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PATHOPHYSIOLOGY OF AGING

A1 The Longevity Associated Variant of *BPIFB4*: A Possible Link Between Human Longevity, Cardiovascular Homeostasis and Cellular Metabolism

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Background: Long-living individuals (LLIs) are genetically predisposed to delay aging and avoid cardiovascular disease. Indeed, our recent genome wide assay (GWA) study on exceptional longevity identified a four-SNPs haplotype (Longevity Associated Variant-LAV) in the *BPIFB4* gene that reverted old mice decline of endothelial nitric oxide synthase activity and endothelial function and potentiated homing of CD34+LSK cells and revascularization in limb ischemia. To be noted, *BPIFB4* overexpression activates heat shock proteins (HSPs), translation, ribosome biogenesis, and spliceosome. These highly energy-demanding processes are typical of cells under stress, stem or tumoural cells, where *BPIFB4* is expressed, and that are exposed to hypoxic environment.
Methods: MitoSOX and Sea Horse for radical stress and cell metabolism. *In vivo* treatment with adeno-associated virus containing GFP or LAV-BPIFB4 in models of atherosclerosis and brain ischemia.
Results: We have found that *BPIFB4* is expressed in hypoxia and its overexpression activates OX phos and aerobic glycolysis, induces reduction of mitochondrial radical stress and differentiation processes. *In vivo*, LAV-BPIFB4 prevents atherosclerosis and reduces brain ischemia damage.
Conclusions: LAV-BPIFB4 is a key protein in the regulation of cardiovascular homeostasis.

A2 Pasta With *Opuntia*: A Functional Food with Hypoglycemic and Antioxidant Effects

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Background: *Opuntia ficus-indica* is a typical plant of the Mediterranean basin. Its leaves, the cladodes, contain mainly pectins, carotenotenoids and polysaccharides, and their extracts, rich in fibers, have health positive effects in animal models. However, little is known in humans. The aim of this study was to evaluate the effect of pasta with *Opuntia* extracts on hematochemical, anti-inflammatory, antioxidant and anthropometric parameters to test its potential hypoglycemic, hypocholesterolemic and weight loss properties and its possible modulator action on gut microbiota.
Methods: We have performed a nutritional intervention on 39 participants with metabolic syndrome, administering 500 gr/week of pasta with *Opuntia* for 30 days. At baseline, and after 30 days, blood tests were assessed. Additionally, qPCR assays were carried out to address any variations in the amount of *Lactobacilli* population from fecal samples.
Results: After 30 days of pasta with *Opuntia* administration, a statistically significant decrease in serum levels of glucose, uric acid, total cholesterol, and nitrogen was observed. The results also showed a statistically significant variation of oxidative stress markers, such as SH protein, paraoxonase, and reduced glutathione, demonstrating its antioxidant effects. However, no changes were observed in anthropometric parameters while the neopterin, a marker of macrophage activation, increased its levels. Interestingly, a statistically significant reduction in *Lactobacilli* amount was measured.
Conclusions: The study suggests hypoglycemic and antioxidant effects of pasta with *Opuntia* can be considered a functional food.

A3 Associations of *MMP-9* and *MMP-2* Polymorphisms with the Risk, Severity, Short- and Long-Term Complications of Degenerative Mitral Valve Diseases: A 4.8-Year Prospective Cohort Study

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Background: Degenerative forms of mitral valve diseases (MVDs) are complex pathologies. Thus, it is difficult to make generalizations about MVD pathways or genetic risk factors. However, a key role of metalloproteinases (MMPs) in their pathophysiology is emerging. Thus, we performed a study to assess eventual associations of some functional SNPs in *MMP-2* and *MMP-9* genes with MVD risk, symptom severity and short- and long-term (4.8 years) complications.
Methods: For this purpose, 90 patients and two control groups were genotyped for *MMP-2* and *MMP-9* gene SNPs, and systemic levels of pro-atrial natriuretic peptide (ANP), and two enzymes were quantified and correlated to the *MMP-2* and *MMP-9* SNPs. In addition, associations between these SNPs and symptom severity and short- and long-term complications were evaluated.
Results: Interestingly, rs3918242 *MMP-9* and rs2285053 *MMP-2* SNPs were significantly represented in cases compared to two control groups, and were associated with a higher MVD risk. Cases stratified for New York Heart Association (NYHA) symptoms, and particularly NYHA III+IV, with rs3918242 CT+TT MMP-9 and rs2285053CT+TT genotypes also showed higher severity related to significant higher systemic levels of MMP enzymes and pro-ANP at enrollment and 4.8-year follow-up times. In addition, cases with these genotypes, and particularly NYHAIII+IV, had a very significant percentage of complications, particularly at the 4.8-year follow-up. Surprisingly, 20% of patient controls developed MVD at 4.8-year follow-up and were carriers of these genotypes.
Conclusions: The associations observed seem to suggest that the two SNPs might represent useful biomarkers and targets for preventing and

monitoring MVDs, leading to a more appropriate management and outcome.

A4 Markers of Plaque Vulnerability Induced by Oxidized Lipids

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Background: The driving force of atherogenesis is chronic inflammation, which promotes the development of a vulnerable plaque responsible for acute cardiovascular diseases. A vulnerable plaque is characterized by a lipid-rich core and a thin fibrous cap. Moreover, this plaque is infiltrated by macrophages that secrete MMPs, such as MMP-9, which degrade extracellular matrix, causing plaque rupture. It is known that oxidized LDLs cause atherosclerosis by accumulating in the arterial intima where they act as stimulators of inflammatory and immune response. In light of these facts, our studies focus on the contribution of oxysterols, derivatives of LDL-cholesterol oxidation, and 4-hydroxynonenal (HNE), an aldehyde generated by breakdown of polyunsaturated fatty acids, in inflammation and plaque instability.
Methods: Proinflammatory molecules and MMP-9 levels were analyzed by qRT-PCR, western blotting, immunofluorescence and colorimetric tecniques.
Results: In U937 cells, both 27-hydroxycholesterol (27OH) and HNE induced the expression of several cytokinesand MMP-9 through TLR4 activation. Moreover, they sustained inflammation by upregulating COX-2 and mPGES-1 levels, as well as iNOS and NO. Of note, inhibition of inflammatory molecule formation decreases MMP-9 release by macrophages, underlying the crucial role of inflammatory response in MMP-9 overexpression. We are now investigating whether an oxysterol mixture could modulate the expression of the proprotein convertase PCSK6, a new marker of plaque vulnerability. Our preliminary results indicate that oxysterols upregulate PCSK6 in U937 cells and smooth muscle cells. We are also investigating the possible link between PCSK6 and MMP-9 activation.
Conclusions: Our results suggest that oxidized lipids promote plaque vulnerability by enhancing inflammation and MMP-9 upregulation.

A5 Cross-Talk Between Androgen Receptor and NGF Receptor (TrkA) In Neuronal Cells

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Background: Steroids and growth factors control neuronal development in physiological and pathological conditions. In addition to regulating neurite outgrowth, dendritic branching and axon regeneration, androgens exert a beneficial effect in the central nervous system. The decrease in male testosterone levels represents, indeed, an age-related risk factor for Alzheimer's disease development and dementia.
Methods: By using biochemical and DNA sequencing approaches we identified for the first time the expression of a classic androgen receptor (AR) in rat adrenal pheochromocytoma PC12 cells and primary mouse hippocampal neurons. siRNA experiments combined with the use of chemical inhibitors or small peptides interfering with AR action, have established a key role for AR in androgen and NGF signaling.
Results: The cross talk between AR, FlnA, and TrkA regulates neuritogenesis in PC12 cells through activation of the PI3-K δ/Rac downstream pathway. Beta 1 integrin represents the link between TrkA and AR, thereby regulating the differentiative effects triggered by androgens or NGF in PC12 cells.
Conclusions: Our study provides new clues that might lead to a better understanding of the complexity of AR and TrkA signaling in neuronal cells. Our investigation into AR-interacting partners may identify promising candidates to track and target in many neurodegenerative disorders and age-related dementia.

A6 The Androgen Receptor In Human Skeletal Muscle Biopsies

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Background: Aging is often accompanied by the loss of skeletal muscle mass, likely caused by deregulation of sex steroids hormones. Androgens increase muscle size through the androgen receptor (AR), which activates both genomic and non-genomic pathways to trigger various biological responses. Non-genomic androgen effects occur through interaction of AR with effectors or scaffolds, including the Src tyrosine-kinase and filamin A. Activation of the downstream effectors (paxillin, FAK, MAPK, Akt) then follows.
Methods: The activation status of key molecules linking the AR non-genomic axis with cytoskeleton organization was analyzed by Western blot of lysate proteins of human skeletal muscle biopsies from young or old women.
Results: Phosphorylation of both Ser-2152 filamin A and Tyr-118 paxillin is stronger in biopsies from old women compared with those obtained from young women. Conversely, the expression of sex steroid receptors, AR and ERα, is weaker in samples from old women, as compared with those obtained from young women.
Conclusions: This pilot study suggests that derangement of the AR axis occurs in skeletal muscle of old women. This event likely leads to excessive metabolic functions and loss of skeletal muscle. Further investigation in cultured cells and mouse models might help us in targeting the skeletal muscle AR axis with new compounds, such as new selective androgen receptor modulators or stapled-peptides, to improve the clinical outcome of age-related frailty and sarcopenia.

A7 Oxysterols Along the Course of Alzheimer's Disease

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Background: Alzheimer's disease (AD) is associated with neuroinflammation, oxidative stress and dysregulated lipid homeostasis within the brain. The molecular mechanisms underlying this disease are not clearand to date no biomarkers for AD early diagnosis are available. Oxysterols could represent new biomarkers for AD since their levels change in brains during AD progression. In particular, oxysterols derived from cholesterol oxidation by CYP46A1 and CYP27A1, respectively 24-hydroxycholesterol and 27-hydroxycholesterol, and oxysterols derived from cholesterol auto-oxidation, such as 7-ketocholesterol, play a key role in AD progression.
Methods: The brains were classified based on the Braak and Braak staging system of neurofibrillary pathology (early AD: stage 1 or 2; late AD: stage 4 to 6). In the control brains the presence of senile plaques and tau pathology was excluded. Oxysterols were measured by isotope dilution mass spectrometry. Inflammatory molecule expression was analyzed by qRT-PCR.
Results: A variety of oxysterols, of both enzymatic and non enzymatic origin were identified and quantified in AD brains taking into account the different Braak and Braak stages of AD. The enhancement of a few inflammatory mediators and the proteolytic enzyme MMP-9 was also demonstrated in the brains in agreement with the progression of the disease. Conversely, a marked reduction of sirtuin 1, an enzyme that regulates several pathways involved in the anti-inflammatory response, was observed with the progression of AD.
Conclusions: The pathogenic correlation between the amount of oxysterols in AD brains and neuroinflammation is highlighted. Oxysterols could represent novel markers for early diagnosis and progression of AD.

A8 Anemia in the Elderly: A Laboratory Evaluation in Hospitalized and Non-Hospitalized Patients

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Background: In the elderly, anemia is reported to be frequent and often not readily attributable to a single cause. This condition is often overlooked in medical records, although substantial evidences support the association of even mild conditions of anemia and increased mortality in older individuals. Aim of the present study was to investigate retrospectively the prevalence and the main laboratory features of anemia in a large cohort of hospitalized and non-hospitalized Calabrian patients aged 65 and over.
Methods: In this observational study, we examined 4,576 consecutive patients, aged ≥65 years, referred to the laboratory of Clinical Pathology, University Hospital of Catanzaro, for red blood cell testing. Anemia was defined according to WHO [Hb <12g/dl in females (f), and <13g/dl in males (m), respectively]. Blood cell analyses were performed by ADVIA 2120 (Siemens).
Results: In the hospitalized (n=2,045), vs. non-hospitalized patients (n=2,531), prevalence of anemia was 35% vs. 21%. In both groups, the majority of patients (80-85%) had mild anemia (Hb:10-13g/dl), whereas severe anemia (<8 g/dl) was present in less than 3%. In both groups, the most common form was normocytic anemia (57-67%); microcytic anemia was prevalent in females [33-40% (f) vs. 20-30% (m)], but macrocytic anemia prevailed in males [7-13%(m) vs. 3-9%(f)]. Hypochromic anemia was more common in females [26-33%(f) vs. 20-29%(m)].
Conclusions: Our findings highlight, in our population, the wide prevalence of anemia in the elderly. While most patients are affected by mild anemia, gender differences among RBC indexes may account for the multiple causes linked to anemia.

A9 MicroRNA Mediated Methylation in Cellular Senescence

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Background: Aging is a multifaceted process characterized by genetic and epigenetic genome modifications, is associated with a gradual decline in the physiological functions of the human body, and is a key risk factor for many diseases. DNA methylation, histone modifications, and noncoding RNA species are the most studied epigenetic marks and have a central role in aging and age-related diseases. MicroRNA (miRNAs) reveal a complicated network of reciprocal interconnections in epigenetics: the epigenetic modifications can affect miRNA expression, but miRNAs can also control gene expression at the post-transcription level, including genes that are implicated in the epigenetic machinery.
Methods: MiRNA profiles were determined from young and senescent primary cultured human umbilical vein endothelial cells (HUVECs) and dermal fibroblasts (HuDE). The protein and mRNA expression levels of DNMT1 were identified by, respectively, Western Blot and real time PCR.
Results: MiR-148a was the most down-regulated mirRNA during replicative senescence of HUVEC and HuDE. DNA methyltransferase 1 (DNMT1) and DNA methyltransferase 3B, are the validated targets of miR-148a. Our results showed a marked up-regulation of DNMT1 in both HUVEC and HuDE senescent cells compared with young cells.
Conclusions: Our results demonstrate a complex relationship between epigenetic mechanisms and the aging process and suggest that experimental approaches should not only focus on DNA methylation but also on non-coding RNAs. DNA methylation and microRNA deregulation are involved in the phenomena known as “epigenetic drift,” which is characterized by gradual extensive demethylation of genome and hypermethylation of a number of promoter-associated CpG islands.

A10 Correlation Between Bone Marrow Adipose Tissue, Aging and Osteoporosis: Is There a Role for miRNAs?

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Background: Marrow adipose tissue (MAT) accumulation is correlated with decreases in cortical bone and low bone mineral density. It is unclear whether MAT has a causal role or simply represents a passive response to bone loss. In any case, MAT accumulation and bone loss occur with age and aging represents a risk factor for osteoporosis. MiRNAs are a broad class of small noncoding RNAs that act mainly modulating gene expression; they can be released by cells and circulate in the bloodstream in a remarkably stable form, thus representing systemic and tissue specific diagnostic/prognostic biomarkers for a number of age-related diseases. Recently, their use as biomarkers of osteoporosis has been suggested.
Methods: Adipocytes were obtained *in vitro* from bone marrow-derived stromal cells (BMSCs). MiRNA expression profiles of BMSCs and adipocytes were performed using human microRNA Array pool A (Applied Biosystems, Foster City, CA). Significantly deregulated miRNAs in adipocytes compared to BMSCs were validated by RT-qPCR.
Results: A miRNA signature associated with bone marrow adipogenesis was identified. Validation analysis confirm the modulation of a set of miRNAs in bone marrow-derived adipocytes compared to BMSCs. A comparative analysis highlighted that some of them are aging- (miR-181a, miR-34a, miR-126, miR-155) and more interestingly osteoporosis- (miR-146a and miR-21) associated miRNAs.
Conclusions: These data suggest for the first time that bone marrow adipocytes could contribute to circulating microRNA signature associated with aging and age-related diseases such as osteoporosis.

A11 Successful Aging and Longevity: The Role of Functional Foods

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Background: During the last two centuries the most elderly are becoming the population with the fastest growth in the Western World. Although the average life expectancy is increasing dramatically, the healthy lifespan is not growing at the same pace. This underscores the importance of studies on the prevention of age-related diseases in order to decrease medical, economic and social problems associated to aging, related to an increased number of individuals affected by incapacitating pathologies. In particular, data from experimental studies have consistently shown that nutrient-sensing pathways are involved in longevity, affecting the prevalence of age-related loss of function, including diseases.
Methods: To discuss the potential relevance of diet and the nutrient-sensing pathway in the attainment of successful aging and longevity, we performed nutritional intervention using Sicilian local foods, analyzing inflammatory and oxidative stress markers.
Results: Data clearly demonstrate the positive effects of nutraceuticals, functional foods, and Mediterranean diet on several biological parameters. In fact, they could represent a prevention for many age-related diseases, and, if not a solution, at least a remedy to alleviate them.
Conclusions: The possibility to use, in combination, both nutraceuticals and functional foods should allow creation of a new therapeutic strategy, based on an integrated approach, using a mixture of foods rich in bioactive molecules, starting from local dietary habits, to create a “nutrafunctional diet” applicable worldwide.

BIOMARKERS FOR MOLECULAR PREVENTION AND THERAPY

BM1 Soluble Receptor for Advanced Glycation End Products: An Early Biomarker of Cardiometabolic Risk in Healthy Women

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Background: The receptor for advanced glycation end products (RAGE) is a cell-surface protein promoting inflammation, adipocyte hypertrophy, and insulin resistance. Besides the cell surface form, RAGE exists as a soluble molecule (sRAGE), a decoy receptor which, sequestering RAGE ligands, acts as a cytoprotective agent. To date, it is unclear whether the lower sRAGE level observed in obesity is a marker of increased overall adiposity or reflects increases in particular fat depots. We evaluated the relationship among sRAGE and indicators of adiposity, including abdominal visceral (VAT) and epicardial visceral (EAT) adipose tissues, to explore the potential role of sRAGE as an earlier biomarker of cadiometabolic risk.
Methods: Forty-seven healthy women (mean age 33.3 yrs) were enrolled. Anthropometric and biochemical data were recorded. Fat mass (FM) was estimated by bioimpedance analysis. Abdominal subcutaneous (SAT), VAT, and EAT volumes were measured by magnetic resonance. sRAGE was quantified by ELISA.
Results: sRAGE was lower in obese compared both to normal- and over-weight women and in women with a waist circumference (WC) larger than the cutoff of 80 cm. A strong inverse association of sRAGE was observed with body mass index and FM. Concerning adipose tissue distribution, sRAGE inversely correlated with WC, EAT and VAT. In a multiple stepwise regression analysis, performed to emphasize the role of fat distribution, EAT volume was the only predictor of sRAGE.
Conclusions: Lower sRAGE level reflects accumulation of visceral fat mainly at epicardial level and is present in advance of the appearance of metabolic complications. sRAGE quantification may be an early marker of cardiometabolic risk.

BM2 cAmpRGD-Sunitinib Conjugates as Integrin Targeted Delivery Strategy for Inhibition of Angiogenesis and Tumor Growth

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Background: New strategies for antiangiogenic treatment of cancer are under continuous investigation. The vascular endothelial growth factor receptor (VEGFR) signaling cascade and integrin $\alpha_v\beta_3$ exert a critical role in tumor angiogenesis both representing valid targets for anti-angiogenic therapies. The $\alpha_v\beta_3$ intergin strongly contributes to neo-angiogenic vascular development directly and indirectly through its crosstalk with VEGFR. Anti-VEGFR therapies and $\alpha_v\beta_3$ integrin antagonist treatments demonstrated efficient anti-angiogenic activity in preclinical models but an inefficient drug administration limits therapeutic efficiency of the treatments. Interestingly, radiolabeled ligands, which target the VEGF receptor or $\alpha_v\beta_3$ antagonist Cilengitide, have successfully been developed for early and sensitive lesion detection by using positron emission tomography. In the present study we introduced innovative dual conjugates in which selective arginine-glycin-aspartic-(RGD) antagonists combine with Sunitinib, a receptor tyrosine kinase inhibitor (TKI) used as frontline treatment for anti-angiogenic and anti-tumor treatment.
Methods: Inhibition of cell growth and adhesion to RGD substrate, apoptosis assay and tubulogenesis.
Results: Different RGD-Sunitinib conjugates showed efficient inhibitory activity on cell adhesion in endothelial precursor cells and melanoma cells, expressing $\alpha_v\beta_3$ integrin. We found inhibition in TK activity in cells exposed to the different conjugates and a strong synergistic effect on the inhibition of tubulogenesis.
Conclusions: A conjugate carrying two RGD moieties showed a significant synergistic effect on $\alpha_v\beta_3$ and VEGFR inhibition. This compound will be used as a new antiangiogenic treatment in tumor bearing mice and, upon radiolabeling, it might be used in preclinical molecular imaging of early cancer lesions.

BM3 Serum Levels of Matrix Metalloproteinase-9 (MMP-9) and Tissue Inhibitor of MMP (TIMP-1) in Metabolic Syndrome Patients Treated with Mesoglycan

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Background: Mesoglycan is a glycosaminoglycan compound with antithrombotic and profibrinolityc activities, which has been shown to be clinically effective on vascular remodeling. Vascular remodeling is prevalently mediated by MMP-9 and TIMP-1. Herein we examined serum MMP-9 and TIMP-1 levels in sera from metabolic syndrome (MetS) patients treated with mesoglycan and compared these parameters with the elastic properties of the arterial wall.
Methods: Thirty consecutive patients with MetS were enrolled. Patients were double-blind randomized in a 2:1 scheme: 20 patients received mesoglycan per os (50mg b.i.d.) and 10 patients received placebo for 90 days. At baseline (time 0) and at the end of the 90-day period, all patients underwent an ultrasound evaluation to assess the elastic arterial wall properties using distensibility coefficient (DC) and brachial artery stiffness (b). Blood samples were collected for measuring MMP-9, by ELISA and zymography, and TIMP-1, by ELISA.
Results: After 90 days, treated patients showed a marked improvement in arterial elasticity, with an increase of DC and decrease of b-stiffness values, compared to both basal levels and placebo. Consistently, we observed a significant decrease of serum MMP-9 ($p=0.035$) and TIMP-1 ($p=0.049$). Moreover, MMP-9 multimeric form (240kDa) activity was increased ($p=0.046$), with a positive correlation with DC values ($p=0.032$, $rho=0.52$) and a negative correlation with b-stiffness values ($p=0.005$, $rho=-0.58$).
Conclusions: This preliminary study suggests that in patients with MetS, mesoglycan exerts relevant effects on vascular remodeling by modulating MMP-9 expression and enzymatic activity. Thus, serum MMP-9 evaluation might give additional useful information in MetS patient management.

BM4 Effect of Dual PI3K and FAK Inhibition in Squamous Lung Carcinoma with Reduced PTEN Level

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Background: Squamous cell carcinoma of the lung (SCLC) is a group of histologically non-small cell lung cancer (NSCLC) that account for 20% of all lung cancers; to date half of these patients present with metastatic disease, and the chemotherapy regimen is the only treatment proposed. Recent efforts to identify molecular "drivers" have shown some altered pathways including PI3K/Akt signaling. The most relevant alterations are the overexpression and point mutations of PIK3CA and loss of expression of the PTEN tumor suppressor gene, pointing out their role as candidate targets for specific drugs.
Methods: SKMES-1 SCLC cells were stably transfected with a pull of four plasmids carrying shRNA directed to PTEN mRNA and clones with stable reduction of PTEN levels were selected for further investigation.
Results: Reduction of PTEN expression caused an increased growth rate, enhanced migration and invasiveness, and the acquisition of a mesenchymal phenotype, confirmed by increased miR-21 expression. Moreover an increased activation of both Akt and FAK kinases was reported. In this context we investigated the effect of the combination of the PI3K inhibitor buparlisib and the FAK inhibitor defactinib: a synergistic inhibition of cell proliferation, associated with a reduction of cell migration and invasion was observed in cells with reduced PTEN level. These

results are related to a reduction of phosphorylation of some kinases (Akt, FAK, JNK, GSK-3 α/β, AMPK-α2).
Conclusions: These data support a role for PTEN reduction as a good predictive factor for the clinical application of PI3K and FAK inhibitors in SCLC.

BM5 Clinical and Technological Output of the Telemedicine Kosmomed Program
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Background: Kosmomed is an integrated project to provide innovative products and services for medical practice based on information and communication technology associated with satellite telecommunications.
Methods: The program proposes a novel paradigm for satellite telemedicine solution by the development of a bandwidth reservation mechanism integrated with a data protection infrastructure. . The network allows coordinated access to the service platform with a guaranteed on-demand bandwidth allocation and associated billing, finally able to provide quality of service for professional and sustainable telemedicine devices. Diagnostic equipment is easily accessible to individuals.
Results: The program improves and integrates new sensors/analyzing equipment for medical use by the creation of innovative acquisition interfaces, to reach improved performance in data acquisition and evaluation. 220 patients were enrolled and distributed along the different program services: a) tele-oncology; b) tele-dermatology; c) teleconsulting for childhood obesity; d) tele-consulting for vascular disease; e) tele-consulting on data acquired by electronic noise. User compliance and approval reached high scores. Cooperativity among clinicians has been highly improved, shortening time for diagnostic assessment and/or for specific treatment settings. Diagnosis has been improved in almost 30% of cancer patients.
Conclusions: Specific technologic tools have been developed to enable: a) wireless control and monitoring of cardiac and pulmonary functions; b) integrated sensory tools in analyzing liquid/volatile parameters; c) fractal and morphological digital image analysis devices. The Kosmomed program is a reliable and affordable instrument for the delivery of health services.

BM6 Evaluation of Multidrug-resistant (MDR) *Klebsiella spp.* Resistance Pattern in Khartoum, Sudan
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Background: Bacterial resistance to antimicrobial drugs has reached alarming levels. Multidrug-resistant (MDR) *Klebsiella spp*. has become a major public health concern in Sudan and many countries, causing failure in treatment with consequent huge health burden.
Objective: To determine the prevalence and susceptibility of MDR *Klebsiella spp*. among different clinical isolates.
Methods: A total of 349 *Klebsiella spp.* strains were isolated between June 2009 and October 2015. Different clinical specimens (mainly swab culture, urinary tract , and respiratory tract isolates) were identified and tested for their antimicrobial susceptibility.
Results: Of the 349 *Klebsiella spp*. isolated, MDR *Klebsiella spp*. was present in 264 (76%). Resistance rates were recorded to: ampicillin-sulbactam (95%), ceftotaxime (93%), tetracycline (82%),tazobactam-piperacillin (59%), chloramphenicol (51%), ciprofloxacin (45%), gentamycin (23%), and amikacin (9%).
Conclusions: *Klebsiella spp*. showed high resistance against ampicillin-sulbacatm and cefotaxime (95% and 93% respectively). Amikacin was the most effective drug among the antibiotics tested (91% of the strains were susceptible). This study showed that *Klebsiella spp.* presents high level of resistance. This issue needs to be addressed in national guidelines. Antimicrobial resistance surveillance and epidemiological analysis of patient data need to be conducted periodically and can be informative for appropriate management of antimicrobial resistance.

BM7 Biological and Molecular Effects of Belimumab in Systemic Lupus Erythematosus Patients: A Pilot Study
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Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with variable clinical presentation and severity. The monoclonal antibody belimumab (BLM) targets B-lymphocyte stimulator (BLyS), a cytokine that is overexpressed in SLE and sustains B-cell proliferation and autoantibody secretion. BLM represents the first innovative SLE therapy in several years.
Methods: The study involves 6 female SLE patients (mean age 50.8±5.5 yrs) and 3 age-sex matched blood donors. Intravenous BLM was administered every 28 days. On day 0 and at 3, 6, 9 and 12 months follow-up, BLyS serum levels, and PBMC mRNA expression of BLyS, April (its cognate cytokine) and their three receptors (BAFFR, TACI and BCMA) were evaluated.
Results: BLM generally induced an upregulation of BLyS serum levels (1390.4±359.3 pg/ml at day 0, 115922.9±7768.3 pg/ml at m6; p=0.03). PBMC of blood donors expressed moderate levels of BLyS and April and, among the three receptors, only BCMA was detectable. At day 0, SLE patients showed heterogeneous patterns of expression of the investigated molecules; however, after BLM treatment, 4/6 patients tended to normalize BLyS, April and BCMA expression resembling those of blood donors. Of note, these patients were those presenting the most significant upregulation of BAFFR at baseline.
Conclusions: This pilot study demonstrates that BLM induces important effects on PBMC expression of BLyS, April and its receptors. Molecular studies may be useful to better explain the mechanism of action in the single patients and to identify new biomarkers that could help select patients more prone to respond to this specific therapy.

BM8 Characterization of the Proinflammatory Profile of Synovial Fluid-Derived Exosomes of Patients With Gonarthrosis
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Background: Synovial fluid (SF)-derived exosomes have been poorly characterized and their role in joint inflammation has never been investigated thoroughly. Our goal was to characterize SF-derived exosomes of patients with gonarthrosis and to investigate their immuneregulatory properties.
Methods: Exosomes were isolated from SF of seven patients with gonarthrosis by polymer precipitation (Exoquick), quantified by acetyl-CoA acetetylcholinesterase activity and validated by exosomes-specific tetraspanins expression by immunoblot and FACS. To evaluate their immunoregulatory properties, exosomes were further purified with affinity capture antibody-coupled magnetic beads and incubated with M1 macrophages, differentiated from blood donor CD14+ monocytes, to investigate the expression of co-stimulatory molecules by flow cytometry and production of cytokines, chemokines and MMPs by multiplex ELISA.
Results: SF-derived exosomes (1.3±1.4x1011/ml) expressed specific markers (CD9, CD63, CD81 TSG101) by immunoblot and FACS. After Exoquick isolation, contamination by immune complexes was detected. To avoid possible interference in functional experiments by immune complexes, we added a further isolation step. SF-derived exosomes significantly stimulated the production of several inflammatory cytokines and chemokines (IL1β, TNFα, IL16, CCL20, CCL15, CCCL1) and the release of specific metalloproteinases (MMP7 and MMP12) by M1

macrophages, although they did not influence the expression of CD80 and CD86 co-stimulatory molecules on M1 macrophages.
Conclusions: We characterized exosomes isolated from SF of patients with gonarthrosis and demonstrated that they are functionally active in their ability to stimulate the release of proinflammatory cytokines and MMPs from M1 macrophages, suggesting that they may play a role in disease progression.

BM9 MicroRNA: A Striking Player in Alzheimer's Disease
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Background: Alzheimer's disease (AD) is a common and complex age-related neurological disorder worldwide. Despite the advancements in understanding the genetic, molecular, and environmental factors of AD, there are no effective treatments to stop or reverse AD related symptoms. Early diagnosis of AD remains a big challenge as it takes a decade or more before the illness appears. Furthermore, post-mortem verification is often required. Therefore, there is an urgent need of biomarkers that can effectively detect early stage AD. MicroRNA (miRNA) is a class of non-coding RNA (~22-nt long) that regulates gene expression post-transcriptionally. Accumulating evidence suggests that miRNA plays a crucial role in neural development and differentiation. About 70% of the currently identified miRNAs are reported to be expressed in the brain. In this study, we systematically evaluated the available literature to understand the role of miRNA in AD pathogenesis.
Methods: The major biomedical databases were searched systematically to identify miRNA expression signatures in AD. Relevant articles were extracted by online searching using a combination of the following MeSH terms: microRNA or miRNA or miR and Alzheimer's Disease.
Results: We found that a number of miRNAs are deregulated in AD patients compared to the normal age-matched controls.
Conclusions: Our data suggest that miRNA signatures may have potential to be considered as a screening tool as well as a therapeutic target for AD.

BM10 Urinary Exosomes as Biomarkers in Prostate Cancer: A Pilot Study
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Background: Exosomes circulating in biological fluids largely mirror the molecular profile of the originating cells. Given the non-invasive nature of urine collection,analysis of urinary exosome content could be useful in the identification of new biomarkers in prostate cancer. This pilot study evaluated the expression of known prostate cancer genes (PSA, PCA3 and PSMA) as potential exosomes biomarkers.
Methods: mRNA and protein contents (PSMA, CD63, CD9) of urinary exosomes collected from 20 pre-and 14 post-radical prostatectomy patients and 8 healthy age-matched men were examined. The correct isolation of exosomes was confirmed by dynamic light-scattering, transmission electron microscopy, and Western-blot analysis. Western-blot analysis, RT-PCR and nested RT-PCR were used to evaluate the expression of PSMA, PSA and PCA3 content, respectively.
Results: Expression of PSA in urinary exosomes displayed 70% sensitivity, 62.5% specificity and an overall accuracy of 67.8% in identifying prostate cancer. PCA3 sensitivity, specificity and overall accuracy were 55%, 87.5%, and 64.3%, respectively. PSMA sensitivity, specificity, and overall accuracy were 76.5%, 57.1%, and 70.8%, respectively. These values were 85%, 87.5%, and 85.7%, respectively, when all three genes were simultaneously detected in extracted exosomes. Moreover, expression of PSA, PCA3, and PSMA were no longer detectable in a significant fraction of patients after surgery (p=0.0005, p= 0.032, p=0.0002, respectively).
Conclusions: This pilot study suggests that urinary exosomes could be used as non-invasive biomarkers for prostate cancer detection, with an overall accuracy comparable to the traditional PSA. Levels of urinary exosomes are strongly related to surgery and could be adopted into post-operative surveillance.

BM11 Identification of Double-strand Genomic DNA In Colon Cancer Exosomes: Is it a Potential Non-invasive Diagnostic Tool?
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Background: Liquid biopsy offers a promising alternative for tumor characterization and disease monitoring. Exosomes shed by cancer cells are attractive for their content stability in biological fluids and ability to mirror the molecular profile of the originating cells. This study aimed to evaluate the possibility to identify exosomes-associated DNA from colon cancer cell lines and from plasma of colorectal cancer patients (CRC pts) and to analyze their potential use in clinical settings.
Methods: Exosomes were isolated by ultracentrifugation from plasma of 10 CRC pts and from the supernatant of human colon cancer cells and were characterized. Mutation analysis of *KRAS* in DNA extracted from exosomes was evaluated by real-time PCR and confirmed by PCR-RFLP.
Results: We set up the protocols for DNA isolation from exosomes and for the analysis of *KRAS* G12V mutation by HRM analysis. *KRAS* mutation was confirmed by PCR-RFLP using *Mva*I endonuclease digestion. The analysis of exosomes DNA extracted from supernatant of colorectal cancer cells or from plasma of CRC pts confirmed that the isolated DNA displays the same mutational status of DNA extracted from cancer cell lines or from the corresponding primary tumors, respectively.
Conclusions: This pilot study suggests that DNA extracted from exosomes can be useful for the analysis of *KRAS* mutational status in colorectal cancer patients. We are currently evaluating the possibility to detect mutations in other genes (i.e., *BRAF*). Further studies are warranted to evaluate the suitability of exosome extracted DNA as a non-invasive diagnostic, predictive, and surveillance biomarker in colon cancer patients.

BM12 Asymmetric Dimethylarginine (ADMA) In Asymptomatic Cerebral Small Vessel Disease
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Background: Cerebral small vessel disease (CSVD), detected as white matter hyperintensities (WMH) on brain MRI, could be a distinct pathological entity within cerebrovascular diseases. However, the several pathogenic hypotheses so far proposed were based on very heterogeneous clinical settings. Endothelial dysfunction seems to play a pivotal role but more studies are needed to clarify it.
Methods: This is a pilot-case control study of young patients with CSVD. WMH severity was graded using the Fazekas score. Clinical history of patients and controls were negative for vascular and heart disease, classical vascular risk factors, autoimmune disorders, and coagulopathies. None of the patients reported family history of cerebrovascular disease. Blood samples were consecutively collected in the early morning and tested for inflammatory, endothelial, and prothrombotic markers.
Results: 14 patients and 12 age- and sex-matched controls were recruited (mean age 52.6±5.9 yrs). Baseline clinical characteristics did not differ between the two groups. The mean Fazekas score in patients was 2.9±0.86. Compared to controls, patients did not display higher levels of common inflammatory markers (C-reactive protein, fibrinogen, IL-6, IL10) and plasma prothrombotic factors (PAI, vWF, tPA, PAF-AH, homocysteine). In contrast, the levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide, were significantly increased in patients compared to controls (122.2±33.7 µmol/l versus 93.4±32.8 µmol/l; p=0.04).

Conclusions: Our pilot study suggests that ADMA, a known marker of endothelial dysfunction, may represent a new biomarker to identify asymptomatic CSVD, indicating the pathway of nitric oxide may be involved in endothelial dysfunction in this clinical setting.

BM13 Platelet Activation Affects Pre-mRNA Maturation of a Group of Transcripts Useful as Markers of Acute Coronary Syndromes

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Background: Platelets represent a major player in the process of intravascular thrombus formation. Despite significant advancements in antithrombotic therapy, current strategies still fail to prevent thrombotic coronary events in a substantial number of patients, indicating that the complex mechanisms modulating platelet response during activation are not fully elucidated. The evidence that platelets are capable of *de novo* protein synthesis has raised the issue of whether and how these resident mRNAs are regulated in circulating platelets. Among the various mechanisms potentially involved, mRNA splicing may be potentially relevant.
Methods: Purified platelet-rich plasma from healthy volunteers were collected and *in vitro* activated with collagen or thrombin receptor activating peptide. Transcriptome analysis by RNA-Seq and *in silico* intron retention analysis were applied to search for splicing events affected by platelet activation. HiRIEF LC-MS allowed platelet proteome characterization at deep coverage to investigate a possible correlation between splicing events and protein levels.
Results: Extensive computational analysis following RNA-Seq revealed several splicing events occurring in activated platelets. By applying unbiased proteogenomics, we correlated intron retention events in quiescent platelets to exon-exon junctions frequency after activation. In this way we identified a set of transcripts presenting reduced intron retention and high peptide representation at exon-exon junctions in activated vs resting platelets.
Conclusions: The observed results indicate that pre-mRNA maturation of platelet-specific transcripts could be monitored and used as marker of platelet activation in acute coronary syndromes.
Acknowledgement: Research supported by MIUR (FIRB RBFR12W5V5_003), MOH (GR-2011-02347781), CNR (Flagship Project InterOmics) and Genomix4Life.

BM14 *Lactobacillus reuteri*: Production and Characterization of Membrane Vesicles for Future Health Applications

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Background: The probiotic *Lactobacillus reuteri* is effective against infantile colic, alleviation of eczema and *Helicobacter pylori* colonization. *L.reuteri* develops biofilm *in vitro*, producing factors that give health benefit to the host although the mechanism by which commensal bacteria communicate with the host remains unclear. The purpose of this study was to detect and characterize membrane vesicles (MVs), bilayer structures containing several molecules generated by bacteria, produced by *L.reuteri* DSM 17938.
Methods: The structure of MVs was evaluated by transmission and scanning electron microscope analysis. MVs were subsequently isolated by biofilm (bMVs) and planktonic (pMVs) phenotypes by ultracentrifugation and physicochemically characterized by dynamic light scattering (DLS) analysis. An enzymatic treatment was performed to determine MVs composition. eDNA was detected and quantified using the Quant-iTTMPicoGreendsDNA assay and NanoDropUV-VIS spectrophotometer. Proteins associated with MVs were extracted and quantified by BCA assay.
Results: PicoGreen showed that eDNA was associated with MVs and that its concentration is higher in bMVs than in pMVs, although an inverse correlation was found in the protein concentration, suggesting a different role of MVs in the two phenotypes. The enzymatic treatment showed that lipids and proteins represent the main structural components of MVs. The DLS analysis demonstrated that *L.reuteri* generates MVs with sizes in the nanometer range and a broad size distribution.
Conclusions: *L.reuteri* produces MVs whose main components are lipids and proteins; eDNA is also associated to MVs. The biological activity and composition of MVs may represent the starting point for future applications in the development of vesicles-based therapeutic systems.

BM15 Methylation Analysis of Protocadherin Genes in Pancreatic Adenocarcinoma

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Background: Pancreatic cancer is one of the most lethal malignancies and somatic mutations of protocadherins have been identified. Some of them were recently identified as tumor-suppressor genes; in particular, *PCDH10* is a functional tumor suppressor gene frequently silenced by methylation in multiple carcinomas. Our goal is to evaluate the prognostic significance of protocadherin methylation in pancreatic adenocarcinoma so we studied methylation in primary pancreatic cancers. Functional assays with a *PCDH10* re-expressing pancreatic cancer cell line is in progress to characterize its biological effects in pancreatic tumorigenicity.
Methods: 38 ductal pancreatic adenocarcinomas were recruited in "Casa sollievo della sofferenza" Hospital, S.Giovanni Rotondo. DNA was extracted from tumor tissue and treated with bisulfite solution. COBRA analysis and bisulfite genomic sequencing were used to determine the presence of methylated CpG in the promoter regions. A functional assay using AspC-1 and Capan-2 pancreatic cancer cell lines transfected with full length PCDH10 will assess the effect of *PCDH10* on pancreatic cancer cell growth.
Results: Cases were studied for *PCDHAC2*, *PCDHGC5* and *PCDH10* methylation status and immunohistochemistry (IHC). Results showed no *PCDHAC2* methylation pattern and ubiquitous methylation of *PCDHGC5* in all cases analysed. *PCDH10* analysis showed a partial methylation pattern in 9/18 and no methylation in 9/18 cases analysed. IHC analysis detected PCDHAC2 expression in 6% of cases analysed and PCDHGC5 in 30%. IHC of PCDH10 is in progress.
Conclusions: The identification of aberrantly hypermethylated and silenced genes will have diagnostic, prognostic, and therapeutic applications. *PCDH10* may be a potential target gene for cancer therapy.

BM16 Primary Pleuro-Pulmunary Synovial Sarcoma: A Single-Center 13-Year Experience

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Background: Primary pleural and pulmonary synovial sarcomas (PPSSs) are very rare and aggressive neoplasms that affect adults. The oncologic characteristics, treatment, and prognosis for PPSSs are not well defined because of a paucity of data. Dysregulation of Wnt/β-catenin and EGFR pathways leads to tumorigenesis with poor prognosis. We investigated the involvement of Wnt/ β-catenin and EGFR signaling in PPSSs.

Methods: This retrospective study identified seven PPSS cases at the Department of Thoracic Surgery, SS. Annunziata Hospital in Chieti from 2002 to 2015. Paraffin tumour tissues were evaluated for protein and gene expression by IHC and RT-qPCR.
Results: The retrospective study for PPSS reveals by IHC 5 biphasic and 2 monophasic phenotypes with minimal focal epithelial component. Mesenchymal component shown vimentin expression in all PPSSs while CkAE1/AE3, EMA, E-cadherin and β-catenin were absent in the two monophasic PPSS. SYT–SSX1 fusion transcripts were found in 3 out of 7 PPSSs. Using one of the pleural normal tissues with a lower Ct value as a calibrator, RT-qPCR showed very strong expression in *LEF1* trascripts in all tumors. *APC* increased in one pulmonary PPSS whereas the other PPSS showed a normal range. EGFR was overexpressed in all samples. Interestingly, gene expression indicate a normal range of ERB-b2 and ERB-b3 expression for one pleural monophasic sample, while ERB-b4 was over expressed.
Conclusions: Preliminary results confirmed the involvement of Wnt/β-catenin and EGFR pathways in PPSSs and highlight unanswered questions, the solutions of which will be imperative in the rational exploration of these pathways in future molecular treatment strategies.

BM17 Molecular Analysis of Base Excision Repair (BER) Genes in Breast and Ovarian Cancer Patients

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Background: The etiology of breast cancer (BC) is multi-factorial. The mammary tissue is particularly exposed to oxidative stress because of specific hormone metabolism. This state can produce oxidized DNA lesions such as 8-hydroxyguanine (8-oxoG), physiologically removed by the base excision repair (BER) pathway. A reduction of activities and/or transcript dosage imbalance in the BER genes may have a role in BC predisposition.
Methods: DNA and RNA from peripheral blood mononuclear cells (PBMC) of 63 BC and/or ovarian cancer (OC) patients, previously screened for *BRCA1/BRCA2*, were studied for *MUTYH* and *OGG1* germline variants using dHPLC and sequencing; TaqMan assays were employed to test mRNA expression levels.
Results: Forty-six patients presented with cancer family history, 28 of them showing vertical transmission of BC; 17 were BC/OC early onset without family history. No cases had a detectable pathogenic mutation in *BRCA1*/BRCA2 genes. Ten sequence variants were identified in *MUTYH* and *OGG1*. Importantly, 2/6 of *MUTYH* missense mutations were found in the same BC patient and not detected in 120 healty controls. Gene expression was analysed on 46 patients with available RNA and 40 healthy donors selected according to similar gender and age. The *MUTYH* gene showed reduced expression in BC patients with and without evidence of direct genetic transmission compared to OC patients.
Conclusions: Overall, significant reduced expression was observed in patient vs control group ($p < 0.05$) only for *MUYH* gene. While not conclusive, this interesting finding suggests clinical criteria for MUTYH- and OGG1-associated BC and OC phenotypes.

PATHOPHYSIOLOGY OF CANCER

C1 A Novel SIRT Inhibitor Inducing a RIP1-Caspase 8-Dependent Apoptosis

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Background: The development and progression of cancer involves both epigenetic and genetic changes leading to the alteration of gene expression and cell phenotype [Sharma S et al. Epigenetics in cancer. *Carcinogenesis*. 2010]. Emerging evidences suggest the role of acetylation as a post-translational modification that plays a critical role in cell fate. The best known epigenetic enzymes in cancer therapy are histone deacetylases. In the last decade interest in Sirtuins as potential promising targets for cancer treatment has grown [Carafa V et al. Sirtuin functions and modulation: from chemistry to the clinic.*Clin Epigenetics* 2016].
Methods: We characterized a new SIRT inhibitor showing a multi-acting inhibition towards SirT1-2-4-5. The new molecule is inactive against HATs and HDACs.
Results: The new molecule displays enhanced tumor-selective potential *in vitro*, in leukemic blasts *ex vivo*, and *in vivo* in both xenograft and allograft cancer models. PK and PD studies support its use *in vivo*. This SIRT inhibitor induces strong proliferation arrest and cell death in many cancer cells but is unable to induce death in normal cells. Gene expression analyses highlighted the induction of the death-receptor pathway, confirmed by the activation of both TRAIL and DR5 promoters.
Conclusions: We defined a new specific death pathway in which Caspase8 and RIP1 have a functional role. Based on our findings, we hypothesize that Sirtuin modulation may play a key role in the induction of this cell death underscoring the action of SIRT1 in tumor cells.
Acknowledgement: We thank the Worldwide Cancer Research (AICR) for support (grant 15 1002).

C2 Hybrid LSD1/JmjC Inhibitor as a Therapeutic Option in Cancers with Deregulated Hormone-Receptor Signaling

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Background: The field of epigenetic-based drug discovery is in a transitional phase where the search for drugs is shifting from single-target-oriented molecules to network-active compounds. In many cancers, lsyine-specific histone demethylase 1 (LSD1) and Junonji C (JmjC) are co-expressed and co-localize with hormone receptors (AR, ER, PML-RAR), suggesting the potential use of hybrid molecules to target both enzymatic functions and to regulate hormone receptor signaling.
Methods: We report a novel "pan-KDM" inhibitor, obtained by coupling the chemical features of tranylcypromine with the 2OG competitive moiety (*J Med Chem* 2014, 57:42-55). The hybrid molecule displays unique features compared with scaffolds and well known single inhibitors. This compound inhibits LSD1 and JmjC enzymes, induces growth arrest and apoptosis in hormone responsive cells accompanied by strong increases in levels of H3K4me2/3, H3K9me2/3 and H3K27me3.
Results: Treatment with the hybrid molecule reduces the level of ER (in MCF-7), AR (in LnCaP) and PML-RAR (in NB4) by both a transcriptional and non-trancriptional manner. The same treatment changes the methylation status of ER/AR/PML-RAR regulated promoter regions, affecting the transcription of several genes. In *ex vivo* blasts and breast cancers, the hybrid molecule reduces cell proliferation, such as in cellular models with acquired resistance to hormone stimuli (LnCaP C4-2) by rebalancing the epigenetic signature of histones.
Conclusions: In these systems the multiple-target inhibitor may overcome potential mechanism(s) of resistance caused by redundancy and robustness of biological pathways, finding application in the treatment of resistant-to-therapy cancers (triple-negative breast cancers and cancers with acquired resistance).
Acknowledgement: This project is funded by Blueprint Project Contract No. 28251 and PRIN2012ZNH9YH_002.

C3 Novel Mechanisms Involved in the Stimulatory Effects Exerted by Zinc in Breast Cancer Cells

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Background: Zinc (Zn) is an essential element required for diverse cellular processes, including DNA and protein synthesis, enzyme activity and intracellular signaling; however, Zn has been also implicated in the progression of breast tumor. Estrogens stimulate the development of breast cancer mainly binding to and activating estrogen receptor (ER)α and ERβ that regulate the expression of genes involved in cell proliferation, migration, and survival. In addition, the G protein estrogen receptor, namely GPER, has been shown to mediate estrogen action in diverse types of normal and malignant cells.
Methods: To ascertain whether zinc chloride ($ZnCl_2$) might trigger the transduction signaling mediated by GPER, we performed co-immunoprecipitation studies, luciferase assays, gene expression experiments, immunoblotting and cell cycle analysis, and proliferation and migration assays in SkBr3 breast cancer cells that lack the classical ERs.
Results: We found that GPER is involved in the $ZnCl_2$ dependent activation of both epidermal growth factor receptor (EGFR) and insulin-like growth factor receptor I (IGF-IR) transduction pathways as well as downstream effectors like ERK and AKT. Moreover, $ZnCl_2$ stimulated a functional interaction of GPER with EGFR and IGF-IR toward gene transcription and biological responses such as cell-cycle progression, proliferation, and migration of the SkBr3 breast cancer cells.
Conclusions: Our data provide novel insights into the molecular mechanisms through which GPER may contribute to the stimulatory effects exerted by zinc in cancer cells toward breast tumor progression.

C4 Effects of m-TOR Inhibitor Everolimus on Human Breast Cancer Cells

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Background: Breast cancer is a heterogeneous disease, divided into 4 categories based on the receptor profile: luminal A, luminal B, basal-like, and HER2-like. Luminal A/B correspond to the hormone-responsive breast cancer phenotypes and their therapy consists of anti-hormonal molecules; however, resistance to these agents may occur. Considering that the signaling pathway of steroid hormones is mediated by PI3k/Akt and that the mammalian target of rapamycin (mTOR) is a downstream mediator of Akt, some mTOR inhibitors, such as everolimus, were developed to overcome the resistance to anti-hormonal therapy. Unfortunately not all hormone-sensitive breast cancer cells have a good responsiveness to everolimus *in vitro*.
Methods: Two human breast cancer cell lines were utilized: BT-474 (luminal B) and MCF-7 (luminal A). Apoptosis was analyzed by Western blot of PARP, BAX/Bcl2 and caspase 8/9 and by caspase 3 immunofluorescence and FACS analysis of annexin V/propidium iodide. Resistance was analyzed by Western blot of survivin.
Results: We chose BT-474 and MCF-7 among other Lumninal A/B cancers because the first responded to everolimus with apoptosis and the second was not sensitive. At the same time everolimus upregulated survivin, an anti-apoptotic protein, in MCF-7 and downregulated survivin in BT-474. Inhibiting this upregulation of survivin on MCF-7 with the utilization of YM155, the apoptotic response to everolimus was replaced.
Conclusions: These data demonstrate that the pro-apoptotic response of hormone-sensitive breast cancer cells to everolimus can be limited by the over-expression of survivin and that the forced diminution of survivin can sensitize tumor resistant cells to everolimus-induced apoptosis.

C5 Ligand-Activated PPARγ Downregulates *CXCR4* Gene Expression through a Novel Identified PPAR Response Element and Inhibits Breast Cancer Progression

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Background: SDF-1 and its cognate receptor CXCR4 play a key role in mediating breast cancer cell invasion. Therefore, drugs able to inhibit CXCR4 activation may add critical tools to reduce tumor progression, especially in the most aggressive form of breast cancer disease. PPARγ, a member of the nuclear receptor superfamily, has been found to downregulate CXCR4 expression in several cancer cells, however the molecular mechanism underlying this effect is not fully understood.
Methods: CXCR4 expression was evaluated by RT-PCR, immunoblotting and immunofluorescence in different breast cancer cells and in cancer-associated fibroblasts (CAFs) treated with PPARG ligand BRL. Transient transfections, DNA-affinity-precipitation-assay, chromatin immunoprecipitation (ChIP) and re-ChIP assays were performed to identify a PPAR response element (PPRE) within the *CXCR4* promoter. Migratory promoting activities were analyzed by wound healing/transmigration/invasion assays.
Results: We found that ligand-activated PPARγ downregulated *CXCR4* transcriptional activity through the recruitment of SMRT corepressor onto a newly identified PPRE within the *CXCR4* promoter. As a consequence, BRL significantly inhibited cell migration and invasion in a PPARG-dependent manner. According to the ability of CAFs to secrete high levels of SDF-1, BRL reduced migratory promoting activities and affected CXCR4 pathways induced by CAF-derived conditioned media. CAFs exposed to BRL showed a decreased CXCR4 expression, a reduced motility and invasion along with a phenotype characterized by an altered morphology.
Conclusions: Our findings provide novel insights into the role of PPARγ in inhibiting breast cancer progression and further highlight the utility of PPARγ ligands for future therapies aimed at targeting both cancer and surrounding stromal cells in breast cancer patients.

C6 Targeting Breast Cancer-associated Fibroblasts with Farnesoid X Receptor Ligands as a Novel Treatment Strategy for Breast Cancer

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Background: Breast cancer-associated fibroblasts (CAFs) play multiple roles in tumor initiation and promotion. Early studies have shown that activation of nuclear Farnesoid X Receptor (FXR) in mammary epithelial cancer cells exerts an oncosuppressive role. However, the detailed functions of this receptor in CAFs remain to be elucidated.
Methods: Human CAFs were isolated from biopsies of primary breast tumors (n=4). FXR expression was evaluated by realtime RT-PCR, immunofluorescence and immunoblotting. FXR functional role in CAFs was tested by evaluating the effects of the selective FXR agonist GW4064 in MTT, wound healing and transmigration assays. RNA sequencing was used to compare the transcriptomes of vehicle- and GW4064-treated CAFs. Ingenuity pathway analysis (IPA) was used to calculate the activation z-score of molecular canonical pathways. FXR impact on tumor-promoting abilities of CAFs was assessed in coculture experiments with human estrogen receptor-positive MCF-7 and -negative SkBr3 breast cancer cells using soft-agar growth assays.
Results: We found FXR mRNA and protein expression in CAFs. GW4064 treatment significantly decreased CAF motility without affecting proliferation. Quantitative transcriptome analysis revealed 1182 differentially expressed genes in response to GW4064 treatment. IPA evidenced a marked reduction in the activity of RhoA signaling, regulation of actin-based motility by Rho and signaling by Rho Family GTPases, (activation z-score: -1, -0.8, -0.8, respectively). Finally, MCF-7 and SkBr3

cells cocultured with GW4064-treated CAFs showed reduced anchorage-independent growth.
Conclusions: The present and our previous studies propose FXR ligands as potential pharmacological tools that, targeting both cancer and activated stromal cells, may represent a more effective approach to treat breast cancer patients.

C7 High-density Lipoproteins Inhibit Oxidative Stress-Induced Prostate Cancer Cell Proliferation
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Background: Oxidative stress plays a role in the pathogenesis and the progression of prostate cancer (PCa). Reactive oxygen species (ROS) are normally generated during cell metabolism and PCa cells generate a higher amount of ROS proportionally to an aggressive phenotype. High-density lipoprotein (HDL) particles are known to prevent atherosclerosis through different mechanisms, including their antioxidant properties. Thus, the aim was to assess in two human PCa cell lines (LNCaP-androgen dependent, and PC3-androgen independent) whether antioxidant properties of HDL were able to reduce oxidative stress and the related cell proliferation.
Methods: HDL isolation from plasma of healthy human volunteers, RNA isolation, qPCR, cell proliferation and cell cycle analysis.
Results: H_2O_2 induced oxidative stress in LNCaP (62.6%) and PC3 (27.7%) cells. This effect was counteracted by HDL. A 72-h treatment with 5M H_2O_2 increased LNCaP (25%) and PC3 (43%) cell growth. HDL pre-treatment abolished H_2O_2-driven LNCaP and PC3 cell proliferation. LNCaP cells seeded at 10% FBS (control) resulted in 82% of cells in G0-G1 phase, 3% in S phase and 15% in G2-M phase. H_2O_2 increased G2 phase (25%), whereas concomitant treatment with HDL restored a condition similar to the control. PC3 cells (control, 5%FBS) showed 64% of cells in G0-G1 phase, 4% in S phase and 32% in G2-M phase. H_2O_2 treatment increased G2 phase up to 52%, whereas the addition of HDL resulted in cell-cycle phase distribution similar to the control.
Conclusions: HDL exerts antioxidant activities on androgen-dependent and castration-resistant PCa cell lines, thus limiting cell proliferation induced by ROS.

C8 Crosstalk Between NF-κB and Shh Pathways in Prostate Cancer
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Background: Prostate cancer (PCa) is the most commonly diagnosed cancer in men. Common genomic alterations in PCa involve AR and PI3K pathways, rearrangements of ETS and loss of function of NKX3.1. In addition, constitutive activation of both NF-κB transcription factor and Hedgehog (Hh) pathway were suggested to play a role during the development and progression of PCa. This project aimed to investigate the crosstalk between NF-κB and Hh pathways in prostate cancer.
Methods: PCa cell lines and BPH-1 were cultured in standard conditions. RelA silencing was performed by lentiviral infection of pLentiLox3.7-human *RelA shRNA*. NF-κB-p65, Shh and Gli1 IHC was performed on prostate cancer-normal tissue array (CA3; SuperBioChips Tissue). Immunofluorescence, Western blots, Q-PCRs, MTS assay, NF-κB-DNA-binding assay (TransAM™) were performed following standard protocols.
Results: Strong correlation between NF-κB activation and Shh and Gli1 expression was observed. Hyperactivation of both NF-κB and Hh pathways were shown in androgen-independent PC-3 and Du145 cell lines, which correlate with the higher proliferative rate of these cell lines compared to androgen-dependent cell lines. Knockdown of RelA significantly decreased Gli1 levels but had no effect on SHh expression. As expected, a significant reduction of proliferative capacity was observed in RelA knockdown cell lines.
Conclusions: We demonstrated a close relationship between Gli1 and NF-κB in human PCa. Knockdown of NF-κB subunits resulted in decreased GLI1 expression, indicating that NF-κB is a regulator of Gli1 expression. Further experiments are needed to demonstrate whether NF-κB regulates Gli1 at a transcriptional or post-transcriptional level.

C9 The Role of *KCTD11* Tumor Suppressor Gene in Prostate Cancer
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Background: The loss of tumor suppressor genes seems to be an important event in the onset of prostate cancer (PCa). An increased frequency of loss of heterozygosity (LOH) in the 17p13 locus suggests that the loss of genes located in this region may have an important role in PCa progression. Interestingly, *KCTD11* maps to 17p13.2 and its expression was reduced in several human cancers by either LOH and methylation. KCTD11 was shown as an inhibitor of the sonic-hedgehog/patched/Gli-1 pathway and as an hypoxia-regulated gene.
Methods: KCTD11 IHC analysis was performed on cancer-normal tissue array (CA3; SuperBioChips Tissue Array). Human prostate cancer cell lines were cultured in standard conditions. KCTD11 overexpression was obtained by retroviral infection. qPCR, proliferation assay, Western blot and LOH analyses were performed following standard protocols. Cell cultures in hypoxic conditions were performed using a Galaxy 48R New Brunswich incubator.
Results: Nuclear KCTD11 expression was strongly reduced in primary prostate cancer and this event correlated with overexpression of proteins acting on the Hedgehog pathway, such as Gli1 and Patch1. Low levels of KCTD11 mRNA have been also observed in PCa cells; conversely ectopic overexpression of KCTD11 led to growth arrest. Notably, hypoxia significantly induced KCTD11 gene expression.
Conclusions: Our study demonstrates that KCTD11 as well as negatively regulated downstream effectors belonging to Hedgehog signaling play a role in the pathogenesis of prostate cancer. All together, these results underline KCTD11 as a possible diagnostic and therapeutic marker in prostate cancer.

C10 HDAC4 and HDAC6 Protect Glioblastoma Cells from Radiotherapy-Induced Cell Death
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Background: Glioblastoma (GBM) radioresistance restricts the curative potential of radiotherapy (RT) and HDACs inhibitors have been shown to affect GBM radioresistance through unknown molecular mechanisms.
Methods: HDAC4 or HDAC6 expression in U87MG p53WT and U251MG p53MT GBM cell lines was silenced by HDAC4- or HDAC6-shRNA. Radiation response and related molecular networks were investigated using *in vitro* experimental assays. HDAC4 and HDAC6 levels were assessed immunohistochemically on paraffin-embedded tumor samples from 31 GBM patients subjected to temozolomide and radiotherapy combined treatment.
Results: *In vitro* experiments show that silencing HDAC4 or HDAC6 radiosensitized GBM cells by promoting DNA double-strand break (DSBs) accumulation, preventing RT-induced DNA-PKcs or -ATM nuclear translocation and finally promoting U251MG apoptosis- or U87MG autophagy-mediated RT-induced cell death. Silencing HDAC4 predisposed GBM cells to RT-induced p21WAF1/CIP1-mediated cellular senescence in a p53WT dependent manner. Furthermore, silencing HDAC4 or HDAC6 reduced GBM stemness potential. HDAC-4 positivity was found in 29 (93.5%) out of 31 GBM lesions, 22 (70.9%) showed high

and 7 (22.6%) low HDAC4 expression. HDAC6 positivity was found in 30 (96.7%) out of 31 GBM lesions, 22 (70.9%) showed high and 8 (25.8%) low HDAC6 expression. By analyzing retrospective data of these patients, we found that high intensity of HDAC4 and/or HDAC6 immunohistochemical staining was shown to predict poor prognosis.
Conclusions: Altogether, these observations suggest that HDAC4 and HDAC6 are guardians of RT-induced DNA damages and stemness, thus promoting radioresistance and represent potential prognostic markers and therapeutic targets for GBM.

C11 NGF Sensitizes TrkA SH-SY5Y Neuroblastoma Cells to TRAIL-Induced Apoptosis

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Background: APO2L/TRAIL is a promising chemotherapeutic agent with pro-apoptotic action on tumour but not non-transformed cells. Neuroblastoma (NB) originates from neuroblasts transformed at different stages along the sympatho-adrenal lineage, blocked in differentiation and selected for apoptosis-resistance by oncogene activation, onco-suppressor inactivation and physiological apoptosis protection mechanism(s), the characterization of which are critical for developing novel pro-apoptotic therapeutic strategies. Therapeutic TRAIL application in NB, however, has been hampered by observations of TRAIL resistance in NB models. Here we characterize a novel mechanism for sensitizing SH-SY5Y NB cells to TRAIL-induced apoptosis, involving nerve growth factor (NGF) and its receptor TrkA.
Methods: cFLIP, Bcl-xL and dominant negative NF-κB expression; siRNA cFLIP and Mcl-1 knockdown; RT-PCR; tumour growth in soft agar; TRAIL-activated TRAIL-receptor complex purification; apoptosis and death assays; immunoprecipitation-Western blotting.
Results: Non-transfected, control and TrkA-transfected SH-SY5Y cells expressed all components for TRAIL-induced apoptosis but exhibited cFLIP-dependent TRAIL-resistance. NGF sensitized TrkA but not non-transfected or control-SH-SY5Y cells to TRAIL-induced type II apoptosis through the intrinsic pathway. NGF induced TrkA binding of cFLIP and reduced cFLIP recruitment to TRAIL-receptor complexes, consistent with cFLIP sequester. NGF effects were temporary and eventually prevented by NGF-stimulated Mcl-1 expression but optimised by siRNA Mcl-1 knockdown.
Conclusions: This novel pro-apoptotic immunological dimension for NGF/TrkA in sensitizing NB cells to TRAIL-induced apoptosis identifies TrkA as a novel competitive regulator of TRAIL-induced apoptosis, depends upon TrkA sequester of cFLIP, helps to explain TrkA association with better prognosis in NB, and provides a potential pro-apoptotic therapeutic strategy for NGF, TRAIL, and MCL-1 inhibitor use in TrkA expressing NB.

C12 Lack of Glutamine Synthetase Marks Glutamine Auxotrophy of Human Oligodendroglioma Cells

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Background: Besides protein synthesis, the amino acid glutamine plays several other metabolic roles, such as precursor of other non essential amino acids or, in several tumor cells, anaplerotic substrate and mTORC1 activator. Glutamine synthetase (GS, coded by GLUL) catalyzes the synthesis of glutamine and may play important regulatory roles. Oligodendroglioma (OD) is a rare brain tumor often characterized by GS negativity. Here we investigate GS expression and the metabolic role of glutamine in cultured OD cells.
Methods: Human OD cell lines Hs683 and HOG were grown at 4 mM glutamine. Intracellular metabolite content was measured with LC-MS/MS. Radiolabeled leucine was used for protein synthesis evaluation and radiolabeled glutamine for transport. Transient expression of GS was obtained with pCMV-*GLUL* vector.
Results: OD cells had a negligible expression of GS, even upon glutamine starvation. This condition severely reduced cell glutamine and glutamate (but not the anaplerotic substrate α-ketoglutarate), hindered protein synthesis, activated caspase-3, and caused massive cell death. However, mTORC1 was inhibited in HOG but not in Hs683 cells. The inhibition of SNAT1/2 glutamine transporters lowered intracellular glutamine and leucine, inhibited mTORC1, and hindered cell proliferation. Non-essential amino acids, but not α-ketoglutarate, partially rescued viability of glutamine-deprived cells. Upon glutamine starvation, viability and protein synthesis were partially restored by *GLUL* transfection.
Conclusions: In GS-negative OD cells glutamine starvation is markedly cytotoxic. Cytotoxicity is neither due to anaplerosis failure nor to mTORC1 inhibition but, rather, to protein synthesis suppression, partially relieved by GS expression. Thus, human OD may exemplify a glutamine-auxotroph tumor.

C13 Glutathione is a Key Player in the Multidrug Resistance of Neuroblastoma

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Background: The availability of antioxidants is recognized as one of the critical factors that are able to make cancer cells resistant to therapies. Several studies have demonstrated that chemoresistant phenotypes display high levels of glutathione (GSH), which is crucial to maintain reactive oxygen species (ROS) under the cytotoxic limit preventing cancer cell death.
Methods: HTLA-230, a human *MYCN*-amplified neuroblastoma cell line, was chronically treated with etoposide at a concentration that *in vitro* mimics the clinically-used dose. The selected cells (Etopo-R) were exposed to higher doses of etoposide or doxorubicin and their multidrug resistance (MDR) was tested. In order to investigate the role of GSH in MDR, Etopo-R were pre-treated with buthionine sulfoximine (BSO, a GSH-depleting agent) or N-acetylcysteine (NAC, able to promote GSH biosynthesis). Cell response was evaluated in terms of cell viability, tumorigenicity, and oxidative status (ROS production, GSH levels, and activity of GSH-related enzymes).
Results: Etopo-R cells are highly tumorigenic and acquire MDR, becoming less sensitive than parental cells to etoposide or doxorubicin. They are characterized by up-regulation of catalase and glutathione-S-transferase activity, have higher levels of GSH and over-express γ-glutamylcysteine ligase, a crucial enzyme in GSH biosynthesis. Treatment of Etopo-R cells with BSO markedly reduces their tumorigenic potential that is instead enhanced by the exposure to NAC.
Conclusions: Collectively, our results demonstrate that GSH and GSH-related responses are crucially involved in the acquisition of MDR of neuroblastoma cells. We propose this antioxidant molecule as a therapeutic and predictive target (*Grants from Genoa University*).

C14 c-Myc Sustains Transformed Phenotype and Promotes Radioresistance of Embryonal Rhabdomyosarcoma Cell Lines

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Background: We have previously reported that the MEK/ERK pathway sustains *in vitro* and *in vivo* transformed phenotype and radioresistance of embryonal rhabdomyosarcoma (ERMS) cell lines. Furthermore, we found that aberrant MEK/ERK signaling activation promotes c-Myc oncoprotein accumulation.
Methods: In this study, the role of c-Myc in sustaining the ERMS transformed and radioresistant phenotype is characterized. RD and TE671 cell lines conditionally expressing MadMyc chimera protein, c-Myc-dominant negative, and shRNA directed to c-Myc were used.
Results: Targeting c-Myc counteracted *in vitro* ERMS adherence and in suspension, growth motility and the expression of pro-angiogenic factors.

c-Myc depletion decreased MMP-9, MMP-2, u-PA gelatinolytic activity, neural cell adhesion molecule sialylation status, HIF-1α, and VEGF, and increased TSP-1 protein expression levels. Rapid but not sustained targeting c-Myc radiosensitized ERMS cells by radiation-induced apoptosis, DNA damage and impairing the expression of DNA repair proteins RAD51 and DNA-PKcs, thereby silencing affected ERMS radioresistance. c-Myc sustains ERMS transformed phenotype and radioresistance by protecting cancer cells from radiation-induced apoptosis and DNA damage, while promoting radiation-induced DNA repair.
Conclusions: These data suggest that c-Myc targeting can be tested as a promising treatment in cancer therapy.

C15 MEKs/ERKs/c-Myc Pathway Sustains EphA2 Receptor Signaling: Effect of Targeting on Growth Arrest, Myogenic Differentiation, and Onco-Phenotype Reversal in Rhabdomyosarcoma Cells

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Background: Expression of EphA2 receptor is frequently aberrant in a variety of malignancies and correlates with poor prognosis. EphA2 acts as a tumor promoter through a ligand ephrin-independent mechanism, which requires phosphorylation of EphA2 on serine 897 (S897). The RAS/MEKs/ERKs/c-Myc axis sustains *in vitro* and *in vivo* growth potential and counteracts differentiation of RAS-constitutive-active embryonal rhabdomyosarcoma (ERMS) cell lines.
Methods: The aim of this study was to investigate the role of EphA2 signaling in sustaining ERMS cell lines RD and TE671 oncogenic phenotype and its relationship with the RAS/MEKs/ERKs/c-Myc axis. A new synthesized antagonist of S897-EphA2 phosphorilation and selective shRNAs were used for the *in vitro* experiments. The EphA2 protein levels of 33 microdissected ERMS patient samples were evaluated by Western blotting.
Results: We demonstrate that EphA2 ligand ephrin-independent signaling inhibition induced G2/M growth arrest, reversal of anchorage-independent growth, and rescued the myogenic program by inducing myogenin expression and the acquisition of the myogenic-like phenotype in ERMS cells.
Conclusions: These results suggest that EphA2 is a key downstream target of the MEK/ERK/c-Myc signaling pathway in the regulation of rhabdomyosarcomagenesis.

C16 Endogenous Lysyl Oxidase in Tumor Progression and Collagen Stiffness: Study Performed In Human Clear Cell Renal Cell Carcinoma Primary Cell Cultures

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Background: In human clear cell renal cell carcinoma (ccRCC), VHL gene inactivation leads to stabilization of hypoxia inducible factors (HIFs). The lysyl oxidase gene (*LOX*), a HIF-1α target, codes the inactive proenzyme (Pro-Lox) which, secreted and proteolysed, produces the active enzyme (Lox) and the pro-peptide (Lox-PP). Lox, increasing extracellular matrix stiffness by collagen crosslinking, promotes tumor progression and metastasis. Lox and Lox-PP can reenter the cells where Lox promotes cell proliferation and invasion, while Lox-PP acts as tumor suppressor. We performed, for the first time in ccRCC, a detailed study of endogenous LOX using ccRCC primary cell cultures.
Methods: 24 ccRCC patients enrolled. Primary cell cultures were obtained from tumor and matched normal renal cortex samples. Protein expression was evaluated by Western blot, IHC, IF. LOX silencing was performed by siRNA. Apoptosis, proliferation, migration, invasion, adhesion assays were performed. Collagen stiffness was measured by atomic force microscopy.
Results: Conditioned media of ccRCC cells, which overproduced and secreted active Lox, increased collagen matrix stiffness associated with activation of FAK/SRC signaling and a more invasive phenotype. The lack of Lox-mediated crosslinking action prevented matrix stiffness increment. The absence of endogenous LOX heavily impaired the invasion capacity of ccRCC cells on collagen. The oncosuppressive action of Lox-PP was not prevailing in ccRCC.
Conclusions: In ccRCC cells endogenous LOX overexpression, favored by constitutive HIF-1α, plays a major action in tumor progression, promoting cellular adhesion, migration, and collagen matrix stiffness. These findings could indicate new therapeutic strategies by targeting selectively the different LOX peptides in ccRCC.
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C17 Keap1/Nrf2 Pathway In Kidney Cancer: Frequent Methylation of Keap1 Gene Promoter In Clear Renal Cell Carcinoma

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Background: The Keap1/Nrf2 pathway is a master regulator of the cellular redox state through the induction of several antioxidant defence genes implicated in chemotherapeutic drug resistance of tumor cells. An increasing body of evidence supports a key role for the Keap1/Nrf2 pathway in kidney diseases and renal cell carcinoma (RCC), but data concerning the molecular basis and the clinical effect of its deregulation remain incomplete.
Methods: Here we present a molecular profiling of the *KEAP1* and *NFE2L2* genes in five different renal cell carcinoma histotypes by analysing 89 tumor/normal paired tissues: clear cell renal carcinoma (ccRCC); oncocytoma; papillary renal cell carcinoma type 1 (PRCC1); papillary renal cell carcinoma type 2 (PRCC2); and chromophobe cell carcinoma.
Results: A tumor-specific DNA methylation of the *KEAP1* gene promoter region was frequently found only in ccRCC subtype (18/37, 48.6%) and a direct correlation with mRNA levels was confirmed by *in vitro* 5-azacytidine treatment. Analysis of an independent data set of 481 ccRCC tumors corroborates these findings and reveals a significant correlation in multivariate analysis of epigenetic *KEAP1* silencing with the stage, Fuhrman grade and overall survival in ccRCC.
Conclusions: For the first time, our molecular results present the epigenetic silencing of *KEAP1*promoter as the possible leading mechanism for modulation of *KEAP1* expression in ccRCC and corroborate the driver role of Keap1/Nrf2 axis deregulation with a potential new function as an independent epigenetic prognostic marker in RCC.

C18 Wnt Signaling Inhibitors as Promising Biomarkers of Osteolytic Bone Metastasis

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Background: Despite the clinical importance of bone metastases, little is known about their onset and progression and current diagnostic tools lack the sensitivity and specificity required for clear early diagnosis. Growing understanding of the interaction between cancer and bone has led to the

identification of innovative diagnostic tools. An emerging family of molecules with a pivotal role in bone tumor progression and metastasis is the Wnt gene family. This study investigated the diagnostic potential of the two Wnt signaling inhibitors, sclerostin and DKK-1, to improve the detection of osteolytic bone metastases.
Methods: We measured sclerostin and DKK-1, MMP-2, MMP-9, the bone resorption marker TRAP5b, and the metastatic marker survivin in 15 healthy patients, in 20 patients with primary tumors, and in 20 patients with bone metastases. The normal distribution was verified by KS normality. Statistical analysis of ANOVA and correlations were performed by PRIMS 3.0.
Results: Sclerostin and DKK-1 were clearly higher in primary tumor patients and even higher in metastatic patients compared to controls. DKK-1 and sclerostin were positively correlated with bone resorption parameters and this link was particularly strong in metastatic patients. A good correlation of survivin was found with DKK-1, and correlation was even higher with sclerostin in metastatic patients, definitely confirming the importance of these two Wnt signaling inhibitors as diagnostic markers of bone metastases.
Conclusions: The close correlations with metastatic markers and bone resorption markers makes sclerostin and DKK-1 new promising biomarkers in the diagnosis of bone osteolytic metastases.

C19 A Rare Case of Carcinosarcoma Ex Pleomorphic Adenoma of the Parotid Gland with No History of Long-standing or Recurrent Pleomorphic Adenoma

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Background: The aim of this report is to describe a rare case of carcinosarcoma ex pleomorphic adenoma involving the left parotid gland with no history of a long standing pleomorphic adenoma, or a recurrent pleomorphic adenoma, and to describe its morphology and important immunohistochemistry findings.
Methods: The histopathologic findings of the left parotid gland are correlated with the clinical history and ancillary procedure, and surgical pathology staging, including assessment of the draining lymph nodes. Immunohistochemistry studies, assessment of proliferative capacity, and possible treatment target were also done. The tumor is also compared with other reported cases with the same diagnosis.
Results: Carcinosarcoma ex pleomorphic adenoma contains features of the two tumors under malignant mixed tumors, which are carcinosarcoma and carcinoma ex pleomorphic adenoma. Immunohistochemistry studies were done to document the epithelial and mesenchymal areas for both malignant and benign sections from the tumor. Immunohistochemistry studies were done to classify the epithelial component, consisting of adenocarcinoma, not otherwise specified, and sarcoma component consisting of myxoid and mesenchymal chondrosarcoma.
Conclusions: Approximately ten cases of carcinosarcoma ex pleomorphic adenoma have been documented in scientific publication, even less with the background of no recurrent or prolonged untreated pleomorphic adenoma. The case contributes to the fund of knowledge for diagnosis and improvement of quality of care.

C20 IGF-I/IGF-IR Transduction System Involves GPER and DDR1 in the Stimulation of Mesothelioma and Lung Cancer Cells

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Background: The insulin-like growth factor-I (IGF-I)/IGF-I receptor (IGF-IR) transduction system cooperates with various pathways toward the stimulation of cancer growth and resistance to antitumor agents. In this regard, it has been recently reported that IGF-IR-mediated signaling triggers a functional crosstalk with the G protein estrogen receptor (GPER) and the tyrosine kinase receptor, collagen discoidin domain receptor 1 (DDR1), in different types of malignant cells, leading to important biological responses like proliferation and migration.
Methods: Real- time PCR, immunoblotting analysis and gene silencing experiments were performed to ascertain the regulation of GPER and DDR1 at both mRNA and protein levels upon IGF-I treatment in IST-MES1 mesothelioma and A549 lung cancer cells. Luciferase assays were also performed to evaluate the ability of IGF-I to transactivate promoter constructs like GPER and its target genes such as CTGF and EGR1. Chemotactic motility and migration were determined as biological responses to IGF-I stimulation.
Results: We found that IGF-I induces GPER expression through the IGF-IR/ERK/p-38 transduction signaling in both IST-MES1 and A549 cells. Next, we ascertained that in these cells IGF-I triggers the expression of GPER and DDR1 target genes like CTGF, EGR1, MATN-2, FBN-1, NOTCH 1 and HES1, stimulating chemotaxis and cell migration as biological counterpart.
Conclusions: Our findings provide new insights on the mechanisms by which the IGF-I/IGF-IR system exerts a stimulatory action in mesothelioma and lung cancer cells, thus suggesting novel potential drug targets to be considered in these aggressive malignancies.

C21 A Knowledge-Base Approach and Bioinformatic Analysis to Highlight the Role of MicroRNAs in the Pathogenesis of Malignant Mesothelioma

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Background: MicroRNAs (miRNAs) are endogenous, non-coding, small RNAs able to modulate gene expression at the post-transcriptional level with a fundamental role in multiple cellular processes and regulatory pathways. Aberrantly expressed miRNAs are correlated to the pathogenesis of numerous cancers including malignant mesothelioma (MM). MM is an asbestos-related lethal cancer, poorly responsive to current treatments. Recently a systematic review and qualitative meta-analysis identified a pool of high-confidence MM-associated miRNAs (mesomiRs) with potential clinical relevance. Herein we aimed to define the role of mesomiRs on the pathogenetic mechanism underling MM tumorigenesis.
Methods: A data mining approach and bioinformatic analysis on mesomiRs were combined to predict their role in the disruption of key pathways in the pathogenesis of MM. The results obtained were integrated with multiple sources of biological data to build the pathways and the global network.
Results: We have developed a knowledge base of the molecular constituents of pathways and networks whose members, both miRNAs and proteins, are mutated in MM. MesomiRs appear strongly involved in multiple pathways deregulated in cancer, such as signaling pathways regulating pluripotency of stem cells, apoptosis, cell cycle, PI3K-Akt, p53, TGF-β, FoxO signaling pathways, focal adhesion and adherens junction. Together, we demonstrated the ability of our approach to prioritize miRNA targets and to identify relevant deregulated pathways. These findings may provide a new rationale for new therapeutic approach and drug discovery.
Conclusions: In this study, we present a reliable method to consolidate a bridge between aberrant microRNA expression and MM pathogenesis as a basis for further investigations.

C22 Expression of p60-CAF-1 (Chromatin Assembly Factor-1) in Patients with Oral Squamous Cell Carcinoma (OSCC)

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Background: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common human cancer, and is a major cause of cancer-related

mortality. HNSCC represents a major current public health problem. Oral squamous cell carcinoma (OSCC) represents the majority of HNSCC. P60-CAF-1 (chromatin assembly factor 1)-protein is a highly efficient epigenetic regulator of cell replication and DNA-repair in eukaryotic cells. We have evaluated the expression of the p60-CAF-1 protein both by IHC, on formalin-fixed, paraffin-embedded (FFPE) tumor tissues samples and in peripheral blood samples by ELISA assay.
Methods: We collected tissue samples from 9 patients who underwent surgery for OSCC of the tongue, floor and palate, and normal control tissue. The protein-expression study was performed by immunohistochemistry on FFPE tumor tissues using anti-CAF-1 p60 antibody. ELISA assay was determined using: primary antibody; HRP conjugated secondary antibody; substratum TMB activity.
Results: CAF-1/p60 protein was found overexpressed on all OSCC tumor tissue samples; the same samples showed high protein levels as detected by ELISA assay. The results observed by ELISA were: controls (as mean ± S.D.) 29,5±8,5 U.I./ml; patients (as mean ± S.E.) 50,2±4,7 U.I./ml.
Conclusions: Preliminary results are the basis to expand the study of further cases with a prolonged follow-up. We can assume that elevated blood levels of p60-CAF-1 protein may correspond to more aggressive tumors with an unfavorable outcome. Such a protein-expression profile could constitute the basis for a novel prognostic and predictive algorithm to be used in addition to the standard diagnostic protocols for OSCCs.

C23 Institution of Head and Neck Paraganglioma Cell Lines and Effects of Drugs Interfering with Tumor Metabolism

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Background: Head and neck paragangliomas (HN-PGLs) are relatively rare tumors that cause important morbidity. At present, surgery is the only therapeutic option. HN-PGLs are often linked to germline mutations in succinate dehydrogenase genes (SDHx), encoding mitochondrial complex II subunits involved in the tricarboxylic acid cycle. No commercial cell line is available.
Methods: HN-PGLs cell lines were established from two patients carrying germline SDHx mutations to test molecules active on tumor metabolism. Viability in treated and untreated cells was tested by MTT. Effects of treatments on cell cycle and apoptosis were analyzed by flow cytometry and Western blot. Clonogenic ability and cell migration after treatment were also examined.
Results: Since both HN-PGL cell lines derived from patients with germline defects in a gene encoding an SDHx enzyme we tested the effects of three drugs active on tumor metabolism: the pyruvate kinase inhibitor dichloroacetate (DCA), the antagonist of the PPARA nuclear receptor GW6471, and the antidiabetic drug metformin. DCA and GW6471 affected viability of both HN-PGL cell lines and reduced clonogenic activity. Treatment with DCA or GW6471 induced apoptosis in both HN-PGL cell lines. However, the effect of the two drugs on cell cycle was different. Also metformin appears to drastically affect viability of both cell lines.
Conclusions: The establishment of HN-PGL cell lines provides the opportunity to study pathophysiology and therapeutic molecules in this rare tumor. DCA, GW6471, and metformin are active in HN-PGLs, providing a rationale for testing the antitumor effect of these and additional metabolic drugs as single agents or in combination.

C24 Glutamine Addiction and Low Expression of Glutamine Synthetase Underlie Glutamine Dependence of Human Multiple Myeloma Cells: A New Attractive Therapeutic Strategy

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Background: Glutamine supports the growth of several types of cancers through glutaminolysis-dependent anaplerosis. However, glutamine metabolism has never been investigated in multiple myeloma (MM), although hyperammonemia, an indicator of sustained glutaminolysis, was described in relapsed/refractory MM patients.
Methods: 64 patients with plasma cell dycrasias were included in this study together with human myeloma cell lines (HMCLs: RPMI 8226, OPM2, JJN3, KMS-12-BM, XG1). NH_4^+ was assessed in bone marrow (BM) plasma with a biochemical assay. Amino acid BM plasma content was determined by HPLC. Either glutamine-free incubation or the glutaminolytic enzyme L-asparaginase from *E. chrysanthemi* was used for glutamine starvation. Intracellular metabolites were measured with LC-MS/MS. L-[4,5-^{3}H] glutamine was used for transport studies. Stable ASCT2 silencing was obtained with an anti-SLC1A5 shRNA vector. SCID-NOD mice were injected subcutaneously with ASCT2-silenced or scramble transfected JJN3 cells.
Results: MM patients have higher BM plasma NH_4^+ and glutamate, but lower glutamine than patients with indolent monoclonal gammopathies. Both CD138$^+$ cells from patients and HMCLs exhibited glutamine-dependent NH_4^+ production. High glutaminase and low-to-absent glutamine synthetase expression were observed in HMCLs and in CD138$^+$ cells. In HMCLs, glutamine starvation decreased intracellular glutamine, glutamate and α-ketoglutarate, inhibited mTORC1, and triggered apoptosis. Furthermore, silencing of the ASCT2 transporter significantly impaired glutamine uptake and MM cell growth, along with JJN3 tumor growth in rodents.
Conclusions: MM cells show features of glutamine-addiction,and, due to low expression of glutamine synthetase, their proliferation depend upon glutamine uptake. Glutamine availability and metabolism may therefore constitute novel therapeutic targets in MM.

C25 ErbB Receptors and BER Pathways in Human Gastric Tumours: Molecular Implication of Oxidative Stres.

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Background: Deregulation and cross-talk of intracellular signals have been shown to be the driving forces in cancers. In this study, ErbB and BER pathways in neoplastic gastric tissues and cells, as a molecular signature for tumor progression, were investigated.
Methods: ErbBs and BER molecular signaling genes and proteins were evaluated in the gastric carcinoma cell line AGS in basal condition and after single or combined treatments with H_2O_2, EGF and LY294002 and in primary gastric carcinoma tissues by RT-qPCR and Western blot.
Results: Increased levels of pEGFR and pMAPK were detected in AGS cells after treatments with H_2O_2, although EGFR expression did not show any significant change. In addition, H_2O_2 treatment downregulated pAKT, as compared to control or EGF-treated cells. PARP-1 expression was decreased after H_2O_2 treatment, while OGG1 protein and gene expression decreased with LY294002 treatment, suggesting an Akt-dependent mechanism. An upregulation of *ErbB2* gene expression was observed after EGF treatment, while *ErbB4* expression increased with H_2O_2 and LY294002. *Nrf2* and *APE1* gene expression levels were reduced by H_2O_2 and LY294002 combined treatments. Data on gastric tissues showed that EGFR, highly expressed in intestinal-type, diffuse-type gastric carcinomas and their normal counterparts, was not phosphorylated in all cases. In contrast, diffuse-type carcinomas showed increased pMAPK, pAKT and OGG1 expression, as compared to control tissues. *ErbB2* and *Mutyh* gene expression levels were higher in intestinal-type than in diffuse-type carcinomas, while *ErbB4* showed an opposite expression pattern.
Conclusions: These data indicate that oxidative stress modify EGFR and BER signaling, suggesting an integrated role between these pathways.

C26 Two Simultaneous and Very Uncommon PI3KCA Mutations in a Liver Metastasis from a Colorectal Cancer Patient with Aggressive and Resistant Disease

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Background: Colorectal cancer (CRC) is a widespread tumor. *KRAS*/*NRAS* genotype drives metastatic CRC treatment with targeted therapies. The prognostic and predictive significance of other genes (*PIK3CA*, *BRAF)* is under investigation. Here, we describe a CRC patient carrying a *KRAS* and two very uncommon *PIK3CA* mutations in liver metastasis.
Methods: Primary CRC and metastatic liver tumor samples were collected from an early-onset patient with synchronous metastases, treated with first-line intensive triplet chemotherapy plus bevacizumab (FIr-B/Fox). *KRAS*/*NRAS* (exons 2, 3, 4), *BRAF* (exon 15), *PI3KCA* (exons 9, 20) were analyzed by direct sequencing. PTEN, Akt and ^{S473}p-AKT were examined by immunohistochemistry.
Results: The *KRAS* exon 2 c.34G>A, G12S mutation was detected in primary tumor and liver metastasis. Two *PIK3CA* exon 9 mutations on the same allele (c.1633G>C, E545Q; c.1645G>C, D549H) were detected only in the latter sample. Interestingly, the c.1633G>C mutation was reported with very low frequency in databases; on the contrary, the c.1645G>C mutation was never described in CRC, although it was reported in just two samples from hepatocellular and cervical carcinoma.
Immunohistochemistry analysis revealed PTEN expression and differences in Akt and p-AKT expression between primary and metastatic samples. The patient showed aggressive and resistant disease (7 months PFS, 15 months OS).
Conclusions: A *KRAS* and two rare *PI3KCA* mutations, one never reported in CRC, are described in liver metastasis from a patient with very aggressive disease. Differences were detected in Akt and p-Akt expression by comparing primary tumor/metastasis, leading to the hypothesis of a functional role of these rare PI3KCA mutations in cancer aggressiveness.

C27 Magnesium Homeostasis Goes Awry in Chemoresistance

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Background: Chemoresistance is one of the most significant factors impeding the progress of cancer therapy. In addition to the over-expression of P-glycoprotein (P-gp), other biochemical mechanisms seem to contribute to render cancer cells resistant to anti-cancer drugs. Since neoplastic cells accumulate magnesium partly through the upregulation of the magnesium transporter TRPM7, we focused on magnesium homeostasis in a model of chemoresistance, i.e., colon carcinoma LoVo cells sensitive (LoVo-S) or resistant (LoVo-R) to doxorubicin.
Methods: We performed real-rime PCR and Western blot to study the expression of TRPM7 and MagT1 in LoVo-S and -R. We silenced TRPM7 and MagT1 and evaluated cell proliferation by a cell counter and sensitivity to doxorubicin by MTT assay. Total Magnesium content was assessed using the fluorescent chemosensor DCHQ5. Magnesium influx in Mag–Fluo-4-loaded cells was performed by live confocal imaging.
Results: In LoVo-R we found higher intracellular concentration of total magnesium but lower influx capacity than in LoVo-S. Interestingly, the distribution of magnesium is different in the two cell types. Moreover, in LoVo-R we detected lower levels of TRPM7, involved in the transport of divalent cations, and higher amounts of another transporter, MagT1, which is highly specific for magnesium. Silencing TRPM7 in LoVo-S retarded cell proliferation and also induced the cells to shift their phenotype into a resistant one. Silencing MagT1 in LoVo-R markedly inhibited cell growth without affecting the sensitivity to doxorubicin.
Conclusions: We conclude that in LoVo cells drug resistance is associated with alteration of magnesium homeostasis.

C28 Biological Function of Oncogenic KRAS In Promoting Angiogenesis

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Background: Ras functions and activation of its signaling pathways is an important mechanism by which human cancer develops. Constitutive activation of the RAS pathways occurs through mutational activation of the RAS oncogene. Among the three RAS genes (*HRAS*, *KRAS* and *NRAS*) *KRAS* seems to be the most predominantly involved in tumour progression because of its molecular nature. It has been demonstrated that distinct *KRAS* mutations associate with specific metabolic phenotypes showing a distinct angiogenic profile. Although the relationship between KRAS and VEGF has been demonstrated, the biological role of KRAS in VEGF production by tumour cells is still not clarified.
Methods: Using *KRAS* mutant colon cancer cell lines (SW480 and LS174T) and wild-type colon cancer cell line SW48, we first evaluated the production of VEGF by a specific ELISA assay. Moreover, to elucidate the function of oncogenic KRAS in promoting angiogenesis we analysed the effect of conditioned media (CM) derived from SW480, LS174T and SW48 cell lines on the ability to induce tubule formation of HUVEC. By Western blotting analysis we also compared the Ras-activated signaling pathways in these cancer cell lines.
Results: Our preliminary results suggest that oncogenic KRAS significantly promotes the production of VEGF from SW480 and LS174T cells lines. *In vitro* biological assays also showed that upregulated VEGF secretion in CM of SW480 and LS174T with respect to SW48 enhances the tube formation ability of HUVEC.
Conclusions: In conclusion, our study established a direct role of KRAS in promoting angiogenesis in colon cancer cell lines.

C29 Wnt Gene Expression in Polyposis and Colorectal Cancer

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Background: Transcript dosage may influence transcriptome. To elucidate the role of altered gene expression in colorectal polyposis we analyzed *APC* gene expression.
Methods: Patients with familial and sporadic polyps were enrolled following inclusion (age≥18 years) and exclusion (inflammatory bowel diseases) criteria. Donors with no family history of cancer were recruited as controls.We planned to recruit 20 familial adeomatous polyposis (FAP) cases, 50 cases of sporadic polyps, 50 cases of colorectal cancer, 50 healthy controls.
Results: We analyzed APC expression in colon tumour tissues and adjacent mucosa from 20 patients with FAP, 20 cases with sporadic polyps, and in normal colonic mucosa from 20 healthy controls. RT-qPCR results showed a reduced expression in colon tumour tissues (0.13 2^{-}DCT) as compared to adiacent mucosa (0.34 2^{-}DCT). APC expression in adiacent colonic mucosa was higher also compared to healthy control colonic mucosa. The differences in APC expression between colon tumour tissues and adiacent mucosa in familial and sporadic cases were statistically significant in the familial group (p=0.0054). We also correlated APC expression with age. In patients the expression levels tend to decrease more rapidly with age (R^2=0.473). In contrast, the control group had a constant APC expression trend in life. Correlation with sex showed that APC reduced expression is more evident in men than women (0.11 vs 0.22 2-ΔcT).
Conclusions: This study showed that the APC gene expression is lower in colon tumor tissue compared to adjacent mucosa but expressed more

in adjacent mucosa compared to mucosa of control subjects. APC seems to restore cellular homeostasis in human colonic epithelium.

C30 NaBu-induced Differentiation Affects the Release of Exosomes from HT29 Colon Cancer Cells and their Ability to Modulate the Behavior of Recipient Cell

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Background: Exosomes are extracellular vesicles involved in intercellular communication and largely mirror the molecular profile of the originating cells. We previously reported that NaBu-induced differentiation of HT29 colon cancer cells is associated with a reduced expression of CD133. This study aimed to analyze the role of exosomes in the differentiation process.
Methods: Exosomes were prepared using differential centrifugations. MicroRNA and mRNA expression levels were evaluated by RT-PCR. Cell proliferation was assessed by MTT assay and confirmed with ECIS system and cell motility was assessed using the scratch test and confirmed by confocal microscopy.
Results: NaBu-induced differentiation was associated with an increase in the release of exosomes and in their expression of CD133 compared to untreated cells. HT29 cells differentiation and the decrease of cellular CD133 expression levels was prevented by blocking multivescicular body maturation with NH4Cl. Exosomes released from differentiated cells carried specific microRNAs at levels higher than those isolated by untreated cells. Exosomes released from differentiating cells increased the proliferation and the motility rate of both normal and cancer cells, increased the colony-forming efficiency of cancer cells, and prevented NaBu-induced differentiation of recipient HT29 cells. Such effects were associated with an increased phosphorylation of both Src and Erk proteins and with an increased expression of genes involved in EMT.
Conclusions: Release of exosomes is affected by differentiation of colon cancer cells. Exosomes might be used by differentiating cells to remove cellular components that are no longer necessary but might continue to exert their effects on recipient cells.

C31 Rapid Detection of CNV Alterations, Point Mutations and Gene Fusions Using the Next-Generation Sequencing Oncomine Focus Assay

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Background: Next-generation sequencing (NGS) provides tools to identify with high accuracy and sensibility alterations in attractive therapeutic targets. The Oncomine™ Focus Assay allows simultaneous analysis of DNA and RNA, detecting SNVs, indels, CNVs and gene fusions in 52 cancer relevant genes. Identification of druggable players involved in cancer progression can provide new therapy options to improve clinical outcome.
Methods: We obtained RNA and DNA from 82 tumors of the gastrointestinal tract: 30 gastric and 52 colorectal carcinoma (CRC). These samples were sequenced in the PGM sequencer (Ion Torrent) using the Oncomine™ Focus Assay and results were analysed using Ion Reporter™ Software. Sanger sequencing was used to validate SNV, FISH or real-time PCR were used to confirm CNV alterations.
Results: We validated experimentally the NGS data and confirmed the alterations identified. As expected *KRAS* mutations were the most frequent alterations among CRC tumors; *ERBB2* amplification or missense mutations were more frequent among gastric tumors. Interestingly we found *FGFR1* amplification in two CRC patients, confirmed by real-time PCR. In our cohort no specific fusions were found but 3 samples presented 3'to 5'imbalance in the *RET* gene.
Conclusions: Using Oncomine ™ Focus Assay we were able to rapidly identify SNVs, CNVs and gene fusions in key targetable genes with high accuracy, demonstrating that it can have an impact in clinical oncology. The identification of *FGFR1* amplification and possible *RET* aberrations indicated by the *3'/5' imbalance assay* in CRC could provide new therapy options for the cancer patients harbouring these alterations.

C32 miRNA Expression Profiling of Mouse Colon Cancer Stem Cells: A Tumour-Specific Signature Traceable along Colorectal Cancer Progression with Prognostic Value in Human Colon Cancer

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Background: The definition of cancer stem cells (CSC) still lacks conclusive experimental evidence. Colorectal cancer (CRC) EphB2 cells have been correlated to stem-like properties and tumor malignancy. Here, we investigated a panel of miRNA involved in the self-renewal and cell fate during cancer development using murine CRC EphB2 sorted cells and tissue of mouse/human CRC and public dataset (TCGA).
Methods: FACS-isolated CD44$^+$ EphB2high cells of the CRC AOM/DSS murine model were analyzed by gene expression and IHC analysis (Lgr5, Ascl2, krt20) to characterize the stem-like/differentiation phenotype. miRNAs expression profiling was performed using TaqMan Low Density Arrays (LifeTechnologies) both on sorted cells and whole tissue samples of AOM/DSS model containing dysplastic ACFs, microadenoma, adenoma, carcinoma. Single real-time PCR for selected miRNAs were performed on 60 FFPE human samples and further validated in The Cancer Genome Atlas (TCGA) stage I–IV CRC (n=130) to determine their potential prognostic role.
Results: The analysis of miRNA expression pattern in the EphB2high sorted stem-like cells in comparison to miRNAs of CRC murine whole tissue showed a significant cancer stemness signature in the initiation/progression steps. EphB2 stemness-related miRNAs, which orchestrate cell cycle, apoptosis, tumorigenesis, progression and metastasis, were further analyzed in human samples at different phases (ACF, adenoma, adenocarcinoma) revealing a pattern similar to the murine model.
Conclusions: These data provide a comprehensive miRNA signature implicated in the regulation of tumorigenesis, stemness, and cell fate determination that could be exploited for diagnosis and therapeutic design, and could be proposed as novel CRC prognostic biomarkers.

C33 Testing of Novel Peroxisome Proliferator-Activated Receptor Antagonists in Colorectal and Pancreatic Cancer Cell Lines

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Background: PPARs are ligand-activated transcription factors that are often deregulated in tumors, where these receptors modulate cell proliferation, differentiation, survival and lipid metabolism. PPAR isoforms play a pleiotropic role in tumors because they may act as tumor suppressors or oncoproteins depending on cancer type. Here, we explored the antiproliferative activity of a series of novel PPAR antagonists synthetized in our laboratories of medicinal chemistry.
Methods: The activity of novel molecules on PPARα/PPARγ was investigated by a Gal4-PPAR transactivation assay in HEK-293 cells. Expression of carnitine palmitoyl transferase 1 (CPT1A), a key gene controlled by PPARα and PPARγ involved in fatty acid transport in mitochondria, was analyzed by qPCR. Effects of novel antagonists on viability in different tumor cell lines were evaluated using MTT. In

particular, HT-29 and SW480 colorectal cancer cell lines, together with Capan-2 and AspC-1 pancreatic cancer cell lines were selected based on their expression of both PPARα/PPARγ (Expression Atlas Database).
Results: The novel molecules showed an antagonistic behavior on PPARα and PPARγ, and were able to repress CPT1A expression. Preliminary experiments were conducted incubating the four cell lines with novel compounds at 75 uM to evaluate their activity on cell viability. Interestingly, one of the tested compounds significantly reduced viability in the colorectal and pancreatic cancer cell lines examined. Based on these results, we further characterized its dose-dependent cytotoxic effects.
Conclusions: This study shows that inhibiting PPARα/PPARγ activities reduces viability of the colorectal and pancreatic cancer cells tested, suggesting an oncogenic role of these nuclear receptors in these cell lines.

C34 Search for Key Targets in the Biology of Pancreatic Cancer

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Background: Patients with pancreatic cancer have an average survival of less than a year. Hence, there is an urgent need to find more effective therapeutic approaches to treat this deadly disease. Clinical trials based on traditional chemotherapy combined with erlotinib, an EGFR inhibitor, reported benefit in pancreatic cancer patients. Our purpose is to identify key targets in the biology of pancreatic cancer using non-cancer drugs. These can be exploited in a treatment strategy based on combination with erlotinib. We are testing the potential anticancer activity in pancreatic cell lines of nelfinavir, an HIV protease inhibitor, and nitroxoline, an antibiotic active on biofilm infections.
Methods: *KRAS* oncogene mutated pancreatic cancer cell lines AsPC-1 and Capan-2 were treated with increasing concentrations of erlotinib, nelfinavir and nitroxoline. In our preliminary experiments we evaluated the effect of treatments on cell viability by MTT assay.
Results: Erlotinib, nitroxoline, and nelfinavir affected viability of both human pancreatic cancer cell lines. Combined treatments with these and other non-cancer drugs will be tested and the effects on cell cycle, apoptosis and other key pathophysiological features of pancreatic cancer will be analyzed.
Conclusions: Successful treatment of advanced human pancreatic tumors may require simultaneous targeting of several distinct signaling cascades including those driven by *KRAS* and *EGFR*. In our preliminary studies erlotinib, nitroxoline, and nelfinavir appear to be interesting drug candidates for new combined treatment strategies in pancreatic cancer. The sensitivity of the tumor to these agents suggests that they affect key targets in the biology of pancreatic cancer.

C35 miR-182 Dysregulation in a Diet-Induced NAFLD-NASH-Hepatocellular Carcinoma Mouse Model

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Background: Nonalcoholic fatty liver disease (NAFLD) is a frequent liver disorder. It can progress through the more severe nonalcoholic steatohepatitis (NASH), fibrosis and, lastly, hepatocellular carcinoma (HCC). miRNAs are small non-coding RNAs acting as regulators of gene expression at the post-transcriptional level. In this study, a mouse model was used to investigate the effects of high-fat (HF) and low-fat/high-carbohydrate (LF-HC) diets on miRNA expression during liver damage progression.
Methods: C57BL/6J mice were HF or LF-HC diet fed for 3, 6, 12, and 18 months. Control mice were standard diet fed. Hepatic tissues were collected. Histological analyses were performed. TaqMan RT-qPCR was used to analyze miRNAs in liver tissues. Target gene protein products were examined by immunoblotting.
Results: Progressive liver damage was observed in HF and LF-HC mice. Tumors were detected in HF after 12 and 18 months and in LF-HC after 18 months. Molecular analysis showed several miRNAs differentially expressed during the disease progression and in tumors. Among them, miR-182 showed early dysregulation, being overexpressed in HF vs LF-HC fed mice after 3 months. The trend was maintained in HF after 6, 12 and 18 months and, in particular, in tumors compared to peritumoral tissues. The transcription factor FOXO1, a miR-182 target, was analyzed by immunoblotting and found ipo-expressed.
Conclusions: miRNA expression was evaluated in livers from mice HF or LF-HC diet fed during the progression of liver damage up to HCC. miR-182 was upregulated in HF tissues and HF/LF-HC tumors. The expression of its target FOXO1 decreased and the role of this will be discussed.

C36 New Bioinformatics Approach to Identify Target Genes for Dysregulated MicroRNAs in a Chemically-Induced Hepatocellular Carcinoma Mouse Model

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Background: Hepatocellular carcinoma (HCC) is an aggressive tumor. miRNAs are non-coding RNAs acting in post-transcriptional gene regulation, whose dysregulated activity plays a role in cancer. A single miRNA can exert its action on hundreds of putative target genes. We used a chemically-induced HCC mouse model to identify miRNA differential expression during the progression of hepatic damage up to HCC. In this context, we generated an original bioinformatic approach to predict putative target genes and protein networks involved in hepatocarcinogenesis.
Methods: C57BL/6J mice were treated with diethylnitrosamine (DEN) and sacrificed after 3, 6, and 11 months. miRNA expression related to controls was evaluated. Dysregulated miRNAs were analyzed by bioinformatics prediction tools (miRanda, TargetScan, PITA and Rna-22) to identify target genes. Genemania software was then exploited for enrichment annotation analysis and protein construction network. Immunoblotting was used to validate target genes' expression.
Results: Bioinformatics tools globally identified 15 putative target genes of four upregulated miRNA (miR-125a-5p, miR-27a, miR-182, miR-193b) subjected to analysis. The protein product of one among them (ankyrin-G) was further validated by immunoblotting to assess the strength of the approach. Enrichment annotation analysis highlighted 26 significant functional clusters putatively involved in DEN-induced HCC, and a network including links between selected miRs, targets, and possible interactions among them and other proteins was built.
Conclusions: We combined the results of microRNA expression analysis from an *in vivo* HCC mouse model with a new bioinformatics approach. Interactions between miRs, target genes and related proteins putatively involved in HCC initiation and progression were identified.

C37 Defective Chromosome Segregation Causes DNA Damage and Structural Chromosome Abnormalities In Human Hepatocellular Carcinoma

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Background: High levels of genomic instability appear to correlate with progression of hepatocellular carcinoma (HCC). The more common form of genomic instability in HCC is chromosomal instability, resulting in ongoing numerical and structural chromosomal aberrations yielding a heterogeneously tumor cell population that has the ability to undergo selective evolution, mainly represented by drug resistance and immune-system escape. Chromosomal instability *per se* is an important factor of DNA damage sustaining numerical and structural chromosome aberrations but the underlying causes and mechanisms are unknown.

Methods: We screened proteins known to be activated in the presence of DNA damage by using an animal model of diethylnitrosamine-induced HCC expressing karyotypic alterations and we identified the DNA damage response checkpoint kinase Chk2. Next, specimens obtained from patients with primary HCC were evaluated for the expression of Chk2 and pathological analysis was carried out. To assess the functional role of Chk2 in HCC, gain and loss-of- function studies were performed *in vitro*.
Results: We demonstrated that defective chromosome segregation induced DNA damage and numerical and structural chromosome aberrations. Overexpression and activation of the DNA damage response protein Chk2 and its mislocalization within the mitotic spindle carried on chromosomes missegregation self-perpetuating DNA damage. Immunohistochemical studies identified the expression of Chk2 in a subset of patients with HCC.
Conclusions: These findings propose Chk2 as a putative biomarker that can be used to detect chromosomal instability and DNA damage in HCC providing a valuable support not only for prognosis but also for identifying patients who are likely to have a response to therapy.

C38 Identification of Specific PIWI-interacting Small Noncoding RNA (piRNA) Expression Patterns During Hepatocarcinogenesis

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Background: Hepatocellular carcinoma (HCC) is the most frequent primary liver malignancy, mostly occurring in the context of chronic liver diseases leading to cirrhosis, more often beginning with development of premalignant lesions, characterized by low- (LGDN) and high-grade (HGDN) dysplastic nodules. P-element induced Wimpy testis (PIWI)-interacting RNAs (piRNAs) represent a large family of small noncoding RNAs, 23-35 nucleotide-long, first identified in fruitfly germline cells by their ability to interact with PIWI proteins and thereby exert epigenetic silencing of retrotransposons. The piRNAs are now also known as post-transcriptional regulators in somatic tissues, including stem and cancer cells, where piRNAs and piRNA-like molecules have been shown to influence key cellular processes.
Methods: We applied small RNA sequencing to search for liver piRNAs and to profile their expression patterns in cirrhotic nodules (CNs), LGDN, HGDN, early HCC and progressed HCC (pHCC), analyzing 55 nodules from 17 patients.
Results: We identified an expression signature of 58 piRNAs and 67 novel piRNA-like molecules that clearly discriminates HCC from matched CNs, correlating also to clinicopathological characteristics of HCC. This result was confirmed by functional analysis of predicted piRNA target mRNAs. Interestingly, 24 piRNAs showed specific expression patterns in dysplastic nodules compared to cirrhotic liver and/or pHCC.
Conclusions: These results demonstrate that the piRNA pathway is active in human liver, where it is likely to represent a new player in the molecular events that characterize hepatocellular carcinogenesis, from early stages to pHCC. Furthermore, they suggest that piRNAs might represent new disease biomarkers, potentially useful for differential diagnosis of dysplastic and neoplastic liver lesions.

C39 FGF2 in Human Melanoma Progression: The Antithetical Functions of Different Isoforms

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Background: Aberrant fibroblast growth factor 2 (FGF2) expression is correlated with different types of human cancer, included melanoma, where it is thought to contribute to its development and progression. An alternative translational process gives rise to five FGF2 isoforms with specific localization and functions: a low molecular weight (LMW, 18 KDa) and four high molecular weight (HMWs, 22, 22.5, 24, 34 KDa) isoforms. Alternative translation of FGF2 mRNA is regulated by an internal ribosome entry site (IRES) that controls the expression of all the isoforms except for the 34 KDa HMW, which depends instead on the canonical cap-dependent mechanism. To date, very extensive studies have been performed on LMW FGF2, while HMWs isoforms have to be better investigated.
Methods: To disclose the differential contribution of FGF2 isoforms in melanoma progression, we forced the expression of IRES-dependent isoforms- LMW (18 KDa) and HMWs (22, 22.5, 24 KDa)- in human melanoma cells. We then evaluated their biological roles by studying migration, anoikis resistance, homing, chemoresistance and tumor angiogenesis.
Results: We demonstrated that while LMW FGF2 expression confers stem-like traits and a pro-angiogenic profile to melanoma cells, HMWs isoforms are involved in the migratory processes and in the maintenance of tumor perfusion, when endothelial cell-driven angiogenesis is lacking, by promoting vasculogenic mimicry.
Conclusions: This comparative study shows the differential contribution of FGF2 isoforms in melanoma progression, pointing out that, even behaving in specific/antithetical manners, they cooperate in different steps of metastatic cascade, providing melanoma cells with higher malignancy and aggressiveness.

C40 Involvement of ζ-Crystallin in Acidic Microenvironment of Melanoma

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Background: Most cancer cells, including those of melanoma, rely on aerobic glycolysis, which causes a chronic acidification of tumor microenvironment. In turn, acidosis induces a metabolic shift toward glutaminolysis and increases the apoptotic threshold of cancer cells, favoring malignant progression and onset of drug-resistant phenotypes. *Bcl-2* gene expression undergoes an intricate post-transcriptional regulation. Among the regulators of Bcl-2 mRNA fate, we identified ζ-Crystallin (CryZ) and highlighted its alteration in leukemia cells. Beside *Bcl-2*, CryZ regulates the expression of glutaminase (*GLS*) and glutamate dehydrogenase (*GDH*), two major genes of glutaminolysis. Furthermore, it has been demonstrated that aspirin-like analgesics are potent inhibitors of CryZ enzymatic activity.
Methods: Biomolecular effects of exogenous modulation, *per se* or in combination with targeted therapies, have been analyzed in A375 and MeWo melanoma cells exposed to extracellular acidosis. In addition, the role of acetylsalicylic acid (ASA) and salicylic acid (SA) on CryZ binding activity to mRNA targets has been investigated.
Results: We demonstrated that: 1) acidosis induced the expression of CryZ; 2) inhibition of p38 pathway prevented acidosis-mediated CryZ upregulation; 3) acidosis conferred CryZ-dependent resistance to vemurafenib; 4) besides *Bcl-2*, CryZ associates with the anti-apoptotic Bcl-xL mRNA; 5) ASA and SA impaired the binding of CryZ to Bcl-2, Bcl-xL and GLS mRNAs.
Conclusions: We disclosed a role of CryZ as an important pH-responsive element that confers a cytoprotective effect on melanoma cells. In addition, the evidence that ASA and SA impaired CryZ binding to its mRNA targets could lay the basis for development of innovative post-transcriptional therapeutic tools.

C41 Subpopulation of Acidic Melanoma Cells as a New Target of Metformin

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Background: Proliferating cancer cells exhibit an increased glycolysis regardless of oxygen tension, a phenomenon known as the Warburg

effect. Further, an abnormal vasculature can result in regional variation in oxygenation implying a dynamic change in metabolism of tumor cells from an aerobic to an anaerobic type of glycolysis. These metabolic programs lead to a change in tumor microenvironment pH, which reduces to acidosis, conferring a new adaptive aspect to cancer cells.
Methods: A375 human melanoma cells were cultivated under acidic conditions, either obtained by enriching culture media with protons or lactic acid. Acidic cells were evaluated for epithelial-to-mesenchymal transition (EMT) program and metabolism. Metformin, a biguanide commonly used for type 2 diabetes, was used as mitochondrial complex I inhibitor.
Results: We found that melanoma cells exposed to an acidic extracellular microenvironment, show a profile associated with a shift to EMT and an oxidative phosphorylation (Oxphos) metabolism. In particular, lactic acidosis contributed to EMT profile of acidic melanoma cells stimulating motility, and a-cyano-4-hydroxycinnamate treatment inhibited lactate-induced migration. Metformin addition to acidic medium reduced EMT profile, such as N-cadherin expression and invasiveness, and Oxphos metabolism. As we may expect, metformin promoted AMPKα expression and phosphorylation in acidic melanoma cells. In addition, when metformin concentration increased to 10 mM, it induced a striking inhibition of proliferation and colony formation of acidic cells.
Conclusions: These findings disclose a new potential rationale for metformin addition to therapy of advanced melanoma, e.g., targeting acidic cells, a most likely drug resistant subset population.

C42 Tumor-tropic Endothelial Colony Forming Cells (ECFCs) Loaded with Near-infrared Sensitive Gold Nanoparticles: A "Cellular Stove" Approach to the Photoablation of Melanom.
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Background: One of the major challenges in cancer treatment is to selectively deliver cytotoxic drugs to the tumor site while avoiding potential side effects to non-diseased organs. Nanoparticles (NPs) are perfect tools for local drug delivery or drug targeting. Even though during the last decade multifunctional nanoparticles have been developed to improve the accumulation of therapeutic drugs in tumor tissues, the massive uptake of NPs by tumor tissues is still a significant challenge.
Methods: Gold (Au) nanoparticles were prepared by chemical reduction of $HAuCl_4$. Transmission electron microscopy and ICP analysis were performed to estimate gold uptake. Capillary morphogenesis and Boyden chamber assay were performed to evaluate the migration properties. *In vitro* and *in vivo* photothermal treatment were conducted with a laser diode emitting at 808 nm wavelength with light intensities, namely 1, W cm−2.
Results: We propose a promising strategy to deliver therapeutic chitosan-coated gold nanoparticles to tumor cells as hidden cargo of endothelial colony forming cells (ECFCs) endowed with an innate tumor-tropism. Remarkably, ECFC gold enrichment doesn't affect cell viability and preserves the endothelial lineage characteristics such as capillary morphogenesis and cell migration. We demonstrate that heavily Au-doped ECFCs are able to efficiently warm up the tumor environment, and kill the cancer cells via hyperthermic heating both *in vitro* as well as *in vivo*.
Conclusions: The therapeutic impact of these results are relevant for the control of melanoma, which is one of the most aggressive skin cancers, notorious for its high multidrug resistance, easy to relapse and low survival rate.

C43 Impact of KSRP on Post-transcriptional Deregulation of IL-8, IL-1b, TNF-α, CXCR2 and IL-1R Expression and Consequently on Cell Motility and Invasiveness in Melanoma Cells
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Background: The pleiotropic far upstream element binding protein 2 or KH-type splicing regulatory protein (KSRP) is considered a checkpoint of inflammation since it regulates the expression of several inflammatory cytokines, binding to their mRNAs and committing them to exosome-mediated degradation. Deregulation of this post-transcriptional control of gene expression is at the basis of a variety of pathological processes. Genes coding cytokines and cytokine receptors frequently undergo post-transcriptional regulation: their altered expression generates a crosstalk between cancer cells and microenvironment, promoting cancer onset and progression. Having observed that KSRP RNA targets identified so far - IL-1β, IL-6, IL-8, iNOS, TNF-α, β-catenin and let7 family - are deregulated in invasive melanoma, we have hypothesized that KSRP could play a key role in melanoma progression.
Methods: Biomolecular effects of positive or negative exogenous modulation of KSRP expression on human melanoma cell lines (A375, A375-M6, MeWo and M21) were analyzed.
Results: Here, we report the following evidence: 1) KSRP lowers expression of IL-8, IL-1β and TNF-α, already known as KSRP targets, by destabilizing their mRNAs; 2) KSRP binds the mRNAs of CXCR2 and IL-1R, two KSRP targets unraveled so far, and reduces their expression by lowering their mRNA stability; 3) KSRP overexpression causes a marked decrease of cell motility and invasiveness, and lowers the apoptosis threshold in response to UV irradiation.
Conclusions: Our results strongly suggest that KSRP could contribute to melanoma progression. The evidence reported here could be the basis for future studies aimed to modulate KSRP in melanoma by interfering with the multiple factors promoting onset and progression of this severe malignancy.

C44 Quercetin Induces Primary Effusion Lymphoma Cell Death through Inhibition of Multiple Cell Signaling Pathways
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Background: Quercetin is a bioflavonoid widely distributed in the plant kingdom, with anti-inflammatory and anti-cancer properties.Quercetin has been reported to inhibit several cancer-related pathways including PI3K/Akt, mTOR and STAT3. Since these pathways are hyperactivated in primary effusion lynphoma (PEL), a B-cell lymphoma associated with the oncogenic virus KSHV, we investigated whether quercetin would reduce their activation and induce PEL cell death. Moreover, since these pathways may regulate the release of cytokines such as IL-10 and IL-6, which are essential for PEL cell growth, we next explored the effect of quercetin tratment on their secretion.
Methods: Western blot analysis was used to evaluate the expression and phosphorylation of P13K/Akt/mTOR and STAT3, and cytokine release was analysed by chemiluminescence (IMMULITE 1000, SIEMENS).Cell survival was investigated by tripan-blue exclusion assay.
Results: We found that quercetin treatment reduced PI3K/ Akt, mTOR and STAT3 phosphorylation in PEL cells, downregulating the expression of the pro-survival cellular proteins such as c-FLIP, cyclin D1 and c-MYC. These effects resulted in the induction of a strong cytotoxicity against PEL cells, also due to a reduction of IL-6 and IL-10 release. These cytokines are indeed essential for PEL survival.
Conclusions: The results obtained in this study indicate that quercetin may represent an ideal molecule for the treatment of this aggressive B-cell lymphoma, for which an effective therapy has not been found yet.

C45 HDAC4 Silencing Sensitizes Resistant Chronic Myeloid Leukemia Cells to Imatinib Mesylate Treatment

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Background: The combination of imatinib (IM) with histone deacetylase (HDAC) inhibitors sensitizes both chronic myeloid leukemia (CML) cells and CD34+ progenitors of IM-resistant patients to apoptosis. The aim of this study was to determine if inactivation of BCR-ABL affects the expression and the activity of HDAC in CML cells to evaluate a therapeutic role for HDAC inhibitors in IM-resistant patients.
Methods: HDAC activity was evaluated employing a fluorescent assay kit. HDAC4 silencing was achieved by shRNA lentiviral infection. The effects of drugs were analysed by proliferation and apoptosis assays.
Results: IM treatment of CML cells increased total HDAC activity as compared to untreated cells. The same results were observed on BCR-ABL-transduced HL-60 cells; no difference was detected in the empty vector transduced control. An immunoblot analysis revealed that variations in HDAC activity after IM treatment were associated with an increase in HDAC4 expression. Interestingly, HDAC4 silencing reduced HDAC activity after exposure to IM. K562-R and LAMA84-R resistant to IM displayed higher HDAC4 expression as compared to their IM-sensitive counterpart. Indeed, HDAC4 silencing restored IM response and determined an increase of apoptosis. We also found that the combination of IM and apicidin, an HDAC4 inhibitor, enhanced the apoptotic rate of IM-resistant CML cells. Experiments performed employing IM-resistant CD34+ progenitors demonstrated that the two-drug association determined a significant increase in cell death.
Conclusions: Results suggest that BCR-ABL negatively modulates HDAC activity mostly by reducing HDAC4 expression. Furthermore, strategies aimed at reducing HDAC4 levels may represent a potential therapeutic option for CML patients unresponsive to IM.

C46 High BCR-ABL/GUS^{IS} Levels at Diagnosis are Associated with Unfavorable Responses to Standard Dose Imatinib

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Background: The approval of second-generation tyrosine kinase inhibitors (TKIs) for the first line treatment of chronic myeloid leukemia (CML) has generated a need for early molecular parameters associated with inadequate responses to imatinib mesylate (IM). We correlated BCR-ABL transcripts at diagnosis with the outcome (defined according to the 2013 European Leukemia Net recommendations) of 272 newly diagnosed CML patients receiving IM 400 mg/day.
Methods: BCR-ABL transcripts were measured from peripheral blood samples at diagnosis using real-time quantitative PCR (qPCR). Determinations were performed twice on the same sample using either *ABL* or glucuronidase-beta (*GUS*) as reference genes. BCR-ABL values were reported on the international scale (IS).
Results: With a median follow-up of 60 months, 65.4% of patients achieved an optimal response, 5.6% presented a response defined as "warning," 22.4% failed IM, and 6.6% switched to a different TKI because of intolerance to the drug. We applied ROC curves to define BCR-ABL/GUS^{IS} expression that would separate patients likely or unlikely to achieve multiple endpoints, namely: optimal response (OR), failure-free survival (FFS), event-free survival (EFS), transformation-free survival (TFS) and overall survival (OS). We found that high BCR-ABL/GUS^{IS} levels at diagnosis were associated with inferior probabilities of OR ($p<0.001$), FFS ($p<0.001$) and EFS ($p<0.001$). Elevated BCR-ABL/GUS^{IS} levels were associated with higher rates of disease transformation to the accelerated phase or blast crisis ($p=0.029$) but not with OS ($p=0.132$).
Conclusions: BCR-ABL transcripts at diagnosis employing *GUS* allow the identification of CML patients unlikely to benefit from standard dose IM that should be considered for alternative forms of treatment.

C47 BCR-ABL1 Doubling Time and Halving Time May Predict CML Response to Tyrosine Kinase Inhibitors

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Background: With the advent of imatinib (IM), patients with chronic myeloid leukemia (CML) are monitored by qPCR to evaluate treatment response and provide interventions for patients failing the therapy. We determined whether BCR-ABL1 doubling times could distinguish inconsequential rises in BCR-ABL1-transcripts from resistance to the treatment and investigated if BCR-ABL1 halving-times could identify patients with deep molecular responses.
Methods: We examined 377 CML pts. 315 received IM as first-line treatment and the remaining received 2G-TKIs.
Results: We classified 315 IM-treated patients into three groups: subjects that failed IM (n=34); subjects that lost a previously achieved MR^3 but maintained a complete cytogenetic response (CCyR) (n=77); and subjects with a confirmed rise in their BCR-ABL-transcripts without MR^3 loss (n=204). Short doubling-times were observed in patients failing IM (34.85 days). Long doubling times were found in subjects losing a MR^3 with CCyR and in individuals with a rise in BCR-ABL-transcript with a loss of MR^3 (52 vs 60.5 days). Doubling times in individuals failing 2G-TKIs as second-line treatment were shorter than those observed for IM-resistant patients (28.5 vs 34.8). Halving times were lower in subjects with MR^3 after 6 months of IM as compared to individuals without MR^3 (21.7 vs 42.7). After 12 months of IM, short halving times occurred in patients with MR^3 as compared to individuals without MR^3 (30.6 vs 97.3). Similar results were found in patients receiving 2G-TKIs as first-line therapy.
Conclusions: Doubling time is a tool that records differences in CML kinetics according to the clinical context and distinguishes patients failing TKIs. Halving times may identify patients with deep molecular responses.

C48 The Metabolically-Modulated Stem Cell Niche: An Energy Restriction Model for Leukaemia Stem Cell Resistance to Therapy

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Background: The concept of stem cell niche was introduced in 1978 to model sites suited to host haematopoietic stem cells (HSC) and favour their self-renewal, restraining clonal expansion and differentiation commitment. Studies of the effects of low oxygen tension on HSC maintenance *in vitro* led us to hypothesize that *in vivo* HSC niches are located within tissue areas where oxygen tension is physiologically lower than elsewhere. We found that HSC are adapted to survive, and selected, in low oxygen, where they are capable to cycle, and that low oxygen, in turn, steers cycling towards HSC self-renewal.

Methods: We then focused on leukaemia stem cells (LSC) of chronic myeloid leukaemia (CML). CML is triggered by a well-identified oncogenetic mechanism, the expression of the constitutively-active BCR/Abl tyrosine kinase, and brilliantly treated with its inhibitors (TKi). However, TKi are extremely effective in inducing remission of disease, but unable, in the majority of cases, to prevent relapse.
Results: We found that metabolic restrictions (oxygen/glucose shortage), while suppressing BCR/Abl protein, do not abolish LSC potential, which is thereby refractory to TKi.
Conclusions: Thus, metabolically-adapted LSC are excellent candidates to sustain TKi-resistant minimal residual disease (MRD) of CML and the related risk of relapse. Current work is directed (a) to establish the role of other "metabolic" players, such as low pH and availability of glutamine, in the definition of LSC microenvironment and in the maintenance of MRD of CML, as well as (b) to determine the sensitivity of metabolically-adapted LSC to non-TKi drugs.

CELL DEATH, TISSUE INJURY AND REPAIR

CDIR1 Crosstalk at the Mouse Neuromuscular Junction during Neurotoxin-Induced Degeneration and Regeneration of Motor Axon Terminals

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Background: The neuromuscular junction (NMJ) is the site of transmission of the nerve impulse to the muscle. This finely tuned synapse relies on at least three components: the motor neuron, the muscle fiber, and Schwann cells, which assist nerve recovery after injury. The molecular mechanisms underlying peripheral neuroregeneration are not fully understood.
Methods: We have set up an innovative experimental model to study the crosstalk that takes place at the neuromuscular junction among its three main components during motor axon terminal degeneration and regeneration. Animal neurotoxins are used here to induce an acute and reversible nerve terminal degeneration, to detect signaling molecules released by degenerating nerve and pathways that become activated in perisynaptic Schwann cells and muscle cells.
Results: We have identified several molecules through which the damaged nerve terminal communicates with nearby cells, activating signaling pathways in Schwann cells that promote nerve regeneration. Among these messengers, mitochondrial hydrogen peroxide appears to be crucial at the initial stages of regeneration, because its inactivation by catalase delays the functional recovery of the damaged neuromuscular junction *in vivo*.
Conclusions: We are currently investigating, by transcriptomic analysis of the NMJ at different stages of the degeneration and regeneration process, the retrograde signals generated at the NMJ that could stimulate nerve regrowth. These findings may provide important indications about the pharmacological treatment of traumatized patients.

CDIR2 TGF-β1 Production is Modulated by Arg Tyrosine Kinase in High Glucose Treated Human Renal Tubular Cells

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Background: Renal tubular cells subjected to hyperglycemia are involved in tubular interstitial fibrosis development observed in diabetic nephropathy (DN) through mechanisms still unclear. It has been described that tubular cells under high glucose (HG) condition overproduce TGF-β1 involved in epithelial mesenchymal transition (EMT) and renal interstitial fibrosis development. The non-receptor tyrosine kinase Arg protein is involved in some aspects of the EMT process through RhoA/ROCK pathway, which is also involved in TGF-β1 secretion by HG treated tubular cells. Our study investigated the involvement of Arg tyrosine kinase in the production of TGF-β1 induced in well-characterized human primary tubular cell cultures by treatment with HG.
Methods: Human primary tubular cell cultures established from normal renal cortex and cultured in control (100 mg/dl glucose) or in HG medium (450 mg/dl glucose) were used. Arg, TGF-β1 precursor, and phospho-p190RhoGAP were evaluated by Western blot; secreted TGF-β1 by ELISA; ROS by fluorescent oxidized DCF quantification; GTP-bound RhoA by G-LISA kit. Arg silencing was performed by siRNA.
Results: Primary tubular cells under HG condition overproduced ROS that downregulated Arg tyrosine kinase, and overexpressed and secreted TGF-β1 that activated fibroblasts. A further TGF-β1 expression increase was induced in HG condition with Arg silencing. In the HG treated cells ROS-dependent Arg kinase downregulation induced RhoA activation through p190RhoGAP modulation.
Conclusions: These data evidence a novel specific involvement of Arg kinase in the regulation of TGF-β1 expression in tubular cells under HG conditions and provide cues for new translational approaches in DN.
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CDIR3 Role of p110δ PI3K in Carotid Restenosis

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Background: Restenosis is a narrowing of arterial lumen that occurs upon endothelial arterial damage and represents a limiting long term success of intervention in the arteriopathy disease. Accumulating evidence indicates that recruitment of inflammatory cells at the site of arterial injury is a critical step in the pathogenesis of restenosis.
Methods: Several signaling pathways are involved in pathogenesis of intima hyperplasia, including phosphoinositide 3- kinase (PI3K). PI3K is a lipid kinase family that controls many biological cellular functions such as proliferation, growth, differentiation, migration. Class IA PI3K is divided into three isoforms. Although p110α and p110β are ubiquitously expressed, p110δ is restricted in white blood cells. To investigate the role of p110δ PI3K in the pathogenesis of restenosis we used mice in which p110δ PI3K has been ($p110\delta^{D910A/D910A}$) catalytically inactivated and in which we performed an arterial injury.
Results: $p110\delta^{D910A/D910A}$ mice show defects in proliferation and differentiation of B- and T-lymphocytes (Okkenhaug, Bilancio, et al: *Science*. 2002, 297:1031-1034; Ali, Bilancio, et al: *Nature* 2004, 431:1007-1011; Bilancio et al: *Blood* 2006,107:642-650) and in migration of macrophages (Papakonstantis, Zwaenepoel, Bilancio, et al: *Journal of Cell Science*. 2008,121:4124-4133). $p110\delta^{D910A/D910A}$ mice were subjected to arterial injury and monitored for 30 days. In this work, we report that p110δ inactivation abolishes inflammatory cell recruitment in an *in vivo* model of arterial injury resulting in strong inhibition of restenosis
Conclusions: This approach might have great pharmacological interest because it allows the use of an isoform-specific inhibitor of PI3K to prevent restenosis.

CDIR4 DDB2 Mutant Protein Unable to Interact with PCNA Influences Cell Cycle Progression

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Background: DNA damage binding protein 2 (DDB2) is a protein involved in the early step of DNA damage recognition of the nucleotide excision

repair (NER) process. Recently, it has been suggested that DDB2 may play a role in DNA replication, based on its ability to promote cell proliferation. We have previously demonstrated that DDB2 binds the proliferating cell nuclear antigen (PCNA), a molecular platform coordinating the activity of several factors during NER; moreover, it also interacts with PCNA in the absence of DNA damage. However, whether and how this interaction influences cell proliferation is not known. In this research, we have addressed this question by using HEK293 cell clones stably expressing $DDB2^{Wt}$ protein, or a mutant form ($DDB2^{Mut}$) unable to interact with PCNA due to a mutation in PCNA interacting protein sequence (PIP-box).
Methods: For the interaction between the investigated proteins, fluorescence microscopy, cytofluorimetric analysis and Western blot techniques were carried out. Clonogenic assays and BrdU incorporation were performed to analyse HEK293 cell proliferation.
Results: We show that overexpression of the $DDB2^{Mut}$ protein provides a proliferative advantage over the wild-type form, by shortening cell cycle length. In particular, an increase in the number of S-phase cells, together with a different progression throughout this phase, and a reduction in $p21^{CDKN1A}$ protein levels have been observed in the $DDB2^{Mut}$ cells.
Conclusions: The results show that mutation in the PIP-box of DDB2 determines a cell cycle perturbation, suggesting that this effect is dependent on DDB2-PCNA interaction.

CDIR5 The Dynamic Adaptation of Macrovascular Endothelial Cells to Microgravity

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Background: After hundreds of space missions it is evident that long duration space flight activates responses that might result in potential hazard for the astronauts. It is well known that microgravity markedly impacts on the performance of the cardiovascular system. Since endothelial cells are responsible for vascular integrity, we studied the adaptive response of human macrovascular endothelial cells to microgravity.
Methods: Human endothelial cells were isolated from the umbilical vein and propagated *in vitro*. Microgravity was simulated by the rotating wall vessel. Reactive oxygen species (ROS) were detected by dichloro-dihydro-fluorescein diacetate (DCFH) assay. A stress protein array was performed and validated by western blot.
Results: We found that early after exposure to weightlessness these cells synthesize higher amounts of HSP70 than controls. HSP70 returns to basal level after 4 days, when paraxonase-2, HSP27 and sirtuin-2 gradually increase and peak at day 10. In parallel, thioredoxin interacting protein (TXNIP) is upregulated after 10 days in real and simulated microgravity. TXNIP is a stress responsive protein that inhibits thioredoxin activity, thus generating oxidative stress, and is known to be involved in mechanotransduction. However, we detected a transient rapid increase of ROS only after a few hours in microgravity, while at later time points ROS generation dropped to control levels.
Conclusions: Adaptation to microgravity is a dynamic process. Indeed, different stress proteins are involved at different times after exposure to microgravity to allow endothelial cells to adapt to the mechanical stress determined by microgravity.

CDIR6 Is Methadone Really Safe in Opioid Maintenance Treatment?

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Background: Opioids are frequently associated with fatal poisonings due to their ability to cause respiratory and cardiovascular depression, and among the numerous reports published on fatal poisoning, methadone is the most frequently found. Because toxicological analysis is the most informative diagnostic tool after autopsy, we decided to review the toxicological analysis of 150 deaths in the Medical Legal Institute of the University of Udine.
Methods: Between 2012 and 2015 over 150 blood samples were analyzed first by screening test by ILAB 650 (Instrumentation Laboratory IL., Freemont CA, USA). Screening test was performed by cloned enzyme donor immunoassay (CEDIA Technology) and positive samples were subsequently analysed by gas chromatography mass spectrometry (GC/MS), (Agilent Technology 4400, Varian BV, Middelburg, the Netherlands).
Results: Among 150 fatalities, 25 deaths (16,6%) were related to fatal opioid intoxication, and among these 13 deaths (50%) were due to methadone poisoning. Methadone concentrations were from 0.1 to 3.5 µg/mL., according to Clarke's (0-33 µg/mL).
Conclusions: The number of fatal opioid poisonings is increasing in Europe and methadone is the drug most involved. The profile of methadone intoxication is related to accidents in over 90% of deaths and suicide in 6% of cases. The median concentration of methadone in fatal cases does not differ from therapeutic concentrations. In spite the paramount progress of laboratory technology over recent decades it is still difficult to assess a clearcut correlation between blood methadone concentration and therapeutic effect. Forensic toxicology does not possess methods to prove that death has been caused by poisoning.

CDIR7 Retinoic Acid Impairs Neuronal Response to Oxidative Stress: Role of Nrf2-Dependent Responses

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Background: The generation of reactive oxygen species (ROS) derived from NADPH oxidase (NOX) by modulating redox balance favors neuronal differentiation induced by all-trans retinoic acid (ATRA) [Nitti M. et al, *Cell Signal* 2010]. In this work, the ability of neuronal cells to balance ATRA-induced ROS generation has been investigated and the Nrf2-dependent antioxidant system was evaluated.
Methods: SH-SY5Y neuroblastoma cells were differentiated for 4 or 7 days with 10µM retinoic acid. Cell viability after exposure to 100-500µM H_2O_2 was measured by trypan blue exclusion test. Gene and protein expression were detected by RT-PCR and Western blotting. Nrf2 binding to DNA was checked by ChIP analysis.
Results: Cell treatment with H_2O_2 decreased cell viability significantly more in the differentiated cells than in the undifferentiated ones. The expression of GCLM and GCLC, key enzymes in the synthesis of GSH, was not modified by H_2O_2 treatment both before and after cell differentiation. However, after H_2O_2 treatment heme oxygenase 1 (HO-1) expression as well as Nrf2 binding to the HO-1 promoter was significant reduced in differentiated cells in comparison to the undifferentiated ones. In addition, exposure to bilirubin prevented cell death induced by H_2O_2 in differentiated cells.
Conclusions: Our data show that neuronal differentiation induced by ATRA impairs Nrf2/HO-1 activation and reduces the availability of endogenous bilirubin, thus decreasing cell adaptation to oxidative stress.

CDIR8 Atomic Force Microscopy (AFM) Investigation of the Mechnism of Transmissible Cytotoxicity by a Protein Isolated from Cerebrospinal Fluid of a Neurological Patient: A Protein Misfolding Hypothesis

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Background: A cytotoxic protein fraction was originally isolated from glial and epithelial cell cultures inoculated with aliquots of cerebrospinal fluid from a neurological patient. The cytotoxicity thus generated is transmissible to other cell cultures with remarkable efficiency and is

maintained through repeated passages. Analytical,ulstrastructural and immunological analyses excluded the presence in the fraction of virus-like particles and of prion proteins. The cytotoxicity is resistant to denaturation by physical and chemical agents. In the advanced stages, the process results in intracellular accumulation of protein fibrils.In view of all these observations, the hypothesis was advanced that this cytoxic fraction is the result of a process of protein misfolding that propagates by transmitting the protein misfolded state to other cell cultures.
Methods: We investigated with atomic force microscopy (AFM) the relative frequency of protein monomeric, dimeric and low-n-oligomeric forms present in the cytotoxic fraction compared to that in the control. In fact, the misfolded proteins first come together to form dimers and low n-oligomers during fibrillogenesis, The use of AFM allows imaging of individual proteins and protein aggregates at high resolution, thus permitting determination of the frequency of specific structural forms of protein present in a sample.
Results: Statistical analysis of AFM images revealed that the frequencies of dimers and of low n-oligomer forms are significantly higher in the cytotoxic fraction than in the control.
Conclusions: These observations substantiate the above hypothesis that the cytoxic fraction under study is the result of a protein misfolding process, capable of being transmitted to other cell cultures.

INFLAMMATION AND IMMUNOPATHOLOGY

INIM1 Epicardial Adipose Tissue Increase as a Potential Mediator of Cardiac Fibrosis through ST2 Overexpression in CAD and Non-CAD Patients

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Background: In coronary artery diseases (CAD) chronic hemodynamic sheer induces cardiac remodeling evolving in tissue fibrosis. The newer cytokine implicated in this process is ST2, an interleukin-1 receptor with two main isoforms, ST2L and soluble ST2 (sST2). The natural ST2L ligand is interleukin-33 (IL-33) and its binding has cardioprotective properties including cardiac fibrosis prevention. This mechanism is blocked by sST2, the receptor decoy of ST2L and its overexpression can inhibit ST2/IL-33 signaling. Different tissues can express ST2, including adipose tissue. Since EAT shares the microcirculation with myocardium our aim is to verify: first if EAT can produce ST2, and then whether increased EAT thickness can be associated to ST2 overexpression in CAD and non-CAD patients.
Methods: We collected EAT and subcutaneous adipose tissue (SAT) biopsies from 22 CAD patients who underwent bypass surgery and 10 non-CAD patients who underwent valvular replacement. All patients were stratified according to median EAT thickness value (8mm). ST2 and IL-33 expression were assayed using microarray technology. ST2 level was determined by Western blotting analysis.
Results: ST2 protein level was higher in EAT than SAT. CAD and non-CAD patients with EAT thickness >8mm have higher expression of ST2 gene than CAD and non-CAD patients with EAT thickness<8mm. IL-33 expression was similar intra and inter CAD and non-CAD groups.
Conclusions: Our results demonstrated that EAT mass increase is associated to ST2 expression and production in CAD and non-CAD patients and may be related to induction of cardiac fibrosis.

INIM2 Characterization of the Proinflammatory Profile of Synovial Fluid-Derived Exosomes of Patients with Gonarthrosis

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Background: Synovial fluid (SF)-derived exosomes have been poorly characterized and their role in joint inflammation has never been investigated thoroughly. The purpose of this study is to characterize SF-derived exosomes of patients with gonarthrosis and to investigate their immunoregulatory properties.
Methods: Exosomes were isolated from SF of seven patients with gonarthrosis by polymer precipitation (Exoquick), quantified by acetyl-CoA-acetetylcholinesterase activity, and validated by tetraspanins expression by immunoblot and FACS. To evaluate their immunoregulatory properties, exosomes were further purified with affinity capture antibody-coupled magnetic beads and incubated with M1 macrophages, differentiated from blood donor CD14+ monocytes, and the production of cytokines, chemokines and MMPs and expression of co-stimulatory molecules were investigated by FACS and multiplex ELISA.
Results: SF-derived exosomes ($1.3\pm1.4x10^{11}$/ml) expressed the specific exosomal markers (CD9, CD63, CD81, TSG101) by immunoblot and FACS. After isolation with Exoquick, a relevant contamination by immune-complexes was detected. To avoid possible interference in functional experiments by immune complexes, we added a magnetic bead isolation step. SF-derived exosomes significantly stimulated the production of several inflammatory cytokines and chemokines (IL1β, TNFα, IL16, CCL20, CCL15, CCCL1) and the release of specific metalloproteinases (MMP7 and MMP12) by M1 macrophages, although they did not influence the expression of CD80 and CD86 co-stimulatory molecules.
Conclusions: In this study, we characterized exosomes isolated from SF of patients with gonarthrosis and demonstrated that they are functionally active in their ability to stimulate the release of proinflammatory cytokines and MMPs from M1 macrophages, suggesting that they may play a role in disease progression.

INIM3 Biological Properties of Wine Grape Polyphenols on Human Dermal Blood Endothelial Cells

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Background: The role of cutaneous microvasculature is crucial for both inflammatory and neoplastic diseases of the skin. The antioxidant, antiproliferative and antiinflammatory properties of wine grape polyphenols are widely recognized on blood vessels of the main circulatory system; however, little is known about their functions on the cutaneous microvasculature. We sought to analyze the biological effects of selected wine grape polyphenols on primary human dermal blood endothelial cells (HDBECs).
Methods: Primary HDBECs (Promocell, Germany) were incubated for 24 hours respectively with Epicatechin, Quercitin3-glucoside and Malvidin3-glucoside chloride (Phytolab, Germany), dissolved in dimethylsulphoxide. Cells were then irradiated with either UVA or UVB as proinflammatory stimulus. Cytotoxicity was assessed by MTT assay and trypan blue dye, DNA damage was determined by H2AX histone phosphorylation; levels of ICAM-1 were measured by ELISA. Production of reactive oxygen species (ROS) was investigated by flow cytometry analysis.
Results: At the concentrations tested, none of the polyphenols exerted cytotoxic effects, although they significantly prevented UV-induced cell death, as observed mainly after UVA exposure. Similarly, UVA-induced DNA damage was decreased after treatment with each polyphenol, as compared to control. Dose-dependent inhibition of ROS production and ICAM-1 expression were also observed on UVA-irradiated HDBECs. These findings suggest that polyphenols exert an anti-inflammatory effect on HDBECs through their ability to interfere with ROS and cytokine release.
Conclusions: Polyphenols derived from wine grape could be candidate antioxidants and anti-inflammatory compounds for skin diseases with involvement of the blood microvasculature.

INIM4 Elevated Plasma Platelet-Activating Factor Acetylhydrolase Activity is Significantly Associated with High Risk Anti-phospholipid Antibody Antigenic Specificities

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Background: Elevated plasma platelet-activating factor acetylhydrolase (PAF-AH) activity was associated with increased risk of cardiovascular events. Our goal was to study the association between PAF-AH activity and the presence of different anti-phospholipid antibodies (aPL) antigenic specificities.

Methods: Plasma PAF-AH activity was measured in 72 female patients (50±15 yrs) positive for at least one anti-phospholipid antibody in a context of thrombotic events or risk of thrombosis and in 21 age/sex matched blood donors (BDs). aPL antibodies (anti-phosphatidylserine/prothrombin IgG/IgM antibodies - aPS/PT, anti-β2-glycoprotein I IgG/IgM antibodies - aB2GpI, lupus anticoagulant - LAC), were analyzed by diagnostic methods.

Results: PAF-AH activity was significantly more elevated in patients than in BDs (18.6±4.7 vs 13±2.4 nmol/min/ml; p<0.0001). Patients presenting only aB2GpI+ antibodies or only aPS/PT+ antibodies did not express different PAF-AH activity (17.8±3.4 vs 16.5±3.5 nmol/min/ml), although patients positive for both aB2GpI and aPS/PT antibodies showed significantly more elevated PAF-AH levels (20.9±5.6; p=0.03 versus aB2GpI+ and p=0.008 versus aPS/PT+). LAC+ showed higher PAF-AH plasmatic activity than LAC-negative patients (p=0.035). Patients disclosing IgG or IgM positive aPS/PT antibodies did not differ significantly, although aB2GpI IgG+ patients disclosed higher PAF-AH activity than aB2GpI IgM+ patients (21.8±6.4 vs 18.2±3.4 nmol/min/ml; p=0.07). Accordingly, aB2GpI IgG+ patients were more frequently associated to aPS/PT+ antibodies than aB2GpI IgM+ patients (85% vs 35%; p=0.0006).

Conclusions: PAF-AH activity is particularly elevated in patients showing both aB2GpI (especially IgG isotype) and aPS/PT antibodies, supporting the prominent role of this aPL pattern in anti-phosholipid antibody syndrome and possibly identifying PAF-AH as a factor involved in the pathogenic process.

INIM5 Antioxidant Effects of Extra Virgin Olive Oil Polyphenol Extracts on PBMCs

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Background: The traditional Mediterranean diet is characterized by the use of extra virgin olive oil (EVOO), which plays a key role in the prevention of age-related diseases and in the attainment of longevity due to the high levels of phenols. In fact, in particular oleuropein and hydroxytyrosol phenols seem to have the capacity to scavenge ROS and to act as anti-inflammatory molecules. Thus, they can positively affect the pathogenesis of age-related diseases, characterized by a low-grade inflammation and accumulation of ROS. The aim of the study was to establish the antioxidant activity of oleuropein, hydroxytyrosol and derivates, using human peripheral blood mononuclear cells (PBMCs) as an *in vitro* cellular screening system.

Methods: We tested the two native molecules and their paracetylated derivates on PBMCs. The cells were cultured and incubated with three polyphenol concentrations (1, 10 and 100 mM). ROS production was detected by Synergy-HT plate-reader (Ex/Em 488/528nm), after treatment with 20mM of 2',7' –dichlorofluorescin diacetate.

Results: Preliminary results demonstrate that the paracetylated molecules work in a dose-dependent manner, scavenging the ROS. This is in agreement with the datum that peracetylation of oleuropein and hydroxytyrosol may improve the ability to permeate the cellular membrane, hence performing the antioxidant action.

Conclusions: The use of supplements is widely popular but the real effects on humans are not always tested. Their application in terms of potentiation of therapies or prevention is interesting but, because they are chemical molecules, it is important, to first well characterize all their possible effects using human *ex vivo* models.

INIM6 The Crosstalk Between Fibronectin-Elicited Signals and the Receptor for the Colony-Stimulating Factor 1 (CSF-1R) Sustains Macrophage Migration

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Background: Integrins, following binding to proteins of the extracellular matrix, including fibronectin (FN), regulate several biological functions including migration. Besides activation of adaptor molecules and kinases, integrins can transactivate receptor tyrosine kinases (RTK) in the absence of growth factors. Colony stimulating factor-1 receptor (CSF-1R) is a RTK that supports the survival, proliferation, and motility of monocytes/macrophages, which are essential components of innate immunity and cancer development. Macrophage interaction with FN is recognized as an important aspect of host defense and wound repair. The aim of the present study was to investigate a possible crosstalk between FN-elicited signals and CSF-1R in macrophages.

Methods: We used the BAC1.2F5 and J774 murine macrophage cell lines and human primary macrophages derived from peripheral blood of healthy donors. Cell migration was assessed using 48-well modified Boyden chambers.

Results: FN induced migration in BAC1.2F5 and J774 cells as well as in human primary macrophages. Adhesion to FN determined phosphorylation of the focal adhesion kinase (FAK) and Src family kinases (SFK) and activation of the FAK/SFK complex as witnessed by paxillin phosphorylation. SFK activity was necessary for FAK activation and macrophage migration. Moreover, FN-induced migration was dependent on FAK in either murine macrophage cell lines or human macrophages. FN also induced CSF-1R and β1 integrin interaction and FAK-dependent CSF-1R phosphorylation. FN-induced CSF-1R phosphorylation was functional to migration. Indeed, genetic or pharmacological inhibition of CSF-1R prevented FN-induced macrophage migration.

Conclusions: Our results identified a new SFK-FAK/CSF-1R signaling pathway that mediates FN-induced migration of macrophages.

INIM7 $CD161^{++}CD8^{+}$ Mucosal-Associated Invariant T Cells (MAIT) are Reduced in Peripheral Blood of Healthy Elderly People

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Background: MAIT cells represent the most abundant innate-like T cell population in humans involved in anti-bacterial immunity. These cells have been associated with numerous diseases, such as bacterial infections and many proinflammatory diseases. Reduced number of circulating MAIT cells have been demonstrated in different autoimmune pathologies, as well as in HIV-infected patients. It is known that elderly people are characterized by a chronic, low-grade, proinflammatory status associated with the remodeling of the immune system. These changes are strictly associated with the increased susceptibility to infectious disease, other than chronic inflammatory diseases, of elderly people. In the present study, we investigated the percentage and the absolute number of circulating MAIT cells in healthy elderly people compared with young subjects.

Methods: Peripheral blood mononuclear cells were isolated from whole blood of 25 healthy elderly and 20 young subjects. To characterize the phenotype of MAIT lymphocytes, extracellular labelling was performed with anti-CD8-FITC, CD161-PE and CD3-ECD. Cells were acquired on an

Epics XL-MCL (BeckmanCoulter) four-colour flow cytometer and analyzed with FlowJo software (TreeStar).
Results: The results obtained show a significant decrease, both in percentage and in absolute number, of CD161^{++}CD8^{+} MAIT cells in the elderly compared to young subjects.
Conclusions: The age-related reduction of MAIT cells could be due to the chronic proinflammatory status of elderly people. Moreover, the reduction of these cells could explain the impaired ability of elderly subjects to respond to bacterial infections, e.g. *S. pneumonia*, which is one of the causes of morbidity and mortality in elderly people.

INIM8 Immunomodulatory Effect on CXCL10 of Selen-methionine in Association with Myo-Inositol in Patients with Euthyroid Autoimmune Thyroiditis

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Background: Different experimental researches and clinical trials have shown that myo-inositol and phosphatidylinositol(s) are involved in physiological and pathological conditions of the thyroid. The IFN-γ-inducible protein 10 (CXCL10, or IP-10) was initially identified as a chemokine induced by IFN-γ. Determination of high level of CXCL10 in peripheral fluids is considered a marker of a Th1 orientated immune response. The immune-modulating effect of selenomethionine in association with myo-inositol in patients with euthyroid autoimmune thyroiditis (AT) has been evaluated.
Methods: We evaluated 21 consecutive Caucasian patients with newly diagnosed euthyroid chronic AT. All patients were treated with selenomethionine plus myo-inositol (600 mg/83 mg) tablets, twice per day, for 6 months. A complete thyroid assessment was done before the treatment and after 6 months. Serum CXCL10 levels were assayed by a quantitative sandwich immunoassay using a commercially available kit.
Results: After treatment, thyroid-stimulating hormone (TSH) levels significantly declined compared to basal values, particularly in patients with an initial TSH value in the high normal range (2.1<TSH<4.0), suggesting that the combined treatment can reduce the risk of a progression to hypothyroidism in subjects with autoimmune thyroid diseases (AITD). After treatment, antithyroid autoantibody levels declined, as did CXCL10 levels, confirming its immunomodulatory effect.
Conclusions: We first show an immunomodulatory effect of selenomethionine in association with myo-inositol in patients with euthyroid AT. Further researches are necessary to extend the observations in large population, to evaluate the effect on quality of life, and to study the mechanism of the effect on chemokines.

INIM9 CD3^{+}CD56^{+}cells, a Novel Regulatory Subset, Modulates the Effector Function of Human CD8^{+} T Cells: Implication for Type 1 Diabetes

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Background: Orchestration of immune response is a complex phenomenon aimed to maintain tissue homeostasis. The key role of regulatory populations in the prevention of autoimmunity and immune mediated diseases has been largely shown. A number of observations indicate that normal peripheral blood contains a small population of lymphocytes that express both CD56 and CD3 phenotypic markers, which usually characterize human natural killer (NK) and T (CD3) cells, respectively. The biological hallmarks and functional characteristics of CD3^{+}CD56^{+} cells are not completely understood; experimental evidence associated this cell subset with different pathophysiological conditions.
Methods: CD3^{+}CD56^{+} cells were isolated from peripheral blood mononuclear cells (PBMCs) of human healthy subjects (HS) and type 1 diabetes subjects (T1D), by high-performance cell sorting. The phenotype and functional activity of human CD3^{+}CD56^{+} cells was assayed by cytofluorimetric analysis.
Results: We found that flow-sorted CD3^{+}CD56^{+} cells inhibited proliferation, cytoxicity and interferon (IFN)-γ production of activated CD8^{+} T cells, both in autologous and allogeneic conditions. Furthermore, it has been observed that down-modulation of CD8^{+} T cell activity was not dependent on FAS-FASL-mediated apoptosis and required cell-cell contact. Finally, absolute cell numbers and suppressive capability of CD3^{+}CD56^{+} cells were reduced in T1D compared with HS.
Conclusions: Our data unveil a novel lymphocyte subset with regulatory properties that specifically modulate CD8^{+} T cell response, which numbers and function were impaired in T1D. The comprehension of the precise cellular and molecular mechanisms that lead to suppressive activity of CD3^{+}CD56^{+} cells may contribute to the understanding of their role in the pathophysiology of T1D.

INIM10 Role of Annexin A1 (ANXA1) in the Modulation of Immune Response and Inflammation During Multiple Sclerosis

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Background: Annexin A1 (ANXA1) is an endogenous anti-inflammatory molecule, whose activity is mediated by glucocorticoids. It is mainly a cytoplasmic molecule but can be either membrane-associated and secreted as soluble factor. It exerts its anti-inflammatory action by limiting neutrophil extravasation and blocking monocyte migration via α4β1 integrin. ANXA1 also regulates blood-brain barrier (BBB) integrity in brain microvascular endothelial cells, and its expression is selectively lost in BBB of multiple sclerosis (MS) subjects. While the role of ANXA1 in innate immunity is well known, its action on the modulation of adaptive immune response is not completely understood.
Methods: ANXA1 expression in Treg and T conv cells from MS and healthy subjects was measured by Western blot and flow cytometry. Migratory capacity of Treg and T conv cells, from MS and healthy subjects, was tested using a transmigration assay with normal and human brain microvascular endhotelial cells silenced for ANXA1.
Results: We found that ANXA1 intracellular levels were significantly lower both in Treg and Tconv cells from MS compared to healthy subjects, also upon T cell receptor (TCR) activation. Moreover, we observed an impaired expression of ANXA1 levels in Th17 cells from MS compared to healthy subjects. Finally, Treg and Tconv cells from MS subjects showed a higher degree of adhesion and migration compared to healthy subjects and this condition is exacerbated on endhotelium lacking of ANXA1.
Conclusions: Together these findings suggest that the differential expression of ANXA1, in Treg and Tconv cells, could have relevance to the resolution of inflammation in MS subjects.

INIM11 Association of Uncoupling Protein Polymorphisms with Oxidative Stress and Implications for Atherosclerosis in Systemic Lupus Erythematosus Patients

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Background: Uncoupling proteins (UCPs) are a family of inner mitochondrial membrane carriers, implicated in inflammation and atherogenesis through the control of reactive oxygen species production. In this study we investigate the association between genetic variants in the *UCP1*, *UCP2*, and *UCP3* genes with the presence of carotid plaque and oxidative stress in systemic lupus erythematosus (SLE) patients.

Methods: To date, 48 SLE patients and 29 age/sex-matched healthy individuals were enrolled. All samples were genotyped for the following SNPs, *UCP1* -3826 A/G, *UCP2* -866 G/A, UCP2 Ins/Del, and *UCP3* -55C/T using RFPL-PCR. Plasma oxidative stress was evaluated measuring plasma levels of malondialdehyde (MDA) and thiols by laser-induced fluorescence capillary electrophoresis. Carotid plaque was evaluated using echo-colour-Doppler examination.
Results: Statistical analyses of the SNPs showed no significant difference in genotype and allele frequencies between cases and controls. Increased risk for carotid plaque has been observed in SLE patients ($p< 0.05$), but it seems to not correlate with UCP SNPs. Oxidative stress data showed higher levels of MDA, cysteine, and taurine in SLE patients compared to controls ($p< 0.05$). Particularly, the increase of MDA is associated with the mutated allele G of *UCP1* gene ($p= 0.035$).
Conclusions: In our case-control study, we observed no significant associations between genetic variants in UCP genes and SLE pathogenesis. In contrast, we found that *UCP1* -3826 A/G polymorphism is associated with increased levels of MDA in SLE. Several studies suggest MDA as a predictor of cardiac events; further studies are needed to confirm our observations.

INIM12 ANA Testing and Anti-DFS70 Antibody Detection in Clinical Practice: Experience with DFS70-Ko HEp2

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Background: When an ANA pattern suggestive of anti- DFS70 antibodies (dense fine speckled) is detected, it should be confirmed. Several confirmation methods are available, including a new ANA-IIF using HEp2 cells knocked-out for the DFS70 antigen (DFS70-Ko) and mixed at 1:9 ratio with normal HEp2 cells. The aim of this study is to evaluate the possibility of using this test as a first level ANA screening test.
Methods: We performed the ANA test using the IMMCO HEp2 DFS70-Ko ANA-IIF method in 3 different series of sera: 90 previously tested positive for anti-DFS70 by chemiluminenscent immunoassay (CLIA, BioFlash, INOVA); 108 high positive for the most common rheumatic disease-associated autoantibodies; and 131 consecutive routine samples screened for ANA on HEp2 INOVA.
Results: The HEp2 DFS70-Ko test enabled recognition of anti-DFS70 antibodies in 85/90 (94.5%) DFS70 CLIA-positive sera. The 131 sera with defined antibody specificity displayed their expected HEp2 pattern, both on the standard and DFS70-Ko subtrates. We found an elevated concordance (95.5%) between ANA results on Inova and on IMMCO DFS70-Ko HEp2 cells. 3/5 suspected DFS70 sera on Inova were confirmed by IMMCO.
Conclusions: The IMMCO HEp2 DFS70-Ko method could be used for routine ANA screening as it presents sensitivity and specificity comparable to the standard HEp2 substrate, keeping the expression of autoantibody markers intact. Moreover, it offers an excellent opportunity allowing the simultaneous identification and confirmation of the presence of anti-DFS70 antibodies. This could prove to be extremely important regarding organization, turnaround time, and laboratory economy.

INIM13 LPS Adsorption to Titanium Dioxide Nanoparticles Shifts the Proinflammatory Transduction Pathways Triggered by Endotoxin: A Biomimetic Effect of the Complex

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Background: Engineered nanoparticles (NP) have been recently proposed as powerful tools for immunomodulating approaches although they may also cause unwanted inflammatory responses. We recently observed that TiO_2 NPs synergize TLR4-dependent proinflammatory effects of LPS in macrophages (Bianchi et al., *Toxicol Res* 2015, 4:385-398). Our task is to identify which TLR4-dependent inflammatory pathways are triggered by the LPS-NP complex.
Methods: Raw 264.7 (murine) cells were treated with TiO_2 NP (20 nm, 80 $\mu g/cm^2$) and LPS O55:B5 (0.5 ng/ml) alone or in combination. The endpoints evaluated were *Nos2, Tnf* and *Ifnbeta1* expression (RT-PCR), MAPK and IRF-3 phosphorylation (Western blot). MyD88 adaptor function was inhibited with specific peptides.
Results: Compared with LPS or NPs alone, co-incubation with TiO_2 NPs and LPS potentiated either NF-κB- (*Tnf, Nos2*) or TRIF-dependent (*Ifnbeta1)* gene expression, changing also the kinetics of induction. The phagocytosis inhibitor cytochalasin B partially hindered the LPS-dependent induction of *Ifnbeta* and *Nos2*, but abolished the synergistic effect. NP-LPS complex increased IRF-3 phosphorylation and nuclear translocation more than LPS alone. MyD88 adaptor inhibition reduced the LPS-NP dependent overstimulation of *Nos2* and *Ifnbeta1* and prevented the LPS-dependent induction of *Nos2* but not that of *Ifnbeta1*. LPS-NP co-treatment mainly involved p38 phosphorylation, but LPS alone had a prevalent effect on JNK and ERK1/2.
Conclusions: The synergistic effect of TiO_2 NP and LPS on macrophage activation is attributable to the endosomal targeting of the complex, which, due to NP aggregation, may assume bacteria-like shape and size and become endowed with biomimetic properties.

INIM14 Induction of Arginase Pathway in Human Monocytes: Does Celiac Disease Matter?

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Background: Celiac disease (CD) is an immunomediated enteropathy, involving both adaptive and innate responses against ingested gluten in susceptible individuals. We have demonstrated that the activation of the arginase metabolic pathway in murine macrophages by gluten peptides contributes to the modulation of intestinal permeability *in vitro*. The aim of this study is to verify 1) whether a comparable gluten-driven induction of arginase also occurs in human monocytes; 2) if the activation of arginase pathway by gluten corresponds to the onset of an inflammatory phenotype in immune cells; and 3) if the expression of genes belonging to this pathway could be used as serological marker of CD.
Methods: Circulating monocytes were isolated from blood samples collected from 15 CD patients on gluten-free diet, 15 naïve patients and 15 healthy controls. After treatment with enzymatically digested gluten (PTG), the expression of arginase and of the proinflammatory marker IL1β was measured in all samples.
Results: An induction of arginase transcript was observed in PTG-treated monocytes, confirming data obtained in murine cells; a concomitant increase of IL1β also occurred, indicating the inflammatory effect of the treatment. However, no significant difference was detected between healthy or pathological samples, neither CD nor CD_GF. Hence, neither arginase nor IL1β in circulating monocytes appear to be good candidates for diagnosis of CD, being equally expressed in all groups.
Conclusions: In human monocytes, gluten activates arginase pathway and increases IL1β mRNA, confirming the onset of an inflammatory phenotype. However, these genes do not appear to be good candidates for diagnosis of CD.

INIM15 Human Macrophage Differentiation Induces an Increase of Carnitine Transport

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Background: L-carnitine, besides playing a fundamental role in the β-oxidation of fatty acids, has been recently identified as a modulator of immune function, although the underlying mechanisms remain to be clarified. In this study, we addressed the modulation of L-carnitine transport and the expression of the related transporters during the differentiation of human monocytes to macrophages.

Methods: Macrophages were obtained incubating human monocytes, isolated from buffy-coat, with GM-CSF for 5d. L-carnitine uptake was determined by incubation of the cells in EBSS containing L-[^{3}H]carnitine. The expression of the transporters was determined by RT-PCR and by Western blot analysis
Results: Although monocytes display a very modest uptake of L-carnitine, GM-CSF-induced differentiation massively increased the saturable Na^+-dependent uptake of L-carnitine. Kinetic and inhibition analyses demonstrate that in macrophages, L-carnitine transport is mediated by a high affinity component (K_m ~ 4 μM) identifiable with the operation of OCTN2 transporter and a low affinity component (K_m > 10mM) identifiable with system A for neutral amino acids. Consistently, both SLC22A5/OCTN2 and SLC38A2/SNAT2 are induced during the differentiation of monocytes to macrophages at gene and protein levels. The elucidation of GM-CSF signaling demonstrates that the cytokine causes the activation of mTOR kinase leading to the phosphorylation and activation of the transcription factor STAT3, which is, in turn, responsible for the OCTN2 induction.
Conclusions: Monocyte-to-macrophage differentiation associates with the upregulation of carnitine transport due to the mTOR-dependent activation of STAT3. SLC22A5/OCTN2 emerges therefore as a novel member of the set of genes markers of macrophage differentiation.

INIM16 Comparison between Anti-β2Glycoprotein I Antibody and Anti-Phosphatidylserine/Prothrombin Antibody Biological Effects on Peripheral Blood Monocytes

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Background: Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by vascular thrombosis and/or pregnancy morbidity and the persistent presence of antiphospholipid antibodies. The aim of this study was to compare the biological effects of anti-β2Glycoprotein I (aβ2GpI) and anti-phosphatidylserine/prothrombin (aPS/PT) antibodies on peripheral blood monocytes.
Methods: Peripheral blood monocytes isolated from five blood donors (BD) were incubated per four hours with lypopolysaccarides (LPS) alone or in combination with IgG fractions obtained from the serum of six APS patients who were positive either for aβ2GpI or aPS/PT antibodies. As controls, monocytes were incubated with LPS plus the IgG fractions isolated from the serum of five different BD. The mRNA expression of tissue factor (TF), interleukin 1β (IL1β), inflammasome 3 (NLRP3) and inflammasome 1 (NLRP1) were measured using real-time PCR.
Results: Compared to the non-treated cells, no differences were found between treatment with LPS alone (6.4±1 fold) or LPS plus the IgG fraction from BD (6.5±1.7 fold), although LPS plus aB2GpI or aPS/PT IgG fraction isolated from APS patients demonstrated an increased expression of TF mRNA, without significant difference (respectively 8.2±0.9 fold and 9.4±1.3 fold). LPS upregulates NLRP3 expression (but not NLRP1), but the addition of aB2GpI IgG fraction did not differ from LPS alone, while aPS/PT inhibits both LPS-induced NLRP3 and IL1β expression.
Conclusions: aβ2GpI and aPS/PT upregulate TF expression in monocytes pre-activated with LPS, in line with their pathogenic role in APS. However the different effect on IL1β and NLRP3 LPS-induced expression could indicate that aβ2GpI and aPS/PT activate different inflammatory pathways.

INIM17 Simultaneous Detection of Hepatitis B Surface Antigen (HBsAg) and Surface Antibody (Anti-HBs) in Acute Hepatitis B Infection

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Background: We report a case of transient coexistence of hepatitis B surface antigen (HBsAg) and surface antibody (anti-HBs) in a 50-year old patient with acute hepatitis B infection. Usually, anti-HBs is only detected in serum after HBsAg clearance, and a literature review did not provide clear evidence of transient coexistence.
Methods: Chemiluminescent immunoassay ADVIA Centaur XP (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) for qualitative determination of HBsAg and quantitative determination of anti-HBs in human serum and plasma. Chemiluminescent immunoassay Liaison XL MUREX (DiaSorin S.p.A., Saluggia, VC, Italy) for quantitative determination of HBsAg in human serum and plasma.
Results: On 13th May 2016, the patient tested positive for both HBsAg and anti-HBs. Qualitative determination of HBsAg had an index of 19.7. Quantitative determination of HBsAg confirmed reactivity with a reading of 0.3 IU/mL. Anti-HBs quantitative result was 81 IU/L. Other markers for hepatitis B infection, such as total core antibody (anti-HBc), IgM antibody to core antigen (IgM anti-HBc), envelope antigen (HBeAg) and envelope antibody (anti-HBe), were also positive. Quantitative HBV-DNA testing showed a reading of 80490 IU/mL. Four days later, HBsAg test resulted non-reactive with index dropping to 0.48, whereas anti-HBs remained reactive (53 IU/L). On 19th May, non-reactivity of HBsAg was confirmed with an index of 0.47, as well as anti-HBs positivity (45 IU/L).
Conclusions: In our patient, HBsAg and anti-HBs were simultaneously detected, and they coexisted in the serum for four days before HBsAg clearance.

INIM18 Correlation of Glycemic Control with Inflammatory and Endothelial Markers in Patients with Type 2 Diabetes

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Background: Type 2 diabetes mellitus (T2DM) is a chronic disorder characterized by insulin resistance and by an increased risk of cardiovascular morbidity and mortality. Several studies have shown the relationship between insulin resistance, endothelial dysfunction, and systemic low-grade inflammation in the development of chronic complications in T2DM. This study aims to evaluate the correlation of the levels of some circulating adhesion molecules and neutrophil-lymphocyte ratio (NLR), an independent predictor of cardiovascular events, with glycemic control in T2DM patients.
Methods: The study cohort consisted of 133 patients (71♀ and 62♂, 57-74 years), divided into two groups according to their HbA1c (<7% and ≥7%, n= 58 and n=75, respectively). HbA1c was measured by HA-8160 (Menarini); leukocyte count was performed using ADVIA 2120 (Siemens) and NLR was calculated as the ratio between the absolute values of neutrophils and lymphocytes. Serum levels of adhesion molecules (V- and I-CAMs, E-, P-, and L-selectins) were measured by a multiplex biochip array (Randox).
Results: Statistical analysis (Mann-Whitney test, SPSS 20.0) showed significantly higher levels of E- and P-selectins in patients with poor vs good glycemic control [16(IQR 11-22) ng/ml vs 11(IQR 4-18) ng/ml, p= 0,003 and 66(IQR 42-97) ng/ml vs 53(IQR 24-78) ng/ml, p=0,024, respectively]. In the HbA1c ≥7% group, we found increased NLR values vs the HbA1c <7% group [2,1(IQR 1,6-2,9) vs 1,8(IQR 1,3-2,2), p=0,013].
Conclusions: E-, P-selectins and NLR significantly correlate with glycemic control. Further studies are needed to assess these parameters as adjuvant prognostic markers for vascular complications in diabetic patients.

INIM19 Increasing Levels of Sputum Gamma-Glutamyltransferase May be a Contraindication for Glutathione Inhalation Therapies In Cystic Fibrosis

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Background: The antioxidant glutathione (GSH) is decreased in cystic fibrosis (CF) airways. GSH resupply by inhalation has become popular in CF patients, however conflicting results were reported to date.

Interestingly, CF airways present with increased levels of gamma-glutamyltransferase (GGT), the enzyme capable of degrading GSH.
Methods: We have determined GGT activity in sputum samples included in a previously published large study on GSH inhalation (*AJRCCM 2013, 188: 83*). The biochemical parameters measured in that study were re-evaluated for their potential correlations with sputum GGT levels.
Results: Highly varying levels of GGT were detectable in CF sputum and strong correlations between GGT and markers of inflammation (neutrophil count, neutrophilic elastase) were found. Variations in GGT activity correlated with variations in levels of cysteinyl-glycine (i.e., the specific GSH metabolite), thus confirming that inhaled GSH was in fact undergoing GGT-mediated degradation. Only in the presence of decreasing levels of GGT did GSH inhalation result in lower levels of the proinflammatory cytokines IL-8, TNF-α and IL-1β; on the other hand, in samples with increasing GGT, higher levels of carbonylated proteins (an oxidative stress index) were detected.
Conclusions: Data indicate that GGT activity – increasing in the airways of CF patients along with exacerbations of inflammation – can predict the expected outcome of GSH inhalation therapies. The latter appears capable of producing real – but at best, limited – benefits only in patients already presenting with a regression of lung inflammation.
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INIM20 Comparison of Two Methods of Urine Electrophoresis for BJ Detection: Preliminary Results

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Background: In plasma cell dyscrasia (PCD), mutations in immunoglobulin genes induce a super production of light chains (Bence-Jones proteins) that pass from serum to urine. Because electrophoresis is the best technique for identifying abnormal proteins in the urine, it is the first-choice screening test in patients with PCD diagnosis. Samples with abnormal electrophoretic patterns are further analyzed by immunofixation to assess their biochemical identity. This test combines electrophoresis of proteins with immunoprecipitation using specific antisera that present different binding specificity to protein domains of immunoglobulins. The aim of the study was to compare two different methods of urinary immunofixation.
Methods: Between January-February and April-May 2016, we analyzed 388 and 454 urine samples respectively, to compare Bence-Jones proteinuria and to perform urinary immunofixation. The former method (IFE 6, Interlab G26 Easy Fix) provided a urine electrophoretic step combined with immunofixation; the current method (IFE BJ 6, Interlab G26 Easy Fix) is characterized by an additional step of antisera deposition, as to improve the diagnostic performance.
Results: In the first group: 17.2% of the samples were positive for urinary free light chains, 11% showed weakly positivity and 71.8% were negative. Likewise, in the second group: 18.3% of the samples were positive, 10.8% were weakly positive and 70.9% resulted negative.
Conclusions: The preliminary results of our study confirm that there is no statistically significant difference between the two methods. To define the highest sensitivity of the innovative method a wider cohort of patients or a longer follow-up are needed.

INIM21 Low Level of Vitamin D is a Predictor of Cardiovascular Risk

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Background: Patients with Vitamin D deficiency show higher levels of interleukin-6 (IL-6), IL-10, and C-reactive protein (CRP). Our study aims to demonstrate that the majority of patients enrolled in cardiac rehabilitation, though relatively young and with no apparent metabolic problems, could show low levels of vitamin D, not always related to CRP.
Methods: The study enrolled 79 patients (59 M and 20 F) with previous SCA, aged 37 to 76 years (average 56.3). We excluded patients with a history of endocrine and metabolic bone disease . All patients had blood tests: Dosage vit D (DIASORIN) PCR (COBAS -Roche) .
Results: The dosage of vitamin D at entry showed a mean value of 21.2 ng / ml (vn> 25ng/ml), with a minimum value of 9.3 and a maximum of 38. In the face of low values of vitamin D, CRP averaged 3.2 (vn 0-5), not suggestive of inflammatory process in place.
Conclusions: A low value of vitamin D could be considered a marker of cardiovascular risk more sensitive than CRP but well correlated to the ratio ECM / BCM. Our data are too small to be significant. The Vitamin D and Omega-3 Trial (VITAL) should provide a clearer picture of the role of vitamin D and omega-3 fatty acids in the prevention of cardiovascular disease.

INIM22 Cholinergic System Dysfunction in Multiple Sclerosis Patients

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Background: Acetycholine (ACh) in the modulation of neuroinflammation has been described. Butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE) hydrolyze the neurotransmitter acetylcholine (ACh). Recently, it was hypothesized that BuChE and AChE genes could be considered as candidate genes for association studies in multiple sclerosis (MS). The aim of this study is to evaluate cholinergic system dysfunction in MS
Methods: In CSF of patients with MS and with other neurological diseases (OND), ACh was measured by fluorimetric commercial kit and AChE and BuChE by enzymatic test of Ellman. AChE and BuChE polymorphisms were analyzed by RFLP analysis.
Results: CSF levels of AChE were higher in relapsing-remitting (RR) MS than OND (22.4±9.5 vs 9.0±2.2; $p<0.05$), although the BuChE levels were not significantly different ($p=0.48$). ACh levels in CSF of RR-MS patients were 124.3±56.4 pmol ACh/mL compared to 393.6±142.5 pmol ACh/mL in OND ($p=0.03$). We found a negative correlation between levels of AChE activity and levels of ACh in CSF of RR-MS patients. AChE rs2571598 and BuChE rs1803274 polymorphisms found in RR-MS patients may explain the altered activity of these enzymes.
Conclusions: Our results suggest that decreased levels of ACh in MS may be related to increased activity of its hydrolytic enzymes. Our observations, together with the evidence that AChE inhibitors ameliorate the clinical symptoms in experimental autoimmune encephalomyelitis (EAE) models, suggest a new possible role for the cholinergic pathway in the demyelination and neuroinflammatory process characteristic of MS.

INIM23 Age-related Expression of Cholinergic Markers and Cytokines in the Olfactory Bulb of a Rat Model of Alzheimer's Disease

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Background: Alzheimer's disease (AD) is characterized by amyloid peptide formation and neurofibrillary tangle deposition, cholinergic dysfunction, neuroinflammation, and dementia; however, the interactions between these hallmarks remain poorly defined. In AD, impaired olfactory function appears earlier than memory loss, personality modification, and dementia. The aim of this study was to determine the age-related expression of nicotinic acetylcholine receptors, cholinergic enzyme, and

inflammatory cytokines and relationship with presence of Aβ in Olfactory Bulb (OB) of young and older transgenic mice.
Methods: Wild-type C57BL/6 and 8- and 24-month old APPswe/PSEN1dE9 mice were obtained from Cajal Institute Animal House Facility and genotiped by PCR. Behavioral tests were performed to test learning and memory abilities. Mice were sacrificed by cervical dislocation and the OB was dissected out. Tissue was immunostained to detect Aβ plaque burden, and fluorescent images were acquired using a confocal laser-scanning microscope. To determine a7nAChR, a4nAChR, b2nAChR, AChE, BuChE, MCP-1, IL-1β and TNFα, RT-qPCR experiments were performed.
Results: All cholinergic markers and cytokines analyzed were higher in transgenic mice than in wild-type. Aβ plaque load increased with age in OB of APPswe/PSEN1dE9 mice. The expression of nAChRs, AChE, BuChE, MCP-1 is higher in OB of 6-month old APPswe/PSEN1dE9 mice, although the expression of IL1β is lower than in 24-months old mice. In OB of 24-month old mice a significant correlation between AChE and TNFα was observed.
Conclusions: In APPswe/PSEN1dE9 mice, Aβ accumulation in OB is age-dependent and correlated to AChE and BuChE and cytokine expression, suggesting a possible causal relationship between these factors.

INIM24 Cytokine Levels and UVr Sensitivity in HaCaT Cells and Cetuximab Treated Patients.
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Background: Cetuximab, a chimeric monoclonal anti-EGFR, is used for the treatment of colorectal cancer. Cetuximab treatment increases the percentage of survival patients, but causes skin reaction of varying intensity. Ultraviolet radiation (UVr) is related to skin response as a result of anti-EGFR therapy, with conflicting results. The aims of this study are the evaluation of inflammation in cetuximab treated patients and the investigation of the UVr effect on cetuximab stimulated keratinocytes.
Methods: In immortalized human keratinocytes (HaCaT cell line) we analyzed the expression and release of cyto/chemokines by real-time PCR and ELISA assay after stimulation with serum of cetuximab treated patients and UV irradiation.
Results: Our results demonstrate that UVr causes significant activation of EGFR and an increased expression of proinflammatory interleukins, such as IL-1β, IL-6, IL-8, CCL-5 and CL-2 compared to non-exposed cells. IL-1β, IL-6 and IL-8 serum levels in cetuximab treated patients were higher than in healthy controls. Expression of IL-1β, IL-6, IL-8, CCL-5 and CCL-2 in HaCaT cells cultured with cetuximab treated patients' serum and exposed to UVr was reduced compared to non-exposed cells.
Conclusions: Cetuxima, promotes the increase of inflammatory cytokines and treatment with UV decreases systemic cytokine levels, playing a potential immunomodulatory role. Our data seem to rule out that adverse skin reactions due to the use of cetuxima, are increased by UV radiation. Therefore it is possible to use UV exposure to treat the adverse cutaneous reactions to avoid suspension of cetuximab therapy.

INIM25 Circulating Adipokine Levels in Mild Psoriatic Patients
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Background: Increased prevalence of metabolic syndrome in psoriasis patients has been reported, and adipokines, independently or in combination with obesity, may be involved in the pathogenesis of psoriasis and its comorbidities. Thus, the aim of this study is to compare the levels of adipokines in patients with psoriasis and in healthy volunteers in relation to their body mass index (BMI), insulin resistance and beta-cell function (HOMA).
Methods: Twenty-two consecutive patients with mild psoriasis assessed by PASI admitted to the dermatology outpatient clinic in the School of Medicine and 16 age-, sex-, and BMI-matched controls were included in the study. Venous blood samples were drawn from the subjects in the morning following a 12-h fasting period. Serum HMGB1, HMW-adiponectin, apelin, visfatin and RBP4 levels were determined by commercial ELISA kits, according to the manufacturer's instructions.
Results: The mean values of HMW-adiponectin, visfatin and RBP4 were higher in psoriasis patients, whereas the mean value of apelin was lower. We showed that serum HMGB1 levels were elevated in patients with psoriasis compared with those in control subjects. There was not a significant linear correlation between adipokines and HMGB1 levels with HOMA and BMI.
Conclusions: We suggest that in patients with mild psoriasis the imbalance of proinflammatory adipokines may contribuite to the pathogenesis of the disease and to the potential psoriatic march to psoriasis comorbidities. Moreover, the u-regulation of HMW-adiponectin might represent a protective mechanism to the inflammatory state present in psoriasis, in accord with the results found in other inflammatory diseases.

METABOLIC DISORDERS

MD1 Adipose Tissue Related Adipokines and Cytokines Regulate PCSK9 Expression in HepG2 Cells
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Background: Proprotein convertase subtilisin/kexin type 9 (PCSK9) represents a key-regulator pathway for hepatic LDL receptor degradation. Clinical and experimental evidence indicates that PCSK9 may be either a cause or an effect of metabolic syndrome. PCSK9 levels correlate with metabolic syndrome features, namely atherogenic dyslipidemia and insulin sensitivity indices. The aim was to study the possible molecular mechanisms linking the effects of cytokines (TNF-α and resistin) and adipokines (leptin) on PCSK9 expression and *de novo* lipogenesis.
Methods: Human hepatocellular liver carcinoma cell line (HepG2) and HepG2 overexpressing PCSK9 (HepG2PCSK9) were used as *in vitro* tools. qPCR, Western blot, ELISA and luciferase reporter assays, together with siRNA directed to STAT3 and SOCS3, were used.
Results: HepG2 expresses leptin (ObRl) and resistin (adenylyl cyclase-associated protein 1, CAP1) receptors. HepG2PCSK9 expresses higher levels of ObRl and CAP1. 24-hour treatment of HepG2 with TNF-α (10 ng/mL), and 48-h treatment with leptin (200 ng/mL) and resistin (50 ng/mL) induced the expression of both PCSK9 (2.3-, 2.0- and 3.5-fold, respectively) and SOCS3 (3-, 1.8- and 1.9-fold, respectively). TNF-α and leptin increased the secretion of PCSK9 (+15% and +20%, respectively) but only leptin stimulated PCSK9 promoter activity (+20%). TNF-α, leptin, and resistin induced the gene expression of sterol regulatory element-binding protein 1 (SREBP1), stearoyl-CoA desaturase-1 (SCD-1), fatty acid synthase (FAS) and microsomal triglyceride transfer protein (MTP). The TNF-α mediated effects were inhibited by transfection with siRNA anti-STAT3, suggesting the involvement of the JAK/STAT pathway.
Conclusions: Proinflammatory cytokines and adipokines upregulate PCSK9 expression and the key genes involved in *de novo* lipogenesis.

MD2 Hepatic Inflammation in Metabolic Syndrome
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Background: Hepatic steatosis, also known as nonalcoholic fatty liver disease (NAFLD), is a feature of metabolic syndrome. Because of the increased incidence of obesity and type II diabetes, hepatic steatsosis has become the most common chronic liver disease in Western countries, affecting up to 30% of the general population. About 20-30% of NAFLD patients develop steatohepatitis, or NASH, that can progress to liver fibrosis/cirrhosis.

Methods: Although NASH is a recognized leading cause of cirrhosis, the mechanisms involved in sustaining hepatic inflammation are still incompletely understood.
Results: Several studies have implicated oxidative stress, inflammasome activation, lipotoxicity, and changes in gut microbiota as possible triggers of steatohepatitis. However, these factors do not fully account for the large inter-individual variability in NAFLD/NASH progression. Growing evidence points to the possible role of adaptive immunity as an additional factor in promoting hepatic inflammation in NASH. In fact, patients with NASH, but not those with steatosis only, are characterized by the presence of circulating antibodies and lymphocyte-mediated responses triggered by antigens originating from oxidative stress. Similar immune responses are also detectable in experimental models of NASH and interference with oxidative stress or helper $CD4^+$ and effector $CD8^+$ T-lymphocytes ameliorates steatohepatitis. In these settings, T-lymphocytes contribute to sustain lobular inflammation and fibrosis by stimulating macrophage activation as well as by promoting natural killer T-cell recruitment.
Conclusions: Altogether, these results open the possibility of using immune-related markers to detect NAFLD patients at risk of progressing to fibrosis/cirrhosis.

MD3 Is it Possible to Change Lifestyle Habits, Prevent Disease, and Reduce Health Spending?
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Background: In 2016, the estimated Italian public health spending will reach 113.2 billion euro, a 1.9% increase compared to 2015. In 2012 it was 111 billion, equivalent to 7% of GDP (about 1,867 euro per year per inhabitant). Although health expenditure is increasing, the average life span is decreasing, even if only slightly.
Methods: Physical inactivity is a health risk, producing 2 million deaths annually worldwide. In particular, physical inactivity is believed to cause 10-16% of breast cancer, colon cancer, and diabetes cases and 22% of heart attacks. Regular physical activity is critical to prevention.
Results: The health benefits brought by changes in lifestyle habits are proven by a 25-year study in which it was shown that change in lifestyle has reduced deaths from cardiovascular disease (-68%), stroke (-73%), and cancer (-44%). A more active lifestyle would lead to the prevention of at least 2 million premature deaths and 20 million disability-adjusted life years (DALYs) in the world. Increasing the number of active people by only 1%would save 80 million euro per year in healthcare spending.
Conclusions: In short: increasing physical activity would improve the health of the country, would reduce health spending, would give new opportunities of employment, and may also be a small step forward in the field of security.

MD4 Serum ACTH in Children Affected with Type 1 Diabetes Mellitus: Preliminary Results
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Background: Type 1 Diabetes Mellitus (DM1) is one of the most common endocrinopathies in childhood and adolescence, with a higher risk of other organ-specific autoimmune disorders, including Addison's disease (AD). AD is a rare condition caused by immune-mediated destruction of the adrenal cortex, with a prevalence of about 1.2% in DM1 patients. AD is characterized also by increased serum levels of adrenocorticotropic hormone (ACTH). The addisonian crisis can be fatal if not precociously recognized and treated because there are no specific symptoms. AD is diagnosed by the presence of autoantibodies against 21-hydroxilase (21 OH-Ab); however, 21 OH-Ab can remain undetected for a few years after diagnosis. Actually, there is no international consensus on screening for AD in young patients with DM1. Despite this, some authors are in favour of screening. Adrenal function can be evaluated by annually measuring serum ACTH, independently of the presence of adrenal autoantibodies.
Methods: Considering that this topic is still debated, we evaluated serum ACTH in 7397 patients admitted to the Paediatric Diabetology Unit from 2010 to 2015 (mean age 14.08 yrs) affected with DM1 and evaluated 21 OH-Absin patients with high levels of ACTH.
Results: Out of 7397 patients affected with DM1, 142 (1.9%) had elevated ACTH serum levels, and 5/142 patients with high serum ACTH values were positive for 21 OH-Abs (3.5%).
Conclusions: Considering the severity of Addison's disease and the simple diagnostic assay, we suggest ACTH assessment in DM1 patients .

MD5 β-Glucans and Postprandial Satiety: The Role of Gastrointestinal Hormones in Healthy Volunteers
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Background: It is well known that Mediterranean diet can positively influence the health of each individual. In particular it is know that fibers have an important role. In our study, we considered fibers like β-glucans that have been added to pasta aiming to evaluate the effects of β-glucan intake on levels of gut hormones known to modulate glucose tolerance and food intake through a variety of mechanisms. The effect on gut microbiome, known to be involved the pathogenesis of numerous common metabolic disorders, was also evaluated.
Methods: This was a 30±3 days longitudinal, randomized, intervention study on the effect of consuming 80 g pasta supplemented with 6% of β-glucan intake 4 times a week. At the beginning and at the end of the study a standardized test meal was administered in order to measure blood concentrations of GLP-1, ghrelin, PYY, CCK, and GLP-2. Body weight, height and blood pressure of all participants were measured and blood samples for haemato-chemical analysis were collected at the beginning and at the end of the study. Stool specimens were studied for the presence of *Lactobacillus fermentum*, *L. acidophilus*, *L. salivarius*, *Bifidobacterium longum,* and *Enterococcus faecium* before and after 30 days of pasta intake.
Results: Pasta induced a significant increase of plasma GLP-1 ghrelin and PYY levels, whereas plasma levels of GLP-2 and CCK were not affected. In addition microbiome changes were observed.
Conclusions: Pasta prepared from barley flour enriched with β-glucan exhibited promising responses on gut hormone responses and on microbiome modification. Hence, β-glucan enriched pasta may influence metabolic diseases such as diabetes and obesity.

REGENERATIVE MEDICINE AND STEM CELLS

RMSC1 Transcriptomic Profile of PKH^{high} Multipotent Renal Cells for the Identification of a Renal Stem Cell Signature
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Background: In renal tissue the existence and the potential localization of an adult stem cell are still debated. We isolated a population of adult renal stem cells by sphere forming assay and self-renewal and differentiation ability evaluation. We showed that within our nephrospheres the cells with stem capacities are $PKH^{high}/CD133^+/CD24^-$. To better characterize the PKH^{high} status we aim to find the molecular signature of these cells.
Methods: Transcriptomic and bioinformatic analysis performed on stem, progenitor and differentiated cells. Immunofluorescence performed to validate markers at protein level.
Results: Differentially expressed genes (DEGs) between different cell types and exclusive genes for each cell type were evidenced. According

to gene set enrichment analysis, our stem cells positively correlated with $CD133^+/CD24^+$ renal cells and with mammary stem cells; they negatively correlated with embryonic stem cells. Therefore, our stem cells were similar to other adult stem cells but different from embryonic ones.To obtain stem cell signatures we crossed DEGs with stem cell exclusive genes and we selected 4 potential markers for validation based on fold change and rare expression in adult renal tissue.
Conclusions: The stem cell signature will allow localization and quantification of adult renal stem cells in normal and disease kidney and their direct isolation from tissue by FACS sorting.
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RMSC2 Bone Demineralization in Space: Don't Blame Mesenchymal Stem Cells
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Background: Bone demineralization is well documented in astronauts. It is a key medical concern and a limiting factor for space exploration. Several studies demonstrate that simulated or real microgravity alters the balance between osteoblast and osteoclast activity, but less is known about the behaviour of human bone mesenchymal stem cells (bMSC) in microgravity. bMSC are the precursors of osteoblasts and play an important role in the tissue homeostasis and repair after injury.
Methods: We used the random position machine (RPM) to simulate microgravity. We performed a stress protein array to compare cells in the RPM vs controls. Cell differentiation was studied by real-time PCR for Runt-related transcription factor 2 (*RUNX2*) and osterix (*OSX*). Alizarin red staining was used to detect calcium deposition.
Results: We observed the upregulation of the stress proteins HSP60 and HSP70, both involved in regulating bMSC performances. In particular, HSP70 induces the expression of *RUNX2* and *OSX*, crucial transcriptional factor genes for osteogenic differentiation; HSP60 maintains cell viability. Indeed, we found that culture in the RPM for 4 days upregulates *RUNX2* and *OSX*. However, only the addition of an osteogenic cocktail activates the full differentiation process both in simulated microgravity and in the corresponding control.
Conclusions: Simulated microgravity does not inhibit bMSC osteogenic differentiation in the presence of an osteogenic cocktail, thus suggesting that an impairment of the behavior of other bone cells is involved in space-associated osteopenia.

NON-CANCER TISSUE AND ORGAN PATHOPHYSIOLOGY

TOP1 Role of Proline-rich Tyrosine Kinase 2 (Pyk2) in the Metabolic Control of Prostate Cells Proliferation
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Background: Proline-rich tyrosine kinase 2 (Pyk2) is a calcium and ROS-sensitive kinase, involved in MAP kinase activation and in actin cytoskeleton organization, a critical process for cell migration and tumor metastasis. EPN-PKM3 cells, bearing a Pyk2 kinase-negative mutant, derived from non-transformed prostate cells (EPN), acquired increased cell motility and migration, both hallmarks of an aggressive phenotype. The aim of this study was to evaluate the role of Pyk2 in the modulation of metabolic machinery of prostate cells.
Methods: Cell cycle, proliferation, ROS production, autophagy of both cell lines were assessed by cytofluorimetric analysis and western blotting. Metabolic assays were performed by extracellular flux analyzer.
Results: Here we found that EPN-PKM3 cells are more glycolytic than EPN cells. This phenomenon leads to higher level of intracellular ROS, possibly responsible for the slow growth rate of EPN-PKM3 cells. Inhibition of glycolysis, by 2-deoxyglucose (2-DG), exerts dual and dose-dependent effect on EPN and EPN-PKM3 cells. At high concentration (20 mM), 2-DG-induced glycolysis inhibition leads to cell death in both cell lines, whereas low doses (1mM) of 2-DG, although reducing glycolysis and ROS production, significantly increases cell proliferation in EPN-PKM3, but not in EPN wild-type. These events associated with activation of the autophagic machinery. Inhibition of autophagy by NH_4Cl treatment or ULK1 silencing, reverted the effects of 1mM 2-DG, indicating that 2-DG increased proliferative rate of EPN-PKM3 cells is dependent on autophagy.
Conclusions: Taken together these data suggest that Pyk2 is involved in metabolic control of prostate cell proliferation through modulation of autophagic machinery and ROS release.

TOP2 Rare Hemoglobin Variants
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Background: The consolidation of laboratory activities optimizes resources and improves the expertise of the staff that encounter rare and exceptional cases. In the recent years, the Romagna Greater Area Laboratory, which provides diagnostic services to over a million inhabitants in North Italy, detected two hemoglobin (Hb) variants investigating an alarm of Sysmex Hematology Analyzers.
Methods: Cell blood count was carried out using XE-2100 and XS-1000 Sysmex Hematology Analyzers. Blood smear morphology was examined, after May-Grunwald Giemsa staining, with Olympus BX41 microscope. High performance liquid chromatography (HPLC) was performed by Tosoh-G8 system. Molecular analysis was performed by direct sequencing of the β-globin gene (*HBB*) on ABI PRISM 3130xl Sequencing Analyzer.
Results: From January 2013 to May 2016 14 patients were detected with a reduced fluorescence signal in the DIFF graph of XE-2100 and XS-1000. All patients presented with irregular contracted red cells and target cells in the blood smear. HPLC detected the presence of Hb variants in all these cases; molecular analysis was performed in 4 of them, revealing the presence of Hb Leiden and Hb G-Ferrara.
Conclusions: Our findings demonstrate that it is necessary to investigate samples with a persistent low fluorescence signal in DIFF graph when assayed using Sysmex analyzers. Heterozygotes for the Hb variants identified in this manner are asymptomatic but in specific clinical settings combined with other Hb variants could experience complications. The reported cases confirm the important role of tight integration between huge routine workload and specialized competence for the assessment of unusual patterns.

TOP3 Characterization of Parotid Gland Epithelial Cell Primary Cultures from Patients Affected by Sjögren's Syndrome
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Background: It is well known that it is very difficult to obtain a primary culture of parotid gland epithelial cells (PGEC) isolated from patients affected by Sjögren's Syndrome (SjS). Nonetheless. primary cultures of PGEC represent a unique opportunity to study the pathogenesis of this complex disease.
Methods: Parotid gland biopsies were obtained from four patients with SjS diagnosed at the Clinic of Rheumatology of Udine. Tissue fragments underwent microdissection and enzymatic digestion. Cells were then collected and seeded in 100-mm culture dishes. The culture medium consisted of F12 and M199 (1:1) supplemented with antibiotics, fetal bovine serum (FBS; 3 to 5%), freshly frozen bovine hypothalamus and pituitary extracts, epidermal growth factor, and a particular mixture of hormones designated 5H. Histologic characterization was performed by

immunofluorescence on cell monolayer. Cytokeratin 7 (CK7), vimentin, E-cadherin and smooth muscular actin (SMA) expression were investigated.
Results: In all four cases, a stable PGEC culture was obtained. Morphologically, cells appeared as a mixed epithelial- fibroblast population. From the second passage on, by adjusting FBS, bovine extracts and hormones concentrations, it was possible to obtain a population enriched in epithelial/myoepithelial cells, as demonstrated by the expression of CK7, E-cadherin and SMA.
Conclusions: Several fundamental factors are implicated in isolation and survival of PGEC, including tissue fragment selection and media composition. We were able to stabilize PGEC in culture for at least three passages, maintaining their phenotypic and morphologic features. Our approach may be very useful for future studies.

TOP4 Plasticity in Endothelial Cells and Endothelial Progenitor Cells: uPAR-mediated Amoeboid Angiogenesis

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Background: Mesenchymal migration requires the activity of extracellular matrix (ECM) degrading proteases and depends on Rac-driven actin cytoskeleton contractility, while amoeboid motility is a Rho-ROCK-dependent movement, which allows cells to glide through, rather than degrade, ECM barriers. Here we show that mature endothelial cells (ECs) and endothelial progenitor cells (EPCs) are able to migrate by mesenchymal and amoeboid style. We used a cocktail of physiologic inhibitors (Ph-C) of serine-proteases, metallo-proteases and cysteine-proteases, thus mimicking the physiological environment that ECs and EPCs encounter during their migration within the angiogenesis sites.
Methods: To evaluate the mesenchymal-ameboid transition we performed RhoA and Rac1 activation assay, immunofluorescence analysis of proteins involved in cytoskeleton organization. Cell invasion was studied in Boyden chambers.
Results: RhoA and Rac1 activation assay showed, in both EPCs and HMVECs, a decrease of activated Rac1 and an increase of activated RhoA upon shifting of cells to the amoeboid conditions. Then we showed that under Ph-C inhibition both cell lines acquired a round morphology and showed a Matrigel invasion that was greatly enhanced with respect to that observed in the absence of protease inhibition. We didn't see differences in tubular-like structures formation under mesenchymal or amoeboid conditions. uPAR silencing and uPAR-integrin uncoupling with the M25 peptide abolished both mesenchymal and amoeboid angiogenesis of ECs and EPCs, indicating a role of the uPAR-integrin-actin axis in the regulation of amoeboid angiogenesis.
Conclusions: The receptor of the urokinase plasminogen activator receptor (uPAR) is indispensable for ECs and EPCs to perform an efficient amoeboid angiogenesis, in terms of cell migration and capillary morphogenesis.

TOP5 Hsp90, Thioredoxin, and Thioredoxin Reductase Form a Chaperone-Redox Machinery Enabling the Catalytic Activity of Clostridial Neurotoxins inside Nerve Terminals

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Background: Clostridial neurotoxins (CNTs) are the etiologic agents of botulism and tetanus. They are composed of a metalloprotease light chain (L) linked via a disulfide bond to a heavy chain (H). H mediates the binding to nerve terminals and the membrane translocation of L into the cytosol, where its substrates, the three SNARE proteins, are localized. Translocation is accompanied by unfolding of L that, once delivered on the cytosolic side of the endosome membrane, has to be reduced and refolded into the native structure for protease activity.
Methods: Use of inhibitors of the thioredoxin-thioredoxin reductase system (TrxR-Trx) and of Hsp90 *in vitro* and *in vivo*. Brain subfractionation. Synaptic vesicle immunoisolation. Immunoblotting. Immunohistochemistry. Immunoprecipitation.
Results: TrxR-Trx is responsible for the reduction of the interchain disulphide bond. Hsp90 aids L in reacquiring a cleavage-competent folding. CNTs toxicity is potently hampered by inhibitors of TrxR-Trx or of Hsp90 *in vitro* and *in vivo*, which strongly synergise, suggesting that the processes of L chain refolding and interchain disulphide reduction are strictly coupled. TrxR-Trx and Hsp90 physically interact on synaptic vesicles, the organelles from which L translocates in the cytosol
Conclusions: TrxR-Trx and Hsp90 orchestrate a chaperone-redox machinery which is exploited by CNTs to deliver and activate their catalytic part in the cytosol. This clue offers a rational target for the development of new antitoxins.

TOP6 EGFR and BER Pathways in Human Thyroid Goiter: Role of Oxidative Stress

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Background: Thyroid nodules occur in over 50% of the Italian population. In thyroid, H_2O_2 is largely produced and DNA lesions, generated by ROS excess, are physiologically removed by the BER system. Since EGF is a physiological regulator of thyroid functions, this study investigated the effect of oxidative stress on EGFR and BER signaling in goiter.
Methods: Normal human thyroid cells, Nthy-ori3-1, were treated with H_2O_2, EGF, LY294002, and PD98059 alone and in combination. Goiter tissues were obtained from patients undergoing thyroidectomy. EGFR and BER pathways were analyzed by Western blot and RT-qPCR.
Results: A time-dependent increase of *OGG1* and *MUTYH* gene expression was observed after H_2O_2 treatment and was reversed by EGF and LY294002 alone or combined with H_2O_2. EGF and LY294002 induced EGFR overexpression, alone and combined with H_2O_2. H_2O_2 treatment, alone or combined with EGF, LY294002, and PD98059 resulted in increased EGFR and MAPK activation. In addition, H_2O_2, alone and combined with EGF, induced a rapid increase of *ErbB2* gene expression, while LY294002, alone and combined with H_2O_2, reduced its expression. Surprisingly, PD98059 enhanced ErbB2 expression compared to H_2O_2-treated and control cells. Tissue analysis showed a strong individual variability among patients. Preliminary data displayed a downregulation of *OGG1* in pathologic tissue compared to control. The opposite trend was observed with the *Nrf2* gene.
Conclusions: These results indicate that EGFR and ErbB2 are upregulated in response to oxidative stress, suggesting an Akt-dependent mechanism. Our observations present the first evidence that pronounced H_2O_2 tress may directly interact with ErbB2 and BER downstream signaling.