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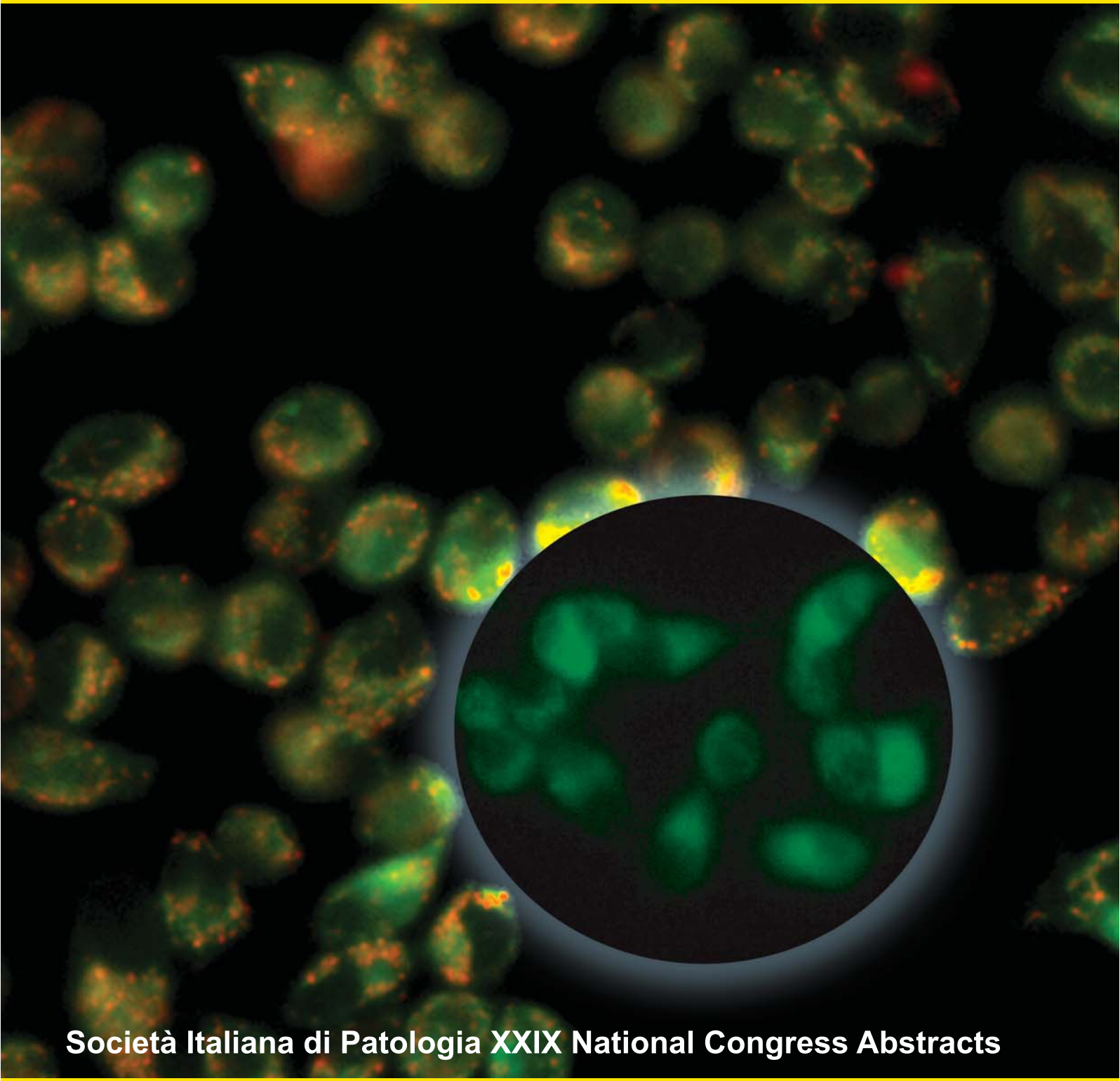

Società Italiana di Patologia XXIX National Congress Abstracts

September 2008 Volume 173, Supplement

April 18 - 22, 2009
New Orleans, Louisiana, USA

ASIP 2009 Annual Meeting at Experimental Biology

GUEST SOCIETIES

- American College of Veterinary Pathologists (ACVP)
- American Society for Matrix Biology (ASMB)
- International Society for Analytical and Molecular Morphology (ISAMM)
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SATURDAY, APRIL 18, 2009

Achieving Work-Life Balance
Chaired: Marion Cohen and Vallie M. Holloway
Sponsored by the ASIP Committee for Career Development, Women & Minorities and the FASEB Minority Access to Research Careers (MARC) office

Biology of Aging
Chaired: David A. Sinclair and Ivonne Ronchetti
Sponsored by ASIP and SIP

ASMB Lecture: Matrix Metalloproteinases as Effectors of Mucosal Immunity
Speaker: William Parks
Sponsored by ASIP and ASMB

Highlights: Graduate Student Research in Pathology
Chaired: James R. Stone
Sponsored by the ASIP Committee for Career Development, Women & Minorities

Cancer Stem Cells
Chaired: Stewart Sell

Eaten Alive: Autophagy in Cardiac Disease and Atherosclerosis
Chaired: Jonathon W. Homeister and Zhelong Xhu
Sponsored by ASIP and SCVP

PPS Symposium: Stem Cells in Lung Development and Disease
Chaired: Sem Hin Phan
Sponsored by ASIP and PPS

KEYNOTE LECTURE: Normal and Neoplastic Stem Cells
Speaker: Irving L. Weissman

SUNDAY, APRIL 19, 2009

ISAMM Symposium: Circulating Tumor Cells
Chaired: Larry E. Debault and Raymond R. Tubbs
Sponsored by ASIP and ISAMM

Patrolling the Vascular Interface by Leukocytes
Chaired: Myron I. Cybulsky and Francis W. Luscinskas

The Road to Independence – Careers in Pathology
Chaired: Tara L. Sander
Sponsored by the ASIP Committee for Career Development, Women & Minorities

9th Annual Career Development Program and Lunch: Winning in the Granting Process – Pathology
Chaired: Dani S. Zander and Jayne Reuben
Sponsored by the ASIP Committee for Career Development, Women & Minorities, the American Association of Anatomists, and the FASEB Minority Access to Research Careers (MARC) office

Liver Pathobiology Symposium: Interdisciplinary Approaches to Liver Disease
Chaired: Harriet C. Isom
Sponsored by the ASIP Liver Pathobiology Scientific Interest Group

Mechanisms of Tumorigenesis in the Phakomatoses
Chaired: Steven L. Carroll

BLOOD VESSEL CLUB: Genetic Approaches to Vascular Disease
Chaired: Luisa Iruela-Arispe and Douglas A. Marchuk
Sponsored by ASIP and the North American Vascular Biology Organization

Rous-Whipple Award Lecture: Liver Regeneration
Award Recipient: George K. Michalopoulos

MONDAY, APRIL 20, 2009

ACVP Symposium: One Medicine: Canine Genomic Models of Human Disease
Chaired: Elizabeth Whitley and John Erby Wilkinson
Sponsored by ASIP and ACVP

Pathobiology of Angiogenesis: Update 2009
Chaired: Harold F. Dvorak
A tribute to Judah Folkman

Trends in Experimental Pathology: miR'ely Making Sense of It All: Novel Implications of Micro RNA in Disease and Therapies
Chaired: Wing C. Chan and Mark Alan Feitelson
Supported by an unrestricted educational grant from the Robert E. Stowell Endowment Fund

ASIP Outstanding Investigator Award Lecture: New Approaches to the Pathology and Genetics of Neurodegeneration
Award Recipient: Mel Feany

ASIP Presidential Symposium: Resolving Cell Death and Inflammation: Implications in Disease
Chaired: Linda McManus

ASIP Awards Presentation and Membership Business Meeting
Chaired: Linda McManus

Awards Reception
All EB attendees are welcome to attend

TUESDAY, APRIL 21, 2009

Beyond Genomics: Epigenetic Pathogenesis of Cancer
Chaired: Ashley G. Rivenbark and Timothy H. Bestor
Supported by educational grants from Active Motif® and Zymo Research®

Molecular Mechanisms and Dynamics of Leukocytes Breaching Tissue Barriers
Chaired: Sean P. Colgan and William A. Muller

Which Way the Wnt Blows: Implications in Tissue Pathobiology
Chaired: Asma Nusrat and Youhua Liu

WEDNESDAY, APRIL 22, 2009

Pathobiology: Genetically Engineered Mouse Models
Chaired: Alexander Nikitin and Robert D. Cardiff

The American Journal of Pathology

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On the Cover: Combined exposure of MCF7 breast cancer cells to low doses of rosiglitazone and 9-*cis*-retinoic acid disrupts mitochondrial membrane potential and leads to apoptosis. Within these apoptotic cells, the dye JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) remains in the cytoplasm, which stains green (foreground), whereas in control cells, aggregation of JC-1 within mitochondria results in orange-red fluorescence (background). (See abstract NC 01 on page S8.)

Meeting Abstracts

Società Italiana di Patologia XXIX National Congress Abstracts

The XXIX National Congress of Società Italiana di Patologia (Italian Society of Pathology),
in collaboration with the American Society for investigative Pathology,
was held at the University of Calabria, Arcavacata di Rende, Cosenza, Italy, September 10-13, 2008.

To cite the Abstracts published here, please use the following format:

Maletta R, Anfossi M, Bernardi L, Colao R, Frangipane F, Gallo M, Geracitano S, Puccio G, Tomaino C, Bruni AC: Familial Late Onset Dementia: From Complex to Monogenic Model. XXIX National Congress of Società Italiana di Patologia, 2008 September 10-13, Cosenza, Italy. Am J Pathol 2008, 173(Suppl):S1 Abstract A 01

These abstracts were reviewed by the Società Italiana di Patologia (Italian Society of Pathology) and the American Society for Investigative Pathology.
The American Journal of Pathology was not involved in the peer review process.

Società Italiana di Patologia

XXIX National Congress Abstracts

September 10-13, 2008
University of Calabria, Arcavacata di Rende, Cosenza, Italy

AGEING

A 01. Familial Late Onset Dementia: From Complex to Monogenic Model

R. Maletta[1], M. Anfossi[2], L. Bernardi[2], R. Colao[2], F. Frangipane[2], M. Gallo[2], S. Geracitano[2], G. Puccio[2], C. Tomaino[2], A. C. Bruni[2]
[1]Centro Regionale di Neurogenetica. [2]ASP Catanzaro Ospedale Lamezia Terme, Lamezia Terme, Italy.

Background: Studies on early onset (EO) familial dementia led to identify APP, PS1 and PS2 genes in AD, MAPT and PGRN in FTD patients. Heritability in late onset (LO) forms of dementia is now more evident, exactly due to longer life expectancy in all countries. Aim: To report genetic mutations in familial AD and LOFTD cases. Methods: All above reported genes have been screened in a total of 150 patients with LO familial dementia. Results: Mutations in PS2, APP and PGRN genes have been found in 5 unrelated LOAD and 1 LOFTD patients. Conclusions: Mutation frequency in LO forms has been underestimated due to the large variability of the phenotype caused by longer life expectancy.

A 02. A Proteomic Approach for investigating the Aging Process: The Human Fibroblast Model

D. Quaglino[1], F. Boraldi[1], G. Annovi[1]
[1]Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy.

Aging is defined as the accumulation of deleterious changes occurring in tissues and cells. Fibroblasts are important stromal cells synthesizing the structural components of the extracellular matrix, and migrating within the stroma in order to interact with cells and the extracellular milieu, according to different stimuli. Analysis of the protein profile of fibroblasts isolated from young and old individuals demonstrated the role of synthetic and degradative pathways in modulating the whole cell machinery. In the light of these results we have compared fibroblasts from aged individuals and in vitro aged, focusing on changes in the expression of proteins related to stress response, protein synthesis and folding, cytoskeleton, membrane stability and extracellular matrix.

Work supported by ELASTAGE #18960 and PXE International.

A 03. Molecular Characterization of Thirty-Five Italian Patients with Niemann-Pick C: Identification of 21 NPC1 and 1 NPC2 Novel Alleles.

P. Tarugi[1], E. Pinotti[1], T. Fancello[1], C. Romano[3], M. Filocamo[2]
[1]Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy, [2]IRCCS G. Gaslini, Genova, Italy, [3]Bioinformatica, Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy.

Niemann-Pick C, the autosomal recessive neurological disease resulting from a failure of cholesterol trafficking within the lysosomal pathway, is due to mutations in NPC1 or NPC2 genes. We identified thirty-five patients including 32 patients with mutations in NPC1 gene and 3 others in NPC2 gene and found 33 genotypes. Among the twenty-one unpublished NPC1 alleles, 15 were due to point mutations resulting in thirteen codon replacements (p.C100S, p.P237L, p.R389L, p.L472H, p.Y634C, p.S636F, p.V780G, p.Q921P, p.Y1019C, p.R1077Q, p.L1102F, p.A1187V, p.L1191F) and in two premature stop-codons (p.R934X, p.Q447X), respectively; 4 other NPC1 alleles were small deletions/insertions leading to premature protein-truncations (p.C31WfsX26, p.F284LfsX26, p.E1188fsX54, p.T1205NfsX53). Finally the new intronic c.464-2A>C change at the 3' acceptor splice site of intron 4 affected NPC1 mRNA processing. We also found a new NPC2 mutant caused by a change of the first codon (p.M1L). The novel missense mutations were further investigated by two bioinformatics approaches. Panther classification system protein computationally predicted the detrimental effect of all new missense mutations occurring at evolutionary conserved positions. The other bioinformatics approach was based on prediction of structural alterations induced by missense mutations on the NPC1 atomic models. This analysis predicted malfunctioning and/or local folding alteration of the protein for most missense mutations. Moreover the effects of the missense mutations (p.Y634C, p.S636F, p.L648H, p.V780G) affecting the sterol-sensing domain (SSD) was evaluated by a docking simulation between the atomic coordinates of SSD model and cholesterol.Heterozygotes for NPC1 mutations may be more prone to develop neurodegenerative disease late in life.

A 04. Oxidative Stress and UV Radiation-induced Effects on Human Primary Thyroid Cell Proliferation

I. Kostic[1], B. Toffoletto[2], M. Toller[3], M. Moretti[3], C. A. Beltrami[2], F. S. Ambesi Impiombato[3], F. Curcio[3]
[1]Institute of Pathophysiology, School of Medicine, Kragujevac, Serbia, [2]Dipartimento Scienze Morfologiche, sez. di Anatomia Patologica, Università degli studi Udine, Italy, [3]Dipartimento di Patologia e Medicina Sperimentale e Clinica, Università degli studi Udine, Italy.

It has been reported that UV-radiation and oxidative-stress-inducers (responsible for the ageing process), provoked apoptosis in FRTL-5 cells. This study examined how UV-radiation and buthionine-sulfoxymine (BSO) affect human primary thyroid cells. Cell cycle analysis 24h after irradiation showed a G0/G1 block, while after 48h, S-phase significantly increased compared to the control, non irradiated cells, indicating possible DNA repair during S-phase of the cell cycle. The number of apoptotic cells, estimated by annexin V test, increased gradually over time after the UV radiation. The significant difference was seen after 40-48h of UV radiation in comparison with the control, non irradiated cells ($p < 0.05$). However, the number of apoptotic cells after treatment with BSO was significantly higher as compared to control cells ($p<0,05$). The difference was even more pronounced after exposing BSO treated cells to UV radiation when about 74% of cells entered apoptosis 96h after treatment. Younger-actively proliferating cells, were more sensitive to UV radiation or BSO treatment as compared to older-less proliferative cells. Results indicated that human primary thyroid cells were more resistant to UV-radiation than to oxidative stressors. Differently from FRTL-5 cells that almost all entered apoptosis 48h after irradiation, human cells reather inhibited cell proliferation trying to repair damaged DNA. However, exposing cells to additional oxidative stress induced earlier appearance and higher percentage of apoptosis. In conclusion, the human primary thyroid cells could be a good model to study the influence of different factors that are involved in the ageing process in human tissues.

A 05. Polymorphisms of the Eicosanoid Enzymes in Myocardial infarction and Longevity: Implications for Pharmacogenomics

F. Listi[1], C. Caruso[1], M. Caruso[1], E. Incalcaterra[1], E. Hoffmann[1], G. Caimi[1], D. Di Carlo[1], S. Vasto[1], D. Lio[1], G. Candore[1]
[1]University of Palermo, Palermo, Italy.

Cyclo-oxygenases (COXs) and 5-lipoxygenase (5-LO) are implicated in a wide variety of inflammatory disorders, including atherosclerosis. We have tested the hypothesis that anti-inflammatory variants of these genes confer genetic resistance to myocardial infarction (MI) and conversely favor longevity. So, we analyzed MI patients, age-related controls and centenarians. The pro-inflammatory alleles were overrepresented in MI and under-represented in centenarians whereas age-related controls displayed intermediate values. In conclusion, these studies are meant to detect and utilize a risk profile which allows early identification of individuals susceptible to disease and design of the right dose for a desired effect, a pharmacogenomic approach for age-related diseases, i.e. a preventive treatment with specific inhibitors of eicosanoids or their enzymes.

A 06. Involvement of Myosin II-B and Protein Kinase C in the Processing of the Alzheimer Amyloid Precursor Protein

F. Argellati[2], S. Massone[2], C. Domenicotti[2], B. Marengo[3], U. M. Marinari[2], M. A. Pronzato[2], R. Ricciarelli[1]
[1]Department of Experimental Medicine, University of Genoa, Genoa, Italy, [2]University of Genoa, Genoa, Italy, [3]University of Genoa and G. Gaslini Institute, Genoa, Italy.

Alzheimer's disease (AD) is characterized by extracellular deposits of amyloid-beta peptides cleaved from the amyloid precursor protein (APP); however, why and where this processing takes place in neurons remains unknown. Our previous data indicated that the processing of APP involves myosin II-B, important contributor to

the cytoskeleton of neuronal cells (Massone et al. 2007). The results presented here are consistent with a model in which PKC-mediated phosphorylation of myosin II-B regulates shuttling of APP between the cell periphery and the perinuclear region, shifting APP away from the compartments where its amyloidogenic processing is favored or, alternatively, moving APP toward a non-amyloidogenic pathway.

A 07. Evidence for Multiple Modifications of Albumin Exposed to Malondialdehyde

S. Millanta[2], A. Furfaro[2], E. Balbis[2], D. Cottalasso[2], U. M. Marinari[2], M. A. Pronzato[2], N. Traverso[1]
[1]Experimental Medicine, University of Genova, Italy, Genova, Italy, [2]University of Genova, Genova, Italy.

Peroxidation generated aldehydes bind spontaneously to proteins, generating adducts whose characteristics are still incompletely known. Here some properties of the adducts between malondialdehyde (MDA) and bovine serum albumin (BSA) have been studied. MDA-BSA was produced by simple incubation of BSA with various MDA concentration for different times. Aldehyde excess was then removed by gel filtration. Modification of tryptophan absorbance and fluorescence, generation of fluorescence and SDS-PAGE behaviour of the modified proteins were analysed. The obtained results indicate that multiple modifications occurs in MDA-BSA interaction, depending on MDA concentration and incubation time. In particular, it is not guaranteed that short incubations at high MDA concentration mimic long incubations at low MDA concentrations.

Grants from Genoa University and PRIN 2006065711_002

A 08. Cystatin-C, Apo-E Polymorphisms and Alzheimer'S Disease: An Association Study in Southern Italy.

V. Andreoli[1], F. Trecroci[1], A. La Russa[1], N. Romeo[1], G. Di Palma[1], G. Nicoletti[1], R. Cittadella[1]
[1]ISN-CNR, Mangone, Italy.

Cystatin C is a cysteine protease inhibitor which is found to colocalize with Aß in plaques and cerebrovascular deposits in Alzheimer's Disease (AD). Recent studies have reported a genetic association between the 73 G/A polymorphism within exon 1 of the cystatin C gene (CST3), a common Ala/Thr substitution in the signal peptide, and AD with conflicting results. To further investigate the proposed association in our population, we analyzed this variant in a population based group of 171 Italian patients with sporadic AD from southern Italy (Calabria region) and 190 healthy controls subjects from the same geographical area. All 361 subjects were genotyped for CST3 and APOE polymorphisms but our data showed no association between AD and CST3. We therefore stratified our samples based on age (of controls) or age of onset (of cases): <65-69, 70-79, and 80+ years. After this stratification according to age, in older patients (80+ years) the GG frequency resulted over-represented when compared to controls, but far from statistically significant. There was also no evidence of a statistical interaction between CST3 and APOE polymorphisms. In conclusion, our data suggest that the 73 G/A polymorphism within exon 1 of the cystatin C gene is not a susceptibility factor in AD and nor mitigate the effect of the ApoE -e 4 allele in the risk of developing AD in our population but further studies will be necessary to clarify the CST3 polymorphism position among AD risk factors.

A 09. A Clinical, Pathological and Molecular Study of A Frontotemporal Dementia Sporadic Case.

M. A. Losso[1], R. Vuono[4], M. Anfossi[2], L. Bernardi[2], R. Colao[2], C. Duyckaerts[6], J. F. Foncin[7], F. Frangipane[2], M. Gallo[2], S. Geracitano[2], F. Lamenza[3], R. Maletta[2], M. L. Panno[4], G. Puccio[2], M. G. Spillantini[5], C. Tomaino[2], A. C. Bruni[2]
[1]Cell Biology, Department of Cell Biology, Arcavacata di Rende, Italy, [2]Regional Neurogenetic Centre, AS 6, Lamezia Terme, Italy, [3]Geriatric Unit, AS3, Rossano, Italy, [4]Department of Cell Biology, University of Calabria, Arcavacata di Rende, Cosenza, Italy, [5]Centre for Brain Repair, University of Cambridge, Cambridge, United Kingdom (Great Britain), [6]Laboratoire de Neuropathologie R. Escourolle, Hôpital de La Salpêtrièe, Paris, France, [7]Laboratoire de Neurohistologie, Ecole Pratique des Hautes Etudes, Brie Comte Robert, France.

Frontotemporal dementias (FTDs), a heterogeneous group of progressive neurodegenerative disorders, are caused by several mutations in the MAPT gene. Alternative splicing of exon 10 produces six tau isoforms with either four (4RTau) or three (3RTau) repeats. In normal human brain the ratio of 4R:3R is ~1. Here we describe two novel intronic mutations, the IVS10+4A>C in intron 10 and the IVS9-15T>C in intron 9, identified in the MAPT gene of a FTD ascertained patient. Neuropathological studies have shown a diffuse atrophy of the brain with severe neuronal loss. Molecular in vitro and brain tissue studies show an altered 4R:3R ratio. We hypothesize that for the clinical expression of this condition both MAPT mutations may have contributed to the neurodegeneration process.

A 10. CXCR3 Chemokine Receptor Facilitates Dermal and Epidermal Maturation in Aged Associated Wounds

C. C. Yates[1], D. Whaley[1], A. Wells[1]
[1]University of Pittsburgh, Pittsburgh, PA, United States of America.

Failure to heal wounds is a major problem in persons of advanced age, compounded by pathologies the cause limited mobility or metabolic imbalances, which are common in advanced age. In skin wounds, the regeneration of the ontogenically distinct mesenchymal and epithelial compartments must proceed coordinately to restore proper functionality. We have recently found that the orchestration of repair is, to a significant extent, coordinated by ELR-negative CXC chemokines (PF4/CXCL4, IP-10/CXCL10, MIG/CXCL9, and IP-9/CXCL11) that bind the common CXCR3 receptor. These ligands affect not only the cells of the innate and acquired immune system but also the fibroblast, endothelial, and keratinocytes critical to tissue regeneration. To investigate the effects of CXCR3 signaling in wound healing of the aged population full thickness excisional wounds were created on CXCR3-/- or wild-type (12mo-old) mice and examined for 30-days. Wounds were histologically analyzed for re-epithelialization, epidermal and dermal maturation, collagen remodeling and organization. The CXCR3 -/- mice exhibited a significant delay in healing in all areas compared to the wild type mice. Interestingly, a lack of vascularization was seen in CXCR3-/- mice; there was also apoptosis in the dermis during the stage in which one normally sees fibroplasia. Combined, the lack of blood vessels coupled with fibroblast death would cause the delay seen in granulation tissue accumulations, compared to the wild type. Taken together, these results suggest the aged-associated healing delay may be regulated at least in part by CXCR3. All of these finding have intriguing implications for rational interventions aimed at promoting wound healing.

A 11. Loss of CXC Chemokine Receptor 3 Signaling Causes a Delay in Epidermal and Dermal Maturation and Leads to Hypertrophic Scarring

C. Yates[1], D. Whaley[1], R. Bodnar[1], A. Wells[1]
[1]University of Pittsburgh, Pittsburgh, PA, United States of America.

It is important to identify potential mechanisms of signaling dysfunction that leads to a failure to 'stop healing' resulting in excessive and hypertrophic scarring. Wound maturation, at least in part, is mediated by ELR-negative chemokines and their receptor CXCR3. CXCR3 and its ligands modulate the timing of keratinocytes migration, fibroblasts and endothelial cell immigration into the wound. This study investigates the long term effects of aberrant CXCR3 in wound healing. Full-thickness excisional wounds were created on CXCR3-/- or wild-type mice and examined at 180-days postwounding. Grossly the CXCR3-/-mice presented a visibly thickened, keratinized scar compared to the wild-type mice, in which the scar was scarcely noticeable. Histological analysis of the CXCR3-/-mice also revealed a thicker epidermis that was hyperproliferative as visualized by Ki67 staining. Additionally, the dermal layer appeared disorganized with thick and long collagen fibrils and contained excessive collagen content in comparison to the wild-type mice. Interestingly, the CXCR3-/-wounds were weaker as determined by tensile-strength in contrast to wild-type wounds as a result of decreased cross-linking of collagen-fibers. ECM turnover and maturation was limited in these mice, denoted by the elevated expression of matrix proteins of immaturity, tenascin-C and fibronectin, and an imbalance of MMP-9 expression; whereas these markers of an immature dermis are long gone in wounds in wild type mice. These in vivo studies establish that the absence of the CXCR3-signaling network results in hypertrophic scarring by delaying epidermal maturation and altering the synthesis and degradation of collagen in addition to other matrix components during wound healing.

HOST PATHOGENS

HP 01. Pseudomonas Aeruginosa induction of IL-8 Promoter in Human Conjunctival Cells.

I. Venza[1], M. Cucinotta[1], M. Visalli[1], G. De Grazia[1], S. Oliva[1], D. Teti[1]
[1]Policlinico Universitario, Messina, Italy.

Pseudomonas aeruginosa is a causative agent of conjunctivitis and in some systems induces ulceration via IL-8. Here we investigated the IL-8 secretion by human conjunctiva challenged with *P. aeruginosa*. IL-8 protein and mRNA were determined by ELISA and RT-PCR. Cells were transfected with IL-8 promoter constructs and the DNA binding of transcription factors were investigated through EMSA, ChIP and Re-ChIP. Our results show that *P. aeruginosa* induces a significant increase of IL-8 expression in these cells by activating C/EBPβ via p38 MAPK and NF-κB, and inducing the *in vivo* and *in vitro* binding of RelA and C/EBPβ to the IL-8 promoter.

Re-ChIP demonstrated that RelA and C/EBPβ functionally cooperate in the *P. aeruginosa*- induced activation of the IL-8 gene expression.

HP 02. HP-NAP, a Key Factor in the Helicobacter Pylori Chronic inflammation

A. Cappon[1], L. Cancian[1], G. Codolo[1], M. De Bernard[1]
[1]University of Padua, Padua, Italy.

The *Helicobacter pylori* neutrophil-activating protein (HP-NAP)is a virulence factor of *H. pylori* that stimulates in neutrophils high production of oxygen radicals and adhesion to endothelial cells. Here we report that the exposure of monocytes to HP-NAP resulted in a significant increase in cell viability; while culture monocytes undergo spontaneous apoptosis after 48 h, HP-NAP-treated cells survived up to 7 days. Accordingly, HP-NAP prevents the cleavage of poly-ADP-ribose polymerase (PARP) in monocytes at the same time. Furthermore, we determined that HP-NAP acts indirectly through the secretion of survival factors from monocytes. Thus, HP-NAP, influencing the life-span of the cells involved in the inflammation, might create an appropriate environment, in the gastric mucosa, for the *H. pylori*-associated inflammation.

HP 03. Chlamydia Pneumoniae, Helicobacter Pylori and T Cells in Atherosclerosis

M. M. D'Elios
Internal Medicine, University of Florence, Firenze, Italy.

Atherosclerotic lesions invariably consist of monocyte-derived macrophages and T lymphocytes. We studied in vivo activated T lymphocytes that infiltrate atherosclerotic plaques of *Helicobacter pylori*-infected patients with or without anti-*Chlamydia pneumoniae* antibodies. In human atherosclerotic lesions we showed predominance of T cells producing T helper1 cytokines. We detected *C. pneumoniae* DNA and *C. pneumoniae*-specific T cells but not *H. pylori*-specific T cells in atherosclerotic plaques of anti-*C. pneumoniae* seropositive patients infected by *H. pylori*. Plaque-derived T cells, either specific for *C. pneumoniae* or not, exhibited a predominant Th1 cytokine profile, helper function for tissue factor production by monocytes, proapoptotic activity, perforin-mediated cytotoxicity. Thus, atherosclerosis, although multifactorial, can be regarded as an antigen-driven immuno-pathological condition, at least in part related to infections.

HP 04. The Neutrophil Activating Protein of Helicobacter Pylori (HP-NAP) as an Immune Modulating Agent

M. De Bernard
Venetian Institute of Molecular Medicine, Padova, Italy.

The Neutrophil Activating Protein (HP-NAP) is a virulence factor of *Helicobacter pylori* that stimulates in neutrophils high production of oxygen radicals and adhesion to endothelial cells. We found that HP-NAP is a Toll-like receptor (TLR)-2 agonist able to induce the expression of interleukin (IL)-12, and IL-23 by neutrophils and monocytes. Addition in culture of HP-NAP, as immune modulator, to antigen-induced T-cell lines resulted in a remarkable increase of interferon-gamma-producing T cells and decrease of IL-4-secreting cells, thus shifting the cytokine profile of antigen-activated human T cells from T helper (Th) 2 to a Th1 cytotoxic phenotype. We also found that HP-NAP elicited in vivo antigen-specific Th1-polarized T-cell response in the gastric mucosa of *H. pylori*-infected patients.

HP 05. Novel insight into T Cell Immune Response Against Mycobacterium Tuberculosis.

N. Caccamo[1], S. Meraviglia[1], A. Salerno[1]
[1]University of Palermo, Palermo, Italy.

The majority of individuals, upon exposure to *M. tuberculosis*, develop protective immunity and successfully contain infection. Acquired resistance to *M. tuberculosis* depends on the interaction of CD4 and CD8 T cells and infected macrophages and granuloma formation, which serves to contain pathogens, although *M. tuberculosis* bacilli are not always completely eradicated and may remain dormant for decades. One of the major roles of the granuloma is to localize and contain not only the bacteria but also the inflammatory response to the bacteria. Thus, rigorous control of the organization of granulomas is likely necessary to prevent immunopathology. Therefore, understanding of the immune responses that control *M. tuberculosis* are important not only for achieving optimal immunity, but also for avoiding dangerous immunopathology.

HP 06. NapA of Borrelia Burgdorferi Drives Th17 inflammation in Lyme Arthritis

G. Codolo[1], A. Amedei[2], A. Polenghi[3], A. C. Steere[4], C. T. Baldari[5], G. Zanotti[6], C. Montecucco[1], M. M. D'Elios[2], M. De Bernard[3]
[1]Department of Biomedical Sciences, University of Padua, Padova, Italy, [2]Department of Internal Medicine, University of Florence, Florence, Italy, [3]Venetian Institute of Molecular Medicine, Padova, Italy, [4]Center for Immunology and Inflammatory Diseases, Division of Rheumatology, Boston, MA, United States of America, [5]Department of Evolutionary Biology, University of Siena, Siena, Italy, [6]Department of Chemistry, University of Padua, Padova, Italy.

Human Lyme arthritis (LA) caused by *Borrelia burgdorferi* is characterized by neutrophils and T cells infiltrate. The Neutrophil Activating Protein A (NapA) is essential for *B. burgdorferi* persistence within ticks, but its role in immune response of LA is unknown. Here, we report that it is a major antigen of the humoral response in patients with LA. We show that T cells from synovial fluid of those patients produce Interleukin (IL)-17 in response to NapA. NapA is a Toll-like receptor (TLR)-2 agonist able to induce the expression of IL-23 in monocytes and neutrophils, and IL-6, IL-1b and TGFβ in monocytes, and elicits a synovial T helper (Th)-17 response that might play an important role in the pathogenesis of LA.

HP 07. Molecular Cloning and Characterisation of Helicobacter Pylori L-Asparaginase: A Cytotoxic Factor with Potential Biomedical Applications

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[1]Università di Pavia, Pavia, Italy.

H. pylori is a Gram-negative bacterium which remains the major pathogenic inhabitant of the human stomach. Epidemiological evidences suggest the relationship between *H. pylori* infection and gastric cancer, probably due to cell cycle alterations induced by the bacterium. We recently found that the cell-cycle block of cultured cells is related to *H. pylori* L-asparaginase. We report the molecular cloning of the *H. pylori* ansB gene, the biochemical characterisation of the recombinant enzyme purified to homogeneity, and its effects on cultural cell viability. Our results show that *H. pylori* L-asparaginase might represent both an interesting pathogenetic factor able to explain some as yet unclarified features of *H. pylori* infection, and a new potential drug broadening the spectrum of available chemotherapeutic agents.

HP 08. The HIV-1 Matrix Protein p17 interferes with the PI3Kinase-AKT Pathway in p17R-Expressing Cell Lines.

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The HIV-1 p17 is a structural protein that may also act as a virokine able to deregulate several immune functions through a interaction with a cellular surface receptor(p17R). Here we demonstrated that, in different p17R-expressing cell lines, p17 can interfere with the PI3-kinase-AKT pathway through AKT inactivation. This evidence was enforced by the finding that p17 induces an upregulation of PTEN, a specific AKT-phosphatase, and a decrease of PI3-kinase activity. To better define the intracellular pathway involved by the p17 activity we also evaluated the level of MAPK (ERK1/2) phosphorylation and we showed that p17 can promote MAPK dephosphorylation probably though a tyrosine-phosphatase activation. These data support the possibility that p17 may hamper physiological activation pathways through the p17/p17R interaction.

HP 09. Chlamydophila Pneumoniae infection in Cardiovascular Diseases

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Chlamydophila (former Chlamydia) *pneumoniae* (CP) infection is strongly associated with atherosclerosis of the coronary artery disease, carotid artery, aorta, and peripheral arteries. This association has been shown in seroepidemiologic studies and by direct detection of the organism in atherosclerotic lesions by immunohistochemistry, polymerase chain reaction, electron microscopy, and tissue cultureRecently, we examined presence and quantity of the CP DNA in carotid endoartherectomy biopsies by laser microdissection (LCM) and Real-time PCR. Two different sites, intra-plaque and plaque-adjacent areas, were taken from each lesion, and the bacterial DNA was exclusively found in patients with unstable angina, and most of them were localized only in the intra-plaque area. Nonetheless, some other data also suggest an interesting extra-plaque localization of CP , which could play a critical role in acute myocardial events. In fact, CP was detect in autoptic samples of almost all examined subjects who died of acute infarction not only in whole coronary tree but also in distant myocardial tissue, totally unrelated to the plaque area. CP presence in the autoptic tissue was strongly associated with a T-cell inflammatory infiltrate. These results suggest that CP may underlie both coronary and myocardial

vulnerabilities and corroborate the notion that CP may act by reducing cardiac reserves, thus worsening the ischemic burden of myocardium.

HP 10. CPS Genes of Streptococcus Pneumoniae Modulate inflammatory Response of Neutrophils

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Streptococcus pneumoniae is the most common cause of community acquired pneumonia with high morbidity and mortality worldwide. A major feature of pneumococcal pneumonia is an abundant neutrophil (PMNs) infiltration. We have found that the wild-type D39 strain and the ΔCPS2E strain, a non encapsulated isogenic mutant of D39 lacking only the gene cps2E, stimulated superoxide anion (O_2^-), evaluated by cytochrome c reduction, similarly. But a non-encapsulated R6 strain, containing also a deletion starting from cps B to cps G genes, amplified the oxidative burst. The R6 strain activated neutrophil NADPH oxidase to produce reactive oxygen intermediates, as demonstrated by the traslocation of its cytosolic subunits p47phox to the plasma membrane. This activity was completely inhibited by wortamannin and bisidolylmaleimide but not by SB-203580. Taken together these findings strongly suggest that R6 strain activates pathways involving PKC and PI-3 Kinase dependent reactions. In order to know whether modulation of neutrophil functions induced by R6 strain was selective for oxygen metabolism we studied secretion of β-glucoronidase, a marker of degranulation. We have found that PMNs incubated with D39 and R6 strains secreted similar levels of β-glucoronidase and their effects were lower than that of the standard agonist fMLP. On the basis of these observations, we conclude that pneumococcal expression of genes cps B, C and D modulates neutrophil respiratory burst activity and thus may facilitate chronic persistent infection by *Streptococcus pneumoniae*.

INNATE IMMUNITY AND INFLAMMATION

IN 01. Do Voltage-Gated Proton Channels Play a Role in the Microbicidal Activity of Neutrophils?

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The molecular mechanisms governing the antimicrobial activity of neutrophils are still under debate. H+ efflux via voltage-gated proton channels has been proposed to regulate the respiratory burst and microbicidal activity of neutrophils. This conclusion rests on experiments using Zn2+, the best known and most potent inhibitor of these channels. We tested the effect of Zn2+ on killing of *S. aureus* and *C. albicans* by human neutrophils. Zn2+ (10-100 μM) did not affect killing of both microorganisms. At higher concentrations (up to 2 mM) Zn2+ inhibited killing of *C. albicans* while killing of *S. aureus* could not be assessed because of Zn2+ toxicity to the microorganism. High Zn2+ concentrations also inhibited PMA-induced respiratory burst measured as H_2O_2 production. The inhibition of candida killing and respiratory burst appears to be secondary to a cytotoxic effect of Zn2+ towards neutrophils. In fact, we found that Zn2+ at concentrations > 100 μM, interfered with Ca2+ movements, caused cell aggregation and led to cell death as detected by light microscopy, propidium iodide staining and LDH release. Our results do not support an involvement of voltage-gated proton channels in the regulation of the respiratory burst and killing activity of neutrophils. More importantly, the observed toxicity of Zn2+ undermines previous conclusions on the role of H+ efflux in neutrophil functions based on the effects of this cation, and calls for a reevaluation of the results of experiments in which Zn2+ has been used to study functional activities and electrophysiology of voltage-gated proton channels of neutrophils.

IN 02. F2-Isoprostane Receptors and Signal Transduction Pathways on Hepatic Stellate Cells

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F2-isoprostanes are markers of oxidative stress and mediators of important biological effects. Previously, we provided evidence that F2-isoprostanes, generated during CCl4-induced hepatic fibrosis, mediate hepatic stellate cell (HSC) proliferation and collagen hyperproduction. We suggested the involvement of a modified form of isoprostane receptor, homologous to the classic TxA2 binding site. The stimulatory effects of 8-epi-PGF2α on DNA and collagen synthesis are mediated by TxA2 receptor (TP). Moreover, western blotting and immunocytochemistry analysis revealed the expression of TP on HSC both on plasma membranes and within the cells. Experiments on the signal transduction pathways showed that 8-epi-PGF2α increase Ins(1,4,5)P3 and MAPK. Thus, it is likely that the fibrogenic effects induced by 8-epi-PGF2α in HSC are mediated by these transduction pathways.

IN 03. NK Cell Recognition and Functions

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NK cells participate in innate immunity and in early defense against infections and tumors by cytotoxicity and secretion of cytokines and chemokines. They are regulated by activating and inhibitory receptors engaged by ligands expressed on target cells. These include pathogen-derived molecules recognized by activating receptors or Toll-like receptors; self proteins which are up-regulated on “stressed” or damaged cells and that activate the receptor NKG2D. Inhibitory signals are delivered by MHC-I molecules binding to receptors of the killer cell immunoglobulin-like (KIRs) and C-type lectin families. In most instances the inhibitory signals override the triggering ones. However, induction of activating ligands on stressed or injured cells may overcome the inhibition, thus leading the NK cells to kill these targets.

IN 04. Polymorphisms of TLR4 and CD14 Genes and Alzheimer’s Disease Risk

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TLR4 and CD14 gene polymorphisms and Alzheimer’s disease risk
Alzheimer's disease (AD) is neurodegenerative disease. Inflammation plays a key role in AD. So, in this study we evaluated whether, +896A/G TLR4 and -260C/T CD14 polymorphisms, are AD risk factors. The study performed in 626 AD patients from Northern Italy and age and gender matched controls. Our results demonstrated that +896A/G TLR4 polymorphism is associated with AD. Subject’s carrier of high responder TLR4 SNP might be selected for a clinical trial.

IN 05. B7h Costimulation induces Caspase-1 Activation and IL-1ß Secretion in LPS-Activated Dendritic Cells and Modulates their Capacity to Drive T Cell Differentiation

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B7h is constitutively expressed by B-cells, macrophages, and dendritic cells (DCs) and binds ICOS expressed by activated T cells. This work investigates the effect of B7h triggering by a soluble form of ICOS (ICOS-Ig) on DCs. DCs were differentiated from monocytes to immature DC (iDC) and then stimulated with LPS to obtain mature DC (mDC). To assess the role of B7h in this we treated iDC with LPS in the presence and absence of ICOS-Ig and analyzed the capacity to stimulate T cell proliferation in mixed lymphocyte reaction (MLR). Results showed that B7h triggering is inable to drive iDC activation but modulates LPS-induced maturation by changing the secreted cytokines. The most striking effect was a 80-fold increase in IL-1b secretion. IL-1b is synthesized as an inactive precursor and is activated and secreted after caspase-1 activation. In macrophages, IL-1b secretion requires two signals: the first initiates the synthesis of IL-1b and the second activates caspase-1 that cleaves the IL-1b precursor into the mature form that is then secreted. In DCs, LPS stimulation induced intracellular expression but not secretion of mature IL-1b, whereas ICOS-Ig was uneffective on IL-1b synthesis but induced the activation of Caspase-1. Interestingly, stimulation with LPS plus ICOS-Ig induced both intracellular expression and secretion of large amounts of IL-1b. These data show that B7h triggering can act as a second signal to induce release of the active form of IL-1b in LPS-DCs and substantially changes their capacity to drive T cell differentiation.

IN 06. Advances on Neutrophil-Derived Cytokines in Inflammatory and Immune Responses

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Neutrophils represent a key component of the inflammatory response and act as a powerful defensive system against invading bacteria. Following activation by various stimuli, neutrophils have the capacity to release lytic enzymes with potent antimicrobial potential and to generate reactive oxygen intermediates, both essential for pathogen killing. Neutrophils can also produce, upon appropriate stimulation, a variety of proteins in vitro and in vivo, including cytokines and chemokines, both involved in their effector functions, as well as in the recruitment, activation and programming of other immune cells. In this latter context, recent studies have shown

that neutrophils, through secreted products and/or cell-cell contact, might potentially interact with monocytes, DC and NK cells, B and T lymphocytes. The latter will be discussed.

IN 07. The Activation of the DNA Damage Response Regulate the Expression of DNAM-1 and NKG2D Activating Receptor Ligands on Human Multiple Myeloma (MM) Cells.

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Multiple Myeloma is a plasma cell disorder in which abnormal plasma cells proliferate in the bone marrow. It has been demonstrated that cellular stress and induction of DNA damage response can up-regulate NKG2D ligand (NKG2D-L) expression. We show that MM cell lines treated with low doses of chemotherapeutic drugs up-regulate DNAM1-L and NKG2D-L. NK cell-mediated killing of myeloma cells was increased by drug treatment, and NKG2D and DNAM1 activating receptors were the major triggering molecules of NK cell lysis. These data have been confirmed on plasma cells derived from patients with MM. DNAM1-L and NKG2D-L up-regulation was abolished by treatment with inhibitors of ATM and ATR. These findings reveal a common pathway that regulate NK cell activating ligands expression.

IN 08. Down-Regulation of the Activating Receptor NKG2D by its Ligands Expressed on T Lymphocytes: A New Mechanism to Limit CD8+ T Cell Immune Responses.

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Since antigen-activated T cells express the NKG2D ligands (NKG2DLs) MICA and ULBP1-3 on their cell surface, we investigate if this would affect NKG2D expression and functions. NKG2D levels were reduced on activated CD8+ T cells and were dependent on the presence of CD4+ cells. NKG2D expression was decreased by receptor internalization and not by mRNA regulation. Soluble NKG2DLs were found in the supernatants of antigen-activated T cells. Moreover, MICB and ULBP1 were released at higher amounts when CD4+ cells were present in the cell cultures. The functional consequence was a decrease in NKG2D costimulatory activity upon CD3 triggering. These findings show that activated CD4+ T cells expressing NKG2DLs can prevent NKG2D-mediated CD8+ T cell responses.

IN 09. Meta-Analyses of Association Between Cytokine Polymorphisms and Alzheimer's Disease

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Background: Inflammatory processes are associated with Alzheimer's disease (AD). Studies which reported associations between pro- and anti-inflammatory cytokines polymorphisms and AD are controversial. Aim: Verify the association between pro and anti inflammatory cytokines polymorphisms (IL-1ß -511, IL-1-ß +3953, IL-6 -174, IL-10 -1082) and AD by meta-analysis. Methods: Computerized bibliographic searches on PUBMED and AlzGene database (http://www.alzgene.org) supplemented with manual searches of reference lists. Results: 1) TT genotype of IL-1ß +3953 polymorphism is associated with AD, 2) IL-6 -174 polymorphism is not associated, 3) IL-1ß -511 and the IL-10 -1082 polymorphisms are associated only when we considered studies with specific characteristics.Conclusions: These results support a significant association between IL-1ß +3953 polymorphism and AD.

IN 10. Oxidative Stress and Lung Fibrosis

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On the basis of the results obtained in experimentally induced liver fibrosis, we investigated the possible relevance of F2-isoprostanes (IsoPs) in a rat model of bleomycin-induced lung fibrosis.Bleomycin-induced fibrosis is accompanied by an increase in plasma IsoPs and appearance of cells expressing alpha-SMA.Moreover, lung fibroblast cultures treated with IsoPs showed a more prompt expression of alpha-SMA, compared to control, indicating that IsoPs can readily activate fibroblasts to myofibroblasts. IsoPs also increase DNA and collagen synthesis. These effects may be mediated by TxA2 receptor, since it is present on fibroblasts and myofibroblasts in which it co-localizes with alpha-SMA.Our data suggest that IsoPs could play an important role in the onset of the alterations leading to lung fibrosis.

IN 11. Inflammatory Mediators: A Pharmacogenomic Approach in Alzheimer's Disease

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Inflammation and genetics play an important role in the pathogenesis of Alzheimer's Disease (AD). A critical search of published literature has suggested few inflammatory genes directly involved in the risk to develop AD. The selected genes are the pro-and anti-inflammatory cytokines, Toll-like receptor 4 (TLR4), cyclooxygenases (COXs) and lipoxygenases (LOXs). The associations between candidate gene polymorphisms and AD are of difficult and complex interpretation as a consequence of gene pleiotropy and genes and environmental factors. However, current data indicate that screening for interleukin (IL)-1, IL-10, TLR4, COX and LOX polymorphisms are likely to be a useful tool for AD risk assessment.We believe that dissecting out the influence of genetics polymorphism in AD provides a more complete risk risk assessment.

IN 12. Receptor Signaling and Migration Pathways of Dendritic Cells

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Dendritic cells (DC) are professional antigen presenting cells which play a pivotal role in the activation of adaptive immunity. Tissue invasion by pathogens induces the recruitment of blood DC to the site of infection and promote their subsequent migration to secondary lymphoid organs. This complex process relies on the expression and regulation of chemotactic receptors on the surface of migrating DC and on the activation of adhesion molecules that allow DC to properly interact with both blood and lymphatic vessels. In the absence of correct tissue localization, DC fail to promote proper immune responses. Recent work has documented that in addition to chemokines, a number of nonchemokine chemotactic factors play a crucial role in DC accumulation to pathological tissues. In addition, several agonists devoid of chemotactic activity are known to regulate DC migration to peripheral inflammatory tissues and to regional secondary lymphoid organs. These concepts will be discussed in the context of recent acquisitions on the differential migration behavior of DC subsets. Furthermore, we will provide evidence for the role of theChemR23/chemerin chemotactic axis in the trafficking of DC subsets in autoimmune pathologies.

IN 13. Chemokine Decoy Receptors

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Chemokines sustain leukocyte recruitment during inflammatory reactions through the interaction and activation of G protein coupled chemokine receptors. A set of seven transmembrane domain receptors recognizes chemokines that do not elicit migration or conventional signaling responses has also been identified. These "silent" receptors include DARC, which is expressed on erythrocytes and vascular endothelium and recognizes chemokines of different structural and functional families, and D6 and CCX CKR, which selectively recognize CC chemokines of the inflammatory and homeostatic realm respectively, and are strategically expressed on lymph nodes and lymphatic endothelium. We recently demonstrated that D6 acts as a chemokine scavenger receptor, which internalizes and targets the ligand to degradation and cycles back to the membrane. In vivo results with gene-targeted animals now indicate that D6 has a major role in tuning tissue inflammation and in the control of inflammatory chemokines transfer to draining lymph nodes in several inflammatory and infectious conditions. This non-redundant function is supported by biochemical properties unique of this receptor, including constitutive cycling and agonist-dependent upregulation, whose molecular basis have recently been identified by structure-function studies.

IN 14. Granulomatous Experimental Autoimmune Thyroiditis Induced by Immunization of CBA/J(H-2K) Mice with Hydrophobic Intermediates of the Dissociation/Unfolding of Human Thyroglobulin in Urea

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Rationale: Experimental autoimmune thyroiditis (EAT) can be induced in genetically susceptible mice by immunization with thyroglobulin (Tg). A granulomatous EAT (G-EAT) is obtained by the adoptive transfer of splenocytes sensitized in vivo with murine Tg, restimulated in vitro with murine Tg, plus anti-IL-2R or anti-IFN-gamma antibodies and IL-12. Aim: Evaluating whether the hydrophobic domains exposed

during the unfolding of Tg may modify the histopathology of EAT towards G-EAT. Methods: Human Tg (hTg) was denatured in 0.120 M Tris/HCl, 3.5 M urea, pH 9.0, yielding a mixture of partially unfolded monomers (UM), with increased hydrophobicity, in equilibrium with non-native dimers (NND), reassociating by virtue of a hydrophobic effect, which were separated by sucrose density ultracentrifugation. Female CBA/J(H-2k) mice were immunized with 100 µg of hTg, UM or NND in CFA, followed by 50 µg in IFA 8 days later. On day 30, we evaluated thyroid histology and proliferative and secretory responses of splenocytes. Results: The immunization of hTg was associated with focal, low-grade, mononuclear infiltration of thyroids. Aspects of granulomatous infiltration, with widespread follicle disruption and fibrosis, were observed in animals immunized with NND and, especially, UM. Proliferative responses and secretion of IL-12 and IFN-gamma by splenocytes were maximal in mice immunized with UM. Conclusions: Unfolded, monomeric hTg displayed a greater ability, compared with native hTg, to elicit autoimmune thyroid damage. The present data shed light on the possible mechanisms by which the unmasking of intracellular unfolded hTg, following cell thyroid damage, may incite thyroid autoimmunity.

IN 15. Interleukin-1 Receptor Antagonist in Ischemic Cardiomyopathy

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Interleukin-1 receptor antagonist (IL-1Ra) plasma levels have been proposed to monitor the risk of coronary artery disease. We investigated the actual source of IL-1Ra in heart samples taken at time of explant for heart transplant in subjects with ischemic cardiomyopathy. IL-1Ra production was evaluated using in situ hybridization for IL-1Ra mRNA and immunostaining. IL-1Ra was prevalently expressed by cardiomyocytes, the more in peri-infarct regions where ongoing cell apoptosis was also evident. Increased synthesis of mRNA for both secreted (sIL-1ra) and intracellular (icIL-1ra) isoforms was confirmed in the same areas of explanted hearts. In order to investigate a potential role for icIL-1Ra isoform production in the ischemic myocardium we tested the effect of IL-1ra on mitochondria-activated caspases. In fact, recombinant IL-1ra inhibited the in vitro activity of recombinant caspase9, behaving as a non-competitive enzyme inhibitor, with an estimated Ki of 0.3uM. Moreover, in vitro binding experiments indicated that recombinant IL-1ra bound avidly to caspase9. In contrast, IL-1Ra was found to alter the activity of caspase9-activated terminal effector cell death caspases-3,-6, and-7 only at much higher concentrations (>1uM). These findings point to IL-1Ra as a very important mediator within heart remodeling, promoting survival of cardiomyocytes in ischemic regions.

IN 16. Role of Chemochines/Chemochine Receptors on Leukocyte Recruitment by Lymphatic Microvessel Endothelial Cells.

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We used a previously developed model system for the induction of a lymphatic vessel endothelium hyperplasia in the peritoneum of mice (Exp. Cell Res., 246: 368-375, 1999) to investigate the profile of chemokine expression in murine lymphatic endothelial primary cultures in the presence or absence of interleukin-1 (IL-1), gamma interferon (gI), or gram negative bacteria endotoxin (LPS). A functional chemotactic assay on purified populations of murine PMN, lymphocytes, macrophages and dendritic cells using culture supernatants from these endothelial cells evidenced significant changes of leukocyte chemotaxis after exposure of leukocytes and/or lymphatic endothelial cells to inflammatory stimuli. Inhibition experiments using blocking moAbs directed against CXC, CC, and C type chemokines, or leukocyte pretreatment with iRNA to chemokine receptors CCR1, CCR2 and CCR3, evidenced a variable role of specific chemokines/chemokine receptors in leukocyte chemotaxis in the different in vitro/in vivo conditions. Consistent with these observations was the expression of the apparently most involved chemokines C10, JE, SLC, MIG and MIP-2, as studied by quantitative PCR analysis on total lymphatic endothelial cell RNA before and after exposure of the cells to cytokines or LPS. These studies provide new indications about the mechanisms leading leukocyte recirculation within the lymphatic system.

IN 17. Human Airway Epithelial Cells Exhibit Contact-Mediated Cytotoxicity Upon Exposure to Multi-Walled Carbon Nanotubes: A Mechanism for Granuloma formation induced By Biopersistent Materials?

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The biological effects of nanomaterials are only incompletely characterized, although it is known that the exposure of rodents to airborne carbon nanotubes (CNT) causes epithelial hyperplasia and the formation of interstitial granulomas around CNT aggregates. Recently, we demonstrated that human airway epithelial monolayers (Calu-3 cells) exhibit increased permeability upon the exposure to multi-walled CNT (MWCNT), although no marked decrease in cell viability was detected with standard biochemical methods. Here we have observed in confocal microscopy confluent Calu-3 cell monolayers grown on permeable filters and exposed to MWCNT, which formed aggregates on the monolayer surface. Calcein-loaded live cells adhered to MWCNT, climbed onto the aggregates and, eventually, covered them, although incompletely. While most of the monolayer was fully viable, several cells on the top of the aggregate were rounded and positive to propidium iodide, indicating that the contact with MWCNT triggered cytotoxicity. Consistently, several MWCNT-adherent cells exhibited high caspase activity. Nevertheless, the tight junction protein occludin was found expressed also in cells adherent to MWCNT aggregates. It is concluded that airway epithelial cells tend to cover MWCNT aggregates and to include them in the monolayer, but undergo an enhanced rate of apoptosis. While these data confirm that exposure to MWCNT affects the barrier function of airway epithelia, they suggest a thus far unknown mechanism for the entry of MWCNT aggregates in the lung tissues independent from cell uptake. Contact-dependent cytotoxic effects and inclusion in the epithelial monolayer may thus constitute one of the first steps towards epithelial hyperplasia and granuloma formation.

IN 18. Affinity Maturation of Antibodies in the 2-Phenyl-5-Oxazolone System

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Affinity maturation of antibodies is the process whereby more efficient antibodies are produced through somatic hypermutation and antigen-guided selection. The antibody response to 2-phenyl-5-oxazolone has been thoroughly investigated and consists of three antibody classes. Our investigation of the structural basis of this process by X-ray crystallography has shown that in class III antibodies the increase in affinity is mainly determined by the improvement in the surface complementarity of the hapten binding site, while a low-affinity class I antibody structure suggests that in this class it depends on a fine reorganisation of the binding site involving both charge changes and surface shape modifications. These results are relevant to determine the principle underlying affinity maturation in both this and other antibody responses.

NOVEL BIOMARKERS IN ONCOLOGY

NB 01. Control of Tumor Growth by the Endogenous Cannabinoid System in Colon Cell Lines

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We investigated the control of tumor growth by a metabolically stable anandamide analog (Met-FAEA) via the cannabinoid CB1 receptor in DLD-1, HT-29 and SW620 colon cancer cell lines. Met-F-AEA dose-dependently inhibited the proliferation of all cell lines exposed for 24 and 48 hours. The inhibitory effect of AEA on proliferation was reversed by co-incubation of cells with AEA and SR 141716A. Our findings indicate that the endocannabinoid system modulates colon tumor growth.

NB 02. Novel Approaches for High-Throughput Production of Mouse Monoclonal Antibodies

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Mouse monoclonal antibodies are key components in basic research as well as in the clinical laboratory but their production remains restricted by low-throughput screening methods and high tissue culture load. A novel, high-throughput and semi-automated method for hybridoma generation was recently developed by using robotic technology.By this strategy it was possible to carry out up to 8 somatic fusions simultaneously where the cell culture operations were executed by an automatic workstation. This hybridoma production method was coupled to multiplexed immunisation protocols and to a novel and high-sensitive screening assay, namely the antigen microarray assay (AMA). Therefore, this novel technology

represents a major advance of the antibody production platform allowing rapid and high-throughput production of murine monoclonal antibodies for biomedical research.

NB 03. Modification of a Specific Protein Pattern as Potential Indicator of Ovarian Cancer Progression

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Background: In this study we identified and characterized potential biomarkers of tumor progression in tissue, tumor and normal interstitial fluid (TIF and NIF), peritoneal effusion and serum of patients with advanced stage ovarian carcinoma. Experimental Design. Biological samples were collected from six patients affected by metastatic ovarian carcinoma. Specific protein pattern of samples was analyzed using two dimensional electrophoresis (2DE) and mass spectrometry (ESI-Q-TOF MS/MS). Differences in protein expression were assessed using PDQuest software. Results: Six proteins showed differential expression in TIF and tumor tissue, compared to NIF and normal tissue. Among these, S100A8 protein resulted significantly up-regulated in pathological samples and in peritoneal fluid. Five proteins were down-regulated and identified as Galectin3, GSTA2, CRBP-1 and ANXA 5. Conclusion: The proteins here identified are known to be involved in tumor progression and secreted in extracellular microenvironment. Even though they cannot be considered as specific biomarkers for ovarian carcinoma, since they have been detected also in other cancer types, we might assume that they could predict tumor spreading and relapse after the primary therapy (surgery and/or chemotherapy).

NB 04. Concentration of Plasma DNA for Monitoring Patients with Renal Carcinoma

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Renal Cell Carcinoma (RCC) lacks precocious symptoms and displays chr-3p deletions exploitable for diagnosis and monitoring follow-up. Preoperative plasma DNA (pDNA) from 74 RCC patients quantified by Real-Time-PCR showed levels higher (27.7 ± 46ng/ml) than controls (3.2 ± 1.5ng/ml). In 39 patients at follow-up (range:14-74months) pDNA concentration decreased after nephrectomy. Patients with postoperative pDNA level persistently below cut-off-value didn't relapse, while 3 patients with increment relapsed. Microsatellite (MS) alterations (LOH) study was performed in 33 tumor DNA and 14 corresponding pDNA. Preoperative and follow-up pDNA evidenced, in 22 out 49 MSs studied, the same LOH of primary tumor DNA and one patient, with LOH in follow-up pDNA, relapsed. Patient number and follow-up period increment will permit to correlate pDNA characterization and patient outcome.

NB 05. Expression Analysis and Role of RIZ Gene in Myoblasts Proliferation and Differentation

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PRDM2(RIZ) gene is expressed as two alternative forms: RIZ1 has a distinctive PR domain with a histone methyltransferase activity and RIZ2 lacking the PR domain, but is otherwise identical to the other. The two alternative products are involved in tumorigenesis in an unusual *yin-yang* fashion: an imbalance in the amounts of the two products, through either disruption or underexpression of the PR+ form or overexpression of the PR- form, commonly occurs in human cancers. We investigated a possible involvement of *RIZ* in proliferation-differentiation switch using C2C12 myoblast cell line. Expression of *RIZ* was assayed by real time reverse transcriptase-PCR of total cellular RNA. Differentiation induces a variation of RIZ1 expression level whereas RIZ2 expression remains unchanged.

NB 06. Prognostic Factors of Multiple Myeloma

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Introduction: Multiple myeloma is a malignant neoplasm originating in plasma cells. It is frequently associated with poor prognosis and characterized by deregulation of some proteins controlling cell the cycle and apoptosis (p53, p16, FGFR3, cyclin D 1, 2, 3, Bcl-2, caspase 3, 8, 9) seem to play an important role in pathogenesis.Bone marrow samples of patients in advanced stages of MM showed high expression of Bcl-2 in tumor cells, in contrast to those in remission which showed weak or no positivity for Bcl-2. p53 and p16 were completely negative. Caspase 8 was negative in most cases.Based on decreased BCl-2 expression these preliminary data suggest that detection of low BCl-2 expression might has positive prognostic factor.

NB 07. Novel Approach to Detect Occult Neoplastic Disease in Head and Neck Squamous Cell Carcinoma Surgical Margins.

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Primary aim of the present ongoing study (Poeta ML et al. NEJM 2007;357:2552-61) is to use TP53 mutations to detect occult neoplastic disease in histologically clear margins. Indeed when they have been diagnosed as clear the local recurrence rate is still 10–30%. We used LigAmp, a novel PCR-based approach to detect low copy number of TP53 mutations in surgical margins of HNSCC. Receiver Operating Characteristics Curve was drawn to describe the performance of light microscopy and molecular evaluation. Area Under the Curve was higher for ligamp assay in comparison to histopathology evaluation. The detection of occult disease may be predictive for local recurrence and potentially serve as an indicator for consideration of surgery extension and/or adjuvant chemo-radiotherapy.

NB 08. Molecular Mutational Analysis of EXT Genes for Genotype-Phenotype Correlation in MO Disease.

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Multiple Osteochondromas (MO), one of the most common hereditary musculoskeletal diseases in Caucasians, is associated with mutations in the EXT1 and EXT2 genes whereas the existence of a third and/or other genes is debated. Our ongoing studies (Genes Chromosomes Cancer, 2007; Int J Cancer, 2001) have been demonstrating that increasing detection analysis sensitivity and widening the spectrum of detectable EXT-causing mutation (point- and midsize- mutations) in the EXT 1/2 coding and splicing regions by complementary high-throughput techniques (DHPLC, MLPA, FISH) greatly reduces EXT-non1/2 cases. Using these techniques, we have achieved a 96% detection frequency of EXT-1 or EXT-2 mutations in MHE families from current 80%. Inclusion analysis of regulatory regions and gross deletions, together with bioinformatics approaches, might further improve detection.

NB 09. Up-Regulation of Beta3-Adrenergic Receptor mRNA in Human Colon Cancer: A Preliminary Study

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Beta adrenergic receptors (ARs) affect several aspects of malignant phenotype in colon cancer. We studied beta1-, beta2- and beta3-ARs gene expression in cancer and normal surrounding mucosa from 41 patients. Comparable levels of beta1- and beta2-ARs subtypes mRNA are expressed in normal mucosa and in cancer tissues. A significant difference of beta3-AR mRNA levels between cancer and normal mucosa was found (1.88±0.24 *vs* 1.16±0.14 target gene/beta actin gene, respectively). Our results indicate that beta3-AR mRNA is up-regulated in human colon cancer.

NB 10. Protein Clusters Predictive of Therapeutic Response in Breast Cancer Patients

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Background: Breast cancer (BC) patients have different response to neoadjuvant chemotherapy (NACT); we analyzed changed expression profile of proteins in Tumor Interstitial Fluids (TIF) and in Normal Interstitial Fluids (NIF) before and after NACT, in patients responder and non-responder to NACT. Methods: Eleven patients with stage II and III BC, treated with 4 courses of FEC90 q3wks (F:500mg/m^2, E:90mg/m^2, C:500mg/ m^2) followed by 4 cycles of docetaxel (TXT) 100 mg/ m^2 q3wks were investigated. 250 mg of tumor and normal tissue were collected before and after NACT. Tissue samples were incubated at 37°C for 1h and proteins present in the supernatant were analyzed by 2D-gel electrophoresis. Spots of potential interest were purified, digested with trypsin and analyzed with HPLC-ESI-MS/MS. PD-Quest

software analysis and statistical analysis were performed. Results: A cluster of interesting proteins come up from the statistical analysis, which expression corresponds to a good response to NACT. Among these we found cell growth and maintenance proteins such vimentines, energetic pathway proteins like ALDO-a, lactic-dehydrogenase, phosphoglicerate-kinase and triose-phosphate isomerise. Proteasome regulators, anti-apoptotic 14-3-3 enzymes, apolipoprotein-A and nucleic acids metabolism proteins, such is DJ-1, were also found. Conclusions: The NIF/TIF study seems to be useful for the detection of markers predictive of response to treatment. In this study, the identification of a combination of proteins differentially expressed will probably be more sensitive and specific than a single molecular marker for screening, diagnosis, prognosis, and prediction of therapeutic response. The final aim will be the identification of prognostic and predictive markers in serum.

NB 11. Molecular and Proteomic Characterization of Primary Cell Cultures from Normal Kidney and Renal Cell Carcinoma

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Renal cell carcinomas (RCCs), 3% of human malignancies, have difficult and late diagnosis and proteomic studies looking for biomarkers are ongoing. To detect RCC biomarkers, overcoming tissue cellular heterogeneity, 39 primary cultures were established from normal and tumor renal tissues. Most of normal cortex and tumor cells expressed proximal and distal tubule markers. 2-DE/MS analysis of cortex and RCC cultures identified 44 differentially expressed proteins. We analyzed, by Real-Time PCR and western blot, the expression of 3 proteins (ANXA3, LASP1 and CTSB) more abundant in RCC cultures, and of non-receptor tyrosine kinase Arg. These proteins are involved either in VHL/HIF-1 angiogenesis or cytoskeleton remodeling pathways. We evidenced a different isoform expression pattern between normal and RCC cultures.

NB 12. Gamma-Glutamyltransferase-Dependent Resistance of Melanoma Cells to Arsenic Trioxide – The Role of Catalase induction.

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Our aim was to evaluate if γ-glutamyltransferase (GGT) expression can modulate cell sensitivity to hydrogen peroxide (H_2O_2), ascorbic acid (AA) or arsenic trioxide (ATO). GGT-overexpression caused increased resistance against H_2O_2 and AA, as judged by levels of apoptosis (TUNEL assay), DNA damage response (c–H2AX and p53 phosphorylation) and activation of cell death pathways (cytochrome c release, caspase activation). Accordingly, a higher resistance against ATO or AA+ATO was observed. A twice higher catalase activity was detectable in the GGT rich clone. GGT-overexpression is associated with increased anti-oxidant defenses, but also with higher levels of DNA-damage (Comet assay), likely with the meaning of a factor in neoplastic progression.

The financial support of the Istituto Toscano Tumori (ITT, Firenze) is gratefully acknowledged.

NB 13. Overexpression of Glutathione-S-Transferases Omega 1 (GSTO1) Enhances the Resistance of Hela Cells to Cisplatin

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Background: GSTO1 may play a role in the acquisition of the chemoresistance to cisplatin. Methods: Immunoblot analysis and RT-PCR studies were performed on HeLa cells seeded at high and low density. Cytotoxicity studies and measurement of glutathione were made on HeLa cells stably transfected with GSTO1. Results and Conclusion: Our results show that cells reaching high degree of confluence overexpressed the GSTO1 either when cells were seeded directly at high density or if they reach the high density after three days of culture. The levels of glutathione were the same in high and low density cells and in stably transfected cells. Cytotoxicity studies on stably transfected cells show an increased resistance to cisplatin.

NB 14. Exploring Mutant P53 Gain of Functions in Vivo Through Conditional RNA interference

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Mutant-p53 proteins are thought to have acquired 'gain-of-function' (GOF) activity that mainly contributes to tumor aggressiveness. In this study, mimicking physiological conditions, we describe the establishment of a lentiviral-based-system for conditional interference with mutant-p53 expression. In vivo studies assessed the efficacy of conditional RNA interference in inhibiting gain-of-function activity of mutant-p53 proteins by reducing tumor growth ability. Moreover by using this system, microarray data were validated in vitro and in vivo and putative mutant-p53 target genes that may contribute to its gain-of-function effects in cancer were identified. Results are confirmatory that depletion of mutant-p53 protein impacts on tumor malignancy and validated the inducible lentiviral-based system as an efficient tool to study the gain-of-function activity of human tumor derived p53 mutants.

NB 15. Imatinib Mesylate Resistance by T315I Mutation and its Prevalence in Indian Chronic Myeloid Leukemia Patients (First Study from India)

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Aim: a) To detect T315I mutation and study its role in Imatinib mesylate resistance. b) To study prevalence of T315I mutation in relapsed CML patients. Methodology: 200 CML patients diagnosed by RT-PCR and treated with Imatinib (400mg to 600mg/day). ASO-PCR was done for T315I mutation. Results: 45/200 developed hematological and molecular resistance, 10/200 were in AP/BC CML. ASO-PCR was done for T315I .37/45 and 8/10 were positive for T315I mutation. T315I positive cases showed poor prognosis. T315I was detected in 80% to 85% of our relapsed cases. Survival and time-to-progression curves were obtained from Kaplan-Meier method. Conclusion: The early detection of T315I mutation proved to be helpful in therapeutic decisions in CML patients. ASO-PCR based detection of T315 proved to be economical, sensitive and rapid.

NB 16. Idiotype-Specific Peptides as Tools for the Specific Delivery of Therapeutic Compounds into Tumors

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B-cell lymphomas are characterized by clonal expansion of tumor cells expressing a B Cell Receptor (BCR) that include idiotypic immunoglobulin (Id). Id is determined by rearrangements of the immunoglobulin V regions that are unique for each clonal B-cell population and functions as a tumor-specific marker. Random peptide libraries (RPL) are important tools to define the antigen specificity and the characterization of Id ligands for tumor B cells. We evaluated the ability of Id-specific peptide (Id-peptides) for B-lymphoma cells selected by screening RPLs as a tool for the specific delivery of a therapeutic cargo into tumor cell. Results can be summarized as follows: a) 3 phage clones selected screening RPLs with immunoglobulins purified from A20 B- lymphoma cells b) Synthetic peptides, corresponding to the insert of phage clones, maintained their antigenic properties c) Id-peptides targeted specifically tumor cells *in vivo* d) Id-peptides were internalized into target tumor cells e) Id-peptides were able to deliver a cargo fluorophore (FITC) or a protein (GFP) into target tumor cells. This evidence shows that Id-peptides are powerful candidates for specific delivery of therapeutic drugs into tumors.

NUTRITION AND CANCER

NC 01. The Apoptotic Effects of Rosiglitazone and 9 Cis Retinoic Acid at Low Dose on Human Breast Carcinoma Cells as Markers of Benefit in Neoadjuvant Therapy of Mammary Cancer.

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Activation of PPARgamma and RXR heterodimer elicits antineoplastic effects on breast carcinoma. We evaluated the anti-tumor efficacy of combining low doses of the PPARgamma agonist rosiglitazone (BRL) and RXR agonist 9 cis retinoic acid (9cisRA) on normal and four malignant breast cell lines. The combined treatment of BRL 100nM and 9cisRA 50nM reduced the vitality only in cancer cells. Functional experiments indicated that NFκB binding site in p53 promoter is the effector of BRL and 9 cis RA signalling in cancer cells. Both ligands activated intrinsic apoptotic pathway in malignant but not in normal breast cells. These data address how combined low dose of PPARgamma and RXR agonists may elicite chemopreventive effect in the treatment of breast cancer.

NC 02. DHA Exerts Pro-Apoptotic Effects in Lung and Colon Cancer Cells Inducing the Expression of the Dual-Specificity Phosphatase MKP-1.

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The over-expression of MAP-kinase-phosphatase-1 (MKP-1) modulates apoptosis, depending on tissues and represents a positive prognostic factor in lung and colon cancer. We studied if docosahexaenoic acid (DHA) may induce apoptosis in lung and colon cancer cells modifying MKP-1 expression. DHA increased MKP-1 expression, caused apoptosis and reduced the levels of pERK1/2 and p-p38 in all the cells. Transfecting the MKP-1 siRNA reverted the effect of DHA on apoptosis and MAPK levels. The results demonstrate that one possible molecular mechanism involved in DHA-induced apoptosis may be the induction of MKP-1 expression and the reduction of ERK1/2 and p38 phosphorylation. Furthermore, these data sustain the hypothesis that DHA may exert chemopreventive and chemotherapeutic action in lung and colon cancer.

NC 03. CLA Response of Human Breast Cancer Cells: Mechanisms Involved

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Conjugated linoleic acid (CLA) has beneficial effects against breast carcinogenesis. In its antiproliferative action we hypothesized a cross-talk between PPARgamma and E-cadherin/ß-catenin systems, important in cellular adhesion and signal transduction. ß-catenin system is regulated by ERK and PI3K/Akt cascades, crucial in growth signal transmission. We tested CLA interference with these pathways in ERα+ (MCF-7) and ERα- (MDA-MB-231) human breast cancer cells. We used immunoblotting, immunofluorescence, immunoprecipitation, zymography, Boyden chamber techniques. CLA is more effective in MCF-7 cells: it transactivates PPARgamma and induces E-cadherin/ß-catenin complex formation at plasmamembrane. In MDA-MB-231 cells CLA upregulates PPARalpha and induces apoptosis. In both cell lines CLA inhibits ERK and PI3K/Akt signalling and reduces cell invasiveness. CLA action involves signalling pathways recently considered as new targets for anti-cancer strategies.

NC 04. Spatiotemporally-Controlled Targeted Somatic Mutagenesis in the Mouse : Functional Analysis of Nuclear Receptor Signalling.

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Genetically engineered mice generated by gene knock-out have proven to be very valuable to analyze gene functions, and to generate mouse models of human diseases. However, this technology has inherent limitations, such as problems associated with embryonic lethality and the occurrence of developmental aberrations. To generate spatiotemporally-controlled somatic mutations, we developed a chimeric Cre-ERT2 recombinase, which results from the fusion of Cre recombinase with a mutated ligand binding domain of the human estrogen receptor a (ERa), and whose activity is induced by Tamoxifen (Tam). Transgenic mice cell-specifically expressing Cre-ERT2 allow, upon Tam treatment, to efficiently induce Cre-mediated recombination at various loci flanked by LoxP sites. Examples of spatio-temporally controlled site-specific somatic mutations generated through this approach will be presented.

NC 05. Antiproliferative Activity of the New Resveratrol-Derivative 4, 4'-Dihydroxy-Stilbene

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The synthesis of resveratrol-derivatives allowed to identify in the 4'-hydroxystyryl-moiety the determinant required for its antiproliferative activity. We have evaluated the effect on cell growth of human fibroblasts of the new derivative 4,4'-hydroxyl-stilbene. This compound showed a 50% inhibition of proliferation at a concentration 4-fold lower than resveratrol. Increasing resveratrol concentrations determined a progressive accumulation of cells at early S-phase, whereas 4,4'-hydroxyl-stilbene arrested cells in G1-phase. Microarray analysis revealed that both compounds increased G1/S-phase cyclins, CDK-inhibitors, and cell cycle-checkpoints protein expression. Analysis of cell-cycle and DNA replication-related proteins suggested that the antiproliferative activity of 4,4'-hydroxyl-stilbene involved different targets than resveratrol. Preliminary results obtained in breast cancer cells seems to confirm that the two compounds have a different mechanism of action.

NC 06. DLD-1 Human Colon Cancer Cells Proliferative Response to Lactobacillus GG

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Lactobacillus species could decrease colon cancer risk, but their mechanisms of action are unclear. We investigated the influence of increasing concentrations of both homogenate and cellular fractions (cytoplasm/cell wall extract) from *Lactobacillus rhamnosus* strain GG (L.GG) on cell growth and proliferation (by MTT and [3H]-thymidine incorporation) in DLD-1 cells. Exposure of DLD-1 cells to L. GG homogenate showed evident anti-proliferative action starting at intermediate concentrations after 48h. The same effects were obtained at the same cytoplasm extract concentrations whereas DLD-1 cells resulted unsensitive to bacterial cell wall components.

NC 07. Magnesium Deficiency Affects Mammary Epithelial Cell Proliferation: Involvement of Oxidative Stress.

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Low Mg availability reversibly inhibited the growth of mammary epithelial HC11 cells by increasing the number of cells in the G0/G1 phase of the cell cycle. In this conditions, cell cycle inhibitors p27 and p21 resulted up-regulated. Despite low Mg has been reported to promote oxidative reactions, dichlorofluorescein-detectable reactive oxygen species and hydrogen peroxide-induced oxidative DNA damage were not increased by low Mg. Gene expression profiling of low-Mg cells showed the modulation of several genes, some regulating cell proliferation. Low-Mg cells also displayed overexpression of glutathione S-transferase (GST) leading to increased enzymatic activity. We suggest that in low Mg cells, GST upregulation might have a dual role in protecting against oxidative stress and in modulating cell proliferation.

NC 08. Insulin Induces Expression of Leptin Gene in Breast Cancer Cells

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Pathologic conditions associated with hyperinsulinemia increase the risk of breast cancer. Here we studied molecular mechanisms by which insulin activates the expression of leptin obesity hormone that has been shown to promote breast cancer progression. Using MDA-MB-231 cells, we found that: 1) insulin stimulated leptin mRNA and protein expression and increased activation of the leptin gene promoter; 2) insulin increased loading of HIF-1a and Sp1 on leptin promoter and this mechanism is partially regulated by the PI-3K and ERK-1/2 pathways; 3) silencing of either HIF-1a or Sp1 downregulated insulin-induced leptin mRNA and protein expression. Our data suggest that hyperinsulinemia could induce breast cancer progression through leptin-dependent mechanisms. In MDA-MB-231 cells, this process requires Sp1 and HIF-1a-mediated leptin gene transcription.

NC 09. PARP-1 Recruitment to DNA Repair Sites is Modulated by p21

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The p21CDKN1A protein plays a fundamental role in DNA damage response. Little is known about its possible role in BER. The known interaction between PARP-1, PCNA and p21, prompted us to investigate the recruitment of these proteins to DNA damage sites. The analysis was carried on both wt- and p21-null human fibroblasts after treatment with the alkylating agent N-methyl-N'-nitro-N-nitrosoguanidine. The p21-null cells were more sensitive to the drug and showed an increased accumulation of PARP-1 at DNA damage sites, concomitantly with an increased synthesis of poly(ADP-ribose). These results suggest that PARP-1 distribution and activity are regulated by its interaction with p21, which is in turn influenced by the association between p21 and PCNA.

NC 10. Insights into the Mechanisms Involved in Magnesium-Dependent Inhibition of Primary Tumor Growth

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Background: A low Magnesium (Mg)-containing diet reversibly inhibits the growth of primary tumors that develop after injecting Lewis lung carcinoma (LLC) cells in mice. Methods: The Mg content was 30 and 1000 mg/kg for deficient and control diet. The experiments were terminated 21 days after cell inoculation. Results: The inhibition of LLC tumor growth in Mg-deficient mice is due to a direct effect of low Mg on LLC cell proliferation and to an impairment of angiogenesis. We also observed an oxidative DNA damage. cDNA arrays reveal that Mg deficiency modulates tumor expression of genes involved in the control of cell cycle, stress response, proteolysis, and adhesion. Conclusions: Our results suggest that Mg homeostasis is relevant in tumor development.

NC 11. Leptin and Estrogen Crosstalk in Breast Cancer

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Insulin resistance as it occurs in metabolic syndrome and the related alterations in cytokine production by adipose tissue are mainly involved in the progression of breast cancer in obese women. Among the different adipokines only leptin is able to amplify estrogen signalling through an increased aromatase gene expression and a direct transactivation of estrogen receptor (ER)-α. On the other hand, estrogens enhance the leptin expression and potentiate leptin transduction pathways in breast cancer cells. Our data in progress evidence how among thiazolidinediones rosiglitazone (BRL), generally utilized to ameliorate insulin resistance, inhibits leptin signalling involving MAPK and PI3K/AKT in MCF7 breast cancer monolayer and in MCF7 cell xenograft. In the in vivo model BRL treatment drastically reduces any stimulatory effects of leptin on tumor growth. Similarly, in three dimensional cultures BRL is able to remove any upregulatory effect of leptin on cell proliferation and aggregation. Besides, BRL treatment reverses the leptin-induced ERα transactivation as well as abrogates the leptin enhanced expression of two classic estrogen genes as pS2 and cathepsinD in both experimental models.On the basis of these findings it emerges that BRL, as molecule able to improve metabolic environment and to break down the crosstalk between estrogens and cytokines, is eligible in antagonizing breast tumor growth and progression in obese women.

NC 12. IGF-I and Insulin Receptors in Cancer Cells

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There is ample evidence that deregulation of the IGF system plays an important role in the development of human malignancies, including cancer of the breast, prostate, colon and kidney. Several epidemiological studies have suggested that small increases of circulating levels of free IGF-I and, especially, hyperinsulinemia, may play a role in the promotion of these malignancies. Tumor cells overexpress receptors for both IGFs (IGF-IR) and insulin (IR) and often produce IGF-I and/or IGF-II in an autocrine manner. A typical change occurs in IR expression, because malignant tumors preferentially express the fetal IR isoform (IR-A), which binds IGF-II with high affinity. IR-A overexpression, therefore, increases cell responsiveness to both insulin and IGF-II. It also increases IGF-I binding sites by forming hybrid receptors with IGF-IR. Sex steroids may induce up-regulation of IGF-IR and downstream substrates (i.e. IRS-1), thus increasing IGFs and insulin effects in estrogen- and androgen-sensitive tumors. These concepts have important implications for both the prevention and treatment of frequent forms of cancer. Moreover, IGF-IR, IR-A and hybrid receptors have been identified as potential molecular targets for novel anti-cancer therapies.

NC 13. Comparison of Colon Cancer and Breast Cancer Incidence Data from the U.S and U.K: Relationships with Folic Acid Fortification?

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Since the mid-1990s, the U.S. food supply was fortified with folic acid to lower the incidence of neural tube defects. Increased folic acid intake however, may affect cancer incidence. While it may prevent the initiation of cancers by maintaining DNA integrity, it may promote progression of an established cancer by "feeding" a proliferating cell clone. We looked for evidence of effects of folic acid fortification by comparing colon and breast cancer incidence rates in the U.S. with the U.K. where folic acid fortification has not been instituted. U.S. data for cancer incidence was derived from National Cancer Institute's Surveillance Epidemiology and End Results (SEER) and analyzed using SEER*Stat statistical software. The U.K data for incidence was derived from Cancer Research U.K. Before 1998, breast cancer incidence in both the U.S. and U.K. increased. After 1998, breast cancer incidence declined in the U.S., but continued to increase in the U.K. The decline in the U.S. coincides temporally with the introduction of folic acid fortification. The incidence of colon cancer in the U.S. steadily declined between 1992-1995, increased between 1995-1998, and then resumed its decline after 1998. In contrast, the incidence of colon cancer in the U.K has been steady with a slight increase between 1996-2000. The epidemiological evidence is consistent with a possible influence of folic acid fortification on colon and breast cancer incidence in the U.S. The nature of these relationships is complex and may reflect a balance between protection against initiation of new cancers and promotion of existing cancers.

REDOX REACTIONS IN HUMAN PATHOPHYSIOLOGY

RR 01. Oxidative Stress Activates a Positive Feeback Loop Between the γ- and ß-Secretases Cleavages of the ß-Amyloid Precursor Protein: Role in the Pathogenesis of Alzheimer's Disease

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Sequential cleavages of the β-amyloid precursor protein by β-secretase and γ-secretase generate amyloid β. We used neuroblastoma cells treated with H_2O_2 or 4-hydroxynonenal.We also used an in vivo model such as transient middle cerebral artery occlusion and reperfusion. We found that the up-regulation of BACE1 induced by oxidative stress is mediated by γ-secretase activity, and that it requires the activation of the JNK pathway. We further characterized the cellular pathways that control BACE1 expression under oxidative stress. The results have two implications for the pathogenesis of sporadic AD. First, they suggest that oxidative stress can increase the expression of both secretases, enhancing Aβ production. Secondly, the positive or negative cellular responses to oxidative stress parallel the activities of the secretases.

RR 02. Reactive Oxygen Species in Breast Cancer Progression

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Accumulating data suggests that growth factor receptor activation and the deployment of downstream effector signaling molecules, such as the mitogen activated protein kinases (MAPKs), are important determinants of hormone resistance. We have shown that resistance can also be associated with oxidative stress and a subsequent antioxidant response. We propose that altered intracellular redox status, independent of membrane receptor activation, engages the MAPK signaling cascade and impacts on resistance. We recently discovered that MAPK phosphatase 3 (MKP3) overexpression in human breast cancer cells leads to resistance to the antiestrogen tamoxifen. In MKP3-overexpressing cells, growth factor receptor-independent activation of downstream MAPK effectors occurs via induction of reactive oxygen species (ROS) with tamoxifen treatment. ROS inactivates MKP3 phosphatase activity, representing a novel mechanism whereby ROS downregulates a negative regulator of MAPK. It is possible that breast tumors compensate for the chronic activation of MAPK by up-regulation of MKP3. The emergence of resistance may therefore involve the disruption of this regulatory compensatory loop by inactivation of MKP3 phosphatase activity by ROS. The role of ROS in modulating other important regulatory and transcriptional networks in breast cancer will be presented.

RR 03. Immune Responses Triggered by Oxidative Stress in the Progression of Nonalcoholic Fatty Liver Disease.

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The factors responsible for the progression of steatosis to nonalcoholic steatohepatitis (NASH) are still poorly understood. Here we investigated the actual role of immune mechanisms in promoting liver injury in an experimental model of NASH based on mice feeding with a choline/methionine deficient diet (MCD). MCD-feed Balb/C and C57B6 mice developed hepatic steatosis and increased liver oxidative damage. However, only C57B6 mice, which have a prevalent Th-1 immune response, effectively develop specific antibodies against proteins adduced by lipid peroxidation products. Histologically, C57B6 mice, but not Balb/C mice showed hepatic necro-inflammation and initial sings of fibrosis. In conclusion, these observations support a role of immune responses triggered by oxidative stress in the progression of steatosis to steatohepatitis.

RR 04. Protective Effect of Arzanol, A Prenylated Pyrone-Phloroglucinol Etherodimer, in Several Models of Oxidative Stress

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Arzanol, a pyrone-phloroglucinol from *Helichrysum italicum* subsp. *microphyllum*, was investigated for its capacity to inhibit the oxidative modification of lipid molecules in different models of oxidative stress. This phenol showed powerful antioxidant activity under conditions of autoxidation and iron (EDTA)-mediated oxidation of linoleic acid at 37 °C, and thermal (140 °C) degradation of cholesterol. In particular, the pre-treatment with arzanol significantly preserved LDL from oxidative damage induced by Cu2+ ions at 2 h of oxidation, and showed remarkable protective effect on the reduction of polyunsaturated fatty acids and cholesterol and the formation of oxidative products. Furthermore this pyrone showed remarkable protective effect on membrane lipid degradation induced by TBH in VERO cells, a line of fibroblasts derived from monkey kidney.

RR 05. PKC-Delta Dependent NADPH Oxidase Activation in Neuron Differentiation or Death.

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NADPH oxidase is known as anion superoxide generating enzyme, widely distributed in different cell types and involved in intracellular redox state modulation. We demonstrated that SHSY-5Y cell differentiation induced by retinoic acid (RA) treatment was accompanied by PKC-delta activation, increased expression of NADPH oxidase and antioxidant enzymes SOD1, SOD2 and catalase. However, glycoxidative stress was able to increase anion superoxide generation through PKC-delta activation, leading to cell death. Moreover, PKC delta overexpression alone was able to induce cell death through NADPH oxidase activation. Thus, we showed that PKC-delta dependent NADPH oxidase activation leads to cell differentiation if parallel increase of antioxidant defences occurs, otherwise it leads to redox state unbalancement and cell death.

Grants from Genoa University and PRIN2006065711_002

RR 06. Fractions of Gamma-Glutamyltransferase in Healthy individuals, Alcohol Addicts and Patients with Hepatic Disease.

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Serum γ-glutamyltransferase activity (GGT), long used as biomarker of liver function and excessive alcohol use, is now recognised as a predictor of cardiovascular events (Emdin et al., Circulation 2005;112:2078) and has been proposed also as marker of oxidative stress, unfortunately, the clinical value of GGT assay is limited by its poor specificity. We recently devised a novel high performance gel filtration chromatography method, allowing the quantification of four GGT activity fractions (b-GGT, m-GGT, s-GGT, and f-GGT, Franzini et al., Anal. Biochem. 2008;374:1) in human blood. By this new procedure, we characterized 200 healthy subjects, 51 subjects with history of alcohol abuse and 50 with hepatic disease. Among healthy subjects, all fractions were positively associated with ALT (P <0.01), whereas b-GGT showed the most extensive association (P <0.01) with established cardiovascular risk factors in both genders (body mass index, diastolic pressure, uric acid, triglycerides, leukocyte count). m- and f-GGT were associated with LDL-cholesterol, and f-GGT only with triglycerides in males (P<0.01). In alcohol addicts, compared to liver disease patients, a difference was found in b-GGT (P < 0.001) and b-GGT/s-GGT (P <0.001), both discriminating better than total GGT the two conditions. GGT fraction analysis can increase the sensitivity and specificity of plasma GGT activity test, possibly allowing the discrimination of underlying risk and/or pathologic conditions.

RR 07. Redox Regulation of Transcription Factors: Implication in Human Pathophysiology

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Reactive oxygen species (ROS) modulate the expression of genes involved in cellular antioxidant defenses, yet the signaling cascades regulating redox status in endothelial and smooth muscle cells remain to be elucidated. We have shown that induction of heme oxygenase-1 and other stress proteins depends critically on the transcription factor Nrf2, a member of the basic-leucine zipper NF-E2 family regulating expression of globin genes during erythroid development. Our studies in pre-eclampsia have established that antioxidant gene transcription and redox signaling are impaired in fetal vascular cells, suggesting that prolonged oxidative stress *in utero* may lead to fetal programming of vascular dysfunction in adulthood (Siow et al., Redox Report 12, 11-15).

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RR 08. Effect of Conjugated Linoleic Acid Isomers on Mitochondrial Respiration

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Background: Conjugated linoleic acid (CLA) is an unusual fatty acid present in ruminant meat and dairy products. It has been demonstrated that CLA reduces total body fat in experimental animals and humans. We investigated whether this effect could be related to an increased mitochondrial beta oxidation. Methods: We used oleic acid, the CLA c9,t11 and CLA t10,c12 isomers, which are equally present in chemically synthesised CLA, or the CLA mixture (50:50 of both isomers). Liver mitochondria were from fasting male Wistar rats; respiration was measured at 30°C by Clark's electrode with fatty acids as substrates. Results: State 1 respiration was not modified in the presence of oleic acid, while CLA mixture caused increased oxygen consumption. When we tested the CLA c9,t11 the rate of respiration was decreased compared to the CLA mixture, while the use of CLA t10,c12 leads to a significant increase in respiration with respect to the other fatty acids. Conclusions: We showed that CLA t10,c12 is more oxidized by mitochondria compared to both oleic acid and the CLA c9,t11. This data are more relevant considering that it is known that its entry into mitochondria is lower than that of CLA c9,t11. The higher oxidation rate of this isomer may enhance UCP3 activity explaining the increased energy expenditure leading to a reduction of body fat.

RR 09. Redox Modulation of Protein Kinase C Isoforms in Cell Survival or Death.

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Reactive oxygen species (ROS) are products of aerobic metabolism which act as mediators of damage and as second messengers in the intracellular signaling pathways. Among redox signaling molecules a central role is played by protein kinase C (PKC). PKCs are a family of isoenzymes differently involved in secretion, proliferation, differentiation and apoptosis. Many studies report PKC sensitivity to oxidant and antioxidant agents, due to thiol groups both at the level of catalytic and regulatory domains. Our studies have demonstrated that ROS production, consequent to GSH depletion, causes DNA oxidative damage, stimulates PKC-δ activity and induces cell death. On the other hand PKC-δ overexpression influences intracellular oxidative status, favoring cell differentiation or apoptosis.

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RR 10. Comparison of Fibroblasts from Patients Affected by Pseudoxanthoma Elasticum (PXE) or by ß-Thalassemia with (ß-Thal-PXE+) and without PXE-Like Clinical Manifestations (ß-Thal-PXE-)

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Fibroblasts from ß-thal-PXE+ behaved similarly to cells from PXE patients, whereas fibroblasts from ß-thal-PXE- patients appeared to be more like controls as far as the level of vitamin E-sensitive reactive oxygen species, the mitochondrial membrane potential; the production of malonaldehyde; merocyanin 540 binding; calcein accumulation and release. By contrast, inhibitors of calcein release (indomethacin, benzbromarone, MK571) had different effects on PXE and on ß-thal PXE+ cells, indicating that MDR/MRP alterations were due to different mechanisms. Therefore, PXE-like clinical manifestations in some ß-thal patients may at least partly depend on the incapacity of fibroblasts to cope with a chronic oxidative stress associated with, or inducing, cell alterations similar to those in PXE.

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RR 11. Chemotaxis in Profibrogenic Human Hepatic Stellate Cells Requires Redox – Dependent Activation of C-Jun N-Terminal Kinase Isoforms 1 and 2

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Migration and chemotaxis are pro-fibrogenic features of activated, myofibroblast-like, hepatic stellate cells (HSC/MFs) during the development of chronic liver diseases. In this study we found that PDGF-BB and other polypeptide factors can stimulate migration/chemotaxis of human HSC/MFs by eliciting a NADPH/oxidase - dependent intracellular generation of reactive oxygen species (ROS), that in turn activate JNK1/2 isoforms. This was unequivocally shown using JNK1/2 siRNAs, pharmacological inhibitors of either JNK1/2 or NADPH/oxidase and other experimental manipulations. Activation of JNK1/2 was also reported in HSC/MFs that, isolated from an "in vivo" pro-oxidant model of liver injury, were showing heme-oxygenase-1 activation (a marker of on-going oxidative stress) and expression of growth factor receptors, suggesting in vivo occurrence of this polypeptide-elicited, ROS-mediated signaling event.

RR 12. Copper-Zinc and Manganese Superoxide Dismutase Activity in Cirrhotic Patients with Different Child-Pugh Degree

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Oxidative stress has been implicated in the liver cirrhosis. The levels of reactive oxygen species (ROS) are regulated by ROS scavengers like superoxide dismutase (SOD) present as the inducible MnSOD and constitutive CuZnSOD. Aim of our study was to evaluate the CuZnSOD and MnSOD activities in 78 cirrhotic patients with different Child-Pugh (CP) grades in relation to the progression of cirrhosis. Serum activities of CuZn and MnSOD were determined spectrophotometrically. CuZnSOD activity decreased significantly from CP *A* to CP *C*. Differently, the activity of MnSOD increased significantly with the severity of cirrhosis. Our preliminary results suggest a opposite behaviour of two distinct inducible and constitutive SOD isoforms in the worsening of cirrhosis.

RR 13. Heme Oxygenase-1 Expression: Possible Therapeutic Target in Neuroblastoma Cell Lines

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Heme Oxygenase-1 (HO-1) expression and antioxidant status are believed to be involved in carcer biology. Here HO-1 expression and glutathione content were studied in neuroblastoma cell lines, with (SK-N-BE-2(C)) or without MYCN amplification (GI-ME-N), a signal of adverse prognosis. MYCN amplified cells showed lower contents of GSH than non-amplified cells. After depleting cells of glutathione with butionine sulfoximine, GI-ME-N showed lower apoptosis rate than SK-N-BE-2(C), while HO-1 expression was induced in GI-ME-N but not in SK-N-BE-2(C). Our results suggest that modulation of HO-1 expression might be a potential therapeutic target against the survival of neuroblastoma cells.

Grants from Genoa University, G. Gaslini Institute and PRIN2006065711_002

RR 14. 4-Hydroxynonenal (HNE), a Product of Lipid Peroxidation, Inhibits Telomerase Activity in Caco-2, a Colon Cancer Cell Line, by the inhibition of E-Box Activity of the hTERT Promoter.

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HNE has been demonstrated to exert antiproliferative effects on several tumour cells. Since reactivation of telomerase is a key event in cell proliferation control, HNE effects on telomerase was investigated. In CaCo-2 cells, HNE was able to inhibit telomerase activity and hTERT expression, according to previous results obtained in leukemic cells. To investigate the molecular mechanism involved in hTERT down-regulation, the expression and DNA binding activity of c-myc, mad1, sp1 transcription factors were also studied. Results demonstrated that Myc/Mad/Max network plays an important role in hTERT expression regulation. Transient transfection with different EGFP-plasmid constructs, containing the proximal region of hTERT promoter, demonstrated the importance of E-box activity (binding Myc/Max or Mad/Max complexes) in controlling hTERT expression in HNE-treated CaCo-2 cells.

RR 15. Microrna Expression Profiling During Human Leukemic HL-60 Cell Differentiation Induced by 4-Hydrononenal, a Product of Lipid Peroxidation.

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Micro (mi)-RNAs are a group of small non-coding RNAs with modulator activity of gene expression, which have been found abnormally expressed in cancer suggesting a role in tumour growth control. 4-Hydroxynonenal (HNE), a lipid peroxidation product, has antiproliferative and/or differentiative properties towards different tumour cell lines, at low concentration. To investigate whether HNE could affect miRNA expression profiling, we analysed the genome-wide miRNA expression profile (Agilent Technologies) in human leukemic HL-60 cells, treated with 1 μM HNE. A set of four miRNAs were up-regulated (miR-339;miR-660;miR-125a;miR-663) and six miRNAs were down-regulated (miR-575;miR-199b;miR-181a*;miR-202;miR-378;miR-454-3p). The identification of specific miRNA targets in HNE-treated HL-60 cells, will add interesting perspectives in new HNE-dependent pathways.

RR 16. Peroxisome Proliferator-Activated Receptor-Gamma (PPARgamma) Ligands Inhibit Telomerase Activity and hTERT Expression in Caco-2 Colon Cancer Cells

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PPARgamma are ligand-dependent transcription factors, involved in cell proliferation control. We investigated, in Caco-2 cells, the effects of PPARgamma ligands rosiglitazone and 15-deoxy-prostaglandin-J2 on telomerase activity and hTERT expression which resulted inhibited after exposition to both ligands. To investigate the molecular mechanism involved in hTERT down-regulation, we analysed the expression and the binding activity of c-myc and mad 1, two transcription factors involved in h-TERT regulation. PPARgamma ligands raised c-myc expression inhibition and mad-1 up-regulation. Moreover, we demonstrated a reduction of c-myc DNA binding activity to the E-box sequence of the hTERT promoter. Since several effects determined by PPARgamma ligands are PPARgamma-independent, the PPARgamma-dependence of inhibition of hTERT expression has been investigated by using the receptor antagonist GW9662.

RR 17. Identification of 4-Hydroxynonenal-Alpha-Enolase Adducts in HL-60 Human Leukemic Cells: Inhibition of Plasminogen Binding.

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4-Hydroxynonenal (HNE), the major active lipoperoxidation product, reacts with lysyl, histidyl, and cysteinyl residues of protein. We demonstrated by mass spectrometry and immunoblotting experiments the formation of HNE-alpha-enolase adduct(s) in HL-60 cells treated with 10μM HNE. HNE adduction didn't affect alpha-enolase activity. Confocal microscopy results demonstrated the co-localization of HNE and alpha-enolase at the surface of cells. We also showed that HNE caused reduction of the plasminogen binding to alpha-enolase. This effect was conceivably due to the reaction of HNE with the C-terminal lysine, which is crucial for plasminogen binding. Since alpha-enolase binding to plasminogen plays an important role in tissue invasion and metastasis, our results suggest a new role for HNE in the control of tumour growth and invasion.

STEM CELLS

SC 01. Withdrawn

SC 02. The Developing Rodent Brain: Hormonal and Environmental Influences.

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The neuronal network organization, leading to the gender-related sexual behaviour is mainly driven by gonadal testosterone, locally activated by aromatase and 5alpha-reductases. Hormones seem to control also neurogenesis in neural stem cells. Widely diffused pollutants, like polychlorinated biphenyls (PCBs) easily cross the placenta, accumulate in pup tissues and brain, exerting detrimental influences on reproduction and sex behavior. Aim: to verify whether 1) PCBs, given pre and post-natally, influence testosterone metabolism in developing rats; 2) testosterone metabolizing enzymes are already expressed in proliferating neuronal stem cells (NSC) and in differentiated neurons or astrocytes. Results: PCBs influence on the normal enzyme expression is time and sex-specific. Aromatase expression is already detectable in NSC and it increases in the differentiating neurons.

SC 03. Cultured Human Stromal Cells of the Limbus and Cornea are Both Able to Express Epithelial Markers.

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Purpose: To examine whether cells from the stroma of human limbus and cornea co-express epithelial and stromal markers. Methods: Human keratocytes were isolated from the limbus and cornea of cadaver donors,cultured and evaluated for CD34, CK3, and vimentin expression by immunofluorescence and RT-PCR. Results: Cells expressed vimentin and some also CD34 and CK3. Immunofluorescence findings were confirmed in all samples by RT-PCR. Double immunofluorescence evidenced three subpopulations: CK3-/CD34+,CK3+/CD34+ and CK3+/CD34-.CD34 yield was higher in the limbus with a long time to confluence; CK3 cells are greater in the cornea with a short time to confluence. Donor age was negatively related to CD34 yield and positively to that for CK3.The CK3+/CD34+ subpopulation was statistically transitional between the two single positive ones. Conclusions: Cells isolated from human limbal and corneal stroma are able to co-expressCK3.

SC 04. Identification and Characterization of Cells with Stem/Progenitor Properties in Normal Kidney and in Renal Cell Carcinoma

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Renal cell carcinomas (RCCs) cellular growth might be sustained by "cancer stem cells". We tried to identify and characterize human normal and tumor renal stem cells. Single cells obtained after collagenase digestion of RCC and normal tissue were cultured in suspension for 12 days in specific medium to form "nephrospheres". The dissociated single cells from spheres formed secondary spheres after 10 days with a sphere forming efficiency (SFE) of 0.6%. Most fluorescent cells (PKHhigh) in spheres have stem properties because slow dividing or quiescent. By immunocytofluorescence and FACS we identified in the spheres cells positive for putative stem cell markers. Also RCC CAKI-1 cell line generated spheres (SFE=1%); CD24 expression was higher in spheres (79%) than in adherent cultures (6%).

SC 05. Bone Marrow – Derived Mesenchymal Stem Cells Engraft Chronically Injured Liver and Differentiate into Pro-Fibrogenic Myofibroblast-Like Cells: The Role of PDGF-BB

L. Valfrè Di Bonzo[2], E. Novo[2], S. Cannito[2], C. Busletta[2], K. Mareschi[3], D. Rustichelli[3], M. Gunetti[3], S. Colombatto[2], F. Fagioli[3], M. Parola[1]
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Myofibroblast-like cells (MFs) are key pro-fibrogenic effectors in chronic liver diseases (CLDs) that can originate from activated hepatic stellate cells, portal (myo)fibroblasts and bone marrow-derived mesenchymal stem cells (MSCs). In this "in vivo" and "in vitro" study we show, using morphological, cell and molecular biology techniques, that: a) transplanted human MSCs can engraft chronically injured liver of NOD/SCID mice and differentiate into PDGF-β receptor positive MFs; b) PDGF, between mediators occurring in CLDs, has a key role in sustaining migration/chemotaxis and proliferation of MSCs through activation of Ras/Erk, PI-3K and JNK1/2 signaling pathways, as shown by employing specific siRNAs and pharmacological inhibitors. PDGF may effectively recruit MSC, precursors of pro-fibrogenic MFs, into chronically damaged liver during CLDs.

SC 06. Membrane Expression of the CD133 Stem Cell-Marker is Reduced During Sodium Butyrate-Induced Differentiation of HT29 Human Colon Cancer Cells

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The HT29 human colon cancer cells undergo differentiation following treatment with various agents such as sodium butyrate which is a short chain fatty acid and histone deacetylase inhibitor. Recently, a subpopulation of cells which appear to be tumor-initiating have been identified in the majority of human cancers and shown to be characterized by the surface marker CD133. This study evaluated the behaviour of *CD133* during sodium butyrate-induced differentiation of HT29 cells. We found that treatment with sodium butyrate induced a progressive decrease of CD133 expression, as assessed by flow cytometry. Indeed, expression of CD133, which was about 38% in control cells, gradually decreased down to about 3% after 72 h in a time- and dose-dependent fashion. No relationship was observed between CD133 protein and mRNA expression level, as evaluated by Real-time PCR. The promoter region of the CD133 gene contains a CpG island but no changes were detected in its methylation status during HT29 differentiation. Studies are ongoing to analyze how and whether anti-CD133 specific siRNA can affect differentiation of HT29 cells. Our findings demonstrate that membrane expression of CD133 stem cell marker decreases during differentiation of colon cancer cells and suggest that HT29 cells are a useful in vitro model to study the mechanisms involved in this regulation which likely occurs at a post-transcriptional level.

SC 07. Dental Pulp Marrow Similar Cells: A New Source of Stem Cells for Clinical Applications

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Adult stem cells have been proposed as an alternative to embryonic stem cells to study multilineage differentiation and therapeutical applications. While bone marrow is an excellent source of stem cells, collecting bone marrow is invasive, and recent data implicate bone marrow stem cells in cancer development. The recent discovery stem cells in the dental pulp of human exfoliated deciduous teeth (SHED) has offered a potentially non-invasive source of stem cells. SHED showed rapid expansion and proliferation in vitro while expressing several mesenchymal stem cell markers, such as STRO-1 and CD146, but they were not shown to express Oct-4, SSEAs, Nanog, or any other hallmarks of totipotent stem cells, while their multilineage differentiation was marginally successful. SHED are highly heterogeneous, because only 9% of SHED express markers of undifferentiated cells, and it is not clear if clones obtained from SHED maintain expression of these markers. We report the isolation of a population of exfoliated dental pulp stem cells (DPMSC) cultured by using a medium formulation containing very low percentage of human serum 1.25% and without immunoselection methods, which express high levels of embryonic stem cell markers Oct-4, Nanog, Sox-2, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81 and several other mesenchymal and embryonic cell markers. In addition, DPMSC are a highly uniform population, maintain normal karyotype and show a rate of expansion characteristic of stem cells. In vitro these cells can be induced to undergo differentiation into muscles, neurons, liver and bone using chemically defined culture conditions.

SC 08. Subpopulations of Stem Cells Exist in Bone Marrow Which Show Specific Homing Abilities in Uninjured Hosts.

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Recently, the ability of adult stem cells to contribute to regeneration, repair but also to play a role in cancer development has aroused great interest. In particular, bone marrow derived stem cells (BMSCs), the most well known population of multipotent stem cells in adults, have been shown to be able to generate many different cell types. Understanding biological significance and molecular mechanisms underlying adult stem cell trafficking such as adhesion and deadhesion, chemoattraction and chemoretention, movement seems crucial. Currently though, the real potential of BMSCs to replace all cell types is not yet fully accepted and data reported after injection in the general circulation of uninjured animals are contradictory. Such contradictions may be due to the use of total BMSCs, which may contain at variable rate and percentage different subpopulations with specific homing properties.To

address the question, we isolated from the BM of C57B1/6-Tg(UBC-GFP)30Scha/J adult mice, 4 different populations according to molecular markers or culture methods: CD105+, MSC cultured in Mesencult® medium, floaters cultured according to D. Trisler's methods and buffy coat. After extensive immunophenotyping, the 4 populations have been injected in the tail vein of 4 groups of C57B1/6 wildtype mice. Animals have been sacrificed 2 days later and brain, lung, heart, spleen, liver, pancreas and bone marrow harvested. In each tissue, the presence of GFP+ cells has been analyzed by PCR and immunohistochemistry. Here we present data indicating not only specific homing of the subpopulations in different tissues, but even preferential localization within each tissue.

SC 09. ATRA-Induced Differentiation of Glioma Cells is Associated with RAR-Dependent Enhanced Expression of EAAC1 Carrier

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Although recent studies reported the association between defective function of EAAC1, the murine counterpart of EAAT3 carrier, and neurologic disease, the mechanisms underlying EAAC1 regulation during CNS development are not yet fully understood. We have recently found that, in rat C6 glioma cells, a cell population endowed with stem-like features, all-*trans*-retinoic acid (ATRA) induced the over-expression of Slc1a1 gene, encoding for EAAC1 currier, along with oligodendrocytic markers, and caused a four-fold increase of EAAC1-dependent transport Vmax in a dose and time-dependent manner. In agreement with these data, also EAAC1 protein was much more abundant in ATRA-treated cells. ATRA effects on EAAC1 were inhibited by the specific RAR inhibitor LE540 and mimicked by AM80, a RAR agonist, but not by the RXR agonists HX630 and PA024. Slc1a1 mRNA induction was suppressed by cycloheximide, suggesting that a protein intermediate is needed for ATRA effect, and preceded by a marked increase in RARβ expression. The half-life of Slc1a1 mRNA was not increased in ATRA-treated cells compared with ATRA insensitive housekeeping genes. Moreover, preliminary data obtained in the same cell line indicate that a pool of EAAC1 carriers interacts with the cytoskeletal protein α-adducin and that the interaction is increased in ATRA-treated cells. These results suggest that (1) the expression of EAAC1 is induced by ATRA through a RARβ-dependent transcriptional mechanism as a step of the differentiation process triggered by retinoic acid and (2) the increased abundance of EAAC1 in ATRA-treated cells may alter carrier trafficking through the enhanced interaction with cytoskeletal components.

SC 10. Energetic Metabolism and CFTR Expression and Function in Murine Hematopoietic Stem Cells

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Cystic fibrosis (CF) lung disease is characterized by a chronic inflammatory response, whose hallmark is an abnormally neutrophilic infiltrate. The cellular therapy of lung disease may be the resolutive cure for CF. Although neutrophils intensively migrate into the CF airways, the homing efficiency of circulating hematopoietic stem cells (HSCs) into the lung is likely insufficient to give a therapeutical effect. In order to understand the cellular constraints that limit this process, we have studied the mitochondrial status and CFTR expression of murine HSCs. HSCs were isolated and enriched from bone marrow of 5 weeks old male C57Bl/6 mice by means of positive immuneselection against Sca-1. The intracellular content of mitochondria (as studied by confocal microscopy using the MitoTracker dye) was not homogeneously distributed in the Sca-1+ cell population displaying a clear inverse correlation of their density with the expression of the Sca-1 marker. Immunohistochemistry revealed that murine HSCs do express CFTR. Functional characterization of CFTR chloride channel activity was counducted by spectrofluorimetric analysis. Stimulation of PKA by addition of FSK+IBMX increased chloride efflux, and the addition of the specific CFTR inhibitor, glibenclamide or CFTR-172 inhibitor before and during the next FSK+IBMX stimulation almost completely inhibited this increase, suggesting that the PKA-dependent chloride efflux was mainly due to CFTR stimulation. Transplantation of high Sca-1+/low mitochondria should be pursued to verify that less committed HSCs show better performance in homing to the lung.

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SC 11. Plasticity of Third Trimester Placental Stem Cells

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Stem cells can be induced to differentiate into cells with special functions. Two types of stem cells have been described: embryonic stem cells and adult stem cells. Embryonic stem cells are promising, but unsolved technical problems in cell sampling from the embryo make them unusable from an ethical point of view. On the contrary, adult stem cells are a rare population accessible by invasive procedures, whose plasticity relies also upon the site of isolation, the age of the donor and on concomitant pathologies. Therefore, there is need to provide an alternative source for multipotent stem cells without the limitations mentioned above so that a great interest has grown on foetal tissues. We looked at villi of third trimester placentas as a source of multipotent cells. As CD34 expression is characteristic of both EPCs and mature endothelial cells, we immunodepleted CD34+ and CD133+ from the enzymatically digested placental cells: we isolated a cell population whose phenotypic characteristics were similar to those of mesenchymal stem cells derived from other sources,as they were positive for CD13, CD44, CD49d, CD90, CD105 and HLA-ABC and negative for CD14, CD31, CD33, CD34, CD43, CD45, CD52, CD80, CD133 and HLA-DR. The osteogenic potential was assessed by alizarin red staining of mineralized calcium. Adipogenic differentiation led to the formation of intracellular lipid droplets stained by Oil-Red O. Chondrogenic differentiation was achieved by alginate droplet culture and confirmed by alcian blu and PAS staining. Under appropriate culture conditions these cells were able to differentiate into smooth muscle as well as endothelial cells.

SC 12. Evidence of a Conserved Regulatory Circuit Between Mex-3 and GLD-1 Through Analysis of Tino/hMEX-3D Binding Sequence

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We first identified Tino as a novel destabilizing bcl-2 A+U-Rich Element (ARE) binding protein (AUBP). It has been recently recognized as a shorter form of the human Mex-3D (hMex-3D), one of the four members of the family of Mex-3 RNA-binding phosphoproteins. In *C. elegans*, CeMex-3 is a translational regulator involved in the maintenance of worm germline totipotency and interacting with the transcriptional regulator PAL-1 to specify posterior blastomere fate. The high conservation between ceMex-3 and hMex3 suggests that interaction between Tino/hMex-3D and its target mRNAs could impact to early mammalian embryogenesis. We have verified this possibility by identifying Tino bound mRNAs in HEK293 cells by microarrays. Computational analysis of these mRNAs identified an U-rich, 34- to 39-nucleotide long, consensus. Remarkably, more than half of them also contained the consensus for the RNA binding protein Quaking, the human ortholog of nematode gametogenesis regulator GLD-1. All together, our results suggest that Tino/hMex-3D belongs to a regulatory circuit of mRNA trans-acting factors involved in cell fate and differentiationAknowledgements.

Research supported by MUR, AIRC and ECRF.

SIGNAL TRANSDUCTION AND APPROACH TO MOLECULAR THERAPIES

ST 01. Cyclooxygenase (Cox)-2 Regulating Transcriptional and Post-Transcriptional Activation of Aromatase is Involved in Leydig Cell Tumor Proliferation.

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Our recent studies have revealed that estrogens stimulate an autocrine mechanism determining Leydig tumor cell proliferation. Estrogen over-production is due to an elevated SF1 expression and CREB phosphorylation, both inducing aromatase over-expression. In this study, we investigated the role of cyclooxygenase (COX)-2 in CREB dependent-aromatase expression in Leydig tumor cells. We found that COX-2 is expressed in rat and human Leydigiomas as well as in rat Leydig tumor cell line R2C, but not in normal testis. In R2C cells the use of a specific inhibitor for COX-2 (NS398) as well as COX-2 siRNA were able to reduce aromatase expression as a consequence of a decreased CREB activation and recruitment to the PII promoter of the aromatase gene. COX-2 is responsible for prostaglandin E2 (PGE2) synthesis, that binds the PGE2 receptor EP4 activating PKA and ultimately CREB. In fact, the use of inhibitors for EP4 (AH23848), and PKA (H89) reproduced the same effects observed in the presence of NS398, decreasing CREB activation and consequently aromatase expression and activity. Immunoprecipitation experiments demonstrated also the ability of these inhibitors to decrease enzymatic activity interfering with aromatase phosphorylation on tyrosine residues. Indeed, this study demonstrated that the altered expression of COX-2 induces a PGE2/PKA/CREB pathway

responsible for transcriptional and post-transcriptional activation of aromatase determining an increase in estrogen production, estrogen-dependent cell cycle regulator cyclin E and Leydig cell tumor proliferation. The observation that COX-2 signalling inhibitors decrease R2C cell proliferation suggests their potential application as new adjuvant therapies for Leydig tumor cell treatment.

ST 02. Silencing of Survivin induced by a BCR-ABL/JAK2/STAT3 Pathway Kills CML Cells and Sensitizes IM-Resistant Clones To Hydroxyurea

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The BCR-ABL oncoprotein of Chronic Myelogenous Leukemia (CML) induces survivin expression at both the mRNA and protein level. We report that, in murine and human CML cell lines, BCR-ABL-mediated up-regulation of survivin involves the JAK2/STAT3 pathway since silencing of either protein caused a consistent reduction of survivin. We also found that down-regulation of survivin sensitized CML cells to the cytotoxic effect of hydroxyurea (HU). In cells resistant to Imatinib Mesylate (IM), because of point mutations in the BCR-ABL kinase domain, survivin silencing failed to restore sensitivity to the drug but strongly increased HU-mediated death. Finally, incubation of these IM-resistant cells with shepherdin, an inhibitor of heat shock protein 90 that reduces survivin expression, also enhanced HU-induced cell death.

ST 03. Computational and Biological Characterization of Nuclear Import/Export Signals of the Human BCR Protein

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The Breakpoint Cluster Region (*BCR*) gene encodes for a multi-domain protein, which is implicated in the pathogenesis of Chronic Myeloid Leukemia. Previous evidence has suggested that BCR binds to heterochromatin. We performed a computational analysis of the BCR sequence and identified three putative Nuclear Export Signals (NES) and one putative bipartite Nuclear Localization Signal (NLS) detected in the Pleckstrin Homology (PH) domain. We generated a FLAG-tagged BCR construct and multiple cDNAs harbouring either the NES, NLS or PH domain sequences at the carboxy-terminius of GFP. When we expressed either the NLS alone or the entire PH domain, we observed a moderate increase in GFP nuclear localization at different of the putative NES that lack of nuclear export activity.

ST 04. Role of Interferon Regulatory Factor 5 (IRF-5) in Thyroid Carcinoma

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We studied the expression and the role of IRF-5 in four thyroid carcinoma cell lines. IRF-5 is a member of the Interferon Regulatory Factors family and has tumour suppressor properties. We found that IRF-5 is highly expressed in thyroid carcinoma cell lines. In addition, ectopic IRF-5 expression doesn't modulate p21. Recent studies have demonstrated that inactive IRF-5 is localized in the cytoplasm and is phosphorylated on tyrosine but, following exposure to virus or DNA damaging agents, IRF-5 is phosphorylated on serine and threonine and translocates in the nucleus. In our system, IRF-5 localized in the nucleus and was phosphorylated on tyrosine even after doxorubicine treatment. Finally IRF-5 transfection in the thyroid carcinoma cell lines caused an increase in colony formation.

ST 05. Mapping Estrogen Receptor (ER)-α Interactome of Hormone-Responsive Human Breast Cancer Cells

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Estrogens are involved in breast cancer (BC). Their actions are mediated by Estrogen Receptors (ERα and ERβ) and by other receptor interacting proteins, that are partly known. By application of TAP method we investigate the nature of the complexes in which ERalpha is involved in hBC cell nuclei. Therefore, the TAP-tag has been cloned downstream of hERα cDNA in an expression vector, which was stably transfected in MCF-7 to generate clones expressing TAP-ERα. The fusion protein's complexes and its interactors were purified by two specific affinity purification and elution steps. The protein bands visualized by silver staining were identified by mass-spectrometry. This approach provides the identification of novel functional partners of ERalpha, which will be presented.

Research supported by UE IP CRESCENDO contract n.er LSHM-CT2005-018652 MiUR PRIN 2005063915 003 and 2006069030 003 AIRC.

ST 06 . Identification of Estrogen Receptor (ER)-β Interacting Proteins from Breast Cancer Cells

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The aim of this study is to identify interacting partners of ER-β in human breast cancer (hBC) cell nuclei. Tandem Affinity Purification (TAP) allows the rapid isolation of multiprotein complexes. The TAP-tag was cloned to the human ER-β coding sequence and the recombinant proteins were expressed stably in hBC MCF-7 cells. Clones expressing TAP-ER-β were used for TAP. Samples at different stages of purification are being submitted to mass spectrometry, for identification of all the molecules present, the possible partners of ER-β were confirmed by western blotting. This experimental strategy allows us to identify new molecular pathways driven by ER-β in hBC cells.

Research supported by: UE (IP CRESCENDO, contract n.er LSHM-CT2005-018652), MiUR (PRIN 2005063915 003 and 2006069030 003)

ST 07. Genome-Wide Mapping of Estrogen Receptor (ER)-α Binding to Chromatin in Breast Cancer Cells by Massively Parallel Sequencing (ChIP-Seq)

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Estrogen receptors (ER-α and ER-β) are ligand-dependent transcription factors that mediate estrogen effects in hormone-responsive cells. To gain a global view of the genomic targets of estrogen, we mapped 'in vivo' binding of ER-α to its genomic targets by combining chromatin immunoprecipitation(ChIP) with massively parallel sequencing of IPP DNA(ChIP-Seq). MCF-7 cells underwent ChIP analysis after 45 min stimulation with17ß-estradiol. Immunoprecipitation was carried out with anti- ER-α antibody and the IP DNA was sequenced with the Illumina/Solexa Genome Analyzer. Comparison of tag representation and density throughout the genome led to precise mapping of 2.138 ER-α binding sites: 22% located<10kb upstream or downstream of known transcription units, 38% intragenic and 40%>10kb from genes suggesting multiple mechanisms for ER-α regulation of target gene promoters and genome functions.

Research supported by UE (IP CRESCENDO LSHM-CT2005-018652) MiUR (PRIN 2006069030 003) AIRC and Second University of Naples.

ST 08. Classification of Estrogen Receptor (ER)-α Binding Sequences Detected in Responsive Cells by Massively Parallel Sequencing (ChIP-Seq)

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MCF-7 hBC cells underwent ChIP analysis before (-E2) or 45min into stimulation with a mitogenic dose of 17β-estradiol (+E2) and the IPP DNA was sequenced with Illumina's Genome Analyzer.Comparison of tag density throughout the genome in +E2 vs –E2 samples allowed precise mapping of ER-α binding sites. A throughout motif search was performed and resulted in their classification in three groups: sequences containing (1) perfectly palindromic or variations of the ERE (2) half-consensus EREs (3) none of the above elements suggesting indirect interactions of ER-α with the DNA and pointing to several TF as likely partners of this receptor in mediating its interaction with the genome 'via' protein-protein interactions (tethering).

Research supported by: UE (IP CRESCENDO contract n.er LSHM-CT2005-018652), MiUR (PRIN 2006069030 003) and AIRC.

ST 09. A Novel Procedure For Microarray-Based Analysis of Degraded RNA Extracted From Formalin-Fixed, Paraffin-Embedded (FFPE) Tumour Biopsies

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Gene expression profiling of tumour biopsies provides new ways to predict disease outcome. The RNA extracted from Formalin-Fixed, Paraffin-Embedded (FFPE) samples is degraded and thus unsuitable for molecular analysis. We applied and evaluated a microarray-based cDNA-mediated Annealing Selection extension

Ligation (DASL) assay for analysis of 502 mRNAs in degraded total RNA extracted from cultured cells or FFPE cancer biopsies. The study included quantitative and qualitative comparison of data obtained by analysis of the same RNAs with genome-wide oligonucleotide microarrays vs DASL arrays and, by DASL, before and after extensive in vitro RNA fragmentation. Sensitivity, reproducibility and accuracy of the assay indicate that it is suited to identify prognostic and predictive gene expression profiling in archival FFPE tissue banks.
Research supported by UE IP CRESCENDO contract n.er LSHM-CT2005-018652 MiUR PRIN2005063915 003 and 2006069030 003 and AIRC.

ST 10. Integration of MAP Kinase Signaling and Progesterone Receptor Action in Breast Cancer Models

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Recent discoveries suggest that protein kinases are rapidly activated in response to ligand binding to cytoplasmic steroid hormone receptors (SRs), including progesterone receptors (PRs). Thus, PRs act as ligand-activated transcription factor 'sensors' for growth factor-initiated signaling pathways in hormonally regulated tissues, such as the breast. Induction of rapid signaling upon progestin-binding to PR-B provides a means to ensure that receptors and co-regulators are appropriately phosphorylated as part of optimal transcription complexes. Kinases are emerging as key mediators of PR action. Cross-talk between SR and membrane-initiated signaling events suggests a mechanism for coordinate regulation of gene subsets by mitogenic stimuli in hormonally responsive normal tissues, and is suspected to contribute to cancer biology.

ST 11. Role of PKC in Sensitivity and Resistance of Neuroblastoma Cells To Etoposide

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The role of PKC in sensitivity and resistance of neuroblastoma cells to etoposide was investigated. HTLA-230 cells were exposed for 24 h to 10-50 μM etoposide, and 10 μM concentration was monitored for 12-60 h. 50 μM etoposide caused apoptosis and a reduction of cell number, after 24 h. Cell death was accompanied by an enhancement of PKC-α and –ε protein levels. The prolonged treatment with 10 μM etoposide induced a progressive accumulation of apoptotic and necrotic cells and a parallel decrease in PKC isoform levels. These preliminary results suggest that the selective modulation of PKCs might be useful to stimulate apoptosis, thereby sensitizing this tumour to chemotherapy.
(Grants from Gaslini Institute, from Genoa University and PRIN 2006065711 002).

ST 12. Role of Estrogen Receptor Beta in Hormonal Signal Transduction in Human Breast Cancer Cells

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Estrogen receptors ER-α and ER-β are ligand-activated transcription factors that regulate gene expression and cell growth. Contrarily to ER-α and ER-β functions are unclear. It probably inhibits ER-α activity. We analyzed ER-β -mediated functions to characterize estrogen-responsive genes regulated by this receptor. The cell line MCF-7, expressing ER-α, was stably transfected with a vector coding a tagged- ER-β. We studied cell cycle kinetic, nuclear translocation and trans-activating activity of the cell clones compared to wild-type MCF-7. To characterize the effects of ER-β alone we treated cells with different ligands: 17-β-estradiol, which activates both ERs, diarylpropionitrile (ER-β -selective ligand) and propyl-pyrazole-triol (ER-α -selective ligand). Results suggest a complex role for ER-β in hormonal signalling, also depending by the presence of active ER-α in the cell.
Research supported by: UE (IP CRESCENDO, contract n.er LSHM-CT2005018652), MiUR (PRIN 2005063915 003 and 2006069030 003) and AIRC.

ST 13 . Role of Signaling Activation in Steroid Action.

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Steroid stimulation triggers activation of signaling pathways in target cells. This occurs through a rapid and direct interaction of classical steroid receptors with Src, the p85-regulatory subunit of PI3-K and other signaling effectors. Signaling activation controls the steroid-induced DNA synthesis. The cytoplasmic role of steroid receptors has been corroborated by findings showing that estradiol activation of PI3-K/Akt/FOXO pathway modulates estrogen receptor (ER) nuclear export in breast cancer MCF-7 cells. Inhibition of ER exit from nuclei impairs estrogen-induced DNA synthesis. The mitogenic role of Src in sex steroid action has been highlighted by evidence showing that specific interference in the steroid receptor/Src interaction by new molecules inhibits the growth of mammary and prostate tumor cells in vitro and in nude mice.

ST 14. Integrated Analysis of the Estrogen-Responsive Transcriptome and Estrogen Receptor (ER)-α Binding to Chromatin in Breast Cancer Cells

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We studied genome-wide effects of a mitogenic dose of 17β estradiol (E2) on gene expression in the breast cancer cell lines ZR-75.1 and MCF-7. RNA was assayed after 11 time intervals of E2 stimulation (1-32h, unstimulated cells as reference) with Illumina Human WG-6 v2 BeadChips. We identified >1.200 genes similarly regulated by E2 in the two cell lines, 50% of which up-regulated, 43% down-regulated and 7% show bi-phasic changes. We combined these results with ER-α binding regions obtained by ChIP-Seq after 45min stimulation with E2 in MCF-7 by locating the binding regions with respect to the known, detected and regulated (up- or down) genes probed on the microarrays. Binding region sequences are now being analyzed to understand their role in estrogen action on chromatin.
Research supported by: UE (IP CRESCENDO, contract n.er LSHM-CT2005-018652), MiUR (PRIN 2005063915 003 and 2006069030 003) and AIRC.

ST 15. Evidences that Bergapten is an Antitumoral Agent that Enhances p53 Gene Expression Independently of its Photoactivation.

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In this study, we have evaluated in MCF-7 and SKBR-3 breast cancer cells the effects of 5-methoxypsoralen (bergapten) on the signalling pathways involved in cell cycle arrest and in apoptosis. Drug treatment induced a block in the G0/G1 phase and increased mRNA and protein levels of p53 and p21waf. Bergapten can transactivate p53 gene promoter in these cells and site-direct mutagenesis studies showed that the binding sequence of the nuclear factor NF-Y is required for 5-methoxypsoralen responsiveness. Bergapten increases NF-Y nuclear translocation through p38 MAPK activation and the same treatment down-regulated the PI3Kinase/AKT, even with IGF-I preincubation. All these data demonstrate that bergapten, independently of its photoactivation, may be considered a new antitumoral agent for breast cancer treatment.

ST 16. Resveratrol Treatment is Effective for Cell Cycle Arrest of Tamoxifen-Resistant Breast Cancer Cells.

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Treatment of hormone-dependent breast cancer with tamoxifen (T) is responsible for major improvements in cure rates and disease prevention. Despite clinical advances, resistance to this endocrine therapy is a common feature. We have discovered that Resveratrol (R), a nontoxic natural compound, blocks cell cycle at the G(1)-S phase transition in T resistant breast cancer cells (MCF-7 TR); in contrast T and ICI treatment have no effect. The action of R is associated with increased expression of p21 and decreased expression of cyclin D1 concomitantly with dephosphorylation of pRB and it is still present after E2 and IGF treatment. In conclusion R prevents cell proliferation and may be considered a novel pharmacological tool for hormone-resistant breast cancer.

ST 17. MicroRNA Expression Profiling of Hormone-Responsive Human Breast Cancer Cells Lines.

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MicroRNAs (miRNAs) are 18-25nt noncoding segments of RNA that negatively regulate gene expression at the post-transcriptional level. Estrogens are steroid hormones that have wide effects on different cellular and physiological processes and are known to be involved in the development of diseases such as cancer. In order to study whether E2 affects the expression profiles of miRNAs we performed a time course experiment where hBC MCF-7 and ZR-75.1 cells were stimulated with 17beta-estradiol and RNA was extracted before and at different times after

stimulation. Hybridization reactions were performed with the Illumina miRNA panels that measure the levels of 735 miRNA.The estrogen-dependent miRNA expression profiles were then correlated with mRNA expression profiles obtained under identical experimental conditions.
Research supported by UE IP CRESCENDO contract n.er LSHM-CT2005-018652 MiUR PRIN 2006069030 003 and AIRC.

ST 18 . Functional Proteomics to Map Estrogen Receptor Interactomes in Target Cells

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Estrogens act as tumor promoters by their binding to estrogen receptors (ERα and ERβ).The mechanisms are poorly defined and the identification of all functional protein-protein interactions may become useful to discriminate the different pathways of signal transduction in which ERs are involved, providing clues to identify new molecular targets for therapy. We apply the Tandem Affinity Purification (TAP) approach to purify multi-protein complexes comprising ERα in different cell compartments. Such complexes are then analyzed by mono-dimensional (SDS-PAGE) or two-dimensional electrophoresis (2-D PAGE), fluorescent Difference Gel Electrophoresis (DIGE) and Mass Spectrometry. These analyses are holding the promise to uncover novel ER partners, also belonging to still unknown ER pathway in target cells.
Research supported by: UE (IP CRESCENDO, contract n.er LSHM-CT2005-018652), MiUR (PRIN 2005063915 003 and 2006069030 003) and AIRC.

ST 19. Regulation of Interleukin (IL)-8 Gene at a Distinct Site of its Promoter by C/EBP Homologous Protein in Prostaglandin E2-Treated Human T Cells

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PGE2 increases in T lymphocytes IL-8 expression by CHOP phosphorylation. We investigated the CHOP consensus site on IL-8 promoter and the CHOP domain responsible for the IL-8 transcription. Transfection experiments with mutants showed both that CHOP trans-activation domain is essential for IL-8 transcription and that the IL-8/AP-1 promoter mutated in NFκB and NF-IL-6 -but not in AP-1 site- harbours CHOP responsive elements. ChIP showed the *in vivo* binding of CHOP to the IL-8 promoter and EMSA experiments demonstrated that the CHOP responsive element is located immediately upstream of the AP-1 site. Our results suggest that the increased expression of CHOP in response to PGE2 exerts a positive regulation of the IL-8 promoter by direct binding to a novel consensus site.

ST 20. Study of Arg Tyrosine Kinase Expression in SH-SY5Y Neuroblastoma Cell Line Treated with Amyloid Beta-Protein

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Non receptor tyrosine kinase Arg is important in neurulation and dendritic spine morphogenesis. Our study evaluates the involvement of Arg 5'- and 3'- isoforms in neurotrophic/neurotoxic effect of Aβ (1-42) oligomers in SH-SY5Y neuroblastoma cell line. In undifferentiated SH-SY5Y treatment with low concentration (0.1 μM) of A β induces a significant increase in neurites/cell and in neuritic length. These changes correlate with total Arg transcript and protein increment, evaluated by Real Time PCR and western blot, and with a switch of 3'- end different Arg transcripts. An increase of Arg expression has been evidenced also in ATRA-differentiated versus undifferentiated SH-SY5Y cells. The studies on cellular and molecular effects induced by toxic concentration (2.5 μM) of Aβ on ATRA-differentiated SH-SY5Y are ongoing.

ST 21. Enhanced Anti-Tumor Therapy by Inhibition of p21waf1 in Human Malignant Mesothelioma

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Background Information: p21waf1 is expressed in malignant mesothelioma (MM). We assessed the possibility of p21 suppression as a therapeutic target for MM.
Method: We established two different MM-derived clones. Chemosensitivity of these cells was investigated by colony formation assays, the type of cell response induced by drugs was analyzed. Tumor formation in nude mice were also done.
Results: The loss-of-expression of p21 sensitizes MM clones to apoptosis induced by chemotherapeutic agents. This enhancement was achieved by inhibiting a senescence-like growth arrest. Moreover, CPT11 and p21 inhibition co-treatment blocked tumor growth and enhanced apoptotic response. Conclusions: p21 plays an important role in MM chemosensitivity, and the suppression of its expression might be a potential therapeutic target for MM.

ST 22. Inhibition of RET Kinase Activity for the Treatment of Thyroid Cancer

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Some compounds have been described that exert an inhibitory effect on the RET kinase. ZD6474 and BAY439006 function in the nM range and are undergoing phase II testing in patients with thyroid cancer. These compounds are multi-target and share the ability of inhibiting not only RET but also VEGFR. Clinical evaluation coupled with measurement of surrogate phosphorylation levels will be crucial to assess the capability of the compounds to hit the target in human patients and to establish the clinical value of kinase inhibition in thyroid cancer treatment. One problem that could emerge with the use of these inhibitors is molecular resistance formation.

ST 23. Characterization of New Regulators of NF-κB

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Nuclear factor (NF)-κB plays a pivotal role in inflammation, immunity, stress responses, and protection from apoptosis. The Non-Canonical activation pathway of NF-κB regulates the differentiation and development of lymphocytes and secondary lymphoid organs. It is activated by a variety of cytokines (Baff, LTbeta, CD40L, etc.), that activate the kinase NIK and IKKα. The molecular mechanisms regulating activation of this pathway are poorly understood. The purpose of our study is the identification and characterization of new regulators of the alternative NF-κB pathway.

ST 24. Nanotechnology for the Enhancement of Human Health.

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The application of nanotechnology to the prevention and treatment of human diseases holds great promise because it involves the interaction with nanoscale biological materials. Synthetic nanomaterials that are biocompatible, non-toxic and functional in biologic (wet) conditions can be used to engineer and restore cellular function in a manner similar to how artificial joints and heart valves can restore organ function. Early applications of nanomaterials will likely involve the development of medications that take advantage of unique aspects of nanostructures interaction with biological systems to achieve or enhance therapeutic activity. Examples will be provided for the design, synthesis and analysis of therapeutic nanomaterials where distinct kinds of attached molecules allow for unique therapeutic functions. These applications include antimicrobial compounds, vaccines, drug and gene delivery, and functional imaging. These “nanomedicines” all share the capability to uniquely function simply due to their size. Future nanotechnology therapeutic applications such as cellular engineering, human performance augmentation and single molecule manipulation will be reviewed.

ST 25. Role of Differential Phosphorylation of c-Jun N-Terminal Domain in Cancer Cell Death by Genotoxic Stress

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The JNK-c-Jun pathway is involved in opposite biological programs as differentiation, proliferation and apoptosis. Such a functional dichotomy raises problems in using anticancer strategies inhibiting JNK-mediated tumor progression or to sensitise cancer cells to apoptosis. JNK induces c-Jun transactivity through its phosphorylation at S63/S73 and T91/T93. We have recently shown that JNK-dependent phosphorylation of c-Jun at T91/T93 requires a preceding phosphorylation on the adjacent T95 site by a yet not identified kinase. Moreover, alanine substitution of T95 impairs c-Jun biological activity as transactivity and

enhancement of cell death. We present evidence indicating that pharmacological augmentation of T95 phosphorylation may represent a tool to sensitize cancer cells to drug-induced apoptosis, without causing alteration of c-Jun mediated differentiation processes.

ST 26. Tamoxifen Resistance and Growth Factor Signaling in K303R ERα Mutant Breast Cancer Cells

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Estrogens play a crucial role in breast tumor growth and the antiestrogen tamoxifen (Tam) is used to treat estrogen receptor (ER)-α-positive breast cancer, however resistance is a major clinical problem. We have identified a mutation at nucleotide 908 of ERα (A908G), resulting in a K303R transition, in premalignant hyperplasias and invasive breast cancers. The mutation confers hypersensitive growth with estrogen. To determine whether the mutation could play a role in tamoxifen resistance, we used MCF-7 breast cancer cells stably transfected with wild-type (WT) or K303R ERα. Growth assays showed that the mutation alters Tam response, and growth factor stimulation converted Tam into an agonist in K303R mutant-expressing cells. Tam was also less efficient at reducing estrogen and heregulin-stimulated soft agar growth in these cells. Mutant cells exhibited increased phosphorylated levels of HER2, Akt, and MAPK compared to WT cells. Preliminary data suggests that the mutant receptor and HER2 are in a complex. Furthermore, mutant receptor was constitutively phosphorylated at serine 305. We hypothesize that the mutation adapts the receptor for enhanced cross-talk with growth factor pathways, which affects Tam response. These data suggest further that the A908G ERα mutation could be a potential biomarker of hormone response in tumors exploiting ERα growth factor signalling cross-talk to evade treatment.

ST 27. The Role of Sgk1 in the Regulation of Sodium Absorption,Cell Proliferation, Survival and Differentiation.

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Serum glucorticoid regulated kinase (Sgk1) was originally described as a key enzyme in the hormonal regulation of transepithelial sodium transport in kidney principal cells.It is regulated by insulin, IGF1, vasopressin and steroids. The activation of the kinase enhances the sodium absorption in kidney cells by increasing the number of epithelial sodium channels, through the inhibition of ubiquitin ligase dependent degradation. Sgk1 is also involved in transducing growth factors survival signals. We demonstrated that Sgk1 mediates IL2 dependent antiapoptotic signals in kidney cancer cells. The analysis of Hela cell lines expressing wild type and dominant negative Sgk1 demonstrated that Sgk1 is essential for Hela cell survival, cell cycle progression and epithelial to mesenchimal transition by regulating HDM2 dependent p53 ubiquitination.

ST 28. Hedgehog Signaling Pathway and Brain Tumors

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Hedgehog pathway is critical for embryonic patterning, determining cell fate of neural progenitors. Hedgehog signaling keeps cerebellar granule cell progenitors undifferentiated, promotes cell expansion and neural stem-cells maintenance, and is involved in medulloblastoma development. We observed a novel mechanism by which Gli1 transcription factor, effector of Hedgehog signaling, undergoes ubiquitination and degradation by the HECT-type ubiquitin E3-ligase Itch, a process triggered by Numb. Numb is downregulated in medulloblastomas and promotes tumor cell growth arrest and differentiation. Furthermore, neural stem cells from wild type cerebellum and primary cerebellar tumors are responsive to Hedgehog. Additionally Numb is able to control the clonogenic capacity and Hedgehog signaling of cerebellar and medulloblastoma stem cells, revealing its role in neural stem cell self-renewal and tumorigenesis.

ST 29. EDF-1, a Novel Player in Adipogenesis

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Background: PPARγ is the main protein orchestrating adipocyte differentiation. Since EDF-1 is a transcriptional coactivator for PPARγ *in vitro*, we evaluated the role of EDF-1 in adipogenesis. Methods: 3T3L1 preadipocytes were induced to differentiate with a hormonal cocktail. SiRNAs were utilized to silence EDF-1. PPARγ transcriptional activity was determined after transfection with luciferase under the control of a PPARγ responsive consensus. Results: While EDF-1 amounts and subcellular localization did not change in differentiating cells, PPARγ /EDF-1 interactions increased. Silencing EDF-1 markedly retarded 3T3L1 differentiation into adipocytes. In parallel, silencing EDF-1 reduced ligand-dependent luciferase induction and inhibited PPARγ expression. Conclusions: EDF-1 is a positive regulator of adipogenesis by promoting the expression and the transcriptional activity of PPARγ in 3T3-L1 cells.

ST 30. HD-PTP is a Negative Regulator of the Migration of T24 Human Bladder Carcinoma Cells

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Background: HD-PTP is a tyrosine phosphatase which maps on chromosome 3p21.3, an area frequently deleted in aggressive bladder cancers. Methods: HD-PTP was silenced by siRNA. Wound healing assay was used to evaluate the migration. The protein-protein interaction was analyzed by co-immunoprecipitation. Results: HD-PTP silencing induced cell migration in T24 bladder cancer cells. In EGF stimulated cells, Src binds to and phosphorylates HD-PTP on tyrosine residues. HD-PTP also binds to FAK, and this interaction is inhibited after exposure to EGF. The phosphorylation of FAK is enhanced in cells which downregulate HD-PTP. Conclusions: We hypothesize that, following stimulation with EGF, HD-PTP is tyrosine-phosphorylated and releases FAK which will ultimately contribute to the turn-over of focal adhesion and, therefore, to cell motility.

ST 31. Myostatin Blockade by Deacetylase Inhibitors Fails to Counteract Muscle Wasting in Tumor-Bearing Mice

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Background: Muscle wasting is characterized by protein hypercatabolism and increased expression of ubiquitin ligases. Myostatin negatively regulates skeletal muscle mass, whereas histone deacetylase inhibitors (DIs) may increase myofiber size. In this work we evaluated whether DIs can restore muscle mass in C26 tumor-bearing mice. Methods: Tumor-bearing mice received $5x10^5$ C26 cells s.c. Valproate or trichostatin-A were administered daily. Results: C26 growth induced an increase in myostatin and ubiquitin ligases expression. Although only valproate administration proved effective in reducing myostatin by increasing follistatin levels, both DIs were unable to counteract muscle atrophy in tumor bearers. Conclusions: These results show that DIs treatment failed to prevent muscle mass depletion and protein degradation in C26 hosts, despite a reduction in myostatin signaling.

ST 32. Functional Studies in COS-7 Cells Transfected with 8 Different Arg Isoforms

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Arg tyrosine kinase inhibits cellular migration attenuating actomyosin contractility and regulating focal-adhesions (FA) through kinase domain and C-terminal region containing F-actin and microtubule-binding domains. Alternative splicing produces eight Arg isoforms differing in N-termini and C-terminal F-actin-binding domain. We transfected COS-7 cells with eight FLAG-Arg constructs to analyse isoform role in regulating cellular morphology, adhesion and cytoskeleton. Confocal microscopy and ImageJ software analysis evidenced similar isoform cytoplasmatic distribution and colocalization with F-actin and tubulin. Transfected isoforms inhibit stress-fibers and cellular spreading and induce filopodia-like extroflections in different ways. Analysis of fibronectin-stimulation effect on morphology, spreading and FA are ongoing. Arg isoform different expression in normal and neoplastic cells and their different role in cytoskeleton regulation might be important for metastatic diffusion.

ST 33. JNK Inhibition Prevents TNFα-Induced Apoptosis in Huh7 Hepatoma Cells

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Background: The role of JNK in TNFα-induced apoptosis is poorly defined. This study is aimed to characterize JNK involvement in apoptosis induced by TNFalpha and cycloheximide (CHX) in human hepatoma cells. Methods: TNFα +CHX-treated Huh7 cells were incubated with and without SP600125, an inhibitor of JNK. Protein levels were evaluated by western blotting and caspase activity by fluorometric assay. Results: Apoptosis induced in Huh7 cells by TNFα +CHX is JNK-dependent, since it can be partially, but significantly, inhibited by treatment with SP600125. Also caspase 3 activity and PARP cleavage are reduced in the presence of JNK inhibitor. Conclusions: These results suggest that JNK plays a critical role in TNFα -induced apoptosis in Huh7 cells, involving caspase-dependent mechanisms.

ST 34. Sgk1 Promotes Cell Proliferation, Survival and Epithelial De-Differentiation by Activating HDM2-Dependent p53 Degradation.

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Sgk1 is a S/T Kinase, homologous to AKT and activated by serum, steroids, insulin and IL-2 at multiple levels. In the present work we characterize stably HeLa cell lines expressing wild type and dominant negative Sgk1 and show that Sgk1 is essential for survival, cell cycle progression and EMT. We provide evidences that demonstrate how Sgk1 promotes the survival and the clonogenesis, by binding to p53 and activating HDM2. These results are strengthened by the analysis of different cell lines and by the characterization of a mouse model expressing the DN mutant of Sgk1. Since the transcription of Sgk1 is activated by p53, we propose a finely tuned feed-back where Sgk1 down-regulates the expression of p53 by enhancing its ubiquitination.

ST 35. Multiple Molecular Mechanisms Sustain Hypoxia - Dependent Epithelial - Mesenchymal Transition and Increased Invasiveness in Human Cancer Cells

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Epithelial mesenchymal transition (EMT) and hypoxia are considered as a crucial events favouring invasion and metastasis of many tumour cells. In the present study, by using morphological, cell and molecular biology techniques, we investigated whether hypoxia may trigger EMT program and favour invasiveness of human HepG2, PANC-1, HT-29 and MCF-7 cancer cell lines. Hypoxia, as an independent factor and as dissected by employing siRNAs for HIF1a and other specific tools, triggered typical EMT changes and induced increased invasiveness in all neoplastic cells of epithelial origin through a bifasic mechanism involving: 1) early and redox - dependent inactivation of GSK-3ß, followed by SNAIL nuclear translocation and E-cadherin down-regulation; 2) late HIF1a - dependent release of VEGF sustaining invasiveness.

ST 36. Identification of New Sgk1 interacting Proteins by Yeast Two-Hybrid Screening

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Background: Sgk1 is an important molecular target that integrates the multiple endocrine inputs regulating sodium transport. Activated Sgk1 interacts with molecules that mediate Sgk1 action on the ENaC. Method: Yeast Two-Hybrid Screening- *Saccharomyces cerevisiae* AH109 was transformed with pBridge-Sgk1, then mating with a host strain Y187 pretransformed using a MATCHMAKER human kidney cDNA. Results: We found for Sgk1 several new partners: PMM2; Rad50 (non in frame); importin ß; *Homo sapiens* similar to lysophospholipase; RAN (in antisense); TOMM70A. Conclusion: The different interacting proteins that we identified make Sgk1 an enzyme potentially involved in multiple pathways: in glycoproteins and lipids metabolism; in nuclear and mitochondrial transport. This study can contribute to better understand the role of Sgk1 in different cellular compartments.

ST 37. BCR-ABL Tyrosine Kinase Activity Modulates the Phosphorylation and the Localization of Interferon Regulatory Factor 5 (IRF-5) in Chronic Myeloid Leukemia Cells

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Interferon Regulatory Factors (IRFs) are transcription factors regulating cell growth, apoptosis and immune response. Aim of this research was to study the possible functional relationship between IRF-5 and BCR-ABL in Chronic Myeloid Leukemia (CML). We found that IRF-5 is expressed in primary hematopoietic cells derived from healthy donors, CML patients and in immortalized CML cell lines. Co-immunoprecipitation experiments showed that IRF-5 and BCR-ABL have a direct interaction. Interestingly, we detected high levels of IRF-5 tyrosine phosphorylation in CML cells and such phosphorylation decreased after treatment with Imatinib Mesilate (IM) a semi-specific inhibitor of BCR-ABL kinase activity. Finally, cellular fractionation showed that in CML cell lines, IRF-5 localization is modified by IM treatment, suggesting that BCR-ABL alters IRF-5 nuclear-cytoplasmic shuttling.

ST 38. In Vitro Study of Diabetic Epithelial-Mesenchymal Transition of Renal Tubular Cells.

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In diabetic nephropathy (DN) fibrosis arises also from tubular epithelial cells (TECs) through epithelial-mesenchymal transition (EMT) characterized by α-SMA expression, E-cadherin loss and actin reorganization. To understand EMT in DN and to find early markers, human TECs were cultured with normal and high (450mg/dl) glucose for 6-96h. MTT assay measured metabolic activity and annexin V/propidium iodide double staining FACS analysis apoptosis. Microscopy analysis proved EMT development. FACS, Real-Time PCR and Western blot showed α-SMA increase and E-cadherin decrease at 72h with high glucose. Preliminary data show, with high glucose, downregulation of the tyrosine kinase Arg involved in actin reorganization. MUDPIT is ongoing to profile changes in protein expression potentially involved in DN early steps and useful as precocious EMT markers.

ST 39. Ceramide Does Not Mediate TNF Toxicity in Rat Hepatoma Cells

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Background: We have previously demonstrated that, in HTC cells, TNF toxicity requires a functional lysosomal compartment. We further investigated the pathways of TNF toxicity in these cells by focusing on wheter ceramide, which can be generated in lysosomes, mediates TNF toxicity. Methods: HTC cells were treated with ceramide or TNF; cell death measurerd by the Annexin-V/PI technique. Results and conclusions: HTC cells are killed by ceramide, and desipramine attenuates TNF toxicity but altered the lysosomal compartment. However, Bcl-2 ectopic expression or Bcl-xL upregulation by dexamethasone significantly protected from ceramide yet, did not prevent TNF toxicity. These results indicate that ceramide, though able to kill HTC cells, does not account for TNF toxicity in these cells.

ST 40. Induction of ErbB-3 Expression by $\alpha6\beta4$ Integrin Contributes to Tamoxifen Resistance in ERβ1-Negative Breast Carcinomas

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Using a panel of human breast cancer cell lines displaying different levels of $\alpha6\beta4$ and ErbB-3 receptors and a series of 232 breast cancer biopsies from patients submitted to adjuvant tamoxifen monotherapy for five years, we evaluated the functional interaction between both receptors in relationship to tamoxifen responsiveness. In mammary carcinoma cells, we evidenced that the $\alpha6\beta4$ integrin strongly influence Akt phosphorylation through ErbB-3 protein regulation. Moreover, the ErbB-3 inactivation inhibits Akt phosphorylation, induces apoptosis and inhibits in vitro invasion favouring tamoxifen responsiveness. The analysis of human tumors revealed a significant relationship between $\alpha6\beta4$ and ErbB-3 in P-Akt-positive and ERβ1-negative breast cancers derived from patients with lower disease free survival.

ST 41. Is GPR30 a Promiscuous Mediator of Estrogen and Antiestrogen Signals ?

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Estrogen is a crucial hormone involved in both human physiology and diseases including endocrine-related cancer. Although the biological effects of estrogens have traditionally been ascribed to the cognate nuclear receptors ERα and ERβ, recent studies have revealed that the G protein-coupled receptor GPR30 mediates rapid estrogen action via the EGFR transduction pathway. We have provided novel insight into the molecular mechanisms by which estrogen as well as the ER antagonist tamoxifen trigger tumor cell responses through GPR30. Moreover, we have ascertained that EGF-dependent signaling regulates GPR30 expression, thereby allowing estrogen to further enhance the proliferation of breast cancer cells. Our results point to the requirement of a new generation of receptor-specific drugs to fine tune anticancer pharmacological therapy.

ST 42. Regulation of Bruton Tyrosine Kinase Through PKC-Mediated S90 Phosphorylation of Its Repressor IBtk.

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Loss-of-function mutations in the Btk gene lead to X-linked agammaglobulinemia (XLA). We previously identified a novel protein that acts as inhibitor of kinase activity

of Btk, "IBtk" – Inhibitor of Bruton Tyrosine Kinase (Liu et al., Nat. Immunol., 2001). Here, we have investigated the fate of IBtk upon B cell receptor (BCR) stimulation. We show that IBtk is transiently phosphorylated by kinases of PKC family which leads to its dissociation from Btk. Mapping of PKC phosphorylation sites in IBtk identified the critical serine residue as S90. IBtk mutated in S90 constitutively bound to Btk and efficiently inhibited calcium mobilization and NFκB activation upon BCR triggering in B cells, thus preventing activation of primary B lymphocytes and proliferation of B lymphomas.

ST 43. First Evidence of Non-Genomic Estradiol induced Up-Regulation of Aromatase Enzymatic Activity in MCF-7 Breast Cancer Cells.

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In situ estrogen production plays an important role in breast tumor promotion. We demonstrated, in MCF-7 cells, that 17-β-estradiol rapidly enhances aromatase activity in the presence of unmodified enzyme expression. Aromatase kinetics studies showed, upon increasing dose of 17-β-estradiol, decreased Km and unchanged Vmax. 17-β-estradiol phosphorylates in vivo aromatase protein and site-directed mutagenesis experiments revealed that 361 tyrosine residue phosphorylation is crucial in estradiol up-regulation of aromatase activity. We evidenced the involvement of the tyrosine-kinase Src since inhibition of its signalling abrogated the up-regulatory effects induced by 17-β-estradiol on aromatase activity and phosphorylation of aromatase protein. These findings indicate a short non-genomic autocrine loop between 17-β-estradiol and aromatase emphasizing the role of local estrogen production in promoting breast cancer growth.

ST 44. Androgen Receptor (AR) Inhibits Cyclin D1 Promoter Activity. May AR be Considered as an Oncosuppressor in Breast Cancer?

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In the breast, androgens counterbalance positive serum- and estrogen-induced growth stimuli, which are intimately linked to breast tumorigenesis. We demonstrate that in MCF-7 cells, non-aromatisable 5-α-dihydrotestosterone decreases serum- as well as estradiol-induced cyclin D1 expression through inhibition of cyclin D1 promoter transcriptional activity. Mutagenesis, DAPA, EMSA and ChIP analysis indicated that this inhibitory effect is mediated by direct binding of AR to a putative AR response sequence, whose identification allows to define cyclin D1 as an androgen target gene in breast. Moreover AR negatively modulates ER-mediated signalling through competition for AIB1, crucial in the functional coupling of ER with the cyclin D1 promoter, further evidencing AR inhibitory effects and raising a role for AR as a new oncosuppressor in breast.

ST 45. Effects of Selective Cyclooxygenase-2 Inhibitors Etoricoxib and Celecoxib on Colorectal Cancer Cell Lines

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Background: Cyclooxygenase-2 (COX-2) plays a role in colorectal cancer development by inhibiting apoptosis and increasing angiogenesis. Methods: Cytotoxicity (MTT test) and effects on COX-2 and VEGF expression (Real Time-PCR, ELISA) of two selective COX-2 inhibitors, etoricoxib and celecoxib were studied on colorectal cancer cell-lines (CaCo2, HT29, SW480, SW620) at different concentrations. Results: Cytotoxicity and VEGF and COX-2 expression were dependent on drug type (celecoxib >etoricoxib), concentration, cell lines. SW480 and its metastatic counterpart (SW620) from the same patient showed different response. Conclusions: Many potential factors might be involved in biological activity of coxibs. Even if celecoxib appears to be a more potent agent than etoricoxib, therapeutic use of coxibs on colorectal cancer depends on full comprehension of tumor's biology.

ST 46. Insulin Receptor Substrate 1 Modulates the Transcriptional Activity and the Stability of Androgen Receptor in Breast Cancer Cells.

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Although the majority of human breast cancers expresses androgen receptor (AR), its role in breast tumorigenesis remains largely unexplored. Here we demonstrate that 5-α-dihydrotestosterone inhibits MCF-7 cell growth induced by IGF-I. Immuno-precipitation, transient transfection and ChIP assays demonstrate that, upon 5-α-dihydrotestosterone stimulation, IRS-1, the major IGF-1R substrate, interacts and translocates into the nucleus with AR and is recruited to the androgen responsive region of target gene promoters where participates to sustain AR-mediated transcription. Moreover, siIRS-1 suggests that IRS-1/AR interaction decreases the ubiquitin/proteasome-dependent degradation of AR, increasing its stability. Therefore, IRS-1 appears to be a novel AR regulator required to sustain AR activity suggesting the existence of a functional interplay between the IGF system and AR in breast cancer.

ST 47. Different Biological Effects of Chenodeoxycholic Acid (CDCA) in Endometrial Cancer Cells.

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CDCA acts as a tumor promoter in animal models and enhances cell transformation and apoptosis in several tumor cell lines. Here we investigated the biological effects of different concentrations of CDCA on human endometrial cancer Ishikawa cells. [3H]Thymidine incorporation suggests that Ishikawa cell growth was stimulated by CDCA at low concentrations (<30υM) and suppressed at higher (>50υM). Worthily, in all circumstances ERK activation occurred while p38-MAPK was activated only at higher concentrations concomitantly with the cleavage of poly(ADP-ribose)-polymerase, DNA fragmentation, Cyclin D1 down-regulation and p21WAF1/CIP1 up-regulation. Indeed, the crucial role of p38 MAPK pathway, in the above mentioned apoptotic events, rised by their abrogation in the presence of its specific inhibitor, SB203580. All these findings address how CDCA may be perspectively implemented in the novel therapeutic strategies for endometrial cancer treatment.

ST 48. Increased Expression of Dystroglycan Inhibits Growth and Tumorigenicity of Human Prostate Cancer Cells By Sequestering Components of the ERK-MAP Kinase Cascade

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Dystroglycan (DG), a cell adhesion receptor, is a complex composed of two subunits, α and β, linking laminin in the extracellular matrix to the cytoskeleton. We and other demonstrated that DG expression, and mainly the extracellular α-DG, is reduced or lost in a variety of human cancers, including prostate cancer. Overexpression of an exogenous DG cDNA in LNCaP prostate cancer cells was not able to restore α-DG detection although expression of the β-DG was significantly increased. These cells also displayed an induced cell adhesion while cell growth and tumorigenicity were reduced. To investigate the mechanisms underlying these effects, we analyzed the expression of the α-DG core protein in the DG-overexpressing cells and found that its expression is increased confirming that the lack of the α-DG detection is due to post-transductional events. We also analyzed the interaction between β-DG and components of the ERK-MAP kinase cascade including MEK and ERK and found an increased interaction of β-DG with both pERK and pMEK. Interaction with Grb2 was also affected by DG overexpression. We are currently analyzing how these events affect the activity of MAP kinase cascade. Taken together, these findings support the hypothesis that DG might interfere with the Ras/MAPK signaling cascade by sequestering important players of the pathway and might help to understand the significance of the loss of DG expression in cancer cells.

ST 49. Breast Cancer Cells Expressing the K303R Estrogen Receptor (ER)-α Mutant are Resistant to an Aromatase Inhibitor.

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Despite the success of hormonal therapy with aromatase inhibitors (AIs), many patients exhibit de novo resistance. We identified a lysine to arginine transition at residue 303 (K303R) of ERα in invasive breast cancers, which confers estrogen hypersensitivity. We hypothesize that the mutant provides a continuous mitogenic stimulus to the breast even during menopause, thus affording a proliferative advantage during treatment with AIs. To test whether the mutation is resistant to AIs, we utilized MCF-7 parental and MCF-7-K303R-overexpressing cells stably transfected with an aromatase expression vector. Cells were stimulated with the aromatase substrate, androstenedione (AD), with or without the AI anastrazole (AN). We found that AN decreased AD-stimulated growth of WT cells, using MTT and soft agar assays, but AN had no effect on K303R cells. Aromatase activity was reduced by AN, thus resistance cannot be explained by an insensitivity of the aromatase

enzyme to AN. We found constitutively increased levels of pAKT in mutant cells, suggesting Akt signaling as a molecular mechanism of resistance. Indeed, the mutant cells showed increased growth and Akt activation with IGF-1. Blockade of IGF-1R/PI3K/AKT signaling reversed AI-resistance. Our results suggest that the K303R mutation confers resistance to an AI through enhanced cross-talk with IGF1-R/PI3K/Akt signaling.

ST 50. Tamoxifen and Epidermal Growth Factor induce the Aggregation of MCF-7 Breast Cancer Cells Up-Regulating E-Cadherin Expression

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Although many breast cancer patients benefit from tamoxifen therapy in the adjuvant and metastatic settings, resistance is still an important concern. To provide further insights into the molecular mechanisms involved in the tamoxifen resistance, we evaluated the ability of OHT as well as EGF to regulate homotypic adhesion in MCF7 three-dimensional cultures, reproducing somehow tumor growth *in vivo*. Both OHT and EGF, through the increase of E-cadherin expression, induce an enhanced cell aggregation. The up-regulation of E-cadherin induced by OHT and EGF appears to involve a cross-talk between ERα and EGFR since this effect was no longer evident knocking down each receptor. Thus, both ERα and EGFR signaling represent a potential target in the pharmacological management of tamoxifen-resistant breast cancer.

ST 51. Identification and Analysis of Mouse DAG-1 Gene-Promoter and Evaluation of its Activity During Muscle Differentiation

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Dystroglycan (DG) is a widely expressed adhesion complex that anchors the cells to basement membrane and is involved in embryonic development and differentiation. DG expression is frequently reduced in human dystrophies and malignancies and its molecular functions are not completely understood. Several posttranslational mechanisms have been identified and regulate DG expression and/or function. However, since little is known about how expression of the corresponding DAG-1 gene is regulated, we aimed at defining its basal transcriptional regulation. Computational analysis of the 5'-flanking region of mouse DAG-1 gene revealed a TATA box-lacking promoter including a GC-rich region. Transfection studies with serially deleted promoter constructs identified a minimal promoter region containing two Sp1 sites and an E-box. Sp1 binding was confirmed by chromatin immunoprecipitation assay and Sp1-downregulation reduced DG expression in muscle cells. Furthermore, DG promoter methylation was reduced while DG expression increased during differentiation of C2C12 myoblast cells in myotubes. We also demonstrated that treatment with 5-Azacytidine and/or the histone deacetylase inhibitor TSA increased DG mRNA expression levels in myoblasts. In conclusion, we characterized the activity of the mouse DAG-1 gene which will be relevant for a better understanding of the physiopathology of the DG complex.

ST 52. Regulation of Estrogen Receptor (ER) Mediated Transcription By Akt2/FoxO3a Signaling in MCF-7 Breast Cancer Cells

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ERα and the insulin-like growth factor I receptor (IGF-IR) are engaged in a functional cross-talk in breast cancer. Our results indicate that in growth factors deprived ER-positive MCF-7 cells, Akt2 modulates ER function through a dual mechanism: 1) a downregulatory effect on ER expression, and 2) the inhibition of its transactivation. This latter event appears to occur through a direct interaction of ER with one of Akt downstream targets, the forkhead transcription factor FoxO3a at the nuclear level, where it binds to forkhead responsive sequences on ER target gene promoters. As expected, FoxO3a silencing reverses the scenario, addressing how this transcription factor represses ER function. Moreover, E2 upregulation of FoxO3a levels, somehow could represent the basis of a regulatory mechanism on ER homeostasis. In light of these findings FoxO3a has to be implemented in the cross-talk existing between growth factors and ER signaling, and it may be considered a pursuable target in ER-positive breast cancer therapy.

ST 53. Update on Human Sperm Anatomy: New Perspectives in the Treatment of Human Male Fertility.

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Mammalian ejaculated spermatozoa have been studied always with great interest because their function in fertilization and their peculiarities as unique cellular type. Significant strides have been made, but the signalling pathways that govern their physiology as well as their ultrastructural molecular set up is at the beginning of the knowledge. Recently, it has been shown that sperm expresses some nuclear receptors and their classical ligands, discovering that sperm has the ability to modulate its functionalities according to the physiological status, independently by systemic regulation. The spermatozoon leaves the testis, moves through the female genital tract in the host body of the opposite gender, so it needs to be autonomous. Sperm resembles a b-pancreatic cell as it secretes insulin, an adipocyte cell as it secretes leptin and expresses the PPARgamma, showing an autocrine regulation on glucose and lipid metabolism. Sperm contains the aromatase, the estrogen and the androgen receptors regulating through an autocrine short loop both its acquisition of fertilizing ability and survival. Intriguingly, it also possesses the VD3/VDR system that in turn is able to modulate estrogen biosynthesis, linking different steps in sperm physiology. The main line of this review is a reflection about the current researches in the human sperm molecular status that highlights a new system of the response to hormones as well as regulatory pathways controlling sperm cell fate and its basic functions.

ST 54. Genetic and Genomic Studies to Identify Type 2 Diabetes Genes

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Most people with type 2 diabetes are obese. But, most people who are obese do not develop diabetes. We have reproduced this dichotomy in mice by studying a strain that does not develop diabetes when made obese (C57BL/6) and one that is quite diabetes susceptible when obese (BTBR). We have mapped several diabetes loci in an F2 derived from these strains. In one case, we identified a gene by positional cloning; SorCS1. We have also carried out extensive microarray studies and identified genes potentially involved in beta cell function. We have found that cholecystokinin (CCK) is induced in islets of obese mice. When CCK is overexpressed in islets using adenovirus vectors, the cells are induced to proliferate. These studies are being used to identify signaling pathways leading to beta cell proliferation. We have also studied gene expression in a segregating F2 population to map gene loci responsible for gene expression (expression quantitative trait loci; eQTL). These studies identify eQTL in islets for the first time. Of special interest is a set of mRNAs that encode proteins involved in the cell cycle. Our studies identify 3 eQTL that control the expression of these genes. In a similar fashion, we have identified cell a cell cycle module in adipose tissue. Interestingly, this module maps to different QTLs than does the islet cell cycle module. Our studies use data from various sources to identify genes involved in diabetes pathogenesis. These approaches can be applied to any disease syndrome.

ST 55. Erythroid Differentiation of Erythroleukemia Cells is Promoted by the increase of Intracellular Arginine Levels Obtained Through Transporter Silencing

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It has been recently claimed that arginine influx in K562 erythroleukemia cells mainly occurs through CAT1 transporter (system y+) and is needed for the erythroid differentiation of these cells (Shima Y et al, Blood. 2006;107:1352-1356). However, no attempt has been made to verify the contribution of other arginine transporters or to directly assess the role of the intracellular arginine concentration. Here we show that in K562 cells arginine transport occurs through the activity of both systems y+ and y+L, as indicated by a marked inhibition of arginine influx by leucine (70% in the presence of sodium). Consistently, K562 cells express not only SLC7A1, for the CAT1 transporter, but also high levels of SLC7A6 mRNA, for one of the light subunits (y+LAT2) of the heterodimeric transport system y+L. As expected, SLC7A6 silencing abolishes arginine transport through system y+L and doubles the intracellular arginine content, pointing to system y+L activity as an efflux route for arginine. RT-PCR analysis of the expression of genes positively (g-globin) or negatively (the transcription factor PU.1) involved in erythroid differentiation shows that SLC7A6-silencing induces a 5-fold increase in g-globin expression and a significant decrease in PU.1 expression. These results indicate that erythroid

differentiation of K562 cells is favoured by SLC7A6-silencing through changes in the intracellular concentration of arginine.

ST 56. Monoclonal Antibodies Against Ron and HGFI/MSP for Therapeutic Use

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Ron, a tyrosine-kinase receptor belonging to the same family as Met, and its ligand HGFI/MSP are essential for a proper development and for a correct immune response. However, when their activity is up-regulated, they are involved in tumor progression and metastasis. As a result of their implication in several human cancers Ron and MSP are potential targets for therapeutic intervention with monoclonal antibodies, one of the most promising tool developed in the last decade for targeting molecules known to play a role in cancer development. In this project several mAbs have been obtained against both molecules with the hybridoma technology and phage-display screening, and some of them displaying antagonistic activity will be further characterized for their potential therapeutic use.

ST 57. Caveolin-1 as a Target of Therapeutic Activity of Retinoid Derivatives in Osteosarcoma and Glioblastoma in Vitro

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Caveolin-1 (cav-1) is an essential structural constituent of caveolae implicated in mitogenic signalling, oncogenesis, angiogenesis, neurodegenerative diseases and senescence.It acts as an oncosuppressor in some tumors in vivo and as a promoter in several others,where it is associated with high risk of metastasis, apoptosis suppression and acquisition of MDR to chemotherapy. Here we show for the first time a therapeutic activity by Fenretinide, a synthetic derivative of retinoic acid, in MG-63 and HOS osteosarcoma and A-172,LI,CRS-A2 glioblastoma cells, at doses identical to or lower than those detectable in patient's plasma during chemopreventive clinical trials. In each osteosarcoma cell line and in the CRS-A2 glioblastoma cells apoptosis was evident: mediated by pRB dephosphorylation and p53 independent in osteosarcoma, and mediated by caspase-3 in glioblastoma. Cav-1 is expressed either in osteosarcoma and in glioblastoma cell lines and is a molecular target of Fenretinide, which down-regulates its expression in these tumors. As the downstream target of cav-1 downregulation, first results seem to exclude the ERK/pERK MAP kinases pathway. Cav-1 mRNA expression, evaluated by RT-PCR, is not affected by the treatment,indicating an involvement of cav-1 at the protein level. In each cell line cav-1 expression does correlate with a negative ER-α phenotype,indicating a functional relationship between Cav-1 expression and the loss of ER in the cytosol. The capability of fenretinide to target and down-regulate caveolin-1 may have a key role to decrease tumour aggressiveness and to re-establish chemosensitivity in these tumours.

Grants from MIUR, Carisbo Foundation, Pallotti's Legacy for Cancer Research and University of Bologna.

ST 58. The Copper Complex(II) A0 induces Non-Apoptotic Cell Death in Human Cancer Cells Triggering Endoplasmic Reticulum Stress and Unfolded Protein Response

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We have previously demonstrated in HT1080 fibrosarcoma cells that A0, a thioxotriazole copper(II) complex, was able to induce copper overload and a newly described non-apoptotic type of cell death, named paraptosis, hallmarked by endoplasmic reticulum (ER) vacuolization. Cytotoxicity studies in 23 human cancer cell lines and in two pairs of cisplatin-sensitive and resistant models demonstrated that the sensitivity to A0 was always accompanied by paraptotic cell death and was not correlated with cisplatin sensitivity, strongly suggesting different mechanisms of action for the two drugs. Consistently, gene expression profiling of HT1080 cells showed that while cisplatin increased the expression of typical apoptosis-related p53 target genes, A0 induced genes involved in the Unfolded Protein Response (UPR) and in the endoplasmic reticulum stress (ER stress). Microarray results validation by qRT PCR and western blot analysis confirmed that A0, but not cisplatin, activated two pathways of the UPR: i) IRE1 mRNA was up-regulated, resulting in the increased abundance of the spliced form of XBP1 mRNA that codes for the active transcription factor and ii) the translation initiator complex subunit eIF2α was rapidly phosphorylated, with the consequent attenuation of protein synthesis and the concomitant preferential translation of the pro-death ER stress responsive proteins ATF4, CHOP and GADD34. Thus, A0 triggers UPR and ER stress in sensitive cancer cells and leads to their death through a paraptotic process, thus providing a device to overcome apoptosis resistance.

ST 59. Cannabinoid Receptor Activation Commits Colon Cancer Cells to Apoptosis Via Tumor Necrosis Factor A-Induced Ceramide Synthesis

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Recently, CB1 and CB2 endocannabinoid receptor agonists have been shown to exert antitumor activity and, therefore, they are now studied as potential anticancer agents. To clarify mechanism(s) of this phenomenon, we investigated the expression of CB1 and CB2 receptors in colorectal cancer biopsies and cell lines DLD-1 and HT29, and the effects of their agonists - ACEA and the 3g, respectively - on cancer cell apoptosis and ceramide production. CB1 receptor was mainly expressed in normal colonic epithelium whereas CB2 receptor was more expressed in cancer. Activation of CB1 and, still more, of CB2 receptor induced apoptosis and increased ceramide levels in both cell lines. These effects were prevented by ceramide synthesis inhibition as well as by small interfering RNA-induced TNF-α gene knockdown. The CB2 agonist 3g also reduced growth of DLD-1 colorectal cancer cells transplanted in nude mice. Our data indicate that TNF-α mediates signal transduction between cannabinoid receptor activation and ceramide production and that either CB1 or CB2 receptor activation induces apoptosis via ceramide synthesis in colorectal cancer cells.

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ST 60. Newly Synthesized Polyamine Derivatives as Potential Anticancer Agents

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Natural polyamines are nitrogen-bearing aliphatic chains that play an essential role in cell growth and differentiation. Polyamines analogues and derivatives can suppress proliferation of cancer cells by inhibition of the biosynthesis of natural polyamines and can exert cytotoxic activity due to their DNA-binding properties. In the present study, a bis(benzyl)polyamine analogue (MDL 27695), known to exert antiproliferative activity, has been used as a template where to inserte a well-known DNA-intercalator group (aromatic core) to combine the ability to use the polyamine uptake system with the property to intercale and bind tightly the double-stranded DNA. For this purpose symmetric or non-symmetric polyamine derivatives were synthesized, comprising compounds which differ for the number of C atom (n=2-10) in the lateral chain to verify the best lenght of the amino-alkyl chain. All derivatives were tested for antiproliferative activity (MTTassay) in human breast cancer (SKBR-3) and leukemia (CEM) in vitro in a 0.1-10 μM range of concentrations up 72 h of treatment. The symmetric derivatives are more effective than the corresponding non-symmetric ones in each cell line and cause a significant dose- and time-dependent growth inhibition. The symmetric derivative with n=3 is the most potent one, with an IC50 (72h) of 0.35 and 0.17 μM in leukemic and breast cancer cells respectively, significantly more active than known polyamine DNA-Intercalator conjugates against human leukemia or than oxa-polyamines derivatives against breast cancer.

Grants from MUR, Pallotti's Legacy for Cancer Research, University of Bologna.

ST 61. Bcl-2 Over-Expression in Human T-Cell Acute Lymphocytic Leukemias is Consequent to mRNA Stabilization by Increased Binding to Zeta-Crystallin, a New Bcl-2 ARE-Binding Protein

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We previously revealed a mechanism of post-transcriptional control of bcl-2 expression based on interactions between an AU-rich element (ARE) of its 3' UTR and AU-binding proteins (AUBPs) finely modulating bcl-2 mRNA decay. In acute lymphocytic leukemias (ALLs), bcl-2 is very often over-expressed in the absence of evident gene rearrangements accounting for enhanced bcl-2 transcription, which suggests that deregulated post-transcriptional control could be involved. Here, using three bcl-2 over-expressing ALL T-cell lines, we first identified zeta-crystallin as a new bcl-2 AUBP and demonstrated that bcl-2 over-expression resulted from its three-fold increased binding to mRNA of ALL cell lines than of PHA activated T-lymphocytes. Two bits of symmetric evidence demonstrated that bcl-2 and ζ-crystallin expression are causally interrelated. Bcl-2 mRNA stability was significantly

decreased following silencing of zeta-crystallin with siRNA, while it increased following cell transfection with recombinant zeta-crystallin. The relevance of this pathogenetic mechanism in human pediatric T-cell ALLs has been confirmed with highly significant data.
Research supported by MUR, AIRC and ECRF.

TUMOR IMMUNITY AND MICROENVIRONMENT

TIM 01. Oncoantigens as Targets for Preventive Anti-Tumor Vaccination
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Neoplastic transformation is a multistage process. Identification of genes overexpressed at a specific stage provides an unprecedented opportunity to address the immune system against antigens with a driving role in tumor progression (oncoantigens). ERBB2 is a prototype of deregulated oncogenic protein kinase membrane receptors. Mice transgenic for rat ERBB2 (BALB-neuT mice) were used to identify a set of oncoantigens expressed at defined stages by breast carcinomas to be used as vaccination targets. We integrated the transcription data generated by comparing preneoplastic lesions and neoplasia in BALB-neuT mice with a meta-analysis on transcription profiles from normal and breast tumor human specimens. Forty-six oncoantigens identified and prioritized according to their cellular location were chosen for preclinical investigation.

TIM 02. NGAL is an NF-ĸB Dependent Survival Factor in Thyroid Cancer
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Inflammation and cancer are tightly linked and NF-κB plays an important role in both phenomena. We have recently shown that NF-κB is constitutively activated in human anaplastic thyroid carcinomas (ATC). The proteome analysis of conditioned medium of FRO cells revealed the high expression of NGAL (Neutrophil Gelatinase Associated Lipocalin), a protein up-regulated in acute inflammation and expressed in various neoplasms. We found that NGAL was strongly expressed in specimens from human thyroid carcinomas, especially of anaplastic type. Knocking down the expression of NGAL in FRO cells determines a decrease of their oncogenic potential and an increased sensitivity to serum deprivation induced apoptosis. Our data suggest that NGAL is an NF-κB dependent survival factor in thyroid cancer.

TIM 03. Differential Recognition of Human Melanoma Metastatic Cells by Natural Killer (NK) Lymphocytes.
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In spite of the current interest in the involvement of immunological events in the control of melanoma progression, whether NK cells play a role in the spontaneous melanoma metastatic progression and whether this role is influenced by the metastatic route remains to be determined. Melanoma cell lines were established from lymph node, ascites, skin, liver and pleura metastases explanted from 18 patients. Lymph node metastatic cell lines were more susceptible to NK cell recognition than metastatic cell lines derived from other sites. Natural cytotoxicity receptor (NCR) NKp44 and NKp46 ligands were detected only on the lymph node metastatic cell lines. Adoptively transferred NK cells to selectively prevent the death of NOD-SCID mice engrafted with lymph node metastatic cell lines.

TIM 04. Immunopathological Alterations of the Prostate-Associated Lymphoid Tissue (PALT) by Androgen Ablation in Patients with Prostate Cancer
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We reported that human prostate is endowed with a lymphoid tissue (PALT) which allow cellular and humoral immune responses. PALT basically consists of intraepithelial leukocytes and B cell follicles developing GCs. Thus, we have asked whether PALT is altered in patients with prostate adenocarcinoma with or without androgen ablation. We addressed this issue by means of histological, immunohistochemical, and confocal analysis of normal and neoplastic prostate from treated and untreated patients.Prostate adenocarcinoma was lacking in lymphoid follicles, which were still detectable in the remaining non neoplastic tissue as in the normal prostate. CD3+T lymphocytes, mostly CD8+CD27+CD28-, rarely penetrated neoplastic glands and were almost halved in adenocarcinoma from untreated patients while scarcely reduced in adenocarcinoma from treated patients where they may express CD69 but rarely produce granzyme and perforin. The reduced number of B and T lymphocytes was associated with a reduced Bcl-2 expression and increased apoptosis. CD25+Foxp3+Treg cells, scarcely represented in the normal prostate, increased in adenocarcinoma while strongly increased following androgen ablation. In conclusion, tumor onset induces local immunosuppression through significant alterations in the PALT. Hormonal therapy rescue T lymphocyte from depletion observed in cancer while is unable to fully restore their cytotoxic potential and, importantly, promotes intratumoral Treg infiltration. The underlying molecular mechanisms are currently under investigations in our laboratory.

TIM 05. Immunogenic Cell Death Induced by Idiotype-Specific Peptides Treatment of B-Cell Llymphoma.
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Recent studies demonstrated the immunogenic properties of tumor cell death and underline the importance of anticancer therapies that allow the immune system to contribute to the eradication of cancer cells. We have identified Id-specific peptides with anti-tumor activity against a murine B-lymphoma cell line both *in vitro* and *in vivo*. Results showed that the Id-peptide treatment was associated with an increased number of tumor infiltrating macrophages and an enforced antigen-presenting ability of tumor B cells; a reduced expression of immunosuppressive cytokines and Treg cells; and an increased number of activated CD8+ cells with a specific cytolytic activity. These results demonstrated that Id-peptide treatment was associated with a reduced immunosuppression of tumor microenvironment and with a CD8+ antitumor activity.

TIM 06. 50 Hz Extremely Low Frequency Electromagnetic Fields Enhance Sodium Butyrate-Induced Differentiation of HT29 Human Cancer Cell Line
C. Graziani[1], F. Rafanelli[1], M. Maltese[1], A. Galiano[1], B. De Paola[1], A. Boninsegna[1], M. Di Salvatore[1], A. Cittadini[1], A. Sgambato[1]
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Sodium butyrate is a sodium salt of a short-chain fatty acid which can modulate gene expression by inducing several changes within the nucleus such as histone hyperacetylation and DNA methylation. Sodium butyrate has been previously reported to induce differentiation of the HT29 human colon cancer cells. In this study we analyzed the effects of 50 Hz extremely low frequency electromagnetic fields (ELF) on the sodium butyrate-induced differentiation of HT29 cells. We found that ELF were able to potentiate the growth inhibitory and differentiating effects of sodium butyrate, as assessed evaluating the expression of differentiation markers such as alkaline phosphatase. ELF treatment also affected the distribution of cells in the cell cycle with an increase of cells in both G0/G1 and G2/M phase. Moreover, changes observed in the expression levels of cell cycle-related proteins such as cyclin D1, cyclin E, p21, p27 and pRb all occurred at earlier time points in cells exposed simultaneously to sodium butyrate and ELF compared to cultures exposed to sodium butyrate alone. In conclusion our findings demonstrate that ELF can exert a positive effect on the differentiation process of human colon cells and warrant further studies for a better evaluation of their in vivo relevance in view of the wide exposure to electromagnetic fields in the daily life.

TIM 07. Human Breast Cancer-Associated Fibroblasts Show Caveolin-1 Down-Regulation and RB Tumor Suppressor Functional inactivation
M. P.Lisanti[1], F. Sotgia[1], G. Bonuccelli[1], I. Mercier[1]
[1]Cancer Biology, Kimmel Cancer Center / Thomas Jefferson University, Philadelphia, PA, United States of America.
It is becoming increasingly apparent that the tumor micro-environment plays a critical role in human breast cancer onset and progression. Therefore, we isolated cancer-associated fibroblasts (CAFs) from human breast cancer lesions and studied their properties, as compared with normal mammary fibroblasts (NFs) isolated from the same patient. Here, we demonstrate that 8 out of 11 CAFs show dramatic down-regulation of caveolin-1 (Cav-1) protein expression; Cav-1 is a well-established marker that is normally decreased during the oncogenic transformation of fibroblasts. Next, we performed gene expression profiling studies (DNA mircoarray) and established a CAF gene expression signature. Interestingly, the expression signature associated with CAFs encompasses a large number of genes that are regulated via the RB-pathway. The CAF gene signature is also predictive of poor clinical outcome in breast cancer patients that were treated with tamoxifen mono-therapy, indicating that CAFs may be useful for predicting the response to hormonal therapy. Finally, we show that replacement of Cav-1 expression in CAFs (using a cell-permeable peptide approach) is sufficient to revert their hyper-proliferative phenotype and prevent RB hyper-phosphorylation. Taken together, these studies highlight the critical role of

Cav-1 down-regulation in maintaining the abnormal phenotype of human breast cancer-associated fibroblasts.

TIM 08. The Bisphosphonate Zoledronic Acid Affects the Angiogenic Phenotype of Bone Marrow Endothelial Cells in Multiple Myeloma Patients

M. Coluccia[1], A. Boccarelli[1], R. Sasanelli[1], D. Giordano[1], P. De Rinaldis[1], C. Scavelli[1], G. Di Pietro[1], A. Vacca[1]
[1]University of Bari, Bari, Italy.

Angiogenesis is a constant hallmark of bone marrow microenvironment in multiple myeloma (MM). Plasma cells are primary inducers because they secrete major angiogenic factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and hepatocyte growth factor (HGF) (Vacca A. et al. Leukemia 2006;3:193-9). Stromal cells and endothelial cells behave as secondary inducers following activation by plasma cells, being a huge source of these growth factors too (Ribatti D. et al. Oncogene 2006;25:4257-66). Zoledronic acid (ZA) is a bisphosphonate used for MM bone disease. ZA has also a direct cytotoxic activity on tumor cells and suppresses angiogenesis (Wood J. et al. J Pharmacol Exp Ther 2002;302:1055-61), but the associated molecular events have not been fully characterized yet. We show that ZA markedly inhibits *in vitro* proliferation, chemotaxis and capillarogenesis of bone marrow endothelial cells of MM patients. ZA also induces a sizeable reduction of angiogenesis in the *in vivo* chorioallantoic membrane assay. These effects are partly sustained by gene and protein inhibition of VEGF and VEGF receptor-2 in an autocrine loop. Mevastatin, a specific inhibitor of the mevalonate pathway, reverts the ZA antiangiogenic effect, indicating that the drug halts this pathway. On cDNA microarray profiling, ZA exerts a wide antiangiogenic activity through downregulation of multiple genes involved in all phases of the angiogenic cascade. Our results provide evidence of a direct antiangiogenic activity of ZA; we suggest that ZA activity in MM is also sustained by antiangiogenesis, which would partly account for its therapeutic efficacy.

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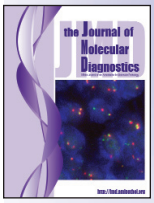

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■ **Participants -** This program is specifically developed for trainees, clinicians and researchers investigating the mechanisms of disease who wish to advance their current knowledge of the cellular and molecular biology of disease.

■ **Examinations -** Each monthly issue of *AJP* will include an Examination comprised of up to 5 questions based on articles appearing in that particular issue. To receive credit for this journal-based CME activity, participants must answer questions based on selected articles in Volume 172 and 173 of *AJP* (calendar year 2008) and achieve a cumulative score of at least 75% (correct answers to at least 38 of the 50 questions in the annual program) in addition to completing an evaluation form.

These activities have been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the Federation of American Societies for Experimental Biology (FASEB) and the American Society for Investigative Pathology (ASIP). FASEB is accredited by the ACCME to provide continuing medical education for physicians. **FASEB designates this educational activity for a maximum of 50 AMA PRA Category 1 Credit(s)™. Physicians should only claim credit commensurate with the extent of their participation in the activity.**

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Keynote Address: New insights into the molecular biology of interstitial lung disease, Robert Homer

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- **The new multidisciplinary classification of lung adenocarcinoma,** William Travis
- **Update on evolving concepts of lymphoproliferative disorders of the lung,** Michael N. Koss
- **Pathology/Radiology correlation of neoplastic and non-neoplastic lung diseases, Part II,** Teri Franks, Jeff Galvin

Update in Pulmonary Neoplasia II
Chairs: Alberto Marchevsky and Osamu Matsubara
- **Classification of neuroendocrine carcinomas,** Douglas Flieder
- **Debate: Consensus classifications revisited: pros and cons of the WHO classification for neuroendocrine tumors,** Pro: William D. Travis; Con: Mary Beth Beasley
- **Panel Discussion: Pulmonary neoplasia,** William Travis, Mary Beth Beasley, Douglas Flieder, Michael Koss, Teri Franks, Masayuki Noguchi

Update on Pulmonary Neoplasia III
Chairs: Tom Sporn and Toshiaki Kawai
- **Molecular targeted therapy of lung cancer and the role of the pathologist**, Elisabeth Brambilla
- **Molecular pathologic diagnosis of lung tumors**, Sanja Dacic

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Chairs: Armando Fraire and Junya Fukuoka
- **New insights in granulomatous lung disease**, Henry Tazelaar
- **Pulmonary vasculitis**, Eugene Mark
- **CT pathology correlation in diffuse lung disease**, Kevin Leslie

Brief Case Presentations I
Chair: Andras Khoor

PPS Dinner Presentation: History of Pulmonary Pathology, David Dail

Update on Non-Neoplastic Lung Disease II
Chairs: Megan Dishop and Aliya Husain
- **Update on idiopathic interstitial pneumonias**, Tom Colby
- **Evolving concepts of small airways disease**, Jeff Myers

Update on Non-Neoplastic Lung Disease III
Chairs: Belinda Clarke and Joanne Yi
- **Respiratory bronchiolitis, airway centered interstitial fibrosis and fibrotic NSIP**, Samuel Yousem
- **New and interesting non-neoplastic pediatric lung diseases**, Fred Askin

Brief Case Presentations II
Chair: Kelly Butnor

Update on Pleural Neoplasia
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- **Update on the diagnosis of mesothelioma - the International Mesothelioma Panel Project,** Francoise Galateau-Salle
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- **Pleural neoplasia: entities other than diffuse malignant mesothelioma,** Tim Allen
- **Molecular pathology and molecular targets suitable for mesothelioma therapy**, Helmut Popper

Update on Asbestos, Asbestosis and Associated Malignancies
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- **Update on the PPS/CAP *Guidelines for the Diagnosis of Asbestosis***, Victor Roggli
- **Environmental exposures, heredity and mesothelioma in Turkey,** Handan Zeren
- **Debate: Asbestos exposure and lung cancer**
  Moderators: Phil Cagle and Koichi Honma
  Pros: Richard Attanoos, Allen Gibbs
  Cons: Sam Hammar, Victor Roggli
- **Panel discussion of mesothelioma and occupational lung disease,** Francoise Galateau-Salle, Andrew Churg, Helmut Popper, Victor Roggli, Douglas Henderson

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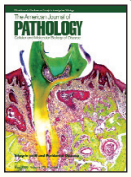

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