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ADVANCES IN MOLECULAR THERAPIES

AGE1. The Role of Dermal Fibroblasts in the Development of Ectopic Calcifications

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Background: Ectopic calcifications (EC) are the most used molecular research of strabismus and eye-movement disorders, as alternative to traditional surgery. However, the temporary effect of BTX treatment requires the research of alternative therapies. To this purpose the in vitro effects of different types of RIFs from plants (lanceolin, stenodactylin and ricin) and of the skeletal muscle-specific immunotoxin saporin-mAb73 were evaluated on muscle cells. Methods: The RIFs and the immunotoxin were tested for their cytotoxicity in three cell lines: L6E9 (myoblasts), TE671 and RD/18 (rhabdomyosarcoma), both undifferentiated and differentiated. The specific toxicity was assessed on conjunctival (IOBA-NHC) cell line. Protein synthesis inhibition, viability and apoptotic changes were assayed. Results: All substances showed a strong cytotoxic effect on protein synthesis and viability, with IC50 and LC50 ranging from 0.1 nM to 0.01 pM. Lanceolin and stenodactylin were 1-2 logs more toxic than ricin and 2-3 logs more toxic than the immunotoxin. Myoblasts were particularly susceptible to stenodactylin (IC50<0.01pM). All RIP-treated cells showed typical morphological apoptotic changes and no signs of necrosis. In further experiments miming in vivo treatment, no toxic effects were reported on conjunctival IOBA-NHC cell line. Conclusions: The strong cytotoxicity observed for stenodactylin at very low dose could be compatible with loco-regional treatments in strabismus and eye-movement disorders. It could be possible to modulate the effect on muscle fibers and to obtain a complete ablation of myoblasts, gaining more durable effects as compared to BTX treatment. Moreover, the absence of necrosis should avoid flogistic side effects.

AGE2. Vascular Aging Effect on Medial Aorta Degeneration: Focus on Blood Leukocyte Telomere Length in Hypertensive and Old Patients with Sporadic Thoracic Aortic Aneurysm

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Background: Aging is a well recognized factor in the development of cardiovascular diseases (i.e. sporadic thoracic aortic aneurysm). The physiological aging process determines various changes and a progressive deterioration in structure and function of heart and vascular system, i.e. thoracic aorta. As consequence of these age-related modifications having catalyst and accelerator effect for sporadic thoracic aortic aneurysm (S-TAA), medial degeneration occurs. This pathological entity leads to weakening of aorta wall, which in turn results in aortic dilatation, aneurysm, increased risk for aortic dissections and ruptures. Thus, S-TAA risk increases with chronological as well as biological aging. One optimal marker of this might be peripheral blood leukocyte telomere content. It accurately reflects that of vascular wall and its decrease is associated with premature vascular disease. Thus, the aim of this study was the evaluation of mean blood leukocyte telomere length as predictor for S-TAA. Methods: Peripheral blood samples were collected from TAA patients and age- and gender matched controls. Genomic DNA was extracted from leukocytes and telomere length was determined using a chemiluminescence technique. We examined patients and controls selected randomly, but considering the same age and gender. Results: A significant lower mean telomere length was detected in TAA group, significantly correlated with age, smoking, hypertension, inflammatory cellular infiltrate and genetic inflammatory variants. Conclusions: Thus, telomere assay could contribute to identify individuals at risk for S-TAA. Accordingly, our results should seem to suggest that vascular biological aging might have a strong role in the S-TAA pathogenesis.
express only CICR3, that leads them to site of inflammation. In the elderly donors this receptor is higher expressed than in young people. Moreover memory switched and DN B cells also express CCR6, which is also involved in the recruitment of cells in the site of inflammation. Conclusions: Our data demonstrate that in the elderly naive/memory B cell populations express differently the studied receptors from those observed in young people. This could be discussed in terms of "inflamm-aging." Our hypothesis is that the inflammatory environment, typical of aging, in some way changes the trafficking ability of B cells rendering them more sensitive to the cytokines and chemokines that are over-produced in the elderly.


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Background: Several studies have examined changes in immune functions with advancing age; in particular, the increase in auto-antibodies production might be a marker of the aging associated with a deregulation of immune system. On the other hand, pro- or anti-inflammatory genotypes (particularly cytokine polymorphisms) might impinge upon successful or unsuccessful aging. Here are reported data on the analysis of the effects of cytokine gene polymorphisms on auto-antibody production in aging. Methods: We evaluated non-organ-specific autoantibodies by an indirect fluorescent antibody test system in a group of ultra-nonagenarians typed for functionally relevant single gene polymorphisms (SNP) of pro- or anti-inflammatory cytokines according to our laboratory procedures. Results: Our results demonstrate a significantly increased frequency of anti-nuclear antibody positivity among ultra-nonagenarians bearing the pro-inflammatory 308A TNF allele. Conversely, the percentage of anti-nuclear antibody positivity was significantly reduced among subjects bearing the anti-inflammatory 1082G IL-10 SNP. Conclusions: Several studies have largely demonstrated the role of an anti-inflammatory genetic background in the achievement of successful aging. Present results indicate that non-organ-specific auto-antibodies production in very old subjects might be an useful marker for the evaluation of the effect of aging associated with restrooping of immune response in subjects bearing a genetically determined pro- or anti-inflammatory profile.

AGE5. Age-related Diseases: Key Role of Insulin Resistance for the Association Between Type II Diabetes and Alzheimer’s Disease

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Background: Alzheimer’s disease (AD) and Type 2 diabetes mellitus (T2DM) present many relationships. Insulin resistance (IR) plays a key role in neuronal degeneration and death. Reduced energy makes neurons more sensible to oxidation causing mitochondrial damages. Moreover AD brain has lower insulin utilization, reduced expression of its receptors and of IGF 1 and 2, all necessary for neuronal survival and learning and memory processes. Hyperinsulinemia is correlated with increase of hyperphosphorylated tau-protein. SHIP2, a phosphatase, is an antagonist of PI3K. Since the PI3K plays a key role in the biological effects of insulin, its attenuation could be associated with IR in T2DM. Methods: We have conducted a case-control study evaluating the association of three SNPs of SHIP2 in T2DM and AD patients and old and young subjects. SNPs study has been developed by ARMS PCR that make it possible to detect a single SNP thanks to the terminal 3-nucleotide of one of the primers that anneal with target mutation. Results: Significant differences were observed for one functional SNP between AD patients and young subjects, old and young subjects but not AD patients and old subjects. Conclusions: Our preliminary results seem to suggest a putative correlation between this SNP and aging thus strengthening the hypothesis of a close relationship among AD and diabetes. In fact, to verify this relationship we are collecting blood from T2DM patients. Moreover we will collect AD samples because to confirm these results a bigger cohort needs.

AGE6. Combination Therapy in Neovascular Age-Related Macular Degeneration

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Background: Pathological choroidal neovascularization (CNV) due to age-related macular degeneration (AMD) is a leading cause of legal blindness in people older than 50 years in the Western world. CNV is a multifactorial condition whose pathogenesis involves angiogenesis, inflammation, and fibrosis. All available monotherapies (anti-VEGF agents, steroids, photodynamic therapy with verteporfin (PDT-V)) are directed specifically to only one part of the CNV process. The purpose of this review is to discuss the current role of combination therapy for the treatment of CNV due to AMD. Methods: A MedLine review via PubMed was performed. Evidence available from clinical studies evaluating the use of the combination of anti-VEGFs, steroids and/or PDT-V and from a selective literature search has been considered for this review. Results: The results of trials focused on the actual options in the management of neovascular AMD are discussed. Anti-VEGF monotherapy results in a significant increase in visual acuity in patients with wet AMD. The combination of anti-VEGFs with occlusive therapies (PDT-V) potentially offers a reduction of re-treatment rate while maintaining long-term visual benefit. Steroids demonstrated an antiangiogenic effect, targeted the extravascular components of CNV such as inflammatory cells and fibrocytes and seems to be efficacious in patients non-responder to anti-VEGF monotherapy. Conclusions: Combination therapy has been proposed to interfere with the multiple stimuli to pathologic vascular proliferation. Many experiences have been conducted and showed encouraging results. Although there is a strong rationale for applying multiple combined therapy in the treatment of CNV, further study is required to determine correct combinations and dosage.

AGE7. Mediterranean Diet and Longevity

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Background: The effect of calorie restriction on human health has been debated due to the lack of information and appropriate study. Furthermore, in many population-based studies and randomized trials there are evidences that a dietary pattern rich in some nutritional food groups such as fruits and vegetables plays a role in delaying age-related diseases. In the inner part of Western Sicily we have some "blue zones" where the ratio of centenaries vs. total population is higher (4.32) than in the Italian population (2.4). Those “blue zones” are located far from the sea in the area of Sicani Mountains. Methods: The people that we interviewed are female and male centenaries belonging to several villages that underwent many analyses: hematological, chemical analysis, complete anamnesis, ADL, MMSE and MNA nutritional assessment tests. Furthermore oxidative stress assessment, such as ROS and NOS, were performed. Also dietary intake, through 24 hours recall has been recorded and different levels of adherence to the Mediterranean diet observed. Results: The results taken together showed a good control of hematological and chemical parameters of healthy status and good adherence to Mediterranean diet, which seems to play a key role in diseases prevention. Conclusions: Mediterranean diet might play a key role in disease prevention and for management of age-related diseases. To reach successful aging it is advisable to follow a diet with low quantity of saturated fat and high amount of fruits and vegetables rich in phytochemicals.

AGE8. Impact of Smoking, Alcohol Consumption and Aging on Antioxidant/Pro-Oxidant Balance in Age-Related Macular Degeneration

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Background: Oxidative stress, inflammation, and genetics are thought to contribute to the development of age-related macular degeneration (ARM), the most common cause of blindness in the elderly. The aim of this study was to determine whether smoking, alcohol consumption and aging, which constitute the main exogenous sources of reactive oxygen species (ROS), affect the balance between antioxidant production and antioxidant levels in ARM. Methods: Superoxide dismutase (SOD), glutathione peroxidase (GSHpX), and catalase (CAT) activities as well as malondialdehyde (MDA), protein carbonyl (PC), 8-hydroxy-29-deoxyguanosine (8-OhdG) and total oxidation status (TOS) levels, were measured in patients with early ARM (n=211) and late ARM (n=205), and control persons (n=262). Results: When compared with healthy controls, early- and late-ARM patients showed significant decreases in the activities of SOD and GSHpX, but not CAT, along with marked enhancements of MDA, PC, TOS and 8-OhdG (P < 0.01). No notable differences were observed in the early- versus the late-ARM group for each of the above-mentioned dependent variables. Multiple regression analysis revealed that in healthy subjects chronic smoking and aging had the strongest impact on oxidative stress parameters, whereas in ARM patients, the combination of smoking, drinking, and aging was the greatest predictor of oxidative DNA, protein and lipid damage. Conclusions: Cigarette smoking, alcohol consumption and aging could be aggravating factors contributing to serious redox imbalance and oxidative damage in ARM. Identification of factors exacerbating ARM-associated oxidative stress can facilitate development and adoption of effective preventative measures for this disease.
have focused on the epigenetic imprinting that could affect the differentiation of mechanisms have been poorly investigated for such tissues, GpC rich motifs regions on the methylation status of promoters driving genes encoding elastic fiber – Conclusions: further studies are needed to understand why these mutations are protective for human health.

Elastic Tissues during Aging

Disease

Background: Centenarians, despite being exposed to the same environmental conditions as members of the average population, manage to live much longer. A recent Genome-Wide Association Study (GWAS) for exceptional longevity in Southern Italian Centenarians (SiCs) identified four SNPs that were either non-

non synonymous or non synonymous taggers with a P < 1x10^-4. Methods: A two stage genetic association study on long living individuals and controls, followed by in vitro and ex vivo kinase activity detection of mutated and wild type BPIFB4. Results: we identified rs2070325 (BPIFB4-Ile268Val) consistently associated with human longevity under recessive model in Italian, German and US cohorts. Rs2070325 is in strong LD (r2=0.93/D=0.98) with rs2889732 (BPIFB4-Asn320Thr) and in vitro studies show that BPIFB4 is detected in cytoplasmic vesicles and secreted together with 14-3-3. HEK293T transfections with Ile268Val/Asn320Thr BPIFB4 activate PKC alpha, AMPK, p65/RELA and improves BPIFB4/14-3-3 cellular secretion, counteracting PKC alpha, AMPK and p65/RELA inhibition observed after wild type BPIFB4 transfection. Conclusions: Further studies are needed to understand why these mutations are protective for human health.

In Situ Determination of Epigenetic Mechanisms at Work in Soft Elastic Tissues during Aging

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Background: Epigenetic modifications, such as DNA methylation and histone modification, are largely responsible for the variable activation and repression of specific genes at specific time points during cell lifespan. Thus, epigenetic mechanisms have been described as crucial processes for embryonic development and deregulation leading to malignant transformation or senescence and aging. More recently, epigenetic imprinting has been associated to stem cell reprogramming and therefore to tissue growth and regeneration. Methods: The methylation of mammalian genomic DNA mainly occurs in GC rich regions termed CpG islands. Methylation of cytosine residues is catalyzed by DNA methyltransferases (DNMTs), which encompass three active isofoms: DNMT1, DNMT3a and DNMT3b. DNMT1 is mainly associated to the maintenance of the methylation pattern on the daughter stand during DNA replication, whereas DNMT3a and DNMT3b are powerful de novo methyltransferases. Results: In our studies, we have focused on the epigenetic imprinting that could affect the differentiation of elastic tissues, as a hallmark of well formed and healthy tissues, a status that must be reached to heal, to reconstruct and to regenerate soft tissues. Though epigenetic mechanisms have been poorly investigated for such tissues, GpC rich motifs allowing Sp1/Sp3-Sp3 binding on extra cellular matrix genes have been documented. Conclusions: Our recent experiments confirmed the importance of such GC rich regions on the methylation status of promoters driving genes encoding elastic fiber related elements. Several technical approaches have been proposed to be as close as possible of the native tissue, including the determination of CpG methylation pattern from embedded human tissues.

Involvement of Oxyesters in the Pathogenesis of Alzheimer’s Disease

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Background: Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by the extracellular accumulation of amyloid-beta (Aβ) in neuritic plaques. Altered cholesterol metabolism in the brain has been suggested to be implicated in pathogenesis of AD. Methods: We have investigated the potential interaction between the oxyesters 24-hydroxysterol (24-OH), 27-

hydroxysterol (27-OH) and 7β-hydroxysterol (7β-OH), present in the brain, and Aβ in human differentiated neuronal cell lines, SK-N-Be and NT-2. Expression and synthesis of CD36 and β1-integrin receptors were analyzed by real time RT-PCR and Western blotting. The necrotic, apoptotic and redox parameters were measured by microscopic and biochemical analysis. Results: Our studies show that all three oxysterols enhance the binding of Aβ to neuronal cells by up-regulating expression and synthesis of CD36 and β1-integrin receptors, which both form, with CD47, the multi receptor complex that mediates the Aβ binding to the plasma membrane of neurons. However, only 24-OH markedly potentiates the proapoptotic and pronecrogenic effects of Aβ on cells, likely through a strong enhancement of reactive oxygen species’ (ROS) generation and impairment of cell redox equilibrium. Cell incubation with antioxidants quercetin or genistein prevents 24-OH’s pro-oxidant effect and potentiation of Aβ induced necrosis and apoptosis. Thus, the presence of 24-OH in the close vicinity of amyloid plaques appears to enhance the adhesion of large amounts of Aβ to the plasma membrane of neurons, then to amplify the neurotoxic action of Aβ by locally increasing ROS steady-state levels. Conclusions: These results support a primary involvement of altered brain cholesterol metabolism in the complex pathogenesis of AD.

Interaction Between 4-Hydroxynonenal and Amyloid-β in Amplifying Neuronal Damage

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Background: Alzheimer’s disease (AD) is characterized by intracellular neurofibrillary tangles made of hyperphosphorylated tau and senile plaques made of extracellular deposits of amyloid β (Aβ). Lipid peroxidation is considered as primarily involved in the pathogenesis of AD. One of its more reactive end-products, 4-

hydroxynonenal (HNE), has been proposed as a candidate biomarker of AD because it was found in the brain of AD patients, in both neurofibrillary tangles and in senile plaques, as well as in the cerebrospinal fluid. HNE production in the brain is stimulated by Aβ and, conversely, Aβ formation is up-regulated by HNE. Methods: We applied a precursor-cell-based approach, comprising primary cultures of human dental-pulp progenitor cells, which spontaneously differentiate to neuron-like cells in vitro. Membrane receptor gene expression was quantified by real time RT-PCR. Amyloid β internalization was observed by Congo red staining. Necrosis was evaluated by measuring the extracellular percentage of lactate dehydrogenase. Results: Our findings point to the ability of HNE and Aβ to interact, with consequent potentiation of Aβ’s cytotoxicity. HNE was found to cause overexpression not only of CD36 but also of another component of the multireceptor complex that binds the Aβ peptide, namely β 1-integrin. The up-regulation of these components allowed the neurotoxic peptide to accumulate in greater amounts within the cells, and to induce much more extensive cell death than occurred in cells challenged with Aβ alone. Cell death was completely prevented by the specific receptor blockade. Conclusions: These results support the involvement of HNE in the pathogenesis of AD.

BM1.

HepG2 Spheroids as in Vitro Model to Study the Release of Gamma-glutamyltransferase Fractions

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Background: Gamma-glutamyltransferase (GGT) have been described in plasma. Clinical study on healthy subjects and patients affected by non-alcoholic or alcoholic liver disease or chronic viral hepatitis showed that s-GGT fraction is a marker of hepato cellular damage, whereas b-GGT is an index of liver metabolic dysfunction. Methods: A aim of this study is to deepen the mechanism of fractional GGT release by hepatocytes. For this purpose we used HepG2 cells cultured as spheroids. Morphology, biochemical parameters (GGT activity, protein content), cellular GGT distribution (immunohistochemistry) were evaluated over a period of 15 days. Secreted GGT fractions were quantified by gel filtration chromatography associated with a GGT-specific post-column reaction. Results: HepG2 spheroid formation can be divided in two stages, immature (1-6 days) and mature (>6 days). In the media of immature spheroids only b-GGT [mean (SD), 1.31 (0.01) U/L] and f-GGT [0.45 (0.01) U/L] were present. Between day 6 and 9, f-GGT [3.49 (0.36) U/L, P < 0.01 vs. day 6] and s-GGT [0.25 (0.02) U/L, P < 0.001 vs. day 6] increased significantly, whereas b-GGT activity was unchanged [1.04 (0.08) U/L]. Morphological and immunohistochemical analysis showed the presence, in mature spheroids, of structure compatible with bile-canaliculi surrounded by GGT protein. s-GGT fraction was non-detectable in media obtained from HepG2 monolayer culture. Conclusions: In conclusion, mature HepG2 spheroid culture is a good model for the in vitro study of fractional GGT release. Obtained results showed that the 3D structure is necessary for the secretion of s-GGT, which could result from an extracellular modification inside bile-canaliculi.

Biomarkers for (Non-Cancer) Disease Detection

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endothelial dysfunction (as shown by increased plasma ADMA levels and reduced L-arginine/ADMA ratio) is present in PsA patients.

Affected by ADHD/ASD Overlapping

Many investigators focused their attention on polymorphisms affecting gene regions coding for dopamine receptors. In this study we evaluate the association of three single-nucleotide polymorphisms (SNPs) with clinically significant level of autistic symptoms in unaffected people and children with ADHD/ASD.

Methods: We enrolled 150 children who were divided into four groups: children with ADHD, children with ASD, children with co-occurrence of ADHD/ASD, and control subjects. We investigated rs265975 C/T (174862195C>T) for dopamine receptor D2 gene, rs1076560 C/A (113283688C>A) and rs1079597 G/A (113296286C>T) for dopamine receptor D2 gene utilizing previous DNA extraction and amplification, restriction enzymes that recognized one of two allelic variants. Results: Our data demonstrated that homozgyosis T/T for rs265975 had a lower frequency in ADHD patients compared to other groups, whereas small differences were seen in heterozygosis C/T. Both heterozygosis C/A for rs1076560 and heterozygosis G/A for rs1079597 showed higher frequency in ASD group with respect to control children and ADHD patients, whereas in ADHD/ASD group a ratio 3:1 vs unaffected people was seen. The same trend, but with slight differences, was observed in homozgyosis A/A for rs1076560 and rs1079597. Conclusions: These preliminary data pointing to differences between ADHD/ASD and other groups must be confirmed and encourage us to enlarge our study populations.

BM5. Performance of CD64 Index and Soluble TREM-1 as Biomarkers in Late Onset Sepsis of Preterm Neonates

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Background: In Neonatal Intensive Care Units, infections remain important cause of neonatal morbidity and mortality. Early and accurate diagnosis improves the outcome. The aim of this study was to explore the performance of CD64 index and soluble TREM-1 as biomarkers of sepsis in a group of preterm neonates.

Methods: We enrolled 54 neonates with gestational age <32 weeks and weight <1500 gr. Sixteen newborns (septic group) developed late onset sepsis (culture positive) during the 16th to 25th days of life (T1); they were also tested between the 5th and 15th days of life (T0, free from infection). Thirty-eight preterm, without clinical and laboratory signs of sepsis, were tested between day 5 to 20 of life as laboratory normal range. Fifty-microliter blood samples were processed using the Leuko64 Assay kit and analyzed with an Abbott Celi-Dyn Sapphire hematolgy analyzer. Soluble TREM-1 was quantified using sandwich immunoassay kit. Statistical analysis was performed with Medcalc. Results: Clinical and laboratory data, whole blood leukocytes, neutrophils, monocytes and platelet counts were comparable in all the groups/conditions, besides a significant increase (P = 0.049) of neutrophil in septic group (T0 vs T1). CD64 index increased significantly in septic group T1 vs T0 (P = 0.0002) and T1 vs control group (P = 0.0001) and ROC curve indicated cut-off 2.86, sensitivity 87.52%, specificity 97.1%, AUC 0.925. Soluble TREM-1 didn’t show any differences in septic group (T1 vs T0 P = 0.8) and T1 vs control group (P = 0.8).

Conclusions: In preterm neonates CD64 index can be useful as biomarker of late-onset sepsis whereas solubleTREM-1 showed less efficient diagnostic role.

BM6. Senescence Markers

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Background: Evolutionary theory and empirical evidence suggest that aging is a process of gradual accumulation of damage in cells and tissues of the body. The progressive loss of ability to interact effectively with environmental stimuli is accompanied by progressive modification and adaptations that are influenced by lifestyle and genetic background of the individual. These affect the ability to age successfully, defined both as longevity and/or escaping the major age-related diseases. Some of the most important characteristics of adaptive immunity in aging are compatible with this assumption. Actually, the antigenic load results in the progressive generation of a chronic low grade inflammatory response involved in body and brain aging. Methods: Data on genetic background and immune system have been obtained in the last 10 years studying Sicilian centenarians and subjects affected by aging related diseases. Results: Studies have been focused on the genetic background predisposing to aging related diseases. Data gathered on gene variants in cytokine, pathogen-related pattern receptors or acute phase response genes allow the research group to define a complex trait in which the antagonistic pleiotropy of regulating immune-inflammatory mechanisms might play a central role in predisposing to a large array of age-related diseases and in determining lifespan expectancy. Conclusions: These findings suggest that different alleles at different immune-related genes coding for pro- or anti-inflammatory molecules may affect individual life-span expectancy and might be useful markers for the evaluation of aging-associated disease risk.

BM7. Training Effects on Laboratory Parameters Are Independent of Genetic Polymorphisms of IL-10 and TNF-alpha (TNF-α) Expression

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Background: It is well known that exercise has beneficial effects on health. Although intense exercise is experienced by the body as a condition of stress, a well designed training has long term beneficial effects on the organism of an athlete. Less is known about the effects that the genetic background might have on training
adaptation and on the consequent modification of laboratory parameters. Methods: In our study we evaluated the blood chemistry parameters of a group of 41 athletes compared with a group of 45 amateur athletes, to assess whether the training has effects on their variation. In addition we typed our subjects for polymorphisms 308 A/G of the tumor necrosis factor-α (TNF-α) and 1082 A/G of Interleukin-10 (IL10). Results: After statistical analysis, performed with Mann-Whitney Test, we observed a statistically significant (p value 0.05) increase of basophilcs, eosinophils, monocytes, and total bilirubin and decreased levels of neutrophils, glucose, electrolytes and AST in professionals compared to amateurs. These parameters were not modified by the genetic background. Actually the training modification observed were independent of the presence of pro-inflammatory (carrier allele A of 1082 A/G of IL10) or anti-inflammatory alleles (subjects A negative for 308 A/G of TNFα).

Conclusions: The genetic polymorphisms analyzed do not influence changes in laboratory parameters values induced by professional training.

BM8. TGF-β Pathway Polymorphisms as Markers for Gender Differential Susceptibility to Sporadic Thoracic Aortic Aneurysm
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Background: It has become increasingly evident that the immune system plays a pivotal role in the development of thoracic aortic aneurysm (TAA). The Transforming Growth Factor (TGF)-β isoforms might be involved in TAA pathogenesis inducing disruption of extracellular matrix, apoptosis of smooth cells in tunica media, metalloprotease production and remodelling tissues after inflammation. Methods: 133 subjects affected by TAA (from Cardiac surgery Unit of Palermo University Hospital), 107 unrelated patients of the same unit without TAA and a group of 91 healthy controls matched for age and gender, all living in western Sicily, were typed for TGF-β1, TGF-β2 and their receptors polymorphisms by a KASPar assay (the KBiosciences Competitive Allele-Specific PCR SNP genotyping system). Genotype and allele frequencies were compared by statistical analysis. Results: No differences in distribution between cases and controls were observed except for TGF-β2 rs900 TT genotype, whose frequency was increased in patients affected by aortic aneurysm in comparison to the controls (P = 0.037). In particular this genotype was significantly increased in women affected by TAA in comparison both to women of control patient group (P value = 0.040) and of health control group (P value = 0.010). Conclusions: TAA is a complex pathology with a greater prevalence among women. Our results suggest that rs900 TGF-β SNP might be a genetic factor involved in women’s susceptibility to TAA.

BM9. Evaluation of Genome-Wide Expression Profiles of Blood Neutrophils in Cystic Fibrosis Patients Before and After Antibiotic Therapy
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Background: Cystic fibrosis (CF) lung disease is characterized by massive extravasation of neutrophils into the airways that undergo apoptosis and thereby do not clear respiratory infections. The surrogate end-points that describe this process and the effect of antibiotic therapy, such as spirometry, are not sensitive and non-specific. We sought to evaluate the gene expression profile of circulating neutrophils in CF patients before and after antibiotic treatment. Methods: Microarray analysis (28,869 genes, Affymetrix GeneChip Gene 1.0 ST Array System) was performed in blood neutrophils from 10 CF patients before and after treatment for clinical exacerbation with antibiotics and 7 healthy control subjects. Results: Blood neutrophils before therapy presented 293 down-regulated genes and 57 up-regulated genes as compared with control subjects (considering as cut-off P < 0.05 by ANOVA). Comparison between the same patients before and after therapy (with the same cut-off by paired t test) showed instead that 1,422 genes were down-regulated and 282 up-regulated following antibiotic treatment. Interestingly, three genes appeared to be sensitive to therapy and returned to “healthy” condition: phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1), hydrogen voltage-gated channel 1 (HVCN1), and drom-3 homolog Z (DOM3Z). The up-regulation of these genes after therapy were confirmed by RT-PCR in blood neutrophils (n=9) and in sputum neutrophils obtained from the same patients (n=7). These results suggest the feasibility of investigating novel biomarkers of therapeutic efficacy by a global gene-wide platform and indicate more specific targets for future interventions involving respiratory burst and apoptosis.

BM10. First Trimester Biochemistry to Predict Cesarean Section for Fetal Distress and Cardiotocographic Alterations During Labor at Term of Gestation
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Background: At this time the literature tries to predict the outcome of pregnancy in the first trimester of pregnancy such as analyzing placental function. Our study compares first trimester clinical and biochemical characteristics between controls and pregnancies affected by cardiotocographic (CTG) anomalies or characterized by urgent cesarean section (CS) during labor at term. Methods: In this study at term pregnancies were considered. Clinical and biochemical characteristics were evaluated during the first trimester. The blood examinations have been performed between 2004 and 2010. We collected data about all CTGs performed during labor classified in three categories based on the National Institute of Child Health and Human Development terminology of 2008: (normal; 2intermediate; 3abnormal). Results: In a multivariate logistic regression reduced PAPP-A is correlated with a higher frequency of urgent CS at term of pregnancy (P < 0.05), regardless of hypertensive disease of pregnancy, IUGR, BMI, maternal age, gestational age at delivery and other obstetric pathologies considered. Abnormal CTGs were associated with older maternal age, higher prevalence of nulliparous women, and lower placenta weight than normal ones (P < 0.05). First minute Apgar scores were lower in category 3 than in 1 and 2 (P < 0.05). Finally, CTG alterations defined in category 3 were correlated with lower free-beta-hCG and PAPP-A values during the first trimester, although without statistical significance. Conclusions: A low PAPP-A in the first trimester of pregnancy appears to be correlated with a higher frequency of urgent CS at term of pregnancy and fetal distress during labour.

BONE METABOLISM

BMT1. Nitric Oxide Mediates Low Magnesium Inhibition of Osteoblast- Like Cell Proliferation
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Background: An adequate intake of magnesium is important for bone cell activity and contributes to the prevention of osteoporosis. Because i) magnesium is mitogenic for osteoblasts and ii) reduction of osteoblast proliferation is detected in osteoporosis, we investigated the influence of different concentrations of extracellular magnesium on osteoblast-like SaOS-2 cell behavior. Methods: SaOS-2 cells were cultured in media containing different concentrations of magnesium. Nitric oxide synthase (NOS) activity was evaluated by mass spectrometry and Griess assay, NOS isoforms were studied by western blot. Results: We found that low extracellular magnesium inhibited SaOS-2 cell proliferation. An additive effect was observed when cells cultured in low magnesium were silenced for the magnesium transporter Transient Receptor Potential Melastatin (TRPM7), which plays a prominent role in intracellular Mg homeostasis. In particular, we found that low magnesium inhibition of SaOS-2 cell proliferation was due to an increase of nitric oxide production through the up-regulation of inducible nitric oxide synthase (iNOS). Indeed, both pharmacological inhibition with the NOS inhibitor L-NIL and genetic silencing of NOS by siRNA restored the normal proliferation rate of the cells. Conclusions: Because a moderate induction of nitric oxide is sufficient to potentiate bone resorption and a relative deficiency in osteoblast proliferation can result in their inadequate activity, we conclude that maintaining Mg homeostasis is relevant to ensure osteoblast function and, therefore, to prevent osteoporosis.

BMT2. Frailty Fractures and High Energy Fractures: Serum Concentrations of IL-6, TNF-α, OPG, RANKL and Their Correlation with Radiographic Assessment
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Background: Stages of bone turnover during fracture repair can be assessed employing serum markers of osteoblastic and osteoclastic activity, inflammatory cytokines, clinical evaluation and imaging instruments. Our study compares the fracture healing process in fragility fractures and high energy fractures by evaluating serum changes of IL-6, TNF-α, OPG and RANK-L in combination with radiographic (RUST) and clinical (LEM) assessments. Methods: Subjects: femoral or tibial shaft fractures (group A,14), femoral fractures (group B,14), healthy (control A,14) and osteoporotic subjects (control B,14). Serum concentrations of IL-6, TNF-α were
quantified by Quantikine R&D Systems, RANK-L by BioVendor and OPG by Biomedica Medizinprodukte. Results: Results showed a significant decrease in IL-6 and TNF-α during fracture healing, with their values higher in group A than B and lower in both controls compared to TO (before surgery). OPG was significantly lower in each control group than that of the respective fractured group. In addition, OPG at TOA was significantly lower than at T0B whereas at T0A (after 10 weeks) OPG was less than at T10B. RANKL was significantly higher at T10 than at T0 only in group B. RANKL/OPG ratio was significantly higher in both controls than in fractured groups and significantly increased at T10. IL-6 and TNF-α correlated with RUST and LEM in fragility fractures and high energy fractures, whereas RANKL/OPG ratio was associated with these parameters only in fragile fractures. Conclusions: Our findings suggest that these serum parameters might be used to assess the stages of fracture healing. Further studies are required to clarify the complex fracture healing process.

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Background: Lumbar disc degeneration (LDD) is the main cause of low back pain and has a complex etiology. Several risk factors likely contribute to the genesis and/or acceleration of disc degeneration, however, there is some evidence for an influence of genetic factors and familiar predisposition. The vitamin D receptor (VDR) gene is one of the most studied genes involved in the predisposition to LDD. However, the data on the interplay between VDR genotypes, environmental factors and specific spine pathologies are still controversial. Methods: By using a case-control design, 234 Italian LDD patients and 70 healthy controls were enrolled. MR-based patients’ clinical assessment was performed and a questionnaire assessing constitutional and environmental risk factors was obtained. Blood samples were collected, genomic DNA was extracted and VDR FokI polymorphism was detected by PCR-RFLP. Results: Preliminary data showed that the genotypes frequencies for FokI polymorphism, in patients versus controls, were 44.9% versus 35.7% for FF homozygosity, 44.9% versus 45.4% for FF heterozygosity, 10.3% versus 12.9% for ff homozygosity, respectively. A significant association was found between the FF genotype and disc herniation (OR=1.82; CI=1.02-3.27), with 50.3% of patient with hernia versus 35.7% of controls presenting the FF genotype. Conclusions: The FokI FF genotype is a putative risk factor for disc herniation, but a larger control group is needed to better define the genotypes frequencies in the normal population. Moreover, study of other VDR polymorphisms (Taq1, Bsm1 and Apal) is in progress together with the evaluation of the vitamin D status and the association with constitutional and environmental factors.

CARDIOVASCULAR BIOMARKERS

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Background: Cardiovascular risk in men rises around the fourth decade of life, whereas women appear to be protected by sex hormones until menopause, which however negatively affects the lipid profile and the related cardiovascular risk. Since the 1980s, the incidence of cardiovascular disease has been reported to progressively decline in men, but it persists almost unchanged in women. Major clinical trials (e.g., on statin therapy) have mostly been conducted in men and have fostered the introduction of these agents into clinical practice worldwide. Only a few reports have however evaluated a possible differential activity of statins in the two genders, providing in some cases divergent findings. Methods: The lipid profile changes in response to 1-year treatment with different statins in 378 dyslipidemic patients (189 men and 189 women, aged 20-82 years) were evaluated. Results: In this large series of patients, a significantly attenuated reduction of total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels in women compared to men upon drug treatment was noted. A gender difference in the reduction of LDL-C was also noted according to baseline high-density lipoprotein cholesterol (HDL-C), which was particularly evident in women with baseline HDL-C > 80 mg/dl (22.1% vs. 26.4%, woman vs. man; p=0.05). Conclusions: The study suggests that statin treatment seems to have a reduced effectiveness in improving the plasma lipid profile in dyslipidemic women compared to men. Whether such gender difference may have an impact on health, leading to a lower preventive activity, remains to be elucidated.

CVBM2. Anti-Lp(a) Antibody: For Diagnosis and Therapy M. Pasquetto2, V. Molina3, A. Dargel4, D. Gerolati5, L. Vial1, C. Scotti7, G. Banfi1, M. Brayda-Bruno1
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Background: The presence of the lipoprotein(a) in plasma was first described by Kare Berg in 1963 as an LDL-like particle. Lp(a) was later identified as a risk factor for atherosclerotic diseases because of its pro-atherogenic, prothrombotic and antifibrinolytic properties. Different epidemiological studies have also suggested that Lp(a) could increase the risk of cardiovascular disease and ischemic stroke if associated with other predisposing factors such as hypercholesterolemia, hypertension, diabetes mellitus and low level of HDL. There is also experimental evidence that lipoproteins have a role in degenerative diseases and not only in atherosclerosis. Today there are few therapeutic approaches for the treatment of hyperlipidemia(a), High-affinity monoclonal antibodies are an attractive therapeutic alternative. Due to their specificity, they have the ability to selectively bind the molecule of interest, and their structure, which includes an Fc region, allows complex binding to the Fc receptor localized on the surface of monocytes and macrophages. Methods: In this study, we sought to characterize the effect of anti-Lp(a) monoclonal antibody 2E8, directed toward K/H2, in an in vitro system. The ability of the antibody to induce internalization of Lp(a) in murine macrophages (RAW cells) has been tested by an ELISA test and by microscopic evaluation of intracellular lipid accumulation.

Results: The number of foam cells had increased five times compared to the non-MAb control. Conclusions: This study will allow selection of new MAbs generated against human-Lp(a), and a fine characterization of the cellular response triggered by Lp(a)-MAb complexes.

CVBM3. Protein Array Analysis of Pro-Inflammatory Status in Patients with Different Heart Diseases E. Vianello1, G. Dogliotti1, E. Galliera1, E. Dozio1, G. Schmirtz1, E. Galletti1, M. Corsi Romaneli2
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Background: Cardiovascular diseases (CVDs) have been associated with inflammation and cytokine modulation and altered levels of these have been observed in the plasma of patients with various heart diseases. Our goal was to investigate new possible markers of heart disease to better understand involvement of these in different pathology such as coronary artery disease (CAD) and cardiac valvular replacement (VR). Methods: We have studied 3 groups of subjects: the first consisted of 20 patient affected by CAD undergoing coronary artery bypass grafting (CABG); the second of 20 patient with no signs of CAD undergoing VR; and the third group of 20 healthy man without apparent pathologies as controls. The analysis of interest were quantified using a biochip array protocol (Evidence, Randol Ltd., Crumlin, UK). Analytes researched in our study were IL-1α, IL-1β, IL-6, IL-10, TNF-α, TNF R1, R2 and INF-γ. Results: Our data showed a statistically significant increase of plasma levels of IL-1α, IL-1β, IL-6, IL-1, TNF-α and INF-γ in CABG and VR patients compared to controls (P < 0.05). Our data also showed a statistically significant increase of IL-1α and IL-6 plasma levels in CABG patients compared to VR, whereas IL-1β, IL-1, TNF-α and INF-γ were not different between the two groups. Conclusions: Our data suggest that CVD patients showed an inflammatory status due to an increase of pro-inflammatory and a reduction of anti-inflammatory mediators compared to healthy patients.

CVBM4. The Epidural Adipose Tissue as a Potential Source of IL-18 E. Vianello1, E. Dozio1, E. Galliera1, G. Dogliotti1, G. Schmirtz1, M. Corsi Romaneli2
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Background: The observation that circulating levels of IL-18 are elevated in human obesity and that weight loss is associated with proportional reduction of its level suggested that a likely site for IL-18 production may be the adipose tissue. Due to the link between obesity, inflammation and cardiovascular diseases, we aimed to measure IL-18 circulating levels in patients with different degree of adiposity undergoing open-heart surgery both to coronary artery bypass grafting (CABG) surgery or to valve replacement (VR) and we also evaluated whether epidural adipose tissue (EAT) may be a potential source of IL-18. Methods: Blood samples of patients undergoing elective CABG or VR surgery and of lean control subjects were collected after an overnight fasting to measure IL-18 levels by immunoenzymatic assay. IL-18, IL-18R1 and IL-18RAP gene expression were evaluated on EAT biopsy harvested from CABG and VR patients. Results: Quantification of IL-18
protein indicated that patients had higher IL-18 level than controls, considered both together (303.76 ± 132.23 pg/mL vs. 124.75 ± 15.03 pg/mL, mean ± SD, P < 0.0001) and after subdivision in CABG (282.89 ± 79.33 pg/mL, P < 0.001) and VR patients (354.79 ± 211.94 pg/mL, P < 0.01). Also after classification of the patients in subgroups according to their body mass index (BMI) (normal-weight and overweight), IL-18 levels were higher than those in control group. Conclusions: It seems that although these two different groups of patients had similar increased circulating levels of IL-18, which were independent of the BMI status of the subjects, a different local biology for IL-18 may exist at EAT level.

CVBV5. Interleukin-15, Coronary Artery Disease and Epicardial Adipose Tissue: Possible Correlation
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Background: The epicardial adipose tissue (EAT) has been shown to increase in obesity and to play a potential role in the development of coronary artery disease (CAD) by secreting different mediators. Interleukin-15 (IL-15) is one of the cytokines expressed by the inflammatory cells located at atherosclerotic plaques. In our study we measured IL-15 plasma level in two groups of patients with different degree of adiposity: 1) patients affected by CAD and undergoing coronary artery bypass grafting surgery (CABG); 2) patients without CAD undergoing valve replacement surgery (VR). We also compared gene expression levels of IL-15 and its receptor (IL-15RA) in EAT samples isolated from CABG and VR patients. Methods: Blood samples of patients CABG or VR were collected after an overnight fasting to measure IL-15 level by enzyme-immunometric assay. IL-15 and IL-15RA gene expression were evaluated on EAT biopsy harvested from CABG and VR patients. Results: IL-15 plasma level resulted higher in CABG than in VR patients (3.70 ± 1.17 pg/mL vs. 2.52 ± 1.04 pg/mL, mean ± SD, P < 0.05). After classification according to BMI, IL-15 level resulted higher in overweight/obese (OB) CABG compared to OB VR patients (4.54 ± 0.30 pg/mL vs. 2.18 ± 0.52 pg/mL, P < 0.01). A trend of increase was also observed in normal-weight (NW) CABG compared to NW VR patients (3.58 ± 0.40 pg/mL vs. 2.63 ± 0.89 pg/mL). Only in CABG group IL-15 level was higher in OB than in NW group (4.54 ± 0.30 pg/mL vs. 3.58 ± 0.40 pg/mL, P < 0.001). Conclusions: The increased IL-15 circulating level observed in CABG vs. VR patients seemed more correlated to the CAD pathology than to the obesity status of the patients. Whether EAT may significantly contribute to increase IL-15 circulating levels in these patients need further investigation.

CVBV6. Pathophysiological Implications of Inflammation and Genetic Immunologic Factors in Hypertensive and Old Patients Affected by Sporadic Thoracic Aortic Aneurysm
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Background: Sporadic thoracic aortic aneurysm (S-TAA) is potentially devastating with severe morbidity and mortality. Current evidence suggests inflammation as main mechanism of its pathophysiology associated with both aging and age-related hypertension. Thus, we assessed whether inflammation is the principal mechanism of its pathophysiology associated with both aging and age-related hypertension. We investigated the role of mrs2 in doxorubicin-induced apoptosis in mammary epithelial cells. Methods: Mammary epithelial cells (HC11) were adapted to grow in low or high magnesium medium. Control HC11, high-Mg and low-Mg HC11 were assessed for sensitivity to doxorubicin-induced apoptosis (annexin-V/PI, mitochondrial membrane potential and cytochrome c release) and mrs2 expression (Western blot). mrs2-siRNA cells were assessed for doxorubicin sensitivity and compared to wild type counterparts. Results: Our data show that sensitivity to doxorubicin depends on magnesium availability. High-Mg cells and magnesium-supplemented HC11 cells (10mM for 48h) are more resistant to doxorubicin-induced apoptosis. Interestingly, both cell lines mitochondrial mrs2 protein was up-regulated compared to control or untreated cells. Silencing of the mrs2 gene enhanced doxorubicin-induced apoptosis in all cells. Conclusions: Our data suggest that increased magnesium availability protects HC11 cells from doxorubicin-induced apoptosis. The expression of the mitochondrial protein mrs2 is involved in apoptosis resistance as mrs2-siRNA increased dox sensitivity also in high-magnesium cells. Since mrs2 overexpression has been associated to multidrug resistance (Chen, 2009) we hypothesise that mrs2 and the associated mitochondrial magnesium uptake have a crucial role in the mechanism of drug resistance.

ENDOCRINE AND METABOLIC DISORDERS
EMDI. Novel Mutations in SIRAR1 and MTPP Genes in Children with Chylomicron Retention Disease and Abetalipoproteinemia
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Background: Monogenic hypobetalipoproteinemias (mHBL) include Familial Hypobetalipoproteinemia (FHL) with a dominant transmission and
Abetalipoproteinemia (ABL) and Chylomicron Retention Disease (CRMD) with a recessive transmission. We investigated three children born from consanguineous parents, presenting with hypobetalipoproteinemia associated with chronic diarrhea and retarded growth. **Methods:** We resequenced the candidate genes for mHDLs and performed in vitro functional studies of mutant alleles. **Results:** Patient HBL-108 had a moderate hypobetalipoproteinemia, apparently transmitted as dominant trait, suggesting the diagnosis of FHBL. However, she had no mutations in FHBL candidate genes (APOB, PCSK9 and ANGPTL3) nor in MTTP gene (ABL). She was found to be homozygous for a novel mutation in SART1 gene resulting in a missense mutation (p.Glu262lys), as a possible cause of CRMD. In patients HBL-103 and HBL-148 the seventy of hypobetalipoproteinemia and its recessive transmission suggested the diagnosis of ABL. The MTTP gene sequencing showed that these patients were homozygous for a nucleotide substitution in the donor splice site of intron 9 (c.1236+2 T>G) (patient HBL-103) and intron 16 (c.2342+1G>A) (patient HBL148) predicted in silico to obliterate the splice site. In vitro assay with splicing mutation reporter MTTP minigene showed that intron 9 mutation caused the skipping of exon 10, whereas intron 16 mutation caused a partial retention of this intron in the mature mRNA. The products of these mRNAs are truncated proteins devoid of function. **Conclusions:** The diagnosis of the rare disorders ABL and CRMD should be considered in children born from consanguineous parents presenting with chronic diarrhea associated with hypobetalipoproteinemia.

**EMD2. Oxidative Stress During Prolonged Exercise in Insulin-Dependent Type 1 Diabetic Patients**

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**Background:** Several studies showed that diabetes mellitus (DM) is accompanied by increased formation of free radicals and decreased antioxidant capacity, leading to oxidative stress. Physical activity is part of the management of DM; however, the impact of exercise on oxidative stress is unclear. We aimed at investigating the oxidative stress during prolonged moderate exercise in a group of type 1 DM patients and a group of well-matched healthy controls. **Methods:** Nine patients (47 ± 10 years, 73 ± 15 kg weight, 170 ± 10 cm stature; Hba1c 7.1 ± 1.1%) and 15 healthy controls (46 ± 10 years, 75 ± 16 kg weight, 174 ± 10 cm stature) performed a 3-hrs constant intensity walk at 30% of the heart rate reserve. Patients were administered appropriate amounts of carbohydrates to avoid an excessive fall of glycemia. Venous blood samples were obtained before and at the very end of the trials for determination of glucose and insulin levels. Capillary blood samples were taken at the start of the walks and thereafter every 30 min to perform the Free Oxygen Radicals Test (FORT, CR-2000 Callegari1930, Italy). **Results:** Glucose and insulin levels were higher in patients than in controls. Type 1 DM patients showed higher oxidative stress values as compared to healthy controls (380.1 ± 14.7 vs 293.1 ± 9.6 arbitrary units; P = 0.05). Nevertheless, oxidative stress remained constant in both groups of volunteers throughout the whole exercise (P > NS).

**Conclusions:** Our data show that, even if type 1 diabetic patients show higher oxidative stress values as compared to healthy people, prolonged moderate exercise does not exacerbate this potentially harmful condition.

**EMD3. Preliminary Evidence of a Peculiar Hormonal Profile in Men with Adverse Effects After Use of Finasteride Against Androgenetic Alopecia**

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**Background:** Finasteride is a 5-alpha-reductase inhibitor that impairs the conversion of testosterone (T) to dihydrotestosterone (DHT). At dosage of 1 mg/day finasteride is successfully used against androgenetic alopecia. In young men finasteride used against hair loss is reported to provoke reversible sexual side effects. However, some very recent reports highlighted long-term persistence of sexual dysfunctions. We aimed to hormonally characterize 9 patients with long-term post-finasteride syndrome. **Methods:** Nine patients (36 ± 5 years old) with persistent (over 6 months) adverse effects including erectile dysfunction, infertility and depression, and 10 healthy matched controls were enrolled. Testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol (E2), progesterone and prolactin levels were evaluated in morning serum of all subjects. **Results:** Testosterone concentrations did not differ in cases and controls, P = 0.74. However, LH was 2-fold lower in cases (P = 0.03), whereas FSH was not statistically different between groups. Interestingly, the ratio of T/LH was 1.5-fold higher in cases than in healthy controls (P = 0.04). **Conclusions:** A concerning evidence is accumulating on severe long-term consequences of finasteride use in less than 50 years-old men. The percentage of subjects having post-finasteride syndrome is still to be determined and reasons of such persistent effects are unknown. We were the first to determine a peculiar hormonal profile in these patients suggesting an impairment of the endocrine interplay of hypofalalimum, pituitary and the tests, which specifically dampens only one of the gonadotrophins released from the pituitary gland, i.e. LH.
identified two mutated samples only on the DNA extracted from the granulocytes. A selection of 10 cases of MM presenting one of the researched genetic anomalies was made. The percentage of anomalous clones encountered within the plasma cells was always higher than the one observed in the bone marrow, but especially identifiable in the first 100 analysed cells. However, 300 to 5,000 cells, should be analysed to observe the anomalous clone in the bone marrow. **Conclusions:** The selection of the cellular population on which to perform specific tests was essential to improve the diagnostic efficiency, either in terms of sensitivity and in the precocity of the diagnosis.

**HEM2. Splenectomy is a Risk Factor for Developing Hyperuricaemia and Nephrolithiasis in Thalassaemia Intermedia Patients:** A Retrospective Study

**Background:** In patients with thalassaemia major and intermedia, hyperuricaemia and nephrolithiasis have been already described; however, reliable data about their occurrence, pathophysiological basis and consequences are lacking. **Methods:** We retrospectively reviewed the charts and radiological studies of 89 patients with thalassaemia intermedia followed at our clinic with routine biochemical examination and radiological imaging of the urinary tract with the aim to analyze both the prevalence of hyperuricaemia and nephrolithiasis and their impact on renal function. **Results:** Renal calculi were identified in 11 patients (12%) and 22 patients (25%) were under uricosuric treatment for hyperuricaemia. The prevalence of nephrolithiasis increased with age but not in a statistically significant manner. Major risk factors for renal stone formation were splenectomy (in 91% of the cases, O.R. = 13.6) and higher number of erythroblasts. Patients with renal stones had lower GFR value and had significantly higher level of uric acid with respect to those observed in patients not affected. Stone formers had higher mean creatinine level and lower GFR value with respect to those observed in patients without urolithiasis although the difference was nearly statistically significant (P = 0.051) only for creatinine level. **Conclusions:** Our data suggest that splenectomy, by further increasing erythrocyte turnover and number, may be directly involved in the pathogenesis of hyperuricaemia and nephrolithiasis observed in thalassaemia intermedia patients; our findings attributed additional disease-related complications to age and to splenectomy in thalassaemia intermedia patients.

**HEM3. Endogenous Thrombin Potential in Trombophilic and Non-Trombophilic Women with History of Complications During Pregnancy**

**Background:** Thrombophilia increases the risk of complications during pregnancy: IUGR, miscarriage, MEF, eclampsia. These complications can be present in healthy women too, and the hypercoagulable state, not detected in common tests, has been invoked as an alternative cause. Endogenous Thrombin Potential (ETP) may constitute a valuable diagnostic aid in defining a hypercoagulable condition. **Methods:** Out of the 84 women with complications, 41 were trombophilic and 43 were not. Thrombophilic condition showed: 7x FV Leiden, 11x F II, 15 with ACA, 1x LA, 1x Protein S deficit, 1x Protein C deficit, 3x F V + FII, 1x FV + hyperhomocysteinemia, 1x ACA + Protein S deficit. In thrombophilic patients the complications were: 2 women with eclampsia, 33 with more miscarriages, 1 IUGR, 4 MEF, 1 premature delivery; in patients with no thrombophilia: 9 women with eclampsia, 17 with more miscarriages, 4 IUGR, 8 MEF, 5 premature delivery. 36 healthy women with normal pregnancy were used as control. No patient in this study had cardiovascular risk or took any antiarthrombotic medication. ETP was measured by the chromogenic method (SIMENS) on platelet-poor plasma. **Results:** ETP evaluation did not show significant differences between thrombophilic women (median 93% [range 74%-130%]) and non-thrombophilic women [median 97% (range 71%-124%)]. ETP in the control group is comparable to the two populations studied [median 108.3% (range 80.9%-127.9%)]. **Conclusions:** ETP shows no correlation between thrombophilia and complications in pregnancy.

**HEM4. Pretest Clinical Score (4Ts) and Laboratory Testing for Reliable Diagnosis of Heparin-Induced Thrombocytopenia**

**Background:** Heparin-induced thrombocytopenia (HIT) is an uncommon but potentially devastating complication of anticoagulation with unfractionated heparin (UHF) or low molecular weight heparin (LMWH). HIT is defined as a decrease in platelets to less than 50% or to less than 100 x 109/L and positive laboratory HIT assay. Early diagnosis of HIT leads to rapid interruption of the anticoagulant treatment, substituted by alternative anticoagulant therapies, reducing mortality from 23 to 1.1%. When HIT is suspected (score ≤4Ts) rapid and highly sensitive diagnostic tests are required. ELISA and functional cytofluorimetric tests are highly specific and sensitive, but are time- and labor-consuming. **Methods:** We re-evaluated 61 samples from patients from 2011, showing high or moderate HIT, as assessed by 4Ts clinic test. The turbidimetric immunological test (Hemosil HIT-Ab-IL) was performed on all of them and showed the presence of anti-PF4/Epine antibodies in 6 patients (9.8%). These samples were subsequently functionally tested at the cyttofluorimetry and by chemiluminescence (Hemosil AcuStar-IL). **Results:** Both tests were positive in only 3 patients (4.9%); there was a strong correlation between the chemiluminescent immunological test and the functional cytofluorimetric one. **Conclusions:** The chemiluminescence test, being as sensitive and specific as the cytofluorimetric one, but easier and faster, might represent the elective test for HIT diagnosis. However, this test is suitable only for patients who scored high or moderate for HIT at the clinical test.

**HEM5. Bioinformatics as a Starting Point for the Analysis of ALK1 Missense Mutations**

**Background:** Activin A receptor, type II-like kinase 1 (also called ALK1), is a serine-threonine kinase predominantly expressed on endothelial cell surface, involved in TGF-β signalling. It is encoded by the ACVRL1 gene (12q11-14), whose mutations cause type 2 Hereditary Hemorrhagic Telangiectasia (HHT2), an autosomal dominant multisystem vascular dysplasia. Although an X-ray structure of ALK1 intracellular domain has recently become available (PDB ID: 3MY0), structure determination of ALK1 ectodomain (ALK1EC) has been elusive so far. **Methods:** We recently described the building of a homology model for ALK1EC, followed by an extensive bioinformatic analysis, based on a set of 38 methods, of the effect of missense mutations at the sequence and structural level. ALK1EC potential interaction with its ligand BMP9 was then predicted combining modelling and docking data. **Results:** Major structural changes and loss of stability of the protein were predicted for several mutations, whereas others were found to interfere mainly with binding to BMP9 or other interactors, like Endoglin (CD105), whose encoding ENG gene (9q34) mutations are known to cause type 1 HHT. **Conclusions:** Building on these predictions, we are now creating a library with the most interesting mutations, both by gene synthesis and site-directed mutagenesis, to try to express them and analyse their effects on endothelial cells by in vitro and in vivo tests, as well as to determine the real crystal structures.

**IMMUNITY AND INFLAMMATION – AUTOIMMUNE DISEASES**

**IASD1. Advances in Anti-Topoisomerase I Antibodies Evaluation**

**Background:** Positivity of Anti-Topoisomerase I antibodies in systemic sclerosis is historically correlated with disease activity and severity. On the contrary, a small number of studies are focused on the "quantity" of these antibodies. Our study aims at evaluating the relations between the "quantity" of antibody and the activity/severity of the disease. **Methods:** In 30 scleroderma patients, anti-topo I antibodies were consecutively measured in an observation time from 2-6 years. Naifold videocapillaroscopy was made at baseline. Clinical evaluation was performed by Eustar score for disease activity and Medsger scale for disease severity. Anti-topo I were tested by ELISA technology (Phadia, Germany). **Results:** For each patient we calculated the median value of anti-topo I. Median values were categorised in 3 groups: lower tertile 164 U/ml. Eustar evaluation: the median values of anti-topo I showed a positive correlation with Eustar score and a significant difference emerged in the patients of lower tertile in comparison with the upper tertile. Medsger evaluation: in presence of low levels of anti-topo I the organ-system involvement is often absent or at least limited. Also videocapillaroscopy findings agree with anti-topo I levels. **Conclusions:** Our data show differences mostly between low and high levels of anti-topo I. Despite this limitation a partly quantitative expression of anti-topo I results seems to be a useful tool for clinical evaluation until the first observation at diagnosis.
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Background: Several recent papers highlighted the possible role of serum matrix metalloproteinase-3 (MMP-3) assessment in rheumatoid arthritis (RA) as a reliable tool in the diagnosis, prognosis and to detect therapeutic efficacy (Hoxussian, Arthritis Res Ther 2012). Here we tested 2 different ELISA methods to analyse MMP-3 serum levels in routinely attending patients with rheumatoid arthritis.

Methods: 23 RA patients were enrolled (17 females, age range 33-79), tested more than three times in the follow-up, total samples n. 95. 42 age and sex-matched healthy donors (HDs - 27 females, age range 20-64); MMP-3 serum levels were analysed by two ELISA kits: DiaMetrà MMP-3 ELISA (DiaMetrà, Segrate, Milano, Italy) and AESKU/ELISA MMP-3 (AESKU diagnostics, Germany). Results: As expected, MMP-3 serum levels in HDs were significantly more elevated in males than in females, as assessed by both methods, even with slightly different ranges (25.4 ± 5 ng/ml vs 12.7 ± 3.4 ng/ml by DiaMetrà; 86 ± 24.2 ng/ml vs 36.3 ± 10.5 ng/ml by AESKU). RA patients generally disclosed higher levels than HDs, either by DiaMetrà (45.5 ± 39 ng/ml) and by AESKU (200 ± 203 ng/ml) and a good correlation between serum MMP-3 and disease activity was found in the follow-ups. A very good correlation was found between the results obtained in the same sera by the two methods (Spearman r = 0.97, 95%CI 0.95-0.98, P < 0.0001). Conclusions: The MMP-3 ELISA methods work well in the assessment of MMP-3 serum levels in routinely attending RA patients and this test may be useful to better characterize disease outcome in the follow-up.

IAD3. A Single Non-Synonymous Polymorphism of TLR2 Is responsible for Variability of Experimental Multiple Sclerosis in SJL and B6 Mice

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Background: Multiple sclerosis is characterized by variability of course and lesions distribution. The complexity of its presentation may reflect differences in either environment or genetic. In rodents challenge with peptides of myelin drives different forms of EAE in each strain/peptide pair, but it is difficult to assess the relative role of genetic background and antigens in determining the course of the disease. In all EAE models the administration of mycobacterium-derived motives is essential for disease development. TLR2 is the main receptor recognizing motives from M tuberculosis. Methods: To study the contribution of TLR2 genetics to EAE, we crossed SJL (TLR2 B136e) and B6 (TLR2 83Met) mice, generating F1 of SJLxB6wt (heterozygous for TLR2 Ile83Met) and F1 of SJLxB6tlr2- (TLR2 83Ile). We then immunized both groups of F1 mice with PLP139-151 and examined course and lesion distribution of EAE. Results: TLR2 83Ile increases secretion of IFN-γ (P = 0.043) and IL-17 (P = 0.041), whereas IL-13 and FoxP3 are similar in both groups. Consequently there are significant differences in the EAE. F1 mice of SJLxB6tlr2- display a more severe EAE (P = 0.0004) in the absence of PTx administration. SJLxB6wt mice are sensitive to PTx administration, whereas F1 mice of SJLxB6tlr2- are not. EAE developed in SJLxB6wt mouse has a clear progressive/chronic clinical course, whereas that obtained in SJLxB6tlr2- mice often show incomplete and of course inversely correlated with the stage/progression of disease.

IAD4. Phenotypic Characterization of Circulating B- and NK-Cell Subsets as a Marker Of Primary Sjögren’s Syndrome (pSS)

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Background: Primary Sjögren’s syndrome (pSS) is an autoimmune disorder characterized by chronic inflammation and loss of function of salivary/lachrymal glands. pSS patients generally disclosed higher levels than HDs, either by DiaMetrà (45.5 ± 39 ng/ml) and by AESKU (200 ± 203 ng/ml) and a good correlation between serum MMP-3 and disease activity was found in the follow-ups. A very good correlation was found between the results obtained in the same sera by the two methods (Spearman r = 0.97, 95%CI 0.95-0.98, P < 0.0001). Conclusions: Both the MMP-3 ELISA methods work well in the assessment of MMP-3 serum levels in routinely attending RA patients and this test may be useful to better characterize disease outcome in the follow-up.

IAD5. Clinical and Serological Response to Tocilizumab in Rheumatoid Arthritis Patients

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Background: Rheumatoid factor (RF) IgM and antibodies to citrullinated proteins (ACPA) are serological markers of rheumatoid arthritis (RA), currently assessed in clinical practice. Yet the measurement of IgG- and IgA-RF is not performed routinely because of conflicting data on the clinical relevance of these isotypes. Data confirming a definite relationship between decreased RF levels and clinical responses are scarce. The aim of the present observational longitudinal study was to investigate whether RF isotypes and ACPA are related to clinical response in RA patients treated with tocilizumab (TCZ, anti-IL6R).

Methods: The study population was composed by 27 subjects (24 females, mean age 56.4 ± 10.7 years). The subjects were studied at baseline (T0), and at follow up visits at 3 (T1) 6 (T2), and 12 months (T3) after the beginning of the treatment with TCZ 8 mg/kg. Each patient was assessed at each time point through clinical scales (VES, PCR, HAO, DAS28- VES, DAS28-PCR, CDAI, and SDAI). IgM-, IgA- and IgG-RFs and anti-CCP antibodies were assessed by enzyme linked immunosorbent assay at T0, T1, T2, T3.

Results: All patients showed a rapid, significant, and sustained clinical response to treatment throughout the observation period. Whereas the clinical scales (except HAQ) significantly decrease during the treatment, the antibody counts do not. We only found a significant correlation (P = 0.03) between ACPA and SDAI changes from baseline at T1 and T2. We found no significant correlation between the antibodies count at T0 and the changes of the DAS-28 VES at T1 and at T2.

Conclusions: Tocilizumab, although effective in treating RA, does not decrease antibody levels.

IAD6. Proliferative Potential of FoxP3+ Regulatory T Cells in Multiple Sclerosis Patients

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Background: In autoimmune diseases there are no reliable markers able to predict either loss of self-antigen tolerance or clinical progression of pathology. We investigated the relationship between the proliferative capacity of regulatory T cells (Treg), a cellular subset that controls the immune response, with clinical progression in multiple sclerosis (MS). Methods: Since proliferation of Treg cells is inhibited by the adipecotein lepine we investigated the capacity of Treg cells to expand upon lepin-neutralization in vitro and correlated this phenomenon with the clinical stage/progression of disease. Results: Proliferated Treg cells from MS patients, showed a reduced proliferative capacity during anti-CD3/CD28-stimulation upon lepin-neutralization when compared with healthy controls. Interestingly the patients clinical stage inversely correlated with the in vitro expanding capacity and proliferative potential of Treg cells. Indeed, patients with a higher Expanded Disability Status Scale (EDSS) showed a reduced expansion of Treg cells upon lepin neutralization. We applied the multinominal logistic model to calculate the relative risk for MS patients to develop a worst clinical progression of pathology in function of the observed expansion index at diagnosis. Thanks to this model we found a higher risk to develop a more severe MS in patients with a lower expansion of Treg cells.

Conclusions: Our findings could be of relevance in understanding the pathogenesis of
of MS and introduce the use of the in vitro Tregs expansion index as marker for evaluation of immunological tolerance and disease progression in MS patients.

IAD7.  Meta-immunological Profile of Children with Type 1 Diabetes: Toward the Possibility to Predict Progression of Autoimmune Diabetes
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Background: Type 1 Diabetes (T1D) is an autoimmune disease characterized by T cell-mediated destruction of pancreatic β-cells. The time course and the precise mechanisms of progressive β-cells failure are still not completely elucidated.

Methods: Nine circulating biological compounds, at the interface between immune and metabolic regulation, and twenty immune cell populations were investigated over time as markers associated with disease progression at different disease stages (high-risk subjects, T1D at onset, 12 and 24 months after diagnosis). The correlation matrix among the different biological parameters was statistically evaluated and visually assessed on 2-dimensional graphs. Finally, we built a multivariate logistic regression model to identify markers able to predict residual β-cell function. Results: We observed that the meta-immunology profile significantly differed among the different study groups and during disease progression, thus defining a specific signature typical of disease progression. Further, we defined a simple and robust decision rule, based on the multivariate logistic regression model, by measuring the number of circulating CD3+CD16+CD56+ cells and the percentage of myeloid Dendritic Cells (mDCs) at disease onset. This model was able to predict pancreatic residual C-peptide production by β-cells up to one year after disease onset.

Conclusions: This study defines a specific meta-immunology asset in T1D changing during disease progression and provides a simple decision rule that predicts residual β-cell function and monitors the meta-immunology status typical of T1D.

IMMUNITY AND INFLAMMATION – HOST DEFENSE

IH01. Hepatitis B Virus Isolates of Different Genotype May Vary in Their Capacity to Limit Effectiveness of Endogenous and Therapeutic Interferon-α
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Background: Several clinical studies suggested that Hepatitis B Virus (HBV) genotype may affect response to IFNα-treatment of chronic hepatitis B (CHB). Aim of our study were: (a) To investigate and compare in vitro the transcriptional and replicative capacity of three wild-type HBV isolates of genotype A, C, and D, respectively; (b) to verify whether these three HBV genotypes display different IFNα-mediated induction of antiviral defence mechanisms in human hepatoma cells. Methods: HBV naïve CHB patients infected with HBV of genotype A, C, and D, respectively, were fully-length amplified and cloned in accordance with the method described by Gunther et al (J Virol 1995). Comparison among their transcriptional/replicative activities (Southern and Northern blot, real time-PCR and ELISA assay), recruitment of Stat1/phospho-Stat1, Stat2/phospho-Stat2 onto the HBVcccDNA (cccDNA-ChIP) and expression profile of innate immune response genes (TaqMan low-density array) were assessed after transfection of untreated- and IFNa-treated HepG2 cells. Results: Genotype A isolate produced higher levels of replicative intermediates and showed a better response to IFNα treatment than genotype C and D ones. Both genotype C and D HBVcccDNAs showed a reduced binding of Stat1/2 and phospho-Stat1/2-Stat2 compared to genotype A both in untreated and IFNa-treated cells. The three genotypes were down-regulated to a greater extent both in genotype C- and D-Duplicating cells. Conclusions: Our data indicate that each HBV genotype may possess different phenotypic and biological characteristics and may differentially impairs pathways of the interferon response.

IH02. Hepatitis B Virus Causes Epigenetic Induction of Interleukin-8 Production which in Turn Inhibits Interferon-α/β Antiviral Activity
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Background: Serum interleukin-8 (IL-8) concentrations are increased in chronic hepatitis B (CHB) patients during hepatic flares. Aims of this study were: (1) to determine IL-8 amounts in sera and liver tissues of CHB patients, inactive HBV carriers (IBC) and healthy controls (HC), (2) to analyse the mechanisms implicated in HBV-induced IL-8 gene expression, (3) to study the possible antagonistic activity of IL-8 on INFα-treatment response. Methods: Serum IL-8 amounts were analysed by an ELISA assay. IL-8 mRNA quantification in liver tissues and HBV-replicating cells was performed by real-time PCR. An IL-8 promoter-driven luciferase assay was used to study IL-8 promoter transactivation. The ChIP-assay was applied to analyse IL-8 promoter acetylation/methylation status. A cell-based HBV replication system was used to test the potential antagonistic effect of IL-8 on INFα-treatment response. Results: CHB patients had higher amounts of IL-8 both in serum and liver tissue compared to controls. In HBV-replicating cells, IL-8 promoter transcriptional activity was stimulated up to 100-fold and IL-8 transcription was significantly increased. The luciferase-reporter assay showed that NFκB and AP-1 are essential for IL-8 induction by HBV. HBV viral protein was recruited onto the IL-8 promoter. Acetylation/methylation status of IL-8 promoter was strongly correlated with IL-8 amounts both in serum and liver tissue. Inhibition of IL-8 by anti-IL-8 monoclonal antibodies or IL-8-siRNA increased IFNα inhibitory action on HBV replication in transfected cells. Conclusions: (1) Highly replicating HBV is able to induce IL-8 transcription by targeting the epigenetic regulation of IL-8 promoter. (2) The induction of IL-8 by HBV inhibits the INFα antiviral activity.

IH03. Local Interleukin-1 beta (IL-1β) Levels in Early Pregnancy and Preterm Birth
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Background: Preterm birth (PTB) is considered a research priority. The rate of PTB has not declined in the last decades. Non-invasive early biomarkers for PTB risk are highly warranted to possibly perform a personalized treatment. Methods: To assess if vaginal interleukin-(IL)-1 beta (IL-1β) in early pregnancy is associated with adverse outcome among bacterial vaginosis (BV)-positive women, 1,836 women were enrolled at <20 weeks' gestation in 5 Philadelphia Hospitals (Philadelphia, PA). 800 women were BV-positive (by Gram evaluation according to Nugent score 7-10), 707 of them had birth outcome data. Vaginal IL-1β concentrations were measured in 105 BV-positive women who had an adverse preterm outcome, including 66 preterm births (20-37 weeks, of which 52 were spontaneous) and 14 late miscarriages (12-20 weeks), and in 295 BV controls (term normal birth weight infants). The upper (>66th percentile) and lower (<33rd percentile) tertiles of IL-1β concentrations were compared with the middle tertile (33rd to 66th percentile) to assess whether the risk profile is U-shaped. Results: None of the IL-1β tertiles was associated with increased risk for any adverse preterm outcome, nor preterm birth and miscarriage with or without exclusion of women with concurrent STDs. Conclusions: Vaginal IL-1β is not a risk marker for preterm birth among BV-positive women in early gestation, likely because of large overlapping of events producing actual levels of the pro-inflammatory cytokine IL-1β in vaginal fluid.

IH04. Viral N-linked Glycans Contribute to Alphavirus-induced Myositis and Arthritis
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Background: Mosquito-borne alphaviruses chikungunya virus (CHIKV) and Ross River virus (RRV) cause outbreaks of debilitating infectious arthritis in humans in the Indian and Pacific Ocean regions, and CHIKV has recently become endemic in parts of Europe. CHIKV- and RRV-induced disease is characterized by severe inflammation and immunopathology. Recent studies demonstrated that the mannose binding lectin (MBL) pathway of the host complement system is essential for RRV-induced immunopathology, MBL binds to terminal carbohydrates, such as mannose found on glycosylated viral proteins, to activate the complement system. There are three N-linked glycosylation sites on the RRV envelope glycoproteins that are glycosylated with high mannose and complex glycans in mammalian cells. We hypothesized that these RRV glycans are ligands for MBL and complement activation, contributing to development of disease. Methods: Using a panel of RRV mutants lacking one or more glycans, we evaluated and characterized the RRV-induced disease in mice infected with the glycan mutant viruses. Results: Mice infected with a virus lacking both E2 N-linked glycans exhibit reduced disease, reduced tissue damage, and reduced levels of MBL and complement activation within infected tissue compared to wild-type RRV infected mice. However, virus-induced inflammation and viral replication within infected tissues were similar between the two viruses. Conclusions: These results suggest that interactions between the N-linked glycans and MBL play a central role in the development of severe alphavirus-induced arthritis and may be an effective target for therapeutic treatment in patients infected with arthritogenic alphaviruses.

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IHDD. Mycobacterium tuberculosis in the Adjunct Modulates Trafficicking of Effector T Cells Through a Polymorphic Site of TL2

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Background: The role of infectious agents in the regulation of T cell trafficking is currently unknown. Methods: We examined this issue using immune response and TCR transgenic mice. Results: The amount of M tuberculosis in the adjacent modulates rapid relocation of PLP139-151 (p139)-specific T cells carrying a public T-beta chain B10V-1/CASS SGS NTE JB.1.1 from draining lymph nodes (LN) to spleen in the SJL mouse. In the presence of low dose of M tuberculosis in the adjacent, T cells mostly reach the spleen by day 28 after immunization (late relocation), whereas the same T cells reach the spleen by day 14 after immunization with high dose of M tuberculosis (early relocation). The B6 background confers a dominant “early relocation” phenotype to F1 (SJL x B6) mice, allowing early relocation of T cells in the presence of low dose M tuberculosis. A single non-synonymous polymorphism of TL2 (Ile36Met) is responsible for ‘early/late’ relocation phenotype. By transferring T cells from F1 mice obtained crossing SJL mice transgenic for the TCR-beta chain indicated above (SJLB/10) with C57/B6 or C57/B6/B24, we determined that egress of antigen specific lymphocytes is modulated by TL2 expressed on T cells. We also examined the expression of some markers regulated by activation and involved in T cell trafficking. Early relocation is associated with an intermediate expression of CD44 and that TL2 also regulates processing of CD44 pre-mRNA. Conclusions: Pathogens engaging TL2 on activated T cells through a polymorphic site modulate expression of activation/adhesion molecules and regulate effector T cells trafficking in vivo.

IHDE. Analysis of the Polymorphisms of TH1 and TH17 Cytokines in Mediterranean Spotted Fever

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Background: We have recently reported that the susceptibility for Mediterranean spotted fever (MSF) caused by Rickettsia conori, is influenced by the TH2 and TH1 cytokine genetic polymorphism profiles. Less it is known on the effect of gene polymorphisms of cytokine produced by the Th17. Methods: 70 Sicilian patients affected by MSF and 239 control subjects matched for age, gender, and geographic origin were typed for functionally relevant single nucleotide polymorphisms (SNPs) of IFN-γ (+874 T/A), IL-18 (-137 G/C and -607A/C) and IL-17 (7488T/C) according to the laboratory procedures. Results: No significant differences in IL-18 -137 G/C, -607A/C and in IFN-γ +674 T/A genotype frequencies were observed. On the contrary a statistically significant (p value 0.0126) increase of the IL-17 TT genotype frequency of in MSF was observed. Conclusions: Cytokines play a crucial role in modulation of the host defense and genetically determined differences in cytokine production seem to influence the extent and severity of a large number of infectious diseases. 7488T/C SNP impinge on IL-17 signaling and might play a crucial role in neutrophil recruitment, induction of IFN-γ and IL-12 production in macrophages and in the induction of T regulatory cells. Our results suggest that a genetically determined increase of IL-17 dependent activation pathways might interfere with R. Conori infection control.

IHDF. The Alarmin Interleukin-33 Is a Notch Target in Quiescent Endothelial Cells

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Background: The molecular mechanisms that drive expression of the alarmin interleukin-33 (IL-33) in endothelial cells are unknown. Because nuclear IL-33 is a marker of endothelial cell quiescence (corroborated in this study by showing co-expression of cyclin-dependent kinase inhibitor p27(KIP1)), we hypothesized that Notch signaling might be involved in regulating IL-33 expression. Methods: Umbilical vein-derived endothelial cells, rPDR, immunocytochemistry, recombinant Notch ligands, siRNA, in vivo experiments. Results: Here we show that activation of Notch1 by immobilized Notch ligands was sufficient to induce nuclear IL-33 expression in cultured endothelial cells. Conversely, IL-33 expression was inhibited by the γ-secretase inhibitor DAPT or by inhibiting the function of DI4, Jagged1, Notch1, or the canonical Notch transcription factor RBP-Jκ. Sensitivity to cycloheximide indicated that IL-33 was a direct target of Notch signaling, well in line with the identification of several conserved RBP-Jκ binding sites in the IL-33 gene. The in vivo expression of DI4 but not Jagged1 was well correlated with expression of IL-33 in quiescent vessels, and subcutaneous injection of DAPT in healthy skin reduced IL-33 expression, indicating that Notch signaling was involved. On the other hand, loss of IL-33 during angiogenesis occurred in spite of sustained DI4 and Notch1 expression, suggesting that other signals may override the IL-33-driving signal in this context. Conclusions: Taken together, our data demonstrate that endothelial nuclear IL-33 is induced by Notch and that DI4 may be the dominant ligand responsible for this signaling in vivo.

IHDD. Recognition of Fungal β-glucan by Human Neutrophil CR3

(CD11b/CD18) Results in Homotypic Cell Aggregation and Rapid Formation of Extracellular Traps by a Mechanism That Depends on cSf1

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Background: The armament of neutrophil-mediated host defense against fungal and bacterial pathogens includes the extrusion of a lattice of DNA and microbialid enzymes known as Neutrophil Extracellular Traps (NETs). The receptor-mediated interactions and intracellular signaling events responsible for elaborating NETs are not well characterized and were determined in this study for the response to Candida albicans. Moreover, as the host response of extravasated neutrophils to deep-seated mycotic infections necessitates contact with ECM, this study identified an important regulatory function for the ubiquitous matrix component fibrinogen (Fn) in anti-fungal NET release. Methods: Global tyrosine phosphotyroplastic analysis mitigated the quantitative analysis of phosphorylated sites of neutrophils adherent to immobilized Fn vs. Fn + β-glucan and was validated via Western blot analysis. Light, confocal and transmission electron microscopy was implemented to observe NET formation after addition of Sytox Green, indicating a breach in membrane integrity. Results: Recognition of the purified fungal cell wall pathogen associated molecular pattern β-glucan by human neutrophils resulted in formation of cell aggregates and NET release that required Fn. NET formation was dependent on CR3 (CD11b/CD18), but not Dectin-1 or reactive oxygen species (ROS). Fifty-four phosphopeptides were differentially regulated by β-glucan and validation revealed a role for ERK in aggregation and NET release. NET formation to C. albicans hyphae was also dependent on Fn, CR3, and ERK but not ROS. We also report a regulatory role for cSf1 in mediating NET release. Conclusions: This study identified key receptor/ligand interactions that induce NET formation rendering insight into understanding host defense mechanisms against fungal infections.

IHDE. Signaling Molecules Involved in Homotypic Aggregation of Human Neutrophils in Response to Fungal β-Glucan

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Background: Complement receptor 3 (CR3), an integrin found on neutrophils, rapidly states firm cell adhesion to the extracellular matrix and serves as a pattern recognition receptor for the fungal Pathogen Associated Molecular Pattern (PAMP) β-glucan. Coincident ligation of CR3 with fibrinogen and β-glucan is possible due to spatially distinct ligand binding domains. Current work demonstrates that dual ligation of CR3 with fibrinogen at its i-domain and β-glucan at its lectin-like site causes homotypic aggregation of primed human neutrophils. Methods: Using phosphoproteomics, we have identified several proteins that are significantly phosphorylated when ligated with fibrinogen and β-glucan but not fibrinogen alone. From these data, we propose a signaling pathway in which increased phosphorylation of phosphoinositide 3 kinase (PI3K), protein kinase C5 (PKCδ), glycogen synthase kinase 3β (GSK3β), and ERK could lead to the observed homotypic aggregation of neutrophils via CR3 on immobilized fibrinogen and β-glucan. Western Blotting validated our initial phosphoproteomic findings for each protein and use of inhibitors obviated homotypic aggregation of neutrophils for all aforementioned proteins except for GSK3β in which a partial inhibition was observed. Results: To quantify such partial inhibition, a computer algorithm was created to help quantify the number of cells clustered in a field. With this program, we were able to determine that despite our observation GSK3β inhibition has no effect on cell clustering. Conclusions: Thus PI3K, PKCδ, and ERK are all components of the signaling pathway of dually ligated CR3 that are also relevant to homotypic aggregation.
**IMMUNITY AND INFLAMMATION – IMMUNITY**

**IMM1. Structural Basis of Affinity Maturation of Antibodies in the 2-Phenyl-5-Oxazolone System**

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**Background:** Affinity maturation of antibodies is the process whereby more efficient antibodies are produced through somatic hypermutation and antigen-guided selection. No extensive study is available at the moment concerning the relationship between somatic mutations and their structural counterpart. The antibody response to 2-phenyl-5-oxazolone has been thoroughly investigated from the genetic point of view. It consists of three antibody classes, with each member of each class derived from a unique pair of VH and VL germline genes by somatic hypermutation. In this project, we are investigating the structure of the VH and VL domains of 10 representative antibodies. **Methods:** The VH and VL domains of each antibody are being expressed as recombinant scFvs, crystallised, and their structure determined by X-ray crystallography. **Results:** The structures and models available allow an initial definition of the strategies adopted. In class I, maturation is bound to improvement of surface complementarity, especially at the top of the binding site, and in surface charge changes. In class II the maturation strategy seems to be based on the increase of the interacting surface, and on the introduction of a specific bond with the oxazolone ring. In class III, where the low and high affinity antibodies differ by 8 mutations, the increase in affinity is mainly determined by the improvement in the surface complementarity by removal of a bulky phenylalanine, which allows a better tightening of the two sides of the binding site. **Conclusions:** These results are relevant to determine the principles underlying affinity maturation of antibodies.

**IMM2. Identification Of CIKS/ACT1 New Interacting Partners**

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**Background:** CIKS (Connection to IKK and SAPK/JNK, a.a. Act1) is a putative E3 ubiquitin ligase required for signaling by IL-17. IL-17 is an inflammatory cytokine (an E3 ubiquitin ligase) by its evolutionary conserved motif at the N-term. **Methods:** Immunoprecipitations of FLAG-CIKS interactors were performed with anti-FLAG antibody. The immunoprecipitation products were electrophoresed by SDS-PAGE and analyzed by mass spectrometry. **Results:** The aim of the present project is the identification of new CIKS interactors. To this purpose we infected CIKS−/−MEFs with a lentiviral expression vector containing the cDNA encoding FLAG-CIKS. The potential CIKS interactors obtained by co-immunoprecipitation were analyzed by mass spectrometry. **Conclusions:** We identified about 30 CIKS interactors whose characterization is currently under investigation.

**IMMUNITY AND INFLAMMATION – IMMUNOPATHOLOGY BIOMARKERS**

**IMBM1. Antiprothrombin/phosphatidylserine Complex Autoantibodies in Antiphospholipid Syndrome: Prevalence in Routinely Attending Patients in Udine**

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**Background:** Antiphospholipids (aPL) are a heterogeneous family of antibodies reacting with serum phospholipid-binding proteins, including prothrombin. Antibodies against prothrombin alone, or the phosphatidylserine antiprothrombin complex may be detected. The prevalence of IgG/IgM prothrombin/phosphatidylserine complex antibodies (aPPT) in consecutive patients, focusing on lupus anticoagulant (LA)-positive patients, was compared with the routinely used test for anti-prothrombin antibodies. **Methods:** 136 patients were enrolled, 78% female, age range 18-89, 156 (78.9%) LA-positive (72.4% of which aCL-negative), 24 LA/aCL-negative, 3 unknown LA, aCL-negative. Clinically: 81 lupus (SLE) or undifferentiated connective tissue diseases (UCTD), 21 with aPL syndromes (APS) (73.3%), followed by SLE/UCTD (70.4%, that were 57.4% aCL-negative) and other systemic AID (53.8%, that were 92.3% aCL-negative). aPPT were positive also in 2/4 seronegative APS, in 31.6% APT and 50% of oncologic patients. In LA- positives, the sensitivity of the new phosphatidylserine-dependent antiprothrombin ELISA was significantly higher than the anti-prothrombin alone ELISA (55.8% vs. 15.4%). **Conclusions:** Testing anti-PPT/PS antibodies improves the capability to identify unrecognized aPL antigens, helping to better characterize APS patients.

**IMBM2. Laboratory Screening of Helicobacter pylori in Stool Samples**

Suggest a Relationship with Graves’ Disease, but Not Hashimoto’s Thyroiditis

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**Background:** Helicobacter pylori (HP) infection has been epidemiologically linked to some extra-digestive conditions, including autoimmune thyroid diseases (ATDs), although there are contradictory data about relationship between HP infection and these disorders. The aim of study was to investigate the correlation between prevalence of Cag-A positive strains of HP in stool samples and ATDs. **Methods:** We investigated 112 consecutive patients: 48 females and 4 males with Graves’ Disease (GD), 54 females and 6 males with Hashimoto’s thyroiditis (HT), at first diagnosis of ATDs. The control group was composed of 100 class-matched individuals. **Results:** Thyroid hormones and autoantibodies for ATDs diagnosis were measured by chemiluminescent immunomassay, using Liaison instrument (Diasorin, Italy). To evaluate HP infection, stool samples were tested by amplified enzyme immunoassay (Amplified IDEA H. pylori STAR, Oxoid, United Kingdom). To detect Cag-A antibodies, serum samples were tested by enzyme-linked immunomassay method (ELISA, Radim, Pomezia, Italy). Results were analyzed by two-sided Fisher’s exact test and the respective odds ratio (OR) calculated. **Conclusions:** The prevalence of HP in stool samples was higher in GD group (43/52, 82%) than in HT group (28/80, 46%) and control group (43/100, 43%). A statistically significant interaction was found between HP positivity and Graves’ disease (P ≤ 0.0001 vs control, OR 6.3), but not Hashimoto’s thyroiditis. **Conclusions:** A possible role of HP infection in GD could be dependent on the different expression of adhesion molecules in the gastric mucosa, but further studies needs to display such hypothesis.

**IMMUNITY AND INFLAMMATION – INFLAMMATION**

**IMIN1. Study of the Modulation of Cytokine Release by Natural Compounds with Pharmacological Properties Using Cell-Based Systems**

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**Background:** The screening of the pharmacological properties of natural compounds (i.e., anti-inflammatory effects) may take advantage of some specific cell-based systems. Panthenolide (PTN) and Copaifera langsdorffii (Copaiba) are natural compounds used to prevent and treat headache and migraine and in inflammatory diseases involving respiratory airways, genital-urinary apparatus and skin, respectively, but their effects at the cellular level are poorly understood. **Methods:** Mouse BV-2 microglia and human THP-1 monocyte cell lines were used. The nuclear translocation of nuclear factor (NF-κB) was evaluated by Western blotting analysis. The secretion of inflammatory cytokines (interleukin (IL)-1, IL-6, tumor necrosis factor-α (TNFα)) was evaluated by immunometric assays (ELISA). **Results:** Treatment of BV-2 cells with 1 μM PTN and of THP-1 cells with 10 μM Copaiba oleoresin (OR), containing diterpene acids, diterpenes and sesquiterpenes, strongly reduced the NF-κB translocation to the cell nucleus induced by 1 μg/mL lipo polysaccaride (LPS). In BV-2 cells, PTN reduced IL-6 secretion in a dose-dependent manner (-29% at 200 nM, P < 0.001; -45% at 1 μM, P < 0.001; -88% at 5 μM, P < 0.001; ANOVA). Moreover, at 5 μM (highest concentration tested) PTN also reduced TNF-α secretion (-54%, P < 0.001). Preincubation of LPS-stimulated THP-1 monocytes with OR (dose-range: 0.1-10 mM), reduced the release of all tested cytokines (IL-1β, IL-6, TNF-α). **Conclusions:** The results obtained provide strong evidence that both cell-based models are useful to validate the anti-inflammatory properties of PTN and OR at the cellular level and suggest that they are related to inhibition of cytokine secretion and NF-κB nuclear translocation.
IMIN2. Origins and Significance of Elevated Gamma-glutamyltransferase in Cystic Fibrosis Sputum
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Background: Cystic fibrosis (CF) is an autosomal recessive disorder characterized by a chronic neutrophilic airways inflammation, increasing levels of oxidative stress and reduced levels of antioxidants such as glutathione (GSH). Gamma-glutamyltransferase (GGT), an enzyme induced by oxidative stress and involved in the catabolism of GSH and its derivatives, is increased in the airways of CF patients with inflammation, but the possible implications of its increase have not yet been investigated in detail. Methods: Sputum samples from 7 CF patients were analyzed by cytchemistry, HPLC-gel filtration, western blot and enzymology techniques.

Results: GGT activity was found both in neutrophils and in ELF fluid, the latter significantly correlating with myeloperoxidase expression. In neutrophils, GGT was associated with intracellular granules. In the fluid, gel-filtration chromatography showed the presence of two distinct GGT fractions, the first corresponding to the human plasma b-GGT fraction, the other to the free enzyme. The same fractions were also observed in the supernatant of ionomycin and FMLP-activated neutrophils. Western blot analysis confirmed the presence of a single band of GGT immunoreactive peptide in the CF sputum samples and in isolated neutrophils.

Conclusions: In conclusion, our data indicate that neutrophils are able to transport and release GGT, thus increasing GGT activity in CF sputum. The prompt release of GGT may have consequences on all GGT substrates, including major inflammatory and other proteins.

IMIN3. Multi-step Regulation of Toll-Like-Receptor 4 Signalling by IL-10-dependent Anti-inflammatory microRNAs
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Background: Toll-like receptors (TLRs) play a key role in detecting pathogens and initiating inflammatory responses that subsequently prime specific adaptive responses. To avoid excessive inflammation and consequent immunopathology, TLR signalling must be tightly regulated. Though microRNAs (miRs) have emerged as important regulators in several biological processes, their functional role in the control of inflammatory responses remains incompletely understood. Methods: Bioinformatic analysis was performed using MiRanda software, luciferase assays were performed on target 3'-UTRs cloned in psi-Check2, THP-1 transduction was achieved using pRLL-based lentiviral constructs. Results: Stimulation of human monocyte/macrophages with LPS induced the expression of mIR-187, mIR-146b, and the cluster 98b/125a-Splele1 at late time-points. Blocking experiments indicated that these miRs are all induced as part of the IL-10-dependent feedback loop, and all substantially decreased under chronicization conditions, mimicked by iNIFy exposure. Bioinformatic analysis predicted and luciferase assays confirmed that receptors (TLR4, CD14), signal transducers (MyD88, IRAK1), transcription factors (Ik-Bzeta), and effector molecules (TNF, IL-6, chemokines) involved in the TLR4 pathway are direct targets of these IL-10-dependent miRs. The biological relevance of this finding was confirmed by the significant reduction of LPS-dependent cytokine production achieved by overexpression of individual miRs in THP-1 cells.

Conclusions: We have identified a set of anti-inflammatory miRs, activated by IL-10 in human monocytes and macrophages, which are able to modulate TLR signalling acting at multiple steps of the signalling cascade, by direct targeting of receptors, adaptor/signalling proteins, and effector molecules. These miR candidate as new modulators of the response to LPS and are potentially involved in the resolution of inflammation.

IMIN4. Recombination as a Major Source of Genetic and Pathogenic Diversification of Group A Streptococcus Serotype M99 Strains
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Background: Group A Streptococcus pyogenes (GAS) is a genetically diverse human pathogen having > 150 serotypes. Serotype M99 strains are a commonly cause of GAS pharyngeal and invasive infections. Recent studies indicate M99 strains may be increasing in prevalence. Over the last 15 years in Italy, two large epidemic outbreaks were caused by macrolide resistant M99 strains. M99 epidemic strains differed in gene content encoding virulence factors and macrolide resistance.

We used comparative genomics to assess serotype M99 genetic diversity and its contribution to GAS pathogenesis. Methods: Next-generation DNA sequencing was conducted for 22 isolates (Italy = 5, Canada = 1, New Zealand = 1, US = 15).

Complete genomes were determined for 1 Italian and 1 US strain. Polymorphisms were identified genome-wide for the cohort and used to infer genetic relationships. Results: The genome of Italian strain 11610 is 1,709,407 bp and lacks prophages. Italian epidemic isolates differed pairwise by ~32 core-genome SNPs. The genome of US strain MGAS11027 is 1,786,881 bp and has 2 prophages encoding secreted virulence factors. US M99 strains differed pairwise by ~397 SNPs. Italian M99s differed from US M99s by ~2064 SNPs. SNPs among US M99s, and differing from Italian M99s, were nonrandomly clustered. Conclusions: SNPs among the M99 strains exceeded those among other GAS serotypes. Most SNPs were attributed to discrete recombination events involving genes encoding virulence factors including surface adhesins, antimicrobial peptides, and secreted anti-immune response proteins. These findings demonstrate that genetic recombination strongly contributes to diversification of M99 pathogenic capacity.

IMIN5. Platelets Amplify IL-6 Trans-signaling
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Background: Human platelets are a key element linking hemostasis, inflammation, and tissue repair. Produced by a broad spectrum of cell types in the cardiovascular system, IL-6 has become a marker of vascular inflammation, associated with a variety of clinically significant outcomes. Recent literature showed a trans-signaling molecular mechanism by which IL-6 could affect leukocyte-recruitment through the release of soluble IL-6 receptor (sIL-6R). We investigated the potential role of platelets in this mechanism. Methods: For each experiment human platelets were isolated from healthy donor single buffy-coat, free of leukocytes. Short time stimulations were performed with thrombine, IL-6, the soluble specific IL-6Rs and Hyper-IL-6, alone or in combination; Hyper-IL-6 is a fusion protein of human IL-6 covalently linked to the human sIL-6R and is 100- to 1000-fold more active than the natural complex, usually used for in vitro experiments. Western blotting, FACS and ELISA were used to analyze platelet reactivity. Results: a) activated platelets can release biologically active sIL-6R, b) the complex IL-6/sIL-6R increases platelet IL-6R and gp130 expression, and c) the complex IL-6/sIL-6R induces STAT3 phosphorylation. Conclusions: We point out the potential role of platelet/IL-6 trans-signaling interaction, which could be another not irrelevant link between inflammation and hemostasis.

IMIN6. Inhibition of Cytokine-Induced Signaling Pathways and Target Gene Expression by Plant Components in Pancreatic Beta Cells
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Background: We have previously found that the extract of Hypericum perforatum (St John’s Wort), SJW) and its component hyperforin (HPF) protect pancreatic beta cells against the cytotoxic effects of cytokines, by acting as powerful inhibitors of the transcription factors STAT-1 and NF-kB. Aims of this study were: a) to explore the time course of STAT-1 inhibition in the INS-1E cell line; b) to further clarify the mechanisms of the regulatory activity of SJW and HPF on the cytokine signaling pathways and expression of target genes. Methods: INS-1E cells, exposed to mixtures of IFN-γ, IL-1β and TNF-a without/SJW or HPF, were used for RT-qPCR gene expression analysis and assessment of phosphorylated components of STAT-1, NF-κB and MAPK pathways by western blotting. Results: SJW and HPF down-regulated STAT-1 even after their removal from the incubation medium before the addition of cytokines or when added 15-30 min following cytokines. The vegetal compounds dose-dependently prevented cytokine-induced STAT-1 phosphorylation in both tyrosine and serine residues. NF-κB activation was hindered through suppression of the p65 subunit phosphorylation and interference with the inhibitory subunit IκB. MAPK cascade was also modulated by SJW and HPF through dose-dependent restriction of ERK1/2 and p38 MAPK phosphorylations. SJW and HPF restrained cytokine-induced mRNA expression of pro-inflammatory genes (e.g., iNOS, CXCL9, CXCL10, ICAM-1) and partially corrected the cytokine-induced unbalance between anti- and pro-apoptotic factors. Conclusions: SJW and HPF, by counteracting crucial mechanisms of cytokine-induced inflammatory and apoptotic alterations, represent interesting pharmacological tools for prevention or limitation of dysfunction and beta-cell loss in diabetes.
**Kidney Diseases**

**KID1. Study of Molecular and Functional Effects Induced in Tubular Primary Cell Cultures by High Glucose Treatment**

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**Background:** Tubulointerstitial fibrosis is an important component of renal injury in diabetic nephropathy. Tubular and tubulointerstitial cells may contribute in different ways to the fibrotic phenotype. We analyzed the effects of high glucose treatment on epithelial tubular cells in vitro to evaluate their contribution to development of renal fibrosis.

**Methods:** Control (DMEM 100mg/dl D-glucose) and treated (DMEM 450mg/dl D-glucose) tubular primary cell cultures have been analysed for gene and miRNA expression by Real Time PCR, FACS, Western blot, Immunofluorescence, and functionally characterized by adhesion and migration assays. Conditioned media from control and treated cells were submitted to Multiplex Cytokine analysis and evaluated for their ability to activate NIH3T3 fibroblasts.

**Results:** Treated tubular cells show a few phenotypic features of EMT, an increase of proliferation and a decrease of migration properties. In these treated cells actin filaments are reorganized in stress fibers with an increase of focal adhesions. The expression of nonreceptor tyrosine kinase Arg, known to regulate cellular morphology and adhesion through RhoGTPases, is decreased and show a different isoform pattern in treated cultures respect to control. Finally, cytokine analysis of culture media was performed.

**Conclusions:** Although high glucose treatment induced cytoskeletal changes and the secretion of pro-inflammatory and fibrotic cytokines in tubular primary cell cultures, it was unable to induce a complete EMT phenotype.

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**Neoplasia – Advances in Molecular Cancer Therapies**

**NAMT1. Photochemical and Photobiological Evaluation of Fluoroquinolones as Anticancer Agents**

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**Background:** Fluoroquinolones are antimicrobial agents used against many infectious diseases. When UVA-irradiated, some of them exert phototoxic and mutagenic effects and those showing the strongest photogenotoxicity have a second fluorine atom in position 8 (other than the one in position 6). The mechanism of action proposed is based on ROS formation, but studies in solution demonstrated a new photochemical reaction producing a triplet-aryl cation able to attack DNA bases. An oxygen-independent photodynamic agent would be a good strategy against cancer, because of its low oxygen pressure. In this study, the photobiological activity of commercially available or newly synthesized fluoroquinolones, optimized to act via any cation, have been investigated.

**Methods:** Ciprofloxacin, lomefloxacin and ofloxacin photoactivity were evaluated in A431 and HeLa cancer cells in terms of intracellular localization, phototoxicity, DNA damage, cell growth inhibition and apoptosis induction. Cells were incubated with each compound for 24h, then UVA-irradiated both at normal oxygen partial pressure and under hypoxic conditions.

**Results:** Fluoroquinolones appeared as small blue spots within the cytoplasm. Most of them colocalized with lysosomes and less with mitochondria. Among the three fluoroquinolones, ciprofloxacin showed the highest phototoxicity, whereas both ciprofloxacin and lomefloxacin markedly impaired cell cycle progression inducing apoptotic cell death. Ciprofloxacin showed a higher damaging potential on DNA plasmid, followed by lomefloxacin, then by ofloxacin. Considerable amount of DNA damage, both as SSBs and oxidized pyrimidine or purine bases, was detected in lomefloxacin- or ciprofloxacin-treated cells.

**Conclusions:** Fluoroquinolones’ photobiological effect was influenced by the oxygen pressure. A bi-hyposensitive mechanism of action can be envisaged.

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**NAMT2. A Novel Molecular Pathway for Cavelin-1 as an Onkocpromoter in Glioblastoma Cells**

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**Background:** Cavelin-1 is an essential structural constituent of caveolae implicated in mitogenic signalling, onco genesis, angiogenesis, neurodegenerative diseases and senescence. Its role as a tumor suppressor gene or as a tumor promoter seems to strictly depend on cell type and tumor stage/grade. The high expression of cavelin-1 in some tumors in vivo is associated with increased tumor aggressiveness, metastatic potential and suppression of apoptosis. **Methods:** In glioblastoma A172, CRS-A2, Li cell lines we found Cav-1 expressed at high levels. The high expression of cavelin-1 in some tumors in vivo is associated with increased tumor aggressiveness, metastatic potential and suppression of apoptosis. **Results:** Fluoquinolones appeared as small blue spots within the cytoplasm. Most of them colocalized with lysosomes and less with mitochondria. Among the three fluoroquinolones, ciprofloxacin showed the highest phototoxicity, whereas both ciprofloxacin and lomefloxacin markedly impaired cell cycle progression inducing apoptotic cell death. Ciprofloxacin showed a higher damaging potential on DNA plasmid, followed by lomefloxacin, then by ofloxacin. Considerable amount of DNA damage, both as SSBs and oxidized pyrimidine or purine bases, was detected in lomefloxacin- or ciprofloxacin-treated cells. **Conclusions:** Fluoroquinolones’ photobiological effect was influenced by the oxygen pressure. A bi-hyposensitive mechanism of action can be envisaged.
proliferation and apoptosis. Then the synergic dose has been used to treat the cell lines to quantify the mRNA of the NIS gene, with real time PCR. Results: On MDA 157 we observed a strong synergy on cell viability between the two compounds. On this cell line synergy between SAHA and PJ34 is observed also at levels of apoptosis. The effect of SAHA and PJ34 was investigated also on the expression of Sodium iodide Symporter (NIS) in MDA 157 and MDA 468 cell line. On MDA 468 cell line synergy among the two compounds, on NIS gene expression, was very strong. Conclusions: These results suggest that combinations between HDACi and PARP inhibitors may be proposed in breast cancer treatment.

NAMT4. PDGFRα Signaling in Liver Regeneration Reveals Novel Redundancies: Implications in Hepatocellular Cancer

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Background: Platelet derived growth factor receptor-α (PDGFRα) is known for its role in mesenchymal cells such as fibroblasts and endothelial cells and other like neurons. We have recently identified low expression of PDGFRα in adult hepatocytes, which is up-regulated in hepatocellular cancer. Methods: To determine the role and regulation of PDGFRα in hepatocyte biology, we generated hepatocyte-specific PDGFRα knockout mice (KO). We examined liver regeneration (LR) in KO and control (WT) mice after partial-hepatectomy (PH). Results: Loss of PDGFRα in hepatocytes was evident at 2 months, albeit no gross, histological or biochemical anomaly was discernible. We identified increased total and active PDGFRα protein at 24hr post PH. Loss of PDGFRα in hepatocytes did not engender any changes in hepatocyte viability (TUNEL) at any timepoint during LR; however, we observed increased hepatocyte proliferation (PCNA) at 72hr during LR after PH. Interestingly, we observed enhanced expression of both EGFR and MET at 24hr post PH. Thus, although PDGFRα blockade enhances, EGF/HGF induced hepatocyte proliferation.

NAMT5. Impact of Histone Deacetylase Inhibitors SAHA and MS-275 on IL-8 Synthesis in Human Melanoma Cells

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Background: Elevated interleukin-8 (IL-8) levels have been observed in patients with metastatic melanoma, and histone deacetylases inhibitors (HDACi) have been shown to influence melanoma progression. In most instances, HDAC inhibitors were positively acting in cooperation with inducers of L-8, whereas in other cases IL-8 expression was down-regulated by HDACi. This study aims at investigating whether the HDACi SAHA and MS-275 affect IL-8 expression in melanoma cells. Methods: Cutaneous and uveal melanoma cell lines were treated with HDACi and IL-8 mRNA and protein were determined by real time-PCR and enzyme-linked immunosorbent assay. The protein content of the main transcription factors involved in IL-8 gene regulation and their binding to IL-8 promoter were evaluated by western blot and chromatin immunoprecipitation assay. Cell proliferation and apoptosis rates were also investigated. Results: HDACi strengthened IL-8 mRNA and protein expression. In parallel, increased cell proliferation and reduced rate of apoptosis were observed. The observed activation of IL-8 by HDACi correlated with increased protein levels of c-Jun. On the contrary, CHOP, RelA- and C/EBPβ synthesis was not affected. Interestingly, SAHA and MS-275 induced c-Jun binding to the IL-8 promoter as well as c-Jun transcription by favoring the recruitment of the preinitiation complex (RNA polymerase II and TFIIB) to the c-Jun promoter. Conclusions: Data reported here indicate that the inhibition of class I HDAC activity is a requisite to activate IL-8 expression in cutaneous as well as uveal melanoma. The increase of IL-8 was mediated by c-Jun promoter activation and was accompanied by enhanced cell proliferation and reduced apoptosis.


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Background: The role of the obesity cytokine leptin in breast cancer progression has raised interest in interfering with leptin’s actions as a valuable therapeutic strategy. Leptin interacts with its receptor through three different binding sites: I-III. Site III is crucial for the formation of an active leptin-leptin receptor complex and in its subsequent activation. Amino acids 39-42 (LDFI) were shown to contribute to leptin site III and their mutations in alamine resulted in mutants acting as typical antagonists. Based on this design strategy, we synthesized the unmodified leptin fragment LDFI and evaluated its activity in both estrogen receptor-positive and -negative breast cancer cells. Methods: The peptide was synthesized by CEMA-Liberty microwave-assisted automated-synthesizer, and characterized by 1H-NMR spectroscopy. We assessed signaling pathway activation by immunoblotting analysis, proliferation by anchorage-dependent and -independent growth assays and migration by wound-healing assays. Results: The LDFI-peptide abolished the leptin-induced phosphorylation of its downstream effectors, as Jak2/STAT3/Akt/MEK, without any agonistic activity. These results correlated with a reduction in anchorage-dependent and -independent growth as well as migration of breast cancer cells. Importantly, the LDFI fragment reversed the leptin-mediated up-regulation of its gene expression, as an additional mechanism able to enhance the peptide antagonistic activity. The described effects were specific for leptin signaling, since the developed peptide was not able to antagonize the other growth factors’ actions on signaling activation, proliferation and migration. Conclusions: We demonstrate that the unmodified LDFI-peptide acts as a full leptin antagonist and could become an attractive option for breast cancer treatment, especially in obese women.

NAMT7. β-Catenin-Mutated Human Hepatocellular Carcinoma (HCC) Cells Show Features of Glutamine Addiction

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Background: High consumption of anaplerotic substrates is a hallmark of cancer cells, which must couple energy production with macromolecular synthesis to sustain a rapid growth. Although most tumors use glucose for anaplerosis, some cancer cells rely on glutamine and, hence, are defined glutamine-addicted. Methods: HepG2 and Huh-7 were grown in low-glucose DMEM supplemented with 10% FBS, 4mM Gl and antibiotics. Glutamine depletion was obtained with L-asparaginase (1U/ml) w/o the GS inhibitor MSO (1mM). Glutamine Synthetase (GS) and SNAT2 mRNA levels were measured with qRT-PCR. Amino acid content was assessed with HPLC. mTOR activation was evaluated determining downstream target phosphorylation with Western Blot. Results: We found that β-catenin-mutated human hepatocellular carcinoma (HCC) HepG2 cells express high mRNA levels for GS and the Gin transporter SNAT2, even when exposed to supra-physiological Gin, and maintain a larger intracellular Gin pool than β-catenin wt Huh-7 counterparts. However, paradoxically, HepG2 cells also exhibit enhanced sensitivity to Gin starvation and increased accumulation of Gin-mimetic GS inhibitors, leading to inappropriate signalling through amino acid dependent pathways, such as mTOR. Consistently, GS inhibitors synergize the cytotoxic effect of the glutaminolytic enzyme L-asparaginase. Preliminary evidence in vivo shows that the combined treatment of nude mice with L-asparaginase and the Gin inhibitor MSO was well tolerated and lowered Gin content in serum, liver and xenografted HepG2 tumors, leading to impaired tumor growth. Conclusions: Thus, the glutamine addicted phenotype, exhibited by β-catenin-mutated HCC cells in vitro, may indicate tumor sensitivity to glutaminolytic treatments in vivo.

NAMT8. Farnesoid X Receptor Activation Induces Apoptosis and Inhibits Leydig Xenograft Tumor Growth

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Background: Leydig cell tumors are the most common tumors of the gonadal stroma and represent about 3% of all testicular neoplasms. In most cases, Leydig cell tumors are benign; however, if the tumor is malignant, no effective treatments are currently available. We have recently reported that farnesold X receptor (FXR) is expressed in R2C Leydig tumor cells, and it reduces the estrogen-dependent R2C cell proliferation by negatively regulating aromatase expression. Methods: R2C tumor xenograft models for in vivo studies. Immunoblotting, DNA laddering and Tunnel assays for apoptosis. Immunoblotting, RT-PCR, reporter-gene assays, mutagenesis experiments, EMSA and ChIP analysis to investigate the molecular
mechanisms. Results: We demonstrated that treatment with GW4064, a specific FXR agonist, markedly reduced tumor growth in R2C xenograft models and induced apoptosis in R2C Leydig cells both in vitro and in vivo. Indeed, FXR ligands induced an enhanced PARP cleavage, a marked DNA fragmentation and a strong increase in the number of apoptotic nuclei. Moreover, FXR activation up-regulated p53 mRNA and protein levels along with an increased expression of its downstream effector p21WAF1/Cip1. Functional experiments showed that FXR ligands up-regulated p53 promoter activity. This occurs through an increased binding of FXR/NF-κB complex to the NF-κB site located within p53 promoter region. Conclusions: These data demonstrate that the induction of apoptotic pathways may represent an additional mechanism through which FXR ligands inhibit Leydig tumor cell growth. From a therapeutic standpoint, strategies aimed to activate FXR might be useful for the treatment of Leydig cell tumors.

NAMT9. Omega-3 Ethanolamides Induce Autophagy through PPAR-gamma Activation in Breast Cancer Cells
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Background: The omega-3 long-chain polyunsaturated fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), elicit antiproliferative effects in cancer cell lines and in animal models. Dietary DHA and EPA can be converted to their ethanolamide derivatives, DHEA and EPEA, respectively; however, few studies are reported on their anticancer activities.
Methods: Proliferation of MCF-7 and MCF-10 breast cells using MTT-assays. Apoptosis and autophagy by DNA fragmentation and monodansylcadaverine labelling, respectively. Transfection experiments using peroxisome proliferator-activated receptor (PPAR)-response-element-reporter plasmid. mRNA and protein levels by RT-PCR, immunoblotting and immunofluorescence analyses. Results: We demonstrated that DHEA and EPEA reduced cell viability in MCF-7 breast cancer cells whereas they did not elicit any effects in MCF-10A non-tumorigenic breast epithelial cells. Since, DHA and EPA are both ligands of PPAR-gamma, we sought to determine whether PPAR-gamma may mediate DHEA and EPEA actions. In MCF-7, both compounds enhanced PPAR-gamma expression, stimulated the transcriptional activity of a PPAR-response-element-reporter plasmid and increased the expression of the oncosuppressor PTEN, a well known PPAR-gamma target gene. PTEN up-regulation caused the inhibition of Akt-mTOR pathways which in turn leads to the activation of either apoptotic or autophagic processes. DHEA and EPEA induced phosphorylation of Bcl-2 promoting its dissociation from beclin-1 which resulted in autophagy induction. We also observed an increase of beclin-1 and LC3 expression. These effects appeared to be PPAR-gamma dependent. Conclusions: DHEA and EPEA acting as PPAR-gamma ligands exert antiproliferative effects by inducing autophagy in breast cancer cells highlighting their potential use in adjuvant breast cancer therapeutics.

NAMT10. Anti-Tumor Activity of Epratuzumab/Saporin-S6
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Background: CD22 represents an attractive molecular target for B-cell neoplasm therapy with immunoconjugates. Epratuzumab, a humanized anti-CD22 mAb has induced tumor regression in preclinical and phase I/II clinical evaluations in patients with indolent or aggressive lymphoma. The results obtained in clinical studies encourage the attempts to improve the Epratuzumab effectiveness, i.e. by conjugation to toxic molecules (immunotoxins). Among plant toxins, the most frequently employed to generate immunotoxins are ribosome-inactivating proteins (RIPs). Saporin-S6 is a RIP often used to construct immunotoxins tested in clinical trials. Methods: The humanized anti-CD22 mAb epratuzumab was conjugated to the toxic enzyme saporin-S6, a type I ribosome-inactivating protein (RIP). The antitumor effect of this immunotoxin has been studied in vitro on CD22+ cell lines and in vivo in an NHL/SCID mouse model. Results: The epratuzumab/saporin-S6 immunotoxin was specifically toxic to five different CD22+ lymphoma cell lines while sparing non-target CD22- cells. The cytotoxic effect was demonstrated in vitro by the complete inhibition of protein synthesis, strong induction of caspase activity, complete loss of viability and total suppression of clonogenic growth of CD22+ cell lines. The immunotoxin showed potent antitumor activity in a SCID mouse Raji xenograft model of human aggressive lymphoma. Conclusions: Our results indicate that it is possible to augment epratuzumab toxicity in target cells by linking the antibody to saporin-S6. An excellent therapeutic index was achieved by this immunotoxin in animals. These results may encourage further evaluation of this conjugate in a phase I clinical trial.

NAMT11. Cell Therapy of Human Cancer: uPAR truncation by Engineered Endothelial Progenitor Cells (EPC) as Intra-Tumoral Shuttles of Anti-Invasion/Anti-Metastatic MMP12
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Background: The onset of angiogenesis (“angiogenic switch”) is a critical step in tumor development and plays a relevant role in cancer progression. The term “angiogenesis” connotes the development of new vessels from pre-existing ones. Identification of bone marrow-derived endothelial progenitor cells (EPCs) in peripheral blood has highlighted an alternative mechanism of angiogenesis (“postnatal vasculogenesis”). However only a subset of EPC, termed endothelial colony-forming cells (ECCFs), have been shown to display the characteristic of a true endothelial cell (EC) progenitor and to possess the ability to form de novo blood vessels in vivo. Many studies suggest an important role of EPC in tumor vascularization and metastasis. ECCFs have been shown to home within tumors and therefore can be used as cellular vehicle to deliver anticancer agents. The membrane-associated plasminogen activation system (uroluinase-type plasminogen activator, uPA; uPA receptor, uPAR) is critical in angiogenesis as well as in invasive properties of cancer cells. Only full length uPAR fosters invasion and angiogenesis. Truncation of uPAR domain 1 by matrix metalloproteinase-12 (MMP12) impairs cell invasion and angiogenesis. Methods: Design and contract of lentivirus encoding MMP12 and delivery of the vector into EFCF. Results: Our preliminary data show that the “gain of function” of MMP12 activity in EFCF shuts can control tumor progression and angiogenesis on several melanoma cell lines. Conclusions: Ex vivo manipulated ECCFs overexpressing MMP12 could be used as cellular vehicle to deliver MMP12 anticancer agent, providing a new way to block growth and metastasis of those tumor which heavily depend on uPAR to perform invasion.

NAMT12. Inhibition of p38MAPK: A Combined Strategy for Sensitizing Neuroblastoma Cells to Etoposide Through the Modulation of Pro-Inflammation Markers
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Background: The tumor-associated inflammatory response has the paradoxical effect of enhancing tumorigenesis and tumor progression. In this regard, there is increasing evidence that the interaction of several pro-inflammatory chemokine receptors with corresponding chemokine ligands are implicated in the growth and invasive phenotype of neuroblastoma (NB). Moreover, it has been reported that in NB cells COX-2 increases migration and modulates the expression of the ICAM-1, an inducible surface glycoprotein that mediates adhesion-dependent cell-to-cell interactions. Methods: The present study has been performed on HTLA-230, a MYCN-amplified NB cell line exposed to etoposide alone or in association with the drugs targeting the intracellular signaling pathways. The oxidative and pro-inflammatory markers have been evaluated by fluorescence microscopy analysis and molecular biology techniques. Results: We provide the evidence that HTLA-230 are highly resistant to etoposide which induces a dose-dependent ROS over-production, DNA double-strand breaks and p38MAPK activation. Therefore, the treatment with etoposide combined with SB203580, an inhibitor of p38MAPK activity has been found to decrease cell viability and tumorigenicity, counteract stem cell development and slow down the cell migration and invasion. In this context, the expression of COX-2, ICAM-1 and CXC4R1 is down regulated, the formation of capillary-like structures is prevented, by generating a phenotype inadequate for tumor development. Conclusions: Collectively, our results suggest that clinical trials of p38MAPK inhibitors, in combination with standard chemotherapy, acting on the modulation of pro-inflammation markers, could be a novel strategy to counteract NB resistance and relapse. (Grants from PRIN 2008N9NLK_002, PRIN 2009MF8BB_002 and Genoa University).

NAMT13. Epigallocatechin Gallate as a New Effective Estrogen Receptor Alpha (ER-α) Down-Regulator in Breast Cancer
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Background: Increasing exposure of the breast to estrogens and other sex hormones is an important cancer risk factor and since estrogens are mitogenic to breast epithelial cells, the magnitude of their effects may be determined by the levels of estrogen receptors (ERs) expressed in the breast. The strong correlation between ER-α expression, breast disease pathophysiology and therapeutic response, make...
ER down-regulators of significant clinical interest. In recent years epigallocatechin gallate (EGCG), a polyphenolic compound found in green tea, evidenced chemopreventive and antitumor properties. **Methods:** Here we investigate whether EGCG effects on breast cancer cell proliferation could be detected following extended treatments with low doses of the catechin. We tested ER+ PR+ breast cancer cell lines, including T47D and MCF-7 cells. **Results:** We report that EGCG causes concomitant PR nuclearization and down-regulation of ER-α protein, mRNA and gene promoter activity. These events appear specifically PR-B dependent, since they are drastically abrogated with PR-B siRNA. EMSA and ChIP assay reveal that, upon EGCG treatment, PR-B is recruited at the half PRE site on ER-α promoter, together with a coexpressor complex containing NCoR and HDAC1. RNA polymerase II is displaced, indicating that the chromatin in this region is in a less permissive environment for gene transcription. Finally we define the functional significance since EGCG produces a significant inhibition of ER+ PR+ breast cancer cell proliferation and anchorage independent growth. **Conclusions:** These results address how EGCG/PR-B signaling may be considered as useful tool to be kept in the adjuvant settings for treatment of breast cancer.

NAMT14. **Generation of Mutants of Helicobacter pylori L-Asparaginase**


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**Background:** Helicobacter pylori L-asparaginase is a recently isolated bacterial factor able to inhibit the cell-cycle of exposed cells, but also a potential platform to develop new anti-cancer drugs, due to its remarkable selectivity for L-asparagine.

**Methods:** To generate, new, useful variants of the enzyme, site directed mutagenesis and random mutagenesis are being used to introduce modifications of the protein. The latter method required finding a powerful selection method to isolate interesting mutants. **Results:** Site-directed mutants generated for L-asparaginase show different levels of activity both towards L-asparagine and L-glutamine. Selection of random mutants is still ongoing. **Conclusions:** Dissection of L-asparaginase activity towards different substrates can be useful to generate better anti-cancer therapeutics.

NAMT15. The Prolyl-His-Isomerase Pin1 Represents a Regulator of Notch3 Protein Functional Activity


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**Background:** Pin1 is a prolyl isomerase involved in the regulation of signal transduction. Its activity is crucial in the control of DNA damage checkpoint pathways and in the modulation of cell proliferation. Alterations of Pin1 are often associated with human malignancies. In particular, a recent report demonstrated the specific role of Pin1 in regulating Notch1 activity, finally contributing to the development of human breast cancer. We thus hypothesized that regulated Notch signaling is regulated by Pin1. We show that Pin1 overexpression in human breast cancer cells causes concomitant PR nuclearization and down-regulation of ER-α protein, mRNA and gene promoter activity. These effects are mediated by direct binding of AR to the breast cancer gene promoter. In turn, increased DAX-1 inhibits SF-1-mediated aromatase expression, thus reducing in situ estrogen production which is responsible for the estrogen-dependent proliferation of breast cancer cells. Our results indicate that the maintenance of gefitinib might be important to control important malignant phenotypes of tumour cells such as loss of epithelial features and the acquisition of invasiveness.

NAMT17. **DAX-1 at the Crossroads Between Androgens and Aromatase:** A Novel Mechanism in the Inhibition of Estrogen-Dependent Cancer Cell Proliferation


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**Background:** Sex hormones, estrogens and androgens, are truly to determine biological response in a tissue- and gender-specific manner. Estrogens, synthesized from androgens by aromatase, influence the pathological processes of several hormone-dependent cancers since they are thought to be the driving force for the formation of breast, endometrial, ovarian and Leydig cell tumors. Likely, the adequate androgen/estrogen ratio represents the most clinically relevant factor in this process; however the molecular mechanism underlying the androgen/estrogen essential balance still needs to be clarified. Here, we investigated the androgen-dependent modulation of DAX-1, a transcriptional co-repressor of genes regulated by steroidogenic factor-1 (SF-1), such as aromatase, as well as of agonist-bound estrogen-receptor. **Methods:** Cell proliferation by MITT-assays. mRNA and protein levels by RT-PCR, immunoblotting and immunofluorescence analyses. Functional studies by luciferase/DAPA/EMSA/ChIP assays. **Results:** Using human breast cancer and rat Leydig tumor cells as experimental systems we demonstrated that ligand-activated androgen receptor (AR) induces the expression of DAX-1 by enhancing its promoter activity. These effects are mediated by direct binding of AR to a newly identified androgen response element within the DAX1-proximal promoter. In turn, increased DAX1 expression inhibits SF1-mediated aromatase expression, thus reducing in situ estrogen production which is responsible for the estrogen-dependent proliferation of carcinoma cells. **Conclusions:** DAX-1 appears to be a specific androgen-target gene in breast and Leydig tumor cells. Since DAX1 expression has been shown to influence cell growth by reversing estrogen-dependent proliferative effects, our study is expected to provide clues for a better comprehension of the AR-dependent inhibition of estrogen-related cancer cell proliferation.

NAMT18. **Effects of New Compounds from Marine Sources in Human Colorectal Cancer Cells**


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**Background:** Several agents originating from marine sources, both plants and animals, have shown anticancer activities. To date, at least 40 compounds isolated from marine sponges have shown anticancer effects, mainly pro-apoptotic, in human. **Methods:** In this preliminary study, we tested the effects of new compounds from marine microorganisms cultured by SZN of Naples and extracted by the Institute of Biomolecular Chemistry ICB, CNR, Naples. In particular, we evaluated the potential antiproliferative effects of five extracts from marine source (MC1, MC2, MC3 MC4 and MC5) in DLD1 and SW620 human colorectal cancer cell (CRC) lines, through MITT and BrdU incorporation assays. **Results:** Results showed that the 24 h and 48h-treatment of DLD1 and SW620 cells with MC5 at concentrations from 1 μM significantly inhibited cell proliferation that was reduced of about 20% by comparison with untreated CRC cells. For MC5 doses higher than 5 μM strongly inhibited colon cancer cell proliferation inducing a percentage of inhibition higher than 90%. Treatment with MC3 10 μM significantly reduced the proliferation after 48h of treatment in both cell lines. Finally, the extract MC2 used at doses higher than 5 μM...
significantly reduced the proliferation in DL1 but not in SW620. Conclusions: Obtained data indicate that M5, M3 and M2 extracts interfere with CRC cell proliferation in a dose- and time-dependent manner. Although further research needs to be focused on clarifying the pathways induced by the observed antiproliferative effects, the results showed that the extracts from marine source evaluated in this study are promising for development of new anticancer drugs.

NEOPLASIA – CANCER STEM CELLS

NSC1. CD133 Protein Regulates Cell Proliferation, Tumorigenility and Drug Resistance in Human Colon Cancer Cells

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Background: CD133 is a transmembrane pentameric molecule considered a putative stem cell marker in several normal and cancer tissues. Surface expression of CD133 identifies a subpopulation of tumor-initiating cells, having the properties of self-renewal, proliferation and multilineage differentiation, in a variety of human cancers, including colon cancer. Although CD133 is considered a useful marker to identify cancer stem cells (CSCs), its exact role(s) in human colorectal tumorigenesis remain unknown. Methods: We found that positive CD133 staining is an independent predictor of shorter survival in colorectal cancer patients. Thus, to shed light on CD133 function(s) and its involvement in the definition of colon CSC phenotype, HCT116 human colon cancer cells were engineered to stably express an exogenous CD133 cDNA, as confirmed by flow cytometry, quantitative real-time PCR and western blot analyses. Results: Increased CD133 expression was associated with an increased anchorage-dependent and independent growth in vitro, an increased mobility and invasiveness and an increased tumorigenicity in vivo. CD133 were also less prone to differentiate when exposed to sodium butyrate. Gene expression profiling identified several genes differentially expressed between CD133-overexpressing derivatives and control cells, including the multidrug resistance-associated protein 2 (MRP2/ABCC2), which was up-regulated in CD133-overexpressing cells, as confirmed by quantitative real-time PCR, and conferred an increased resistance to antineoplastic drugs. Conclusions: These results suggest that, besides its role as a potential CSC marker, CD133 might have an important functional relevance in the definition of colon cancer cell phenotype and might represent a useful molecular target for the development of novel anticancer therapies.

NSC2. MicroRNA Profiles in Different Contexts of Sonic Hedgehog Signaling: Neuronal and Cancer Stem Cells

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Background: Hepatitis B virus (HBV) DNA integration into the host genome is an important pro-oncogenic event in chronic HBV infection. There is evidence that viral integration may occur also in HBsAg-negative patients with occult HBV infection (OBI). Aim of this study was to investigate and characterize HBV DNA integration in OBI patients with hepatocellular carcinoma (HCC). Methods: Tumour specimens from 65 HCC patients were examined (10 HBsAg-positive, 45 OBI-positive and 10 HBsAg-negative/OBI-negative). HBV integration was investigated by Alu-PCR technique. Molecular characterization of virus-genome junctions was performed by cloning and sequencing. Results: Integrated HBV DNA was detected in 31/45 (69%) OBI-positive, 8/10 (80%) HBsAg-positive and 0/10 OBI-negative HCC samples, respectively. In OBI cases, HBV integrants were found both in intergenic (50%) and in intragenic genomic regions (50%). HBV integration frequently targeted genes involved in cell growth and adhesion, angiogenesis and cell signaling (e.g. PHK2A, ADCYS, SCARB1, DNT1P1, TMEM107, CD93). Vital integrants were characterized in 20 cases: HBx gene sequences were found in 12 cases, 4 of whom included viral enhancer-II and basal-core promoter; preS1 region including preS1 promoter was found in 1 case; the carboxy-terminal end of the S region plus a portion of the Pol gene were detected in 2 cases; the preCore region in 1 case; S gene sequences in the remaining 4 cases. Conclusions: HBV DNA integration is a frequent finding in HCC from OBI patients. This evidence leads to hypothesize that HBV DNA integration may play a pro-oncogenic role in all HBV-infected cases independently of the HBsAg status.

NEOPLASIA – HOST PATHOGEN INTERACTIONS IN CANCER

NHP1. Analysis and Characterization of Hepatitis B Virus (HBV) DNA Integration into Chromosomal DNA of Patients with Occult HBV Infection and Hepatocellular Carcinoma.

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Background: Hepatitis B (HBV) DNA integration into the host genome is an important pro-oncogenic event in chronic HBV infection. There is evidence that viral integration may occur also in HBsAg-negative patients with occult HBV infection (OBI). Aim of this study was to investigate and characterize HBV DNA integration in OBI patients with hepatocellular carcinoma (HCC).

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Results: Integrated HBV DNA was detected in 31/45 (69%) OBI-positive, 8/10 (80%) HBsAg-positive and 0/10 OBI-negative HCC samples, respectively. In OBI cases, HBV integrants were found both in intergenic (50%) and in intragenic genomic regions (50%). HBV integration frequently targeted genes involved in cell growth and adhesion, angiogenesis and cell signaling (e.g. PHK2A, ADCYS, SCARB1, DNT1P1, TMEM107, CD93). Vital integrants were characterized in 20 cases: HBx gene sequences were found in 12 cases, 4 of whom included viral enhancer-II and basal-core promoter; preS1 region including preS1 promoter was found in 1 case; the carboxy-terminal end of the S region plus a portion of the Pol gene were detected in 2 cases; the preCore region in 1 case; S gene sequences in the remaining 4 cases. Conclusions: HBV DNA integration is a frequent finding in HCC from OBI patients. This evidence leads to hypothesize that HBV DNA integration may play a pro-oncogenic role in all HBV-infected cases independently of the HBsAg status.
NMTPT3. An Unexpected Role of the Organic Cation Transporter OCTN1 in Autophagy May Underlie Genetic Variant Association with IBD and Colorectal Cancer.

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Background: We have recently demonstrated that the L503F form of the cation transporter OCTN1, a variant conferring genetic predisposition to intestinal inflammatory bowel disease (IBD), is hyper-represented in ulcerative colitis patients progressing to colorectal cancer (CRC), and also in young CRC patients without overt IBD. Unfortunately, molecular mechanisms linking OCTN1 and its variants to bowel inflammation and to its malignant progression are unknown. 

Methods: The two OCTN1 variants 503L and 503F were overexpressed in in 293T cells, and endogenous OCTN1 (SO3L) was knocked down in human monocytic THP-1 cells and colon carcinoma Caco-2 cells. Inflammammasome activation/secretion of IL-1 and autophagy, two phenomena largely involved in IBD genetic risk, were examined in these cell populations. In addition, bone marrow macrophages from wild-type and OCTN1 KO mice were purified and analysed.

Results: 
- Maturation/secreton of IL-1β by 293T cells was significantly increased by OCTN1, with Crohn’s associated SO3F variant having the strongest effect; conversely, IL-1β release was impaired in OCTN1-depleted THP-1 cells and in OCTN1 KO macrophages. Surprisingly autophagy, monitored by western blotting and LC3-GFP immunofluorescence, followed the same trend in the different cell lines. Moreover, in 293T cells, OCTN1 colocalized with the lysosomal marker cathepsin B, and inhibition of autophagy by 3-MA reduced the processing of pro-IL-1β to mature IL-1β.

Conclusions: Taken together these results suggest an unexpected role for OCTN1 in the autophagic control of inflammation. Moreover, since autophagy is often deregulated in cancer cells, our findings may explain OCTN1 association with both IBD-related and sporadic CRC.

NMTPT3. NGAL, a NF-kB-Regulated Gene, Is a Chemotactic Factor in Thyroid Cancer

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Background: NGAL expression is increased in thyroid nodules, which are frequently associated with thyroid cancer.

Results: NGAL was identified as a potential chemotactic factor for inflammatory cells in tumor microenvironment. To test this hypothesis, we down-regulated NGAL expression in the CT26 mouse colon-carcinoma cell line and inoculated parental and NGAL-null CT26 cells in syngeneic mice to analyze the inflammatory infiltrate during tumor development.

The immunohistochemical analysis of tumors from injected mice showed that the number of lymphocytes and macrophages in tumors developed by NGAL-null CT26 mice was strongly reduced with respect to that of parental CT26 mice. 

Conclusions: These data suggest that NGAL secretion by cancer cells could serve as chemotactic factor for inflammatory cells in tumor microenvironment.

NMTPT4. Mechanism of Action of Metformin as a Potential Angiopreventive Compound

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Background: A significantly decreased risk for cancer in diabetics taking metformin, an antiglycemic drug, has been observed. Inhibiting angiogenesis is a key and common effect of many agents able to repress cancer in pre-clinical and clinical studies and appears to be a key strategy for cancer prevention. However, conflicting data concerning the angiogenic action of metformin have been reported and mechanistic aspects remain to be addressed. 

Methods: We investigated the effects of metformin on angiogenesis in vivo with the matrigel sponge assay and in vitro using human umbilical vein endothelial cells (HUVEC) to verify the capability of metformin to interfere with endothelial proliferation, cell death, migration and invasion.

Results: Our data clearly show that in vivo metformin inhibited VEGF induced angiogenesis. To examine the mechanisms underlying this activity, we found that metformin inhibited endothelial cell proliferation without inducing apoptosis by exerting effects on key determinates of cell cycle regulation. Metformin inhibited endothelial cell invasion and repressed the ability of HUVECs cells to organize into capillary-like networks in the presence of angiogenic stimuli. These effects were largely reverted by compound C, a specific inhibitor of AMPK, suggesting that the mechanism involves activation of AMPK signaling. Gene expression profiles of endothelial cells treated with metformin showed down-regulation of several angiogenesis-related genes. Conclusions: Taken together, our results show a clear anti-angiogenic action of metformin that appears to act directly on endothelial cells that may in part explain the reduced tumor incidence in patients on metformin.

NEOPLASIA – NOVEL BIOMARKERS IN ONCOLOGY

NBM1. Molecular and Functional Characterization of Annexin A3 in Human Normal Cortex and Renal Cell Carcinoma Primary Cultures

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Background: Annexin A3 (AnnA3) is a annexin family protein, a cell surface protein and a known regulator of cell membrane. 

Methods: Immunofluorescence. Cell motility and invasion was assessed in the siRNA/sh- RNA transfected cell lines. 

Results: Normal cortex and RCC primary cultures show a differential expression pattern, correlated with HIF-1α level, of two AnnA3 isoforms of 33 and 33 kDa. Moreover, normal AnnA3 protein level results down-regulated in RCC respect to cortex cultures. 

Conclusions: Annexin A3 is a promising target for the treatment of RCC.

NBM2. Kaiso, a Key Regulator in EMT and Cancer Progression

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Background: Advanced stages of cancer are characterized by increased aggressiveness, invasiveness, and the down-regulation of tumor suppressor genes via methylation. Kaiso, a bimodal transcription factor, interacts with DNA through either a DNA consensus sequence or methylated CpG dinucleotides, thus regulating gene expression. A clinical role for Kaiso expression in advanced stages of breast and prostate cancer remain unclear. Here we hypothesize that there will be a correlation between Kaiso expression, localization, and breast and prostate cancer progression. 

Methods: Immunohistochemistry was performed to examine protein expression and localization human breast and prostate tissue. Kaiso-specific siRNA/shRNA small hairpin (sh) RNA was used to decrease Kaiso expression levels in MDA-MB-231, MDA-MB-468, DU-145, and 143B cells. Cells were transfected with a transfection factor, interacts with DNA through either a DNA consensus sequence or methylated CpG dinucleotides, thus regulating gene expression. A clinical role for Kaiso expression in advanced stages of breast and prostate cancer remain unclear. Here we hypothesize that there will be a correlation between Kaiso expression, localization, and breast and prostate cancer progression.
Kaiso to regulate these key characteristics of cancer progression suggests it is a relevant therapeutic target and a potential indicator of breast and prostate cancer.

**NBM3. Blood Cholesterol and Sphingomyelin in Patients with Cancer**

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**Background:** It is generally believed that high levels of blood cholesterol are harmful to health, whereas low levels seem to be positive and therefore are often neglected. Unfortunately this assumption is far from true, since severe hypercholesterolemia can be indicative of cancer. Cancer cells avidly incorporate serum cholesterol, favouring the expression of proteins involved in cell proliferation such as RNA polymerase II, STAT3, PKCz and cyclin D1 (Pugliese et Al. Eur. J. Cancer 46:1735). Numerous studies have shown that a strong interaction exists between unesterified cholesterol and saturated fatty acid sphingomyelin, which arises from the Van der Waals interaction. Since sphingomyelin and cholesterol association is responsible for formation of membrane lipid rafts involved in cell signalling we have studied the possible hypocholesphingomyelinaemia associated to hypercholesterolemia in patients with cancer. **Methods:** The blood of 25 patients with monocular gromapathy were analyzed for their proteins and lipids content. **Results:** The results demonstrated that the patients with high level of gamma proteins present a strong decrease of both cholesterol and sphingomyelin content in blood. **Conclusions:** The results suggested the possible incorporation of cholesterol-sphingomyelin nano-sized vesicles that could change the structure/function of cell membrane or intracellular virstomes. To investigate the reason for increased lipid incorporation by tumour cells, we performed lipidomic study on the serum of the same patients. Initial results show a lipid profile different from that of healthy subjects.

**NBM4. B-Lymphocyte Stimulator Versus Chromogranin A in the Follow-Up of Patients with Neuroendocrine Tumors**

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**Background:** Chromogranin A (CgA) is the most recommended marker in neuroendocrine tumours (NET), but generally presents low sensitivity and specificity. We recently (Fabris et al. Immun Endoc & Metab Agents in Med Chem 2011) described high levels of B-lymphocyte stimulator (BLyS) in patients with neuroendocrine tumours (NET). Here we assessed BLyS role in the follow-up of NET patients compared to CgA. **Methods:** We enrolled 109 NET patients (25.7% lung, 74.3% gastro-enteropancreatics, 56% low grade, 44% high grade), 66.7% with pathological CgA serum levels and 37.6% metastatic. Patients were classified in 3 subgroups: in remission (19), with evidence of persistent but stable disease (42), relapsing or further progressive disease (48). BLyS and CgA were analyzed by ELISA, in 41 patients also after 6 ± 6 months. **Results:** We confirmed BLyS up-regulation in NETs, 65% presenting pathological levels. BLyS did not correlate significantly with CgA. Progressive patients presented higher BLyS than stable and remission (P < 0.0001). Compared to CgA, BLyS appeared slightly less specific (high BLyS in 29.4% vs. CgA pathological in 17.6% of remission cases; P = ns), but significantly more sensible for progression (high BLyS in 87.5% vs. CgA pathological in 68.8% of progressive patients; P = 0.019). In CgA-negative cases, 86.7% of progressive patients presented high BLyS levels compared to 28.6% of remission cases (P = 0.0025). In the follow-up, BLyS remained unchanged in stable patients, decreased in improving patients (P = 0.0078) and increased significantly in relapsing (P = 0.03). CgA did not change significantly in relapsing. **Conclusions:** BLyS appears as a new useful marker in the follow-up of NET patients.

**NBM5. Gelatinolytic Activities in the Sera and in the Urine from Patients with Prostate Diseases**

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**Background:** It is widely recognized that the serum prostate-specific antigen (PSA) as a biomarker of prostate cancer is imperfect, in that it can have many false positive elevations attributable to benign hyperplasia and subclinical prostatic inflammation. There are increasing data that support a positive correlation between gelatinase and Gleason score or pathological findings was found. **Conclusions:** These results suggest that the inexpensive measurement of MMPs may serve as a suitable supplementary tool to distinguish between patients with prostate cancer and patients with BPH, and the addition of this enzyme to currently available PSA and/or f-PSA/f-PSA-ratio might provide clinicians additional objective information on prostate carcinomas.

**NBM6. PGE2 Induces Epigenetic Modifications and Up-Regulation of IL-8 in Gene in Astrocytoma**

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**Background:** It is now well recognized that chronic inflammation is a risk factor for cancer. Several pro-inflammatory gene products, among which interleukin-8 (IL-8), have been linked to tumorigenesis, promotion, proliferation, invasion, angiogenesis, and metastasis. We previously reported that astrocytomas express high levels of this chemokine in response to an increased synthesis of prostaglandin E2 (PGE2). Here, we investigated whether the PGE2-induced IL-8 activation is mediated by epigenetic modifications. **Methods:** DNA methylation status of the 6 CpG sites within IL-8 promoter region and histone acetylation levels were analyzed in two astrocytoma cell lines of different malignancy grade and normal astrocytic cells by bisulphite sequencing and chromatin immunoprecipitation, respectively. IL-8 mRNA was quantized by real-time PCR and protein levels were measured by enzyme immunoassay. **Results:** PGE2 activated IL-8 transcription through specific demethylation of an individual CpG residue (nucleotide -83) located within the C/EBP-beta consensus sequence in the IL-8 promoter and abnormal acetylation of histone H3 in this region of chromatin. These observations promoted the recruitment of C/EBP-beta transcription factor which, in turn, formed a docking platform for p300 coactivator, leading ultimately to enhanced transcriptional potential of IL-8. **Conclusions:** Our findings have elucidated an orchestrated mechanism triggered by PGE2 whereby concurrent association of site-specific demethylation and histone hyperacetylation resulted in derepression of IL-8 gene expression in astrocytomas. These observations imply that anti-inflammatory agents that suppress IL-8 or IL-8-regulated products should have a potential in both the prevention and treatment of this cancer.

**NBM7. Polymorphisms of Genes of TGF-ß Pathway and Susceptibility to Colorectal Cancer**

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**Background:** Genetic background implicated in cytokine network may have a key role in the susceptibility to colorectal cancer (CRC). The TGF-ß pathway is involved in several biological processes, including cell proliferation, differentiation, migration and apoptosis. **Methods:** rs1800471 SNP polymorphism of TGF-ß1 rs334348 and rs334349 of TGF-ß1 rs9100 of TGF-ß2 and rs4522809 of TGF-ß2 were typed in a group of 82 patients affected by sporadic CRC and in 237 age- and sex-matched healthy controls, using a competitive allele specific PCR assays (KASPPar). **Results:** No significant genetic contribution has been observed for 3 of the 5 SNPs tested. Indeed, a significant different allelic distribution between patients and controls has been observed for the polymorphism G—C (rs1800471) responsible for an arginine vs. proline missense change (R25P) in codon 25 of the TGF-ß gene (P = 0.021). By this analysis, a weak protective role would emerge for the minor allele C in the susceptibility to the disease. Furthermore the analysis of genotype and allelic frequencies of rs4522809 showed a statistically significant difference (P = 0.0016 and P = 0.0019 respectively) between patients and controls. **Conclusions:** All together these results, suggest that functional relevant SNPs of TGF-ß pathway might be involved in susceptibility to CRC, influencing the extension and severity of the disease.

**NBM8. Differential Expression Profiling of MicroRNAs in Human Cutaneous and Uveal Melanoma**

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**Background:** Over the past decade, microRNA (miRNA)-mediated epigenetic regulation of tumour suppressor genes and oncogenes has been shown to play a central role in melanogenesis. Here, we examined the miRNA signature discriminating cutaneous and uveal melanoma. **Methods:** Genome-wide profiling of miRNAs was performed in cutaneous and uveal melanoma cell lines by human miRNA microarray platform (Agilent Sanger miRBase-release 10.1) with 723 human
and 76 human viral miRNAs represented. Agilent Feature Extraction Software was used for background subtraction. LOWESS and Quantile normalizations were performed. miRNA microarray expression data were validated by RT-PCR. Results: Relative to normal melanocytes, in uveal melanoma cells, miR-130b, miR-193b, miR-320a, and miR-9* significantly decreased, and miR-654-3p markedly increased, in cutaneous melanoma cells, miR-199a-3p and miR-22 were down-regulated, whereas let-7g was up-regulated. Two of these miRNAs, miR-193b and let-7g, were previously shown as potential regulators in melanoma, whereas the other ones have not been related to melanoma yet. Conclusions: Our analysis enables us to identify miRNAs that have not previously been associated with melanoma. In addition, the comprehensive survey of differentially expressed miRNAs shows remarkable differences between cutaneous and uveal melanoma. Although the study is preliminary, we believe the results add to the present knowledge on miRNA dysregulation in melanoma carcinogenesis. As such the results would serve as a starting point for identifying the direct targets of key miRNAs and elucidating their mechanisms of regulation. Understanding the functional roles of miRNAs in melanoma will contribute to the development of targeted therapy.

NB9. Higher Frequency of Hypermethylation of p16INK4A Compared to p14ARF among Cutaneous Melanoma Patients from Southern Italy
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Background: Epigenetic deregulation due to p14ARF and p16INK4A promoter hypermethylation has been previously reported in many cancers, including cutaneous melanoma (CM). Nevertheless, discriminating the involvement of these genes in CM still remains an open question. Methods: p14ARF and p16INK4A were analyzed in 60 CM formalin-fixed paraffin-embedded tissue sections and in G-361 and GR-M cutaneous melanoma cell lines by methylation-specific PCR and sequencing. Gene expression was evaluated by qReal-time PCR. Cell lines were treated with demethylating agent 5-aza-2'-deoxycytidine. Results: p16INK4A gene promoter methylation was found in 36 of 60 (60%) melanoma tissues, 12 of which were heterozygous and the others homozygous. Conversely, p14ARF was found methylated in heterozygous status in 19/60 (31.67%) cases. Hypermethylation of both genes showed low frequency (10%). G-361 and GR-M cell lines were identified as homozygous methylated in p15INK4A and unmethylated in p14ARF. Loss and decrease gene expression was observed in homozygous and heterozygous status, respectively. All cells exhibited demethylation and induction of gene expression after 5-aza-2’-dC treatment. Conclusions: Here we showed a higher frequency of methylated CpGs in p16INK4A compared to p14ARF in cutaneous melanoma, unlike other studies that have reported the opposite. As this is the first detailed investigation of the frequency of p16INK4A and p14ARF methylation in CM, it is likely that the well-known high occurrence of melanoma in this region may be associated to aberrant methylation of p16INK4A. To note, CM tissue analysis facilitates a heterogeneity of results not otherwise demonstrable in cell lines.

NBM9. A New High-Speed Nested PCR-RFLP Method for the Screening Of V600E BRAF Mutation in Thyroid Tissue and Cytological Samples
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Background: Recent studies have shown that BRAF activation point mutations are present in about 45% (range 29-69%) of papillary thyroid cancers (PTC). Almost 90% of the mutations are BRAF T1799A transversion in exon 15 which results in a (V600E) substitution. Fine needle aspiration biopsy (FNAB) is the primary tool to distinguish benign from malignant nodules. The aim of this study is to establish an accurate and sensitive molecular method to detect BRAF V600E mutation as a biomarker of early PTC. Methods: We analyzed the genomic DNA extracted from 100 PTC patients, previously characterized by cytological examination. BRAF V600E mutation was evaluated by RFLP-PCR, using an artificial HpyCH4-IV restriction site in the PCR product, corresponding to V600E mutation. Our method detected heterozygous V600E BRAF mutation in 40% of PTC samples analyzed. Results: All samples analyzed in heterozygous showed a panel of three restriction fragments (147, 126 and 21 bp), whereas the wild-type samples showed a pattern of two restriction fragments (126 and 21 bp). The results of sequencing are overlapping up to a concentration of pathological cells of 35%, whereas our nested PCR-RFLP method was able to discriminate to a sensitivity of 1% pathological cells. Conclusions: The optimization of the method of analysis performed on all samples by direct sequencing enhances the precision, accuracy, specificity and sensitivity of both the method and results. These data are indicative for an innovative and sensitive technique for the evaluation of the BRAF V600E mutation and can be a useful tool for screening BRAF V600E mutations.

NEOPLASIA – TUMOR IMMUNITY
NTM1. Immuneogenic Dendritic Cell Selection by Natural Killer Cells During Anti-Cancer Immune Response
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Background: Previous studies have reported that activated natural killer (NK) cells can kill autologous immature dendritic cells (DCs) in vitro, whereas they spare fully activated DCs. This led to the proposal that activated NK cells might select a more immunogenic subset of DCs during a protective immune response. However, there is no demonstration that autologous DC killing by NK cells is an event occurring in vivo and, consequently, the functional relevance of this killing remains elusive. Methods: NK cells were activated in a mouse model by injecting MHC-devoid cells. Draining lymph nodes were collected and lymph node DC functions analyzed. Finally, in a model of anti-cancer vaccination, the functional relevance of DC editing by NK cells was investigated. Results: A significant decrease of CD11c+ DCs was observed in draining lymphnodes of mice inoculated with MHC-devoid cells. Residual lymph node DCs displayed an improved capability to induce T cell proliferation. In addition, during anti-cancer vaccination, the administration of MHC-devoid cells together with tumor cells increased the number of tumor-specific CTLs and resulted in a significant increase in survival of mice upon challenge with a lethal dose of tumor cells. Depletion of NK cells or the use of perforin knockout mice strongly decreased the tumor-specific CTL expansion and its protective role against tumor cell challenge. Conclusions: Our data support the hypothesis that NK cell-mediated DC killing takes place in vivo and is able to promote expansion of cancer-specific CTLs. Our data also indicate that cancer vaccines could be improved by strategies aimed at activating NK cells.

NTM2. Gene Regulation by microRNA Is Associated with CD8+ T Cells Immunodepression in Renal Cell Carcinoma Patients: Role of JAK3 and MCL-1
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Background: MicroRNAs (miRNAs) are important regulators of gene expression and numerous miRNAs are expressed aberrantly and correlate with tumorigenesis. In patients with renal cell carcinoma (RCC), T cell immune dysfunctions have been reported. The aim of our study was to assess gene expression profiles and their regulatory mechanisms by miRNAs on CD8+ T cells from RCC patients, at basal (day 0) and after stimulation against RCC line (day 35). Methods: We compared autologous and allogeneic CD8+ T-cell responses against RCC line generated from RCC patients and their HLA-matched healthy donors. We then analyzed the gene expression profiles of CD8+ T cells by microarray approach and then identified molecular mechanisms of gene regulation by miRNAs analysis. Results: Comparison of gene expression data in allogeneic CD8+ T cell vs autologous RCC-reactive CD8+ T cells demonstrated differential expression of genes involved in apoptosis and regulation of cell proliferation. Among these genes, the down-regulation of JAK3 and MCL-1 gene expression in patient CD8+ T cells versus their healthy counterparts was observed. We found evidence for defective suppressor activity of miR-29b and miR-198 in regulating gene expression of JAK3 and MCL-1 in RCC CD8+ T cells. Transfection experiments on isolated PBMCs from RCC patients using anti-hsa-miR-29b and anti-hsa-miR-198 inhibitors revealed a significant up-regulation of both proteins and a significant improvement of cell survival in vitro. Conclusions: miR-29b and miR-198 play a key role in regulating immune-mediated mechanisms by interfering in CD8+ T cells gene expression of JAK3 and MCL-1 and may have important therapeutic implications.

NTM3. CD40 Cross-Linking Induces Migration of Renal Tumor Cell Through NFAT Activation and Integrin β1 Reorganization
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Background: CD40 cross-linking plays an important role in regulating cell migration, adhesion and proliferation in renal cell carcinoma (RCC). CD40/CD40L interaction on RCC cells activates different intracellular pathways, however the molecular mechanisms leading to cell scattering are not clearly defined. Aim of our study was...
to investigate the principal intracellular factors activated by CD40 ligation and their specific involvement in RCC cell migration. Methods: RCC cell lines were isolated from kidney tissue samples of patients affected by RCC and subsequently stimulated with CD40L. Results: We found that CD40-CD40L interaction induced cell proliferation through a cytoskeleton reorganization and integrin β1 distribution, whereas it did not affect apoptosis. Interestingly, CD40 ligation did not activate the pathway involving phosphatidylinositol 3-kinase (PI3K), Akt and p70 ribosomal S6 kinase, but it increased the phosphorylation of extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK) and p38 MAPK. Furthermore, CD40 crosslinking activated different transcriptional factors on RCC cell lines: AP-1, NFκB and some members of the NFATs family. In particular, the specific inhibition of NFAT factors by cyclosporine A, completely blocked RCC cell motility induced by CD40 ligation. Conclusions: These findings support the hypothesis that CD40 ligation induces cell scattering through cytoskeleton reorganization and activation of different intracellular signalling pathways, in particular the NFATs family. These factors could represent a potential therapeutic target in the setting of patients with metastatic RCC.

NEOPLASIA – TUMOR PROGRESSION

NTP1. Estrogen Induces Looping Between Tumor Suppressor RIZ Gene Promoter 2 with Exon 9a C. De Rosa1, E. Di Zazzo2, E. Todisco1, E. Griffo3, M. Spinelli4, M. Ombra5, B. Monchamont6, N. Medici7, B. Perillo8, C. Abbondanza9 1Seconda Università di Napoli, Naples, Italy; 2Università degli studi del Molise, Campobasso, Italy; 3C.N.R., Avellino, Italy

Background: The dynamic intra- and inter-chromosomal links between specific loci contribute to the creation of cell type-specific gene expression profiles and to gene regulation during differentiation processes. Looping is implicated in bringing together far upstream or downstream regions with the gene promoter and body sites, and in establishing contacts between the 5' and 3' ends of genes, since 3' end-processing factors interact with components of the transcriptional machinery. The tumor suppressor PRDM2/RIZ gene plays a role in controlling cellular processes, such as cell cycle progression and regulation of development. The retinoblastoma protein-interacting zinc-finger gene (RIZ) is estrogen responsive and has two alternative promoters, the more downstream of which, promoter 2, is nearby to an ERE-silencer sequence and is involved in estrogen receptor transcriptional activation. Methods: With the innovative DNA-Picked Chromatin (DPC) assay after timecourse of 3h estrodiol (E2) induction of MCF-7 breast cancer cells, we highlight preferential interaction between hormone-responsive RIZ promoter and the polyadenylation sites. Gene expression analysis of induced cell RNA was performed with qRT-PCR assay. Results: Within 60' of E2 treatment of cells, we have observed increased exon segments, exons 9a and 10 (alternative polyA site), linked to isolated promoter 2 and concomitant decrease of exon910 to RIZ promoter 1. The exon 9a shows a low association to R-PCR without E2. qRT-PCR also demonstrated increased exon 9a-containing transcripts. Conclusions: The DPC model the chromatin architecture of PRDM2/RIZ gene locus to create a loop for the mRNA transcription with polyA-exon 9a, leading to the production of oncogenic variants.

NTP2. Role of ER-α in the Modulatory Effect of Adiponectin on Breast Cancer Cell Growth M. Pellegri1, L. Mauro1, F. De Amicis1, E. Ricchio1, S. Ando2 1University of Calabria, Arcavacata di Rende, Italy

Background: Several studies suggest that adiponectin, a hormone mainly produced by adipose tissue, may influence cancer pathogenesis. Circulating adiponectin levels are inversely associated with an increased risk of breast cancer. Methods: qRT-PCR assay, immunolocalization, RT-PCR, transient transfection, ChIP assay. Results: We demonstrated that adiponectin inhibited proliferation in ER-α-negative cells, whereas it stimulated growth in ER-α-positive cells. Adiponectin is able to reproduce the classic features of ER-α transactivation in MCF-7 cells: nuclear localization, down-regulation of its mRNA and protein levels, and up-regulation of the estrogen-dependent genes, catepsin D and p52. In MCF-7 cells, adiponectin up-regulated mRNA and protein levels of cyclin D1 (CD1), which, in contrast, appeared down-regulated in MDA-MB-231 cells. Similar opposite effects were elicited by adiponectin on CD1 promoter activity. Mutagenesis studies revealed that the modulation of CD1 promoter activity by adiponectin was mediated mainly by the Sp1 motif. Moreover, adiponectin induced Sp1 nuclear localization and its phosphorylation. To provide insight into the molecular mechanism by which the Sp1 motif modulates CD1 promoter activity, we performed ChIP experiments. In MCF-7 cells adiponectin increased Sp1/ER-α complex, enhanced Pol-II and pCAF recruitment, addressing the involvement of an activator complex that mediated the adiponectin-induced transcriptional activation of CD1. In contrast, in MDA-MB-231 cells adiponectin recruited Sp1, displaced Pol-II and recruited a co-repressor complex containing SMRT, NCoR and HDAC1. Conclusions: Thus, on the basis of our findings we suggest that a proper use of novel therapeutic tools potentiating adiponectin signaling in breast cancer may target ER-α-negative tumor growth and progression.

NTP3. Estrogen Receptor-β, through Sp1, recruits a Co-repressor Complex to the Estrogen Receptor-α Gene Promoter in Breast Cancer Cells D. Zito1, P. Rizzà1, V. Bartella1, I. Barone1, F. Giordanà1, C. Giordanà1, L. Mauro1, S. Catalano1, D. Scià1, M. Panni1, S. Fucà2, S. Andò2 1Università Della Calabria, Cosenza, Italy; 2Baylor College of Medicine, Houston, TX, United States of America

Background: In the regulation of mammary gland growth and development human estrogen receptors (ERs) α and β play a crucial role. Normal breast tissues display a relative higher expression of ER-β than ER-α, which drastically changes during breast tumorigenesis. The different ratio of expression of the two proteins may be involved in breast carcinogenesis development. However, the molecular mechanisms underlying the potential opposing roles played by the two estrogen receptors on tumor cell growth remain to be elucidated. Methods: Transient transfection, Western Blotting, EMSA, ChIP, RT PCR, silencing. Results: In this study, we have showed that ER-β overexpression in breast cancer cells decreases cell proliferation and down-regulates ER-α mRNA and protein content, along with a concomitant repression of estrogen-regulated genes. Deletion analysis of the human ER-α promoter region, indicated that elevated levels of ER-β down-regulated basal ER-α promoter activity. Furthermore, site-directed mutagenesis revealed that the proximal GC-rich motifs at -223 and -214 are critical for the ER-β-induced ER-α down-regulation in breast cancer cells. The results indicate an enhancement recruitment of Sp1 and ER-β, together with the corepressor NCoR, to the ER-α promoter region concomitant with the hypoacetylation of histone H4 and displacement of RNA polymerase II. Silencing of NCoR gene expression by RNA interference reversed the down-regulatory effects of ER-β on ER-α gene expression and cell proliferation. Conclusions: Our data suggest a novel mechanism by which overexpression of ER-β through NCoR is able to down regulate ER-α gene expression, thus inhibiting the driving role of ER-α on breast cancer cell growth.

NTP4. Role of Argonaute 2 in Estrogen Receptor-β-Mediated Transcriptional Gene Silencing in Breast Cancer Cells R. Tarallo1, G. Nassà1, M. Ravì1, G. Giurato1, F. Rizzo1, C. Cantarella1, C. Stellato1, A. Weiss2 1Laboratory of Molecular Medicine and Genomics, University of Salerno, Baronissi, Italy

Background: Estrogen receptor (ER) is the primary target for chemoprevention and endocrine therapy in breast cancer and provides prognostic and predictive information about tumor response to endocrine therapies. Expression of ER-β reduces tumor cell proliferation and tumor growth, suggesting an anti-proliferative and a positive prognostic value of this receptor subtype. Although ER-β seems to be a tumor suppressor, its role in human breast carcinogenesis remains to be elucidated. ER-α and ER-β could show the ability to interact with the same proteins resulting, however, in divergent transcriptional effects. Moreover, it has emerged that unilaganted ER-β exhibits an active role in constitutive regulation of target genes transcription. Methods: Tandem affinity purification (TAP) and mass spectrometry (MS) were applied to identify unilaganted ER-β nuclear interacting proteins. Among the over 300 partners identified we investigated the functional significance of ER-β/A-GO2, since this last protein has been directly implicated in transcriptional gene silencing (TGS) induced by microRNAs. Co-immunoprecipitation assays, confocal microscopy (Proximity Ligation Assay/PLA) CHIP-Seq, siRNA-mediated gene knock-down (KD) and gene expression profiling were applied to this end. Results: Co-immunoprecipitation and PLA confirmed ER-β/A-GO2 association in MCF-7 cell clones expressing tagged ER-β. Co-immunoprecipitation was also observed for several known A-GO2 interacting proteins, identified by TAP/MS as ER-β partners. ChIP-Seq allowed the identification of a large number of ER-β and A-GO binding sites and the corresponding target genes, whereas AGO2 KD resulted in significant changes in gene expression profiles. Conclusions: Experimental evidences suggest that AGO2 is a partner of ER-β involved in gene regulation in hormone responsive breast cancer cells. Supported by AIRC; MIUR; Regione Campania; University of Salerno; Fondazione e con il Sud; EU COST Action BM1006 and SeqAhead, FEBS Short Term Fellowship to G.Nassà.
NTP5. Interaction between PPAR \( \beta \gamma \) by the combined treatment, could be involved in these biological responses. Affymetrix analysis in CaCo-2 cells revealed that some genes were highly modulated effects of rosiglitazone (PPAR \( \beta \gamma \)) transcriptional activity can be negatively regulated by JNK-mediated

Background:

We observed that miR-196a is overexpressed in human primary lung cancer cells and investigated, through microarray analysis, the genes involved in these processes. Results: Microarray data analysis identified several differentially expressed miRNAs in lentivirus-infected cells compared to the parental cell lines. In particular, miR-196a was significantly up-regulated in BEAS and down-regulated in H460. Subsequently, we observed that miR-196a is overexpressed in human primary lung cancer samples. miR-196a is predicted, using bioinformatic tools, to target genes, such as FoxO1, FoxO3 and p27, involved in the PPAR\( \beta \gamma \) pathway. The generation of stable cellular clones which overexpress miR-196a, or silenced for this miRNA through the expression of an anti-miR-196a, is important to determine the role of miR-196a in the development of human lung cancer. By cell migration assays we demonstrated the involvement of miR-196a in the increased ability of cells to migrate. Conclusions: Further biological studies are necessary to characterize the role of miR-196a in NSCLC.

NTP7. Rosiglitazone and AS601245 Decrease Cell Adhesion and Migration Through Modulation of Specific Gene Expression in Human Colon Cancer Cells

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Background: PPAR\( \gamma \) are nuclear receptors activated by ligands. Activation of PPAR\( \gamma \) leads to a reduction of adhesion and motility in some cancer models. PPAR\( \gamma \) transcriptional activity can be negatively regulated by JNK-mediated phosphorylation. We postulated that the use of agents able to inhibit JNK activity, could increase the effectiveness of PPAR\( \gamma \) ligands. Methods: We analysed the effects of rosiglitazone (PPAR\( \gamma \) ligand) and AS601245 (a selective JNK inhibitor) alone or in association, on adhesion and migration of CaCo-2, HT29, and SW480 human colon cancer cells and investigated, through microarray analysis, the genes involved in these processes. Results: Cell adhesion and migration was strongly inhibited by rosiglitazone and AS601245. Combined treatment with the two compounds resulted in a greater reduction of the adhesion and migration capacity. Alphometrix analysis in CaCo-2 cells revealed that some genes were highly modulated by the combined treatment, could be involved in these biological responses. Rosiglitazone down-regulated the expression of fibrinogen chains \( \alpha \), \( \beta \), \( \gamma \) which were further down-regulated by the combined treatment. Moreover, rosiglitazone, alone or in association with AS601245, caused a decrease in the fibrinogen release. ARHGEF7/\( \beta \)-PIX gene was highly down-regulated by combined treatment, and western blot analysis revealed that \( \beta \)-PIX protein is also down-modulated in CaCo-2, HT29 and SW480 cells. Transfection of CaCo-2 cells with \( \beta \)-PIX gene completely abrogated the inhibitory effect on cell migration, determined by rosiglitazone. AS601245 and combined treatment. Conclusions: Results demonstrated that \( \beta \)-PIX protein is involved in the inhibition of cell migration and sustain the positive interaction between PPAR\( \gamma \) ligands and anti-inflammatory agents in humans.

NTP8. The Outcomes of Notch3-Dependent T Cell Leukemia Are Modified by NF-\( \kappa \)B Deletion

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Background: The Notch3 deregulation inside T-cell compartment of transgenic (N3-tg) mice, induces an aggressive form of T-cell acute lymphoblastic leukemia (T-ALL), strongly sustained by an NF-\( \kappa \)B constitutive activation, mainly represented by the p50/p65-dependent canonical pathway. To clarify the Notch/NF-\( \kappa \)B relationships in the onset/progression of T-ALL, we decided to inhibit NF-\( \kappa \)B canonical pathway in N3-tg mice. Methods: We generated N3-tg/p50\(-/-\) mice, deleted of the NF-\( \kappa \)B/p50 subunit in a Notch3 transgenic background. The follow-up of N3-tg/p50\(-/-\) versus N3-tg mice was conducted and hematopoietic cell analysis was performed at different ages and in multiple tissues from the indicated animals by flow-cytometry techniques. Results: The p50 deletion inhibited the progression of T-ALL in N3-tg/p50\(-/-\) mice, as defined primarily by the peripheral expansion of immature CD4+CD8+ T cells. Surprisingly, the double mutant mice succumb earlier than N3-tg counterparts. Mortality in N3-tg/p50\(-/-\) mice display the trait of a myeloproliferative disease, with the dramatic expansion of Mac1+Gr1+ myeloid cells in both spleen and blood, as well as granulocyte/monocyte progenitors in the bone marrow. Preliminary data indicate that these cells do not express Notch3, suggesting that in the absence of p50 expression, Notch3 is able to mainly influence the equilibrium of the myeloid compartment in trans. Conclusions: The results presented suggest that the ablation of NF-\( \kappa \)B canonical pathway may strongly impact on the outcomes of a T cell specific deregulation of Notch signaling. Thus, providing a useful experimental model to extend our understanding of Notch/NF-\( \kappa \)B interplay and to unravel novel strategies for the therapy of different hematological malignancies.

NTP9. The Resveratrol-Analogue 4,4'-Dihydroxy-Trans-Stilbene Suppresses Transformation in Normal Mouse Fibroblast and Inhibits Proliferation and Invasion of Human Breast Cancer Cells

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Background: 4,4'-dihydroxy-trans-stilbene (DHS) is a synthetic analogue of resveratrol (RSV). We previously demonstrated that DHS exerts a higher antiproliferative activity than RSV on normal human fibroblasts. Herein, our aim was to investigate the effect of DHS both on transformation of BALB/c 3T3 mouse fibroblasts and on proliferation and invasion of human breast cancer MCF-7 cells. Methods: Trypan blue staining was used for assessing cell death. Chemically induced (MNN+TPA) transformation of BALB/c 3T3 mouse fibroblasts was performed with the Cytoselect 96-well cell transformation assay. MCF-7 cell proliferation was investigated by PI staining, BrdU incorporation, and Western blotting analysis. The Boyden chamber cell migration and invasion, gelatin zymography and wound healing assays were also performed. Results: DHS efficiently suppressed the two-stage chemical induced transformation in BALB/c 3T3 cells. It also inhibited with high efficiency both anchorage-dependent and independent MCF-7 growth. In addition, a reduction in the S-phase cell population, associated with an increase in the p21 and p53 protein levels, and with a strong inhibition of the pRB protein phosphorylation, was evidenced in DHS-treated cells. Furthermore, DHS exerted a strong reduction of the matrix metalloproteinase-2 and -9 activities, concomitantly with a marked reduction of cell-cell and cell-extracellular matrix interaction. Conclusions: These results demonstrate that the two 4,4'-dihydroxy groups on the stilbene backbone make DHS a more active molecule, compared to RSV, in inhibiting neoplastic transformation, cancer cell proliferation and invasion. Further in vivo studies to better characterize DHS absorption and metabolism, biosafety, and the mechanisms of its anticancer properties are in progress.

NTP10. DDB2 Interacts with the Nucleotide Excision Repair Proteins at DNA Damaged Sites

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Background: DDB2 is a 48KDa protein originally identified as a component of the heterodimeric complex UV-DDB, together with DDB1. This complex has affinity for the major cytotoxic-mutagenic types of lesions introduced in DNA by UV irradiation, such as 6-4 photoproducts and cyclobutane pyrimidine dimers. These lesions lead to distortion of DNA, predisposing cells to accumulate mutations increasing susceptibility to cancer. Cells, as a protective mechanism against UV induced DNA damage, utilize nucleotide excision repair process. DDB2 plays an important role in the recognition step of UV-induced DNA damage in non-transformed regions and it is
mutated in xerodermia pigmentosum (group E) patients. In this study, we analyse the possible interaction between DDB2 and other proteins involved in NER process. Methods: We have studied the localization of DDB2 in HeLa cells transiently transfected with pCDNA3-1-DDB2 construct and then irradiated with UV-C at 30 or 100J/m², respectively, for western blot and immunofluorescence analyses. Moreover, to study the direct interaction between DDB2 and other NER proteins, solubilised chromatin fractions were immunoprecipitated with DDB2 antibody. Results: Cellular localization of DDB2 was examined 5, 10, 30 min post-UV irradiation: the confocal analysis showed that DDB2 co-localized with DNA repair proteins recruited to DNA-damage sites after local UV-C irradiation. The results obtained by immunoprecipitation techniques demonstrated the interaction and physical association between DDB2 and cullin 4A, XPC, XPG, p21 and PCNA proteins after DNA damage. Conclusions: DDB2 protein is recruited at local DNA-damaged sites where it directly interacts with NER proteins.

NTP11. A Novel Role for DNA Polymerase Eta (Polη) in Regulating the Translesion Synthesis Pathway of DNA Damage Tolerance

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Background: Cellular DNA is constantly exposed to ubiquitous environmental genotoxins, such as UV irradiation in sunlight, that cause DNA damage and predispose to cancer and other diseases. Translesion synthesis (TLS) is a DNA damage-tolerance mechanism that uses specialized DNA polymerases to replicate genotoxin-damaged DNA when conventional polymerases stall. TLS polymerases replicate DNA with relatively low fidelity, and their activity must therefore be tightly regulated. Defects in the TLS pathway cause excessive mutagenesis, as evidenced by the xeroderma pigmentosum variant (XPV) syndrome, in which Polη, the TLS polymerase DNA polymerase eta (Polη), is non-functional. Lack of Polη, which accurately replicates UV-damaged DNA, results in error-prone replication by inappropriate polymerases, UV-induced mutagenesis, and cancer. TLS polymerases are activated when the DNA replication factor PCNA is mono-ubiquitinated by the E3 ubiquitin ligase Rad18. Although this mono-ubiquitination activates TLS, its regulation is poorly understood. Methods: We used in vitro and in vivo biochemical and imaging techniques to map functional interactions between effector proteins in the TLS pathway. Results: We have uncovered a previously unidentified function of Polη in the regulation of TLS. In addition to its polymerase activity, we show that Polη recruits Rad18 to damaged DNA to promote efficient PCNA ubiquitination and activate TLS. Conclusions: Whereas tumorigenesis in XPV patients has been thought to stem solely from defective Polη polymerase activity, our results reveal that Polη also has non-catalytic roles that regulate TLS. Our findings suggest that imbalanced Polη expression, regardless of exposure to UV light, could misregulate TLS to promote mutagenesis, thus perturbing genomic stability in previously uncharacterized ways.

NTP12. Nottingham Prognostic Index and Triple-Negative Breast Cancer

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Background: Despite its old origin and the limited factors considered, NPI represents a valuable prognostic tool also for TNBC. Methods: The biological material from colon cancer and normal colon has been collected in different institutions, the S Gerardo hospital and Desio-Vimercate hospital that hospitalize patients of a well-defined territory corresponding to Monza-Brianza province. An online questionnaire was produced to collect data on clinical, pathological, and environmental-risk information and the data were centralized in a single database. Models for patient informed consent and regulations to keep the data anonymous were established. The guidelines of standardized operating procedures to collect and store the materials have been tested and made operative. Results: We are collecting 170 colon cancer cases/year in conjunction with clinical data. The biobank is favoring the standardization of clinical-diagnostic procedures in the involved hospitals. Conclusions: In three years the biobank will be able to provide material for oncological researches addressed to: i) discover biomarkers or therapeutic targets, ii) personalized medicine, iii) systems biomedicine. This experience will be also extended to other tumor pathologies.

PM1. Establishment of a Colon Cancer Biobank and Database among Different Institutions

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Background: In the post-genomic era there is still a gap between the available analytical tools and their impact on human diseases. On cancer pathology, that in the next years is expected to be the first cause of death, there is the major need to fill up this gap. In this context the establishment of biobanks in conjunction with databases containing all clinical/pathological/environmental risk data may help achieving the goal of this work. We established a colon cancer biobank and relative database involving different hospitals and university departments. Methods: The biological material from colon cancer and normal colon has been collected in different institutions, the S Gerardo hospital and Desio-Vimercate hospital that hospitalize patients of a well-defined territory corresponding to Monza-Brianza province. An online questionnaire was produced to collect data on clinical, pathological, and environmental-risk information and the data were centralized in a single database. Models for patient informed consent and regulations to keep the data anonymous were established. The guidelines of standardized operating procedures to collect and store the materials have been tested and made operative. Results: We are collecting 170 colon cancer cases/year in conjunction with clinical data. The biobank is favoring the standardization of clinical-diagnostic procedures in the involved hospitals. Conclusions: In three years the biobank will be able to provide material for oncological researches addressed to: i) discover biomarkers or therapeutic targets, ii) personalized medicine, iii) systems biomedicine. This experience will be also extended to other tumor pathologies.

PM2. Identification of a Point Mutation in SMN1 Gene Causing Spinal Muscular Atrophy: Implications for Genetic Counselling and Prenatal Diagnosis

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Background: Spinal muscular atrophy (SMA) is one of the most common autosomal recessive genetic disorders characterized by progressive symmetric muscle weakness and paralysis. Carrier frequency of SMA is approximately 1/50 and therefore the incidence is 1 in 6000-8000 live births. Childhood disease is subdivided into three clinical forms with type I being the most severe. About 95% of SMA cases are caused by homozygous deletion of the SMN1 gene or its conversion to highly homologous SMN2. The remaining cases are heterozygous compound for a deletion/conversion of one SMN1 allele and a small intragenic mutation of the other allele. This work reports the case of a couple with two SMA type I affected deceased children and the research of mutation(s) that determines SMA in this family. The final purpose is to perform prenatal diagnosis in a further pregnancy of the couple. Methods: SMN1 alleles copy number determination is performed by multiplex Real-Time PCR method. Sequence analysis of exons and exon/intron junctions is performed by means of an automated Sanger sequencing. Results: The female of the couple is a SMN1 deletion carrier whereas the male that carries two SMN1 copies harbours the rs1048932 point mutation (G>A), already described as associated to a severe SMA typeI phenotype. The same point-mutation has been identified in some of the healthy siblings. Conclusions: The point mutation identified, together with the more frequent SMN1 gene deletion, allows genetic counselling, carrier testing and prenatal diagnosis for the couple programming future pregnancy and for all relatives at reproductive risk in the family.

PM3. Linked Data and Translational Medicine: The Role of ICD-11

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Background: Internet and digital information enable strong interactions within an information ecosystem of researchers, clinical practitioners and other resources and users of biomedical data. Currently the lack of standards for data representation is an obstacle in translational research, making difficult to extract all potential knowledge from data acquired through experiments and data analysis. However, the concept of linked data has recently gained relevance, indicating the practice of publishing structured data that can be interlinked and become more useful. The WHO International Classification of Diseases (ICD) is the world’s standard tool to capture health information. Methods: An exercise is set up using the content model of ICD-11, adopted for the11th revision of the classification, in which descriptive
characteristics of classification are categories are linked to underpinning standardized terminologies to define information such as signs and symptoms, morphology, causality agents or treatment. Results: ICD-11 represents a good example of linked data exercise, leading the way to better data usability and therefore faster exploitation of information collected in translational research. Moreover the ICD-11 update and revision processes, based on ontological tools, allows for collaborative web-based editing thus opening to all interested parties the possibility to rapidly update the classification and allowing fast transfer of biomedical discoveries into the classifications used in clinical practice. Conclusions: The content model of ICD-11 represents a novel enhancing information transfer in translational medicine, but only a large web-based engagement of users in this domain will determine if the classification will become an effective tool for systematic bench-to-bedside knowledge exchange.

REDOX REACTIONS IN HUMAN PATHOPHYSIOLOGY

RR1. Membrane Oxidative Damage in Intestinal Caco-2 Cells: Protective Effect of a Wine Phenolic Extract
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Background: It has been suggested that the beneficial effects on the cardiovascular system ascribed to mild to moderate wine consumption may also arise from a local action in the gastrointestinal tract, where polyphenols and their metabolites concentrate. We investigated the ability of a red wine phenolic extract, obtained from the most typical and widespread grape variety grown in Sardinia, Cannonau, to exert a direct antioxidant action on the oxidative damage to intestinal mucosa.
Methods: The protective action of the extract, obtained through liquid-liquid extraction with ethyl acetate, was evaluated as ability to counteract the loss of epithelial integrity, measured as transepithelial electrical resistance (TER) in differentiated human Caco-2 cell monolayers, following tert-butyl hydroperoxide (TBH) exposure, and through MDA, fatty acids hydroperoxides (HP) and 7-ketocholesterol (7-koeto) production (detected through HPLC analysis). Results: TBH treatment showed a significant decrease in Caco-2 TER from the lowest concentration tested (0.5 mM) already after 30 min of incubation. TBH 2.5 mM caused the complete loss of membrane integrity after 120 min of incubation. In Caco-2 cells exposed to TBH 2.5 mM for 120 min, a significantly high level of MDA compared to the non oxidized samples, paralleled by an increase of HP and 7-keto values, was detected, indicating an ongoing lipid peroxidation process. Pretreatment with the extract significantly slowed the decrease in TER and inhibited the increase of oxidation products. Conclusions: Our results point out for the first time a direct antioxidant action of the phytochemical fraction from the wine Cannonau on enterocytes exposed to oxidizing species.

RR2. Immune Responses Against Oxidative Stress-Derived Antigens Contributes to Hepatic Inflammation in Nonalcoholic Steatohepatitis (NASH). S. Sutti1, A. Jindal1, I. Locatelli1, M. Vacchiano1, C. Bozzola1, E. Albano1
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Background: Nonalcoholic steatohepatitis (NASH) has become the most frequent chronic liver disease in relation to the worldwide increase of overweight and obesity. As NASH is often associated with the presence of circulating antibodies against proteins adducted by lipid peroxidation products, we have investigated the relevance of these immune responses in the disease pathogenesis. Methods: NASH was induced by feeding C57Bl/6 and Balb/c mice 4 weeks with a methionine-choline deficient (MCD) diet. Results: Upon MCD feeding C57Bl/6 mice showed more liver injury and increased hepatic expression of pro-inflammatory cytokines than Balb/c mice. In C57Bl6 mice NASH was also associated with increased prevalence of B- and T-lymphocyte infiltration and higher expression of lymphocyte chemokines CCL5 and CXCL10. Furthermore, liver histopathological examinations revealed that oxidative stress-driven immunity contributes to hepatic inflammation in NASH.

RR3. Oncostatin M Induces Epithelial-to-Mesenchymal Transition and Increased Invasiveness in Hepatic Cancer Cells Through Redox Mechanisms S. Cannito1, C. Paternostro1, C. Busietta1, D. Povero1, S. Colombato1, E. Novo1, M. Parola2
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Background: Oncostatin M (OSM) can orchestrate hypoxia-mediated liver processes (development, regeneration, angiogenesis) contributing to chronic liver disease progression and hepatocellular carcinoma (HCC) development. Accordingly, OSM and its related LIFR (leukemia inhibitory factor receptor) b subunit are overexpressed in cirrhotic liver. Since OSM can operate through hypoxia-inducible factors (HIFs) and hypoxia has been reported to induce epithelial-to-mesenchymal transition (EMT), this study has been designed to investigate whether OSM may act as a stimulus to induce EMT in human hepatic cancer cells. Methods: EMT, invasiveness and signal transduction were analysed by morphological, molecular and cell biology techniques in HepG2, HuH7 and Madin-Darby canine kidney (MCDK) cells exposed to human recombinant OSM as well as by immunohistochemistry on liver specimens from HCV cirrhotic patients carrying G1 and G2 HCC. Results: OSM induced EMT-related changes in all cells (nuclear translocation of SNAIL1, E-cadherin down-regulation, overexpression of θ-smooth muscle actin and expression of matrix metalloproteases-2) within 48-72 hrs and simulated invasiveness in cancer cells. Data revealed a scenario involving early intracellular generation of reactive oxygen species (ROS), activation of PI3K, ERK1/2, JNK1/2, p38MAPK and STAT3, and phosphorylation/inactivation of GSK-3β. Cancer cell invasiveness was prevented by inhibiting ERK1/2, PI3K or JNK1/2 or by preventing ROS generation. Finally, OSM was expressed in HCC tumor cells in areas also positive for hypoxia-related antigens. Conclusions: OSM, expressed in human HCC and peritumoral cirrhotic tissue, can induce EMT in human hepatic cancer cells and stimulate invasiveness through redox-dependent activation of EMT-related critical kinases and transcription factors.

RR4. Xanthine Oxidase Directly and Irreversibly Modifies BH4 Contributing to Endothelial Dysfunction in Ischemic Rat Hearts
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Background: BH4 is a cofactor of nitric oxide synthase activity. Changes in BH4 bioavailability may affect vascular function. BH4 is also a potent hydroxyl radical scavenger. In cardiac diseases, xanthine oxidase (XO) activity is up-regulated. XO catalyzes the oxidation of xanthine to uric acid, generating superoxide. We hypothesized that enhanced XO activity may affect BH4 availability and function. Methods: To test whether BH4 and different pteridine derivatives have an impact on XO-mediated ROS-production, we evaluated inhibitory effects produced by incremental doses of BH4 and pteridines such as BH2, Bio, PT and XPH2 on Xanthine/XO reaction, measuring the formation of urate as readout. Results: Urate formation from Xanthine/XO was inhibited by all pteridines tested, excepting for XPH2. Bio abolished the formation of urate at 20 μM. Higher concentrations of Pt (100 μM) were needed to achieve the same effect. BH4 and BH2 (200 μM) partially inhibited Xanthine/XO-driven production of urate (approximately 50%). We demonstrated that the production of superoxide anion does not occur when BH4 reacts with XO. Therefore, we exposed increasing concentrations of BH4 to XO (0.1 U / ml) in phosphate buffer at pH 7 for 15 min at 37 ° C and analyzed the products by HPLC. We found that BH4 was converted to XPH2, without BH2 as an intermediate product. The latter was proved by Fenton reaction-derived hydroxyl radical-driven BH4 transformation. Conclusions: Our data show that increased XO activity irreversibly transforms BH4 to XPH2. This alteration may contribute to the onset and/or progression of endothelial dysfunction during the course of acute or chronic cardiac diseases.

RR5. NADH Accumulates During Early Ischemia in the Rat Heart as Detected by a Novel One-step Hplc Approach
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Background: Lack of oxygen due to myocardial ischemia alters the redox status of pyridine-nucleotides. To directly test the latter possibility, we developed a method...
to analyze NADH, NAD, NADPH, NADP levels in rat hearts subjected to ischemia-reperfusion injury. **Methods:** A reaction of the oxidized-nucleotides with cyanide in basic solution leads to two stable fluorescent products, allowing us to separate all four nucleotides (NADH, NAD, NADPH, and NADP) and to quantify them on one single chromatogram. Langendorff-perfused rat hearts underwent global ischemia (15, 30 and 60 min) followed by 30-min reperfusion. The heart-chloroform extracts were analyzed by HPLC. **Results:** The analysis of HPLC chromatogram series of nicotinamide-adene-dinucleotides over different ischemia time points revealed a sustained increase in the NADH level. This rise was already visible 15 min after ischemia (67.5 ± 1.07 nmol/hr/gtissue to 15 min. ischemia vs 55.06 ± 7.46 nmol/gr control). When nicotinamide-adene-dinucleotides phosphates are concerned, ischemia induced a significant NADP decrease starting at 30 min of ischemia (69.55 ± 3.25 vs 79.48 ± 3.81 nmol/gtissue in controls, P = 0.0008). These changes became even more evident after 60 min. NADPH levels dropped to 30.17 ± 3.59 nmol/gtissue (P = 0.00003). **Conclusions:** Our studies show that during early ischemia NADH accumulates in the heart tissue, likely due to an anoxia-induced blockade of the Krebs cycle. When the no-flow condition was longer than 15 min, the capacity of generating NADPH impaired during late ischemia. Altered bioavailability of pyridine-dinucleotides may have repercussions on cellular function and viability during the I/R injury.

**RR6. Polyphenol Compounds in Sardinian Wines Can Modulate Redox Cell Signalling Induced by a Dietary Mixture of Oxysterols in Human Enterocyte-like CaCo-2 Cells**

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**Background:** The beneficial role of polyphenols in human health, acting mainly as direct antioxidants has been widely documented. These compounds are distributed in all foods of plant origin, and exert many biological activities. Polyphenol food concentration is extremely variable due to different seasonal and geographic variations of vegetable growth. Sardinian wines (Cannonau and Vermentino) are particularly rich in flavonoids and phenolic acids, which have long residence time and accumulate in the mucosal intestinal layer where they can exert their beneficial effects. In this study we report the role of Sardinian wine extracts in modulating the redox signaling pathways activated in differentiated CaCo-2 cells by a dietary mixture of the most representative oxysterols found in cholesterol-rich foodstuff.

**Methods:** Differentiated CaCo-2 cells, whose phenotype is similar to normal mucosa of small intestine, were treated with a mixture of oxysterols (7-ketocholesterol, 5α,6α-epoxycholesterol, 5β,6β-epoxycholesterol, 7α-hydroxycholesterol, and 7α-hydroxycholesterol). Cells were pre-treated with Cannonau or Vermentino extracts. The activation of colonic NADPH oxidase (NOX1) and of the main transduction effects. In this study we report the role of Sardinian wine extracts in modulating the redox signaling pathways activated in differentiated CaCo-2 cells by a dietary mixture of the most representative oxysterols found in cholesterol-rich foodstuff.

**Results:** 150 μM deferoxamine, a specific iron chelator. Time-course experiments showed that 150 μM FAC was able to phosphorylate both ERK and AMPK after 10 min treatment. Specific ERK and AMPK inhibitors, U0126 and Compound C (both 10 μM) counteracted FAC-driven phosphorylation of ACC, an AMPK downstream protein. **Conclusions:** These data suggest that iron negatively affects neuron migration via ERK and AMPK. Among the consequences of this effect, iron overload may impair migration of GnRH neurons from the olfactory placode into forebrain and hypothalamus, where they promote reproductive competence.

**ST2. PRDM Gene Products in Testicular Germ Cell Tumors**

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**Background:** Testicular germ cell tumors (TGCT) originate from primordial germ cells blocked at different stages during maturation, reflecting different histological tumor subtypes. A common genetic alteration in TGCT is a deletion of chromosome 1 short arm, where the PRDM2 gene, a member of positive regulatory domain gene family, is located. Moreover recent studies demonstrated that members of PRDM gene family have an essential role in the early stages of testicular development. The aim of this study is to evaluate PRDM gene family members as possible tumor-suppressor function in TGCT. **Methods:** PRDM gene expression was assessed by mRNA RT-PCR. Cells were treated with 100 nM 17β-Estradiol (E2), 100 nM DHT or 10 μM RA in serum free medium for 24h. RNA interference was performed using BLOCK-it™ Pol II mRNA system. Proliferation assay was performed with propidium iodide staining and FACS analysis. **Results:** In GC1 mouse spermatogonial cells treatment with proliferation agents 5α-dihydrotestosterone (DHT) and E2 reduced PRDM2/R12 expression levels whereas PRDM2 total forms showed no variation; the same treatment significantly increased PRDM4 and PRMD10 expression levels. Silencing PRDM2 gene expression by RNA interference increased PRDM10 expression levels and reduced the proliferation rate of spermatogonia. **Conclusions:** In spermatogonia as in MCF-7 cell line, E2 and DHT regulate PRDM2 gene expression suggesting that PRDM2 gene products could mediate the effect of these agents on cell cycle progression. PRMD4 and PRMD10 are also responsive to steroid hormones and PRMD10 probably cooperates with PRMD2, as demonstrated by the increase of its expression levels after PRMD2 gene silencing.

**ST3. NHERF1 and CFTR Overexpression Restore Tight Junction**

 Organisation and Function in Cystic Fibrosis Airway Epithelial Cells Via the Involvement of Erzin and the RhoA/ROCK Pathway

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**Background:** The pathophysiology of cystic fibrosis (CF) lung disease is characterised by abnormal ion and fluid transport across the epithelium with neutrophil-dominated inflammatory response. Tight junctions (TJs) restrict the transit of molecules through the paracellular route and act as a barrier to regulate access of inflammatory cells into the airway lumen. In CF cells, NHERF1 overexpression rescues CFTR-dependent chloride secretion by inducing the formation of the multirion complex NHERF1-RhoA-erzin-actin. In this context, we studied whether NHERF1 and CFTR are involved in the organisation and function of TJs. **Methods:** TJ organisation was studied by confocal microscopy on ZO-1, occludin, Claudin-1 and JAM-1 in polarised wild-type (16HBE) and CF (CFBE) airway epithelial cells. Barrier function was studied by dextran permeability and neutrophil transmigration. **Results:** CFBE monolayers presented a disorganisation of TJ proteins as compared to 16HBE monolayers, paralleled by increased permeability to dextrans and neutrophil transmigration. Overexpression of NHERF1 or CFTR rescued TJ proteins to their proper location and restored the barrier function. Expression of a phospho- deacetyl actin mutant, T67A, increased permeability in 16HBE and in a CFBE clone stably overexpressing NHERF1 (CFBE/NHERF1), whereas a constitutively active form of ezrin, T567D, achieved the opposite effect in CFBE. A dominant-negative form of RhoA (Rhoa-N19) disruptedZO-1 localisation at the TJs and increased permeability in CFBE/NHERF1. The inhibitor Y27632 of Rho kinase (ROCK) increased permeability as well. **Conclusions:** These data suggest a role for the multirion complex CFTR-NHERF1-erzin-actin in maintaining TJ organisation and barrier function, and suggest that the RhoA/ROCK pathway is involved.
ST4. The Akt1 Gain of Function Mutation, E17K, in Lung Epithelial Cells

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Background: Aberrant signalling from the phosphatidylinositol 3-kinase (PI3K)/Akt pathway is frequently observed in human cancer. Different studies have identified a gain-of-function mutation in the pleckstrin homology domain of Akt1 that results in a glutamic acid to lysine substitution at residue 17 (E17K) in multiple cancer types including lung carcinomas. So far the contribution of somatic Akt1 mutations to development of epithelial cancer has remained elusive. 

Methods: Herein, we examined the activity of the E17K mutant using immortalized human bronchial epithelial cells as model system (BEAS-2B cells). 

Results: Expression of Akt1-E17K mutant, but not of wild-type Akt1, in BEAS-2B cells induced multiple phenotypic alterations characteristic of tumour cells, including growth factor-independent DNA synthesis, anchorage-independent growth in soft agar, increased ability to migrate and invade, resistance to anoikis and tumorigenicity in nude mice. In addition, mutant Akt1 induced an expansion of a subset of putative tumour-initiating cells (TICs) as determined by an increase in the efficiency of sphere formation as well as by enhanced expression of stem cell markers, leading to the emergence of a cell population endowed with the capability to form aggressive, undifferentiated tumours at high efficiency (103-104 cells/injection). Knockdown of Oct-4 significantly inhibits the capability of BEAS-Akt1 cells to form spheres and to grow as xenografts in vivo.

Conclusions: In our summary, we indicate that the Akt1-E17K mutant is oncogenic in lung epithelial cells and that the stem cell transcription factor Oct-4 is a key mediator of its oncogenic activity, thus contributing to the pathogenesis of lung cancer.

ST5. Identification of Novel Estrogen Receptor-α (ERα) Protein Interactors Reveals Significant Differences Among Antiestrogen Compounds in Human Breast Cancer Cells

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Background: ERα is a ligand-activated transcription factor that promotes mammary epithelial cell growth and breast-carcinogenesis controlling key cellular pathways via protein-protein interactions within co-regulator complexes. ERα-ligands are classified as agonists (estrogens: 17β-estradiol (E2)), mixed agonists-antagonists (SERMs:Tamoxifen/Tam andRaloxifene/Ral) and pure-antagonists (ICI182,780, Faslodex/Faslodex). Gli proteins display a positive function, but can also be converted into a cleaved form with transcriptional repressor activity. This process is finely tuned by ubiquitin ligase involved on major biological processes. Itch binds SuFu and promotes its ubiquitination, thereby preventing Itch-triggered ubiquitination of SuFu, thus explaining how this process is finely tuned.

Objective: Taken together, we demonstrate that Hedgehog-dependent physiologic and tumorigenic processes require a conserved IRES-dependent translational control. We propose that Hedgehog directly regulates IRES-dependent translation via a protein complex of Sufu and the RNA-binding protein CNBP. CNBP is up-regulated by Hedgehog and then, in a complex with Sufu, is recruited to 5'UTR sequences of target mRNAs where it promotes IRES-dependent protein translation. Sufu protects CNBP from ubiquitination and proteasomal degradation, thus promoting its activity. Consistent with the developmental and tumorigenic role of Hedgehog, CNBP is up-regulated in cerebellar stem cells, medulloblastomas and tumor stem cells, where it mediates self-renewal and cancer growth. Furthermore, this mechanism is also conserved in Drosophila, where CNBP directs wing development in synergy with Ci, the Drosophila Gli homolog.

Results: Taken together, we demonstrate that Hedgehog-dependent physiologic and tumorigenic processes require a conserved IRES-dependent translational control. We propose that the translational mediator CNBP and the IRES-dependent translational control can be considered promising novel targets for anti-cancer approaches.

ST6. A Novel Morphogen-Dependent Control of IRES-Dependent Translation in Cancer and Development

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Background: The Hedgehog morphogen is critical for development, stem/progenitor cell fate and is dysregulated in many cancers, such as medulloblastomas and basal cell carcinoma. Hedgehog signaling leads to the activation of Gli transcription factors and activation of gene expression, which is in part regulated by Suppressor of Fused (Sufu). To date it is not known whether Hedgehog also regulates protein translation.

Methods: We have used biochemical, cell and stem cell culture techniques, as well as in vivo gene knockdown or overexpression studies in Drosophila melanogaster.

Results: We show here that Hedgehog directly regulates IRES-dependent translation via a protein complex of Sufu and the RNA-binding protein CNBP. CNBP is up-regulated by Hedgehog and then, in a complex with Sufu, is recruited to 5'UTR sequences of target mRNAs where it promotes IRES-dependent protein translation. Sufu protects CNBP from ubiquitination and proteasomal degradation, thus promoting its activity. Consistent with the developmental and tumorigenic role of Hedgehog, CNBP is up-regulated in cerebellar stem cells, medulloblastomas and tumor stem cells, where it mediates self-renewal and cancer growth. Furthermore, this mechanism is also conserved in Drosophila, where CNBP directs wing development in synergy with Ci, the Drosophila Gli homolog. Conclusions: Taken together, we demonstrate that Hedgehog-dependent physiologic and tumorigenic processes require a conserved IRES-dependent translational control. We propose that the translational mediator CNBP and the IRES-dependent translational control can be considered promising novel targets for anti-cancer approaches.

ST7. Itch-Dependent Nondegradative Ubiquitination of Sufu Controls Hedgehog Pathway

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Background: Hedgehog (Hh) pathway regulates tissue patterning and cell proliferation through the activation of the transcription factors belonging to the Gli family. Gli proteins display a positive function, but can also be converted into a cleaved form with transcriptional repressor activity. This process is finely tuned by Sufu, which in the absence of signaling interacts and protects Gli full-length from degradation and promotes its conversion into a repressor form, Gli3R. The mechanistic aspects of this process are however poorly understood. 

Methods: Mouse fibroblasts (NH3T3) and human epithelial kidney (HEK293) cells were used in this study. Protein-protein interaction was assessed by immunoprecipitation and GST-pulldown, ubiquitin modification by ubiquitination in vivo and in vitro assays. Depletion of Itch was obtained by siRNA.

Results: We show here that the conversion of Gli3 into a repressor form is regulated by itch, an HECT E3 ubiquitin ligase involved on major biological processes. Itch binds Sufu and promotes its ubiquitination both in vivo and in vitro. Of relevance, this itch-mediated ubiquitination is insensitive to the proteasome activity and does not affect Sufu stability, suggesting that such a process drives a regulatory rather than a proteolytic response. Indeed, Sufu ubiquitination increases the binding of Gli3 to Sufu, thereby leading to Gli3R formation and inhibition of the Hh pathway. Of note, Hh agonists prevent itch-triggered ubiquitination of Sufu, thus explaining how this process is regulated by the signaling pathway. Conclusions: Our findings suggest that itch-dependent Sufu ubiquitination, regulating dynamic protein-protein interaction, plays an important role in the control of Hh signaling.

STEM CELLS IN TISSUE REGENERATION AND REGENERATIVE MEDICINE

SC1. Human Amniotic Mesenchymal Stem Cells Modify the Function and Cytokine Production of F508del Airway Epithelial Cells Upon Coculture

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Background: In cystic fibrosis (CF), there is a lack/dysfunction of CF airway epithelial cells. For this purpose, MCF-7 cells stably expressing ERα fused to TAP-tag at the C-terminus were used to purify reactive receptor-containing nuclear multi-protein complexes from treated with either E2, SERMs or ICI. Isoleted complexes were dissociated in vitro and analyzed by mass spectrometry (nanoLC-MS/MS).

Results: This led to identification of a large number of novel ER partners and revealed significant differences among ligands tested. E2-promoted ERα-interactome (270 proteins) is different and more complex than those elicited by TAM (71), Ral (48) or ICI (54) that, in turn, are significantly different from each other. Conclusions: In silico analysis of molecular functions represented by these interactomes indicates that ER2 induces receptor association with epigenetic, transcriptional and actin-polymerization factors. Moreover, although ICI-induced interaction is involved in negative regulation of biosynthetic processes, Tarn- and Ral-dependent ones comprise regulators of macromolecular-complex synthesis and transcription-linked processes. Supported by AIRC, MIUR, Regione Campania: University of Salerno; Fondazione con il Sud; UICC-IORETT fellowship to F. Cirillo; FEBS Short-Term Fellowships to G. Nassa.
investigated by flow cytometry and confocal microscopy. Chorionic efflux was studied by fluorimetry. ENaC activity was assayed by apical fluid reabsorption. Cytokine secretion was studied in the apical and basolateral conditioned media. Results: hAMSCs expressed at low levels CFTR mRNA and gamma, but not alpha and beta, subunits of ENaC. Cocultures of hAMSCs with CFBE cells demonstrated that at least 50-80% of hAMSCs acquired a detectable CFTR expression on the apical membrane above the background. Fluorimetric measure of ion chorionic efflux allowed to detect an increased function of the CFTR channel in cocultures as compared with CFBE cells and hAMSCs alone. Amiloride-dependent fluid reabsorption decreased when CFBE cells were cocultured with hAMSCs compared to CFBE41o- cells alone. Unexpectedly, IL-1β, IL-8, IL-8 and TNF-α showed an increase depending on the hAMSC-CFBE ratio. Conclusions: Overall, these data show that hAMSCs are capable of resuming some pathological features of CF airway epithelial cells, although the cellular and molecular mechanisms have to be deciphered.

SC2. Stem-Like Cells in Nephrospheres Present Multilineage Differentiative Abilities
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Background: To isolate stem cells from adult kidney, we used the sphere forming assay approach coupled with the evaluation of self renewal and differentiative abilities. Methods: Nephrospheres were obtained culturing the cells from adult kidney in specific conditions. The sphere forming efficiency (SFE) was calculated. To identify and isolate the stem cell population within the nephrospheres the PKH dye, retained in quiescent cells, was used. The ability of nephrosphere forming cells and stem cells to differentiate into epithelial, podocytic and endothelial lineages was evaluated using specific media and 3D cultures performed with semisolid substrates.

The regenerative abilities were evaluated transplanting the cells under the renal capsule of nude mice. Results: After 12 days in culture, we obtained nephrospheres propagable for at least eleven passages, with a SFE of 0.8%. The spheres are composed of a heterogeneous population of stem cells, a more PKH fluorescent (PKHHigh) population, and progenitors that are less fluorescent (PKHlow/neg). Nephrosphere forming cells and PKHHigh cells can differentiate into epithelial, podocytic and endothelial lineage; they can form tridimensional hollow structures miming the tubular in vivo behavior. Nephrosphere forming cells can generate tubular like structure in vivo, whereas PKHHigh cells inserted into the mice parenchyma maintain their undifferentiated status. Conclusions: Nephrospheres contain stem-like cells, as shown by the presence of quiescent cells able to self renew and by the ability to differentiate into renal multilineage phenotypes. This PKHHigh stem cells can be isolated and characterized and can represent a cellular material usable in regenerative medicine.

SC3. Influence of Fibronectin and Collagen as Substrates for the Isolation and Expansion of Endothelial Progenitor Cells from Human Peripheral Blood
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Background: Endothelial progenitor cells (EPCs) have a crucial role in endothelial homeostasis. Among populations of endothelial cells that can be cultured from adult blood, endothelial colony-forming cells (EFCFs) are considered the true EPCs. Different methods can be used to isolate and expand EFCFs from peripheral blood, the main difference consisting in the substrate (fibronectin or collagen) used for cell seeding. The possible impact of the substrates on EFCF cultures has not been investigated, so far. In this study we compared EFCFs obtained using fibronectin or collagen during EFCF isolation and/or expansion. Methods: Healthy donor PBMCs were seeded on fibronectin or collagen and cultured in EGM-2. EFCF colonies were released from the original tissue-culture plates and repleted onto tissue-culture flasks precoated with fibronectin or collagen, for further passages. All cultures were analyzed for isolation of EFCF colonies, cell yield after serial passaging, immunophenotype, cytokine production and in vitro angiogenesis. Results: Fibronectin sustained EFCF isolation more efficiently than collagen because, although similar numbers of colonies were obtained on the two substrates, EFCF colonies appeared more adherent to fibronectin (P < 0.05). Collagen sustained EFCF expansion more efficiently, as EFCFs expanded on collagen showed longer survival (P < 0.02), lower rate of cultures undergoing senescence at early passages (P < 0.02), and a higher cell yield. Preliminary results indicate that EFCFs expanded on fibronectin or collagen have similar immunophenotypes, cytokine profiles and ability for in vitro tubulogenesis. Conclusions: We suggest that isolation on fibronectin, followed by expansion on collagen, may represent the most efficient strategy to obtain EFCFs from peripheral blood samples.

SC4. Mobilisation of Hematopoietic Stem/Progenitor Cells in Acute Lung Injury: Role of VLA-4
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Background: The aim of this study was to determine whether there is a relationship between pulmonary inflammation, expression of VLA-4 (CD49d), LFA-1 (CD11a), and L-selectin (CD62L) and chemotaxis in resident hematopoietic stem/progenitor cells (HSPCs), as well as on their mobilisation in the blood. Methods: At 24, 48 and 72 h following an intratracheal administration of a single LPS bolus in C57Bl/6 mice, pulmonary inflammation was studied in cytokines and bronchoalveolar lavage fluid (BALF) samples. Expression of CD49d, CD11a and CD62L was analysed in Sca-1+ HSPCs and subpopulations as well as in circulating Sca-1- blood cells by flow cytometry. SDF-1-directed transmigration through an endothelial cell sheet was investigated. Results: In coincidence with a peak of neutrophils, cytokine (IL-1, TNF-α, IL-6) and chemokine (KC, MIP-2, SDF-1) levels in BALF at 48 h, the number of marrow HSPCs expressing CD49d increased. The number of CD49d-positive HSPCs dropped at 72 h. The HSPC subset comprising bigger cells behaved the same. A significant decrease of circulating Sca-1+ cells in the mononuclear population, but not in the polymorphonuclear granulocytes, at 72 h following LPS administration was observed. Finally, SDF-1 directed chemotaxis of marrow HSPCs subset of bigger cells was higher in cells obtained from LPS-treated animals than those from controls at 72 h. Conclusions: Our data provide evidence for a temporal relationship between CD49d level fluctuation in HSPCs, their mobilization from the bone marrow and decrease in circulating HSPCs, likely for their influx in the inflamed lung, and show that the HSPC bigger subpopulation is affected by these changes.

SC5. Erk1/2-Oct4A Interaction Mediates Oct4A Phosphorylation and Degradation
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Background: Embryonic stem (ES) cell self-renewal properties are attributed to critical Oct4A amounts. Although the Oct4A transcriptional targets have been deeply studied, little is known about its post-translational regulation. Sequence analysis revealed that Oct4A contains five putative ERK1/2 phosphorylation sites. Methods: We were able to show that Oct4A interacts with ERK1/2 in Ntera2 cell line, using both in vitro GST-pull down and in vivo co-immunoprecipitation assays. To explore the mechanism of ERK1/2-Oct4A interaction, we performed mass spectrometry analysis on HeLa cells transfected with Oct4A and MEX1CA. To investigate the possibility that ERK1/2 activation can enhance Oct4A degradation, we analyzed endogenous ubiquitination in HeLa cells transfected with Flag-Oct4A alone or with MEX1CA. Results: Consistent with the hypothesis that Oct4A is a putative ERK1/2 substrate, we were able to show that Oct4A interacts with ERK1/2 in Ntera2 cell line, using both in vitro GST-pull down and in vivo co-immunoprecipitation assays. To explore the mechanism of ERK1/2-Oct4A interaction, we performed mass spectrometry analysis on HeLa cells transfected with Oct4A and MEX1CA and we identified phosphorylation of Ser 111, one of the previously evidenced phosphorylation sites. When we examined ubiquitination of Oct4A from the FLAG immunoprecipitation, we saw that the extent of Oct4 ubiquitination was clearly increased when MEX1CA was co-expressed and this increase was more evident after MG132 treatment, a proteosomal inhibitor. Conclusions: These results suggest an increase in Oct4A ubiquitination downstream of MEX1 activation. Understanding and controlling this mechanism by which stem cells balance self-renewal would substantially advance our knowledge of stem cells and their clinical application.

SC6. Proteomic Profile of CD24+ CD133+ Renal Multitrophic Progenitors (RMP)
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Background: RMP represent a population of undifferentiated pluripotent cells with both self-renewal and multilineage-differentiation characteristics. A population of CD24+CD133+RMP in adult human kidneys is able to repair injured renal tissue.
Proteomics provides a powerful approach for studying the characteristics of RMP and discovering molecular markers. **Methods:** RMP lines were isolated from normal kidneys of 30 patients undergoing nephrectomy for renal cell carcinoma. We have analyzed proteome profiles of two RMP lines using 2-DE analysis combined to nano-HPLC-ESI-ion trap and MALDI-TOF-MS analysis. The identified proteins were studied by ingenuity pathway analysis (IPA) and validated by Immunoblot analysis.

**Results:** An average of about 1080 spots were detected in the silver stained gels of total protein extract. The protein spots identified were involved in cellular cytoskeleton (28.6%), stress-response (23.8%), cellular metabolism (14.3%), cell-proliferation and differentiation (9.5%). A large number of proteins were identified as chaperones, heat-shock-proteins, ubiquitin/proteasome, and oxidative-stress-responsive-proteins. Functional clustering of differentially expressed proteins by IPA in comparison with the proximal tubular epithelial cell proteome from the same donors revealed that 17β-estradiol-pathway was overexpressed in RMP (IPA score=32). To confirm this observation we investigated the expression of three up-regulated key-proteins of the pathway (17β-estradiol receptor, NME1, Zyxin) by immunoblot analysis and observed a significant increase of their expression in RMP cell lines compared to PTEC from the three donors. **Conclusions:** This study represents the first proteomic dataset for RMP and may provide a better insight into RMP-biology. Several studies explore the direct effects of sex-hormones on kidney and our data may suggest that RMP may represent a key-target for 17β-estradiol. Knowledge of RMP biology may enable a better comprehension of the mechanisms of renal repair.

**SC7. Pharmacologic Attenuation of Cardiac Stem Cell Senescence**

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**Background:** We demonstrated that both age and pathology exert detrimental effects on human cardiac stem cells (CSC) and are associated with reduced telomerase activity and telomere length, telomere erosion, telomere induced dysfunction foci and CSC dysfunction in vitro. Our aims were to investigate whether CSC senescence is associated with their reduced reparative ability in vivo, to identify the molecular determinants possibly responsible for CSC senescence, to screen drugs (i.e. rapamycin, resveratrol, and DETA/NO) able to interfere with CSC senescence, and to verify if CSC drug treatment in vitro is effective in restoring the reparative potential of senescent CSC in vivo. **Methods:** CSCs were isolated both from normal (D) and failing (F) human hearts. The reparative capacity of CSC was evaluated in a SCID/beige mouse model of acute myocardial infarction (AMI). Echocardiography and cardiac catheterization were performed 2 weeks post-AMI. Fibrosis, angiogenesis, myocyte growth, myocyte apoptosis, and myocyte senescence were assessed. Western blot analysis of CSC was performed to identify pathways possibly associated with CSC senescence. Drug-screening assays were performed treating F-CSC for three days and analyzing them in terms of cell senescence, proliferation, and death. **Results:** Pathology attenuates the reparative ability of CSC in vivo. The short-term pharmacological treatment of CSC resulted in a significant reduction in p16+, p21+, and γH2A.X+Ki67- senescent cells, together with an increase in CSC proliferation. Last, in vitro treatment of F-CSC with 10nM rapamycin and 0.5µM resveratrol prior to their in vivo administration to infarcted mice restored their reparative ability in vivo, significantly improving global heart function. **Conclusions:** Pharmacological inhibition of CSC senescence enhances their regenerative capacities.