was preserved equally in both cell line models, compared to controls. Nevertheless, the citrate synthase activity and the OXPHOS complexes expression showed a decrease, but in part sustained in IF1-silenced clones. These data suggested that IF1 could modulate mitochondrial functionality and composition through the mitochondria turnover. Indeed, the analysis of mitophagy and mitochondrial biogenesis markers underlined the activation of both processes in IF1-expressing cells, conversely slowed down in IF1-silenced clones. Taken together, our findings support the hypothesis that, in temporary anoxia, IF1 promotes cell survival by preserving ATP and by promoting the quality control of mitochondria. This occurs with the activation of both mitophagy and mitochondrial biogenesis, in order to eliminate and to replace damaged mitochondria, providing functional organelles available in case of oxygen levels restoration. Conversely, in the absence of IF1, the sustainment of Δψm by ATP hydrolysis may acts as a signal of functionality, leading to OXPHOS complexes preservation and mitochondria turnover inhibition, fictitious advantages for the cells because of the detrimental impact of the energy dissipation on cell survival.

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Sulforaphane influences AQP8–linked redox signaling in a leukemic cell line

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Sulforaphane (SFN), an isothiocyanate compound present in abundance in Cruciferous vegetables, exerts potential benefits for prevention and co-treatment of several health disorders, as reported in both experimental and epidemiological studies [1]. In cancer cells, signalling pathways that promote cell proliferation, survival, angiogenesis and metastasis are hyper-activated because of an increase in localized reactive oxygen species (ROS) production and of an altered redox environment, compared to normal cells. In particular, hydrogen peroxide (H2O2) derived from NOX family is involved in various redox signal transduction pathways and the aquaporin 8 (AQP8) has been identified as a H2O2 transport facilitator across the plasma membrane. Recent evidence demonstrated that many tumor cell types express elevated level of aquaporin isoforms and highlighted a positive correlation between histological tumor grade and the AQP expression [2]. We previously demonstrated that AQP8 funnels NOX-derived H2O2, triggered by endogenously generated VEGF, which, in turn, provokes VEGFR-2 phosphorylation and the consequent modulation of many cellular activities, resulting in cell survival and proliferation in a model of acute myeloid leukemia [3]. Therefore, this study aimed at the evaluation of the potential effect of SFN on the modulation of redox signaling involving AQP8, NOX2 and p-VEGFR-2 expression. The elucidation of AQP8 role in cancer redox signalling and the effect exerted by SFN on its modulation can offer new potential target for anti-cancer therapy, suggesting the importance of anti-tumor effect exerted by dietary compounds.

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Regulatory aspects of the human organic cation transporter OCTN1 (SLC22A4)

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The human organic cation transporter OCTN1 (SLC22A4) belongs to the OCTN subfamily (Organic Cation Transporter Novel) which includes three members. Two of these, namely OCTN2 and OCTN3 are well acknowledged as carnitine transporters. Differently, OCTN1 displays low efficiency in transporting carnitine. In intact cells studies OCTN1 was found to transport the prototype organic cation tetraethylammonium (TEA) and the mushroom metabolite ergothioneine; since the two substrates are not physiological in human metabolism, the role of OCTN1 is still unclear. To get further insights into the possible role of OCTN1 in humans, the transporter has been overexpressed in E. coli and functionally assayed in proteoliposomes by studying uptake and efflux of possible substrates. In this system acetylcholine (Ach) was identified as a substrate. Ach uptake, but not efflux, is inhibited by extraliposomal (extracellular) sodium indicating that the efflux process may be the physiological one. This transport function indicates a possible involvement of OCTN1 in the "Non Neuronal Cholinergic System" that is ubiquitous and requires non quantal Ach release from cells of many tissues. Regulation of Ach transport has been investigated by studying the effect on transport activity of ROS inducing compounds, pH changes and lipid composition of the membrane. Hydrogen peroxide increases Ach transport mediated by OCTN1. This effect is probably mediated by disulfide formation which is induced by the compound. The hypothesis is confirmed by the finding that the C-less mutant of OCTN1 is insensitive to hydrogen peroxide. A pH gradient (acidic inside) imposed in proteoliposomes increases Ach uptake. This indicates the possible involvement of a proton in the transport cycle. Finally, cholesterol included in the proteoliposomal membrane stimulates OCTN1 increasing its transport activity. The site of action of this lipid has been predicted by bioinformatics.

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Altered expression of SLC52A members in human cancer

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Riboflavin, otherwise known as vitamin B2, is an essential dietary component and represents the precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are important enzymatic cofactors required for carbohydrate, amino acid and lipid metabolism, and other cellular regulatory roles [1]. In different cells, riboflavin uptake occurs via specialized carrier-mediated processes supported by three specific members of the solute carrier family 52 (SLC52A), identified and named riboflavin transporter 1 (RFVT1; SLC52A1), RFVT2 (SLC52A2), and RFVT3 (SLC52A3), respectively [2]. Inside the cells, riboflavin is phosphorylated to FMN by riboflavin kinase and it is subsequently metabolized by FAD synthase to FAD, the flavin cofactor mainly located in mitochondria [1]. Alterations of some of these proteins have been correlated with rare inherited neuromuscular diseases [3].
Here we point our attention on the possible involvement of flavin cofactor homeostasis in human cancer. We present evidences in favor of a profound alteration of flavin cofactor level in some types of human cancer accompanied by dysregulation of RFVT expression [4].

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A lysosome-plasma membrane sphingolipid axis linking lysosomal storage to cell growth arrest

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Increasing evidence implicates lysosomal dysfunction in the etiopathology of lysosomal storage diseases, neurodegenerative disorders, and the aging process. Nevertheless, the molecular mechanisms by which the perturbation of lysosomal homeostasis induced by the storage of undegraded metabolites may affect the cell function and viability are still unknown. In this context, altered metabolism of sphingolipids could play an active role in the onset of cell damage. To explore this issue, we used human fibroblasts loaded with sucrose as a simple model of lysosomal accumulation. In sucrose-loaded fibroblasts, we observed a significant increase in lysosomal biogenesis followed by arrested cell proliferation. Lysosome-related genes were the most significantly enriched among the upregulated transcripts, mainly activated by the nuclear translocation of TFEB. However, despite induced lysosomal biogenesis we found reduced lysosomal catabolism and autophagy blockage. These conditions are responsible for the increased content of several sphingolipid species (i.e. sphingomyelin, glucosylceramide, ceramide, and gangliosides GM3 and GD3) both intracellularly and at the plasma membrane (PM) level. Moreover, we observed an increase in the lysosomal membrane protein Lamp-1 on the PM of sucrose-loaded fibroblasts and a greater release of the soluble lysosomal protein cathepsin D in their extracellular medium compared with controls. These results indicate increased fusion between lysosomes and the PM, as also suggested by the increased activity of lysosomal glycosphingolipid hydrolases on the PM of sucrose-loaded fibroblasts. The inhibition of β-glucocerebrosidase and non lysosomal glucosylceramidase, both involved in ceramide production resulting from glycosphingolipid catabolism on the PM, partially restored cell proliferation. Our findings indicate the existence of a new molecular mechanism underlying cell damage triggered by lysosomal impairment.

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Protein purification's method affects the electrophysiological features of yeast VDAC2

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The Voltage-Dependent Anion selective Channel (VDAC) represents the most important pore-forming protein family of the Mitochondrial Outer Membrane. VDACs allow the exchanges of metabolites (ATP, ADP) and ions (Mg, K, Cl) between cytosol and inner of mitochondria, playing an important role in cell metabolism and in the regulation of apoptosis [1]. The yeast Saccharomyces cerevisiae has two distinct genes encoding for two VDAC isoforms. yVDAC1, the main and most abundant porin, shares with human VDAC1 about 70% of sequence homology and the main electrophysiological properties (i.e.: channel activity at the Planar Lipid Bilayer (PLB), voltage-dependent channels of 4 nS in 1 M KCl). Due to its important, cells lacking yVDAC1 displays a strong inhibition of cell growth in non-fermentable conditions, as result of a blocking of mitochondrial metabolism [2]. On the contrary, the role of yVDAC2 was unclear since from its discovery. In fact, the absence of yVDAC2 has no effect on cell growth, indicating that the protein is not involved in mitochondrial metabolism and, possibly, it is devoid of channel activity. Only recently, yVDAC2 was extracted from yeast mitochondria in native condition and its electrophysiology was deeply analyzed at the PLB, revealing a clear pore-forming activity (i.e.: channels of 3.6 nS in 1 M KCl characterized by a reduced voltage sensitivity) [3]. An alternative strategy for yVDAC2 purification, consisting in the heterologous expression in bacterial system, is presented in this work. The encoding sequence of yVDAC2 was cloned in expression vector in frame with a 6xHis-tag, expressed in E.coli and purified by affinity chromatography. Then, the protein was refolded in vitro and characterized at the PLB. Our results displayed that the recombinant yVDAC2 maintains similar features to that of native one but, at the same time, several important differences between the two proteins in ion selectivity and voltage sensitivity have been found. Overall, our results confirm that yVDAC2 is another member of VDAC family and that the methods used for protein preparation is determinant for the electrophysiological properties.

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PLENARY LECTURE

Membrane transport of bilirubin and flavonoids. From kinetics to diet

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Bilirubin is the end product of heme catabolism, with a daily production of about 200–300 mg in a normal adult. In the circulation, bilirubin is transported by albumin as a reversible complex, and then is taken up by the liver, which excretes it as a diglucuronide derivative. Bilirubin uptake into the liver is transporter-mediated, as shown by kinetic data. Despite decade-long efforts to identify the hepatic bilirubin transporter, none have yet confirmed by multi-level characterisation to fulfil this function. Among the studied entities is the bilirubin transporter named bilitranslocase (BTL; TCDB 2.A.65.1.1). The in vitro BTL transport assay uses the pH-indicator dye sulfobromophthalein (BSP) as transport substrate. Dietary anthocyanins (AC), glycosidated flavonoid molecules that have phenolic-quinoidal tautomerism as BSP, are strong competitive inhibitors of BTL transport. AC are transported into various cell types expressing BTL. Similarly to the extremely fast uptake of bilirubin in the liver, AC also distribute from the circulation to the main organs, including the brain, at an extraordinary fast rate. This remains one of the most compelling pieces of evidence about the principal role of membrane transporters in determining the pattern of bioactivity of dietary compounds. The presentation will cover the following: 1) overview of bilirubin membrane transporters [1]; 2) Structural details of BTL [2]; 3) QSAR of BTL substrates [3]; 4) Absorption, distribution and metabolism of AC in the rat: focus on kinetics [4]; 5) From AC kinetics to diet and health [5]; 6) The current work in our lab and network of collaborators to develop new technologies to study the bilirubin-diet interplay.

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Session 4: Nutrition and pathologies

Identification of the antigen recognized by RHIGM22, a remyelination-promoting human monoclonal antibody, and his effect on glial cells

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Recombinant human IgM22 (rHIgM22) binds to myelin and oligodendrocytes (OLs) and promotes remyelination in mouse models of Multiple Sclerosis. rHIgM22 preferentially reacts with sulfatidepositive (O4+) OLs [1], and binding of rHIgM22 is abolished in CNS tissue slices from Cst (-/-) mice [2], suggesting that its binding requires the presence of a product of cerebroside sulfotransferase, possibly sulfatide, highly expressed in OLs and myelin. However the identity of the antigen recognized by this antibody remains to be elucidated. We tested the binding of rHIgM22 to purified lipids and lipid extracts from mouse brain, CNS myelin, mixed glial cells, and O4+ OLs using TLC immunostaining. Our preliminary results show that IgM22 binds to sulfatide in vitro, while it does not bind to other myelin sphingolipids suggesting that sulfatide at the OLs surface might be important for the binding of rHIgM22 to these cells and to myelin. However, IgM22 does not bind structures expressing sulfatide outside the nervous system, so additional factors are likely relevant for the immunoreactivity of IgM22 in CNS. Indeed, in lipid extracts from different sources we found another lipid antigen selectively recognized by rHIgM22. To attempt the identification of the antigen, samples were purified using column chromatography and the second rHIgM22-immunoreactive band enriched fractions were analyzed by ESI Mass Spectrometry. The results obtained led to hypothesize that the unknown antigens could be Phosphatidylinositol (16:0/18:1-PI) and two different Phosphatidylserine species, 18:0/22:6-PS and 18:0/18:1-PS. This lipid is also present in the extracts from mixed glial cultures, which do not contain mature O4+ OLs, suggesting that other glial cells in addition to OLs might be important in the response to rHIgM22. Furthermore, literature suggests that rHIgM22 biological activity is mediated by the reorganization of Lyn, integrin αvβ3 and PDGFRα at the cell surface to form a signaling complex triggering Lyn activation which, in turn, promotes oligodendrocyte precursor cells (OPCs) survival and proliferation [3]. We assessed the effect of a 24 hours, single dose treatment with rHIgM22 on OPC, OL and mixed glial cell (MGC). No significant difference in the lipid pattern of MGC treated cells was observed, while in OPC and OL treated with rHIgM22 there is an increase in GD3 and GM3, supporting the hypothesis that the binding of rHIgM22 on the surface of OL could elicit biological responses mediated by alterations of lipid-dependent membrane organization and/or signaling. We also observed an increased expression of several proteins, including PDGFRα, Lyn, activated Lyn and integrin αV in both OPC and OL.

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Human gingival mesenchymal stem cell trophism is modulated by inflammatory microenvironment: effects of Ribes nigrum bud extract.

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Adult mesenchymal stem cells (MSCs) play a crucial role in the maintenance of tissue homeostasis and in promoting regenerative processes. Among the different MSC types, the gingival mesenchymal stem cells (GMSCs) have arisen as a promising tool to promote the repair of damaged tissues secreting trophic, regeneration-promoting mediators. TNF-α is one of the key mediators of inflammation that could affect tissue regenerative processes and modify the MSC properties in in vitro application. Herein, we investigated 1) the effects of TNF-alpha on GMSC trophism and 2) the ability of Ribes Nigrum bud extract (RBE) to modulate the effect of this cytokine on GMSC properties. GMSC were isolated and characterized from health subjects. TNF-α affected GMSC proliferation and the expression of inflammatory-related protein (IL-6, IL-10, TGF-β, and COX-2) in dependence on its concentration. A high TNF-α concentration decreased the GMSC viability and impaired the trophic effect of GMSCs on endothelial cells, likely by enhancing the amount of pro-inflammatory mediators in GMSC secretome. GMSC incubation with RBE changed secretoma cell composition so restoring the GMSC beneficial effects on endothelial viability and motility. These results demonstrated that a high TNF-α concentration, as occurred under chronic inflammatory conditions, decreased the GMSC well-being and alter their trophic activity impairing GMSC-endothelial cell communication. These data highlight that the control of inflammatory microenvironment is crucial to guarantee MSC-driven reparative processes. Furthermore, the use of natural anti-inflammatory agents restored the GMSC regenerative properties on endothelial cells opening the way to the use and the development of natural extracts in wound healing, periodontal regeneration and tissue engineering application that use MSCs.

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Taste sensitivity and body weight: is there a link?

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Obesity (OB), defined as a clinical condition characterized by an increased Body Mass Index (BMI), is becoming a global epidemic in both children and adults. OB is fueled by individual factors, nutrition transition and increasingly sedentary lifestyles that lead to excess caloric intake. Among individual factors, taste sensitivity plays an important role in food preferences, choices, and thus consumption. The present study was conducted to evaluate the relationship between taste sensitivity and BMI, by studying the response to the administration of different tastant substances in different groups of subjects. Thirty healthy normal-weight volunteers (18 females and 12 males, BMI 21.6 ± 1.7 Kg/m2), nineteen healthy overweight (11 females and 8 males, BMI 27.9 ± 1.4 Kg/m2) and twenty-two subjects with obesity (18 females and 4 males, BMI 36.9 ± 5.7 Kg/m2) were recruited. They were asked to avoid eating and drinking anything except water for one hour prior to testing, not to smoke, and not to brush their teeth. For each subject the lateralization Oldfield score, body weight, height, and blood pressure were determined. The taste test is based on filter paper strips soaked with 4 tastants, presented at different concentrations, evoking the 4 basic taste qualities (salty, acid, sweet, bitter); pure rapeseed oil and water were administered, evoking fat and neutral taste. The stimuli were applied to each side of the protruded tongue. Patients were asked to identify the taste from a list of eight descriptions according to a multiple forced-choice. The results have shown a general decrease of taste sensitivity with the increase of BMI, except for fat taste. Other variables affecting the taste sensitivity are the age (negative association), gender (women generally show higher sensitivity), tastant's concentration (positive association). Our findings could provide important insights for the design of new therapies for weight loss and long-term weight maintenance, and for the composition of diets combining the correct caloric and nutritional supply with the individual taste preferences.

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Intestinal epithelial barrier abnormalities in patients with chronic intestinal pseudo-obstruction

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Chronic intestinal pseudo-obstruction (CIPO) is a rare condition due to severe impairment of gut motility responsible for recurrent sub-occlusive episodes. Altghough neuro-muscular-glial-ICC abnormalities represent the main pathogenetic mechanism, the pathophysiology of CIPO remains poorly understood. Intestinal epithelial barrier (IEB) abnormalities can contribute to neuro-epithelial changes by allowing passage of harmful substances. This study aimed to test whether IEB abnormalities occur in CIPO patients by analyzing the jejunal protein expression of the major components of tight junctions (TJs): occludin, claudin-4 and zonula occludens-1 (ZO-1), as markers of IEB integrity. We also examined the expression of vasoactive intestinal polypeptide (VIP) and glial fibrillary acidic protein (GFAP), as neuronal and glial cells markers. 28 clinically characterized CIPO patients (15F; 16-75 yrs) were studied and subdivided according to gut histopathology: n=7 with an apparently normal (AN) neuro-muscular layer; n=11 with inflammatory (INF) changes throughout the neuro-muscular wall and n=10 with degenerative neuro-muscular alterations (DEG). N=8 (3F, 48-73 yrs) non-CIPO subjects undergoing surgery served as controls. Protein expression was evaluated on jejunal full thickness biopsies with Western Blot. Total occludin was significantly decreased in the intestine of CIPO patients vs. controls ($P=0.002$), particularly in AN ($P=0.007$) and INF ($P=0.004$) subgroups; ZO-1 and VIP expression was decreased selectively in DEG ($P=0.015$ and $P=0.0305$). Occludin/claudin oligomers, an index of TJs assembly, were absent in 81% of CIPO ($P<0.0001$) patients. Claudin-4 was upregulated in CIPO ($P=0.070$), particularly in INF ($P=0.050$) and DEG ($P=0.044$) groups. GFAP resulted ubiquitously increased in CIPO ($P<0.001$). The absence of occludin-claudins oligomers indicates IEB abnormalities in CIPO patients and provides the morphological basis for the hypothesis of noxious agents passing through the intestinal wall. It is likely that barrier dysfunction in AN and INF is occludin dependent, while in DEG is ZO-1-dependent. IEB abnormalities might affect neuronal and glial cells.

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Lactobacillus crispatus interferes with *Chlamydia trachomatis* infectivity through modulation of integrin exposure in cervical cells

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In women, urogenital CT infections are often asymptomatic, thus remaining unnoticed and untreated. This can lead to complications and sequelae including pelvic inflammatory disease, tubal infertility and ectopic pregnancy [1,2]. A normal vaginal microbiota, dominated by lactobacilli, is crucial for the prevention of several urogenital and sexually transmitted infections, including Chlamydia [3-5]. This aspect is strengthened by the demonstration that in case of bacterial vaginosis, a clinical condition characterized by the depletion of lactobacilli, a higher risk of STI transmission and acquisition is reported [6]. This study aimed to elucidate the molecular bases of the interaction among lactobacilli, Chlamydia trachomatis and epithelial cells. We evaluated the capacity of lactobacilli cells and supernatants to interfere with C. trachomatis infectivity in HeLa cells, by means of competition, exclusion and displacement mechanisms. Lactobacilli cells were the most active fraction, by means of an exclusion strategy. We investigated the potential mechanism of protection in Lactobacillus crispatus BC5 (model strain), and we demonstrated that the incubation of HeLa cell line with BC5 cells induces important modifications at the level of the epithelial plasma membrane, by altering lipid composition and α5 integrin subunit exposure. When α5 integrin subunits were masked by a specific blocking antibody, Chlamydia infection was precluded. α5 integrin subunit is thus crucial for the pathogen penetration into HeLa cells, and the anti-Chlamydia activity of BC5 can be directly linked to membrane properties modifications in epithelial cells. In conclusion, we identified a potential molecular mechanism at the basis of the protection exerted by Lactobacillus against the sexually transmitted pathogen Chlamydia trachomatis, getting insights into the role of the vaginal microbiota for the woman's health.

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Effect of 9-hydroxy-stearic acid on glucose metabolism in a human colon cancer cell line

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Recent findings identified a new class of endogenous lipids, branched Fatty Acid esters of Hydroxy Fatty Acids (FAHFAs), able to behave as specific signaling molecules that can regulate the cellular metabolism [1]. Among FAHFAs, the Palmitic-Acid-9-Hydroxy-Stearic Acid (9-PAHSA) seems to exert favorable metabolic effects in obesity-related diseases and type 2 diabetes, causing an increase in insulin sensitivity and glucose uptake. It has been recently reported that the FAHFAs content in human serum of breast cancer patients was significantly decreased compared to healthy controls [2], thus it is very interesting to investigate the possible involvement of these lipid molecules also in cancer transformation and progression. Previous studies of our research group have shown that the administration of 9-hydroxy-stearic acid (9-HSA) to colon carcinoma cells (HT29) induces strong antiproliferative and differentiating effects, with a cell cycle arrest in G0/G1 phase [3]. Since 9-HSA can be produced by the hydrolysis of 9-PAHSA, it is conceivable that also this lipid could act as signaling molecule. Consequently, the aim of this research was the characterization of the glucose metabolism of HT29 cells treated with 9-HSA. As a first step, the effect of 9-HSA on the lipid organization of HT29 cells was studied, showing an increase of the fluidity of plasma membrane. The treatment of HT29 with 9-HSA for 1 h provokes a significant increase of the glucose transporter Glut1 (and in a lesser extent of Glut3) on the plasma membrane, as a result of a translocation from intracellular stores, since the level of expression of the two transporters were unchanged, as determined by RT-PCR. Accordingly, the higher amount of Glut1 on the cell surface caused also an increase in glucose uptake into the cells. RT-PCR analysis showed also a significant increase of MCT1, the monocarboxylate transporter, in HT29 cells treated with 9-HSA. MCT1 is commonly overexpressed by cancer cells to maintain lactate and pH homeostasis [4]. Therefore, lactate production was measured in HT29 cells upon 9-HSA treatment for 1 h. Results show that lactate production was significantly increased, indicating a cellular metabolic shift toward glycolysis. The acute metabolic changes observed in HT29 cells are typical cellular responses to a signal molecule, supporting the hypothesis of a signaling role for 9-HSA in cancer cells.

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Evaluation of endothelial dysfunction markers in children with cardiovascular risk factors: obesity and/or hypertension

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Cardiovascular diseases (CVDs), responsible for more than 30% of annual deaths, are the leading cause of death worldwide [www.who.int]. These diseases arise from a set of risk factors that can occur even at an early age. In the pediatric population incorrect behaviors and eating habits lead to pathologies such as obesity and predispose to cardiovascular damage. Obesity is associated with a number of complications such as hypertension, hyperuricaemia, dyslipidaemia and insulin resistance, which, if not treated, expose the child to a higher risk of developing CVDs at a young age [Bridger T. , 2009; Daniels S., 2011]. Endothelial dysfunction is another risk factor for CVDs. This pathological condition is characterized by a reduced bioavailability of vasodilators, in particular nitric oxide (NO), and an increase in vasoconstriction factors produced by the endothelium (such as endothelin-1) [Lerman A. 1992]. The cause of endothelial dysfunction resides in the inflammatory state of adipose tissue that is triggered in the presence of excess weight; in this condition, the adipose tissue undergoes a macrophage infiltration which induces the production of pro-inflammatory molecules [Tran B., 2012]. At the level of the wall of blood vessels, it seems that this inflammation originates a condition of oxidative stress that damages tissue homeostasis and determines the onset of endothelial dysfunction [Van Gaal L., 2006]. In particular, a depletion of NO and / or a concomitant increase in vasoconstrictors such as endothelin-1 induces an alteration of the release capacity in the smooth muscle cells of the vessels and favours the increase in arterial pressure [Shulz E. , 2008]. This condition of vasoconstriction is initially reversible, but over time it stabilizes and determines the onset of hypertension [Bleakley C., 2015]. Given these premises, the aim of this work is to study the association between the condition of endothelial dysfunction and the predisposing factors cardiovascular risk such as weight excess and hypertension in a pediatric population. We evaluate the production of NO and the release of endothelin-1 in plasma sample of a children population attending a Clinic for Cardiovascular Risk Assessment (Milano). Results show that the markers of endothelial dysfunction are related to 'excess weight more than hypertension and correlate with metabolic alterations such as hyperuricemia and insulin resistance. In conclusion, the cardiovascular risk factors associated with an altered endothelial function are already present in pediatric age, however since the child is a growing organism this mechanism could be reversible.

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Is Notch involved in protective effects of estrogen on endothelial function and angiogenesis?

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Unlike age-matched men, premenopausal women benefit from cardiovascular protection. The mechanisms of action are still poorly understood, although a role for estrogens in stimulation of angiogenesis and protection against apoptosis of endothelial cells (ECs), hallmark of endothelial dysfunction, have been suggested. In estrogen receptor (ER) α positive breast cancer cells, 17-estradiol (E2) treatment inhibits Notch1 activity, however little is known regarding the role of E2 in Notch signaling in endothelium. The aims of this study were to establish i) whether estrogens modulate Notch activity in endothelial cells and ii) the possible consequences of this modulation on endothelium functions. Treatment with E2 activates Notch signaling in human umbilical vein endothelial cells (HUVECs), effect counteracted by ERs antagonist ICI 182.780, suggesting that E2 modulation of Notch1 is mediated by ERs. Our data were in contrast with findings of Notch1 inhibition by E2 treatment in ERα positive breast cancer cells; since ECs differently from breast cancer cells express high levels of ERβ, we hypothesized that these opposite results could be due to the different activity of the two forms of ERs on Notch1. Treatment with ERβ specific agonist (DPN) but not with ERα specific agonist (PPT) induced activation of Notch1 in HUVECs. We next evaluated the role of ER-Notch1 axis in angiogenesis and TNFα-induced endothelial apoptosis. Notch1 inhibition increased ECs sprouting, effect abolished by E2, evaluated by a tube formation assay on 3D Matrigel and in mouse aortic ring explants. Moreover, DPN but not PPT counteracted the increase in ECs sprouting caused by Notch inhibition, suggesting that ERβ but not ERα is involved in Notch1 modulation. TNFα reduced the levels of active Notch1 protein, which were partially restored by E2 treatment. E2 counteracts TNFα-induced apoptosis, effect abrogated when Notch1 is inhibited, whereas ectopic overexpression of Notch1 diminished TNFα-induced apoptosis. Moreover, the E2-mediated regulation of the levels of active Notch1 was abrogated after silencing ER, abolishing the effect of E2 on apoptosis. Interestingly, DPN treatment antagonized TNFα-induced decreased of cleaved Notch1 and apoptosis. In summary, our results indicate that E2 requires active Notch1 through a mechanism involving ERβ to regulate angiogenesis and protect the endothelium against TNFα-induced apoptosis. These findings could be relevant for assessing the efficacy and applicability of menopausal hormone treatment, because they suggest that reduced levels of Notch1 signaling may interfere with the protective action of hormone on the endothelium.

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