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S. Margherita di Pula, June 12-14, 2013

Centro Congressi Hotel Flamingo

President

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Opening Lecture

THE MORPHOFUNCTIONAL ASPECTS OF TUMOR MICROCIRCULATION

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The human vascular system is composed of a network of vessels lined by endothelial cells. In normal condition, the gross vascular anatomy of the vascular system is characterized by a reproducible branching pattern. Control of branch patterning includes both attractive and repulsive guidance signals and is regulated by both positive and negative regulators.

Vascular architecture in tumor is different from normal tissues and is determined by micro heterogeneities in the cellular interactions with the extracellular matrix. In tumors, the organ- and tissue-specific vascular architecture is not retained. The architecture of the tumor seems to be primarily determined by the tumor cells themselves. Tumor blood vessels do not display the recognizable features of arterioles, capillaries or venules, are irregular in size, shape, and branching pattern, form arteriovenous shunts. Moreover, they have uneven diameters, chaotic flow patterns, and increased permeability to macromolecules. Tumor vessel density is very heterogeneous: the highest values are found in the invading tumor edge, where the density is four to ten times greater than inside the tumor and the arrangement of vessels in the centre of a tumor is much more chaotic than at its edges. The molecular mechanisms causing abnormal vascular architecture are not completely understood, but the imbalance of pro- and anti-angiogenic factors is considered to be a key contributor. Moreover, mechanical stress generated by proliferating tumor cells also compress vessels in tumors, with some vessels being oversized, other being more immature smaller vessels. These structural abnormalities result elevated interstitial pressure in many solid tumors, responsible, in turn, of impaired delivery of anticancer drugs as well as oxygen to the tumor site.

Simposium I
Clinical molecular markers predictive of therapeutic response
In memoriam Prof. Giovanni Mazzotti

NEXT-GENERATION HISTOPATHOLOGY OF CANCER

A. Scarpa

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An increasing number of cancers are being treated or in clinical trials with targeted drugs, and the number of additional targeted drugs is expected to rapidly increase. This calls for the implementation of companion diagnostic methods probing the mutational status of multiple genes on the limited cytological or bioptic material available in most cases. Companion molecular diagnostic methods have also to address the issue of cancer heterogeneity. In fact, cancers are composed by diverse clones, and a complete characterizationof a cancer must be based on the morphological and molecular description of these clones. Multiple different tumor areas may be microdissected and subjected to molecular analysis in order to obtain the molecular fingerprint of the diverse clones. Examples of "next generation histopathology of cancers" will be presented, showingthe integration of morphological, immunophenotypical and mutational analysis of multiple genes using routinely processed tissues. Morphology and immunohistochemistry provide the diagnosis and drive the choice of areas to be microdissected and used for multiplex deep sequencing. Methods of next generation sequencing may be used to simultaneously sequence dozens of genes using as little as 10 ng of DNA from paraffin embedded tissues. Such geographical mutational analysis: i) presents as a potent diagnostic complement to histopathological and immunophenotypic diagnosis, able to trace the clonal evolution of the neoplasm and thus permitting the description of cancer heterogeneity in a diagnostic report; and ii) identifies multiple potential therapeutical targets, where agents currently in clinical trials for different tumor types could be of use. Any research effort to discover and validate novel cancer markers should be also based on the study of well characterized cancer tissues, where the descritpion of cancer heterogeneity is taken into account.

SIGNALLING MOLECULAR MARKERS IN MYELODYSPLASIAS

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Lipid signalling in disease is an important field of investigation and stems from the pioneering work from our and Irvine's laboratories at the end of the eighties. Inositides are key cellular second messengers with well established roles in signal transduction pathways. A distinct nuclear inositide signalling metabolism has been identified, thus defining a new role for nuclear inositides, which are now considered essential co-factors for several nuclear processes, including DNA repair, transcription regulation, and RNA dynamics. Imbalances of the major lipid signalling pathways may contribute to disease progression in several disorders, such as chronic inflammation, cancer, metabolic, and degenerative syndromes. Inositide signalling cascades are therefore essential components of the extremely complicated, multistep process that allows one extracellular signal to be transduced inside the cell, to the nucleus. Moreover, these pathways are complex and redundant, and many of the signalling molecules, their modifying enzymes and downstream targets are common to multiple pathways. As a consequence, many signalling pathways can be deregulated in several pathological conditions, as well as in cancer. That is why several signalling lipid-generating enzymes have been and are still being targeted pharmacologically, alone or in combination, to alleviate the symptoms, or even progression of the different diseases. Myelodysplastic syndromes (MDS), clonal hematopoietic stem-cell disorders mainly affecting older adult patients, show ineffective haematopoiesis in one or more of the lineages of the bone marrow. Most MDS are characterized by anaemia, and a number of cases progresses to acute myeloid leukaemia (AML). Indeed, the molecular mechanisms underlying the MDS evolution to AML are still unclear, even though the nuclear signalling elicited by PI-PLCβ1 has been demonstrated to play an important role in the control of the balance between cell cycle progression and apoptosis in MDS cells. Recently it has been shown that monoallelic deletion of the PI-PLCβ1 gene is predictive for evolution towards AML and that the methylation state of the promoter is predictive for the success of the demethylating therapy. We shall discuss both the role of epigenetic therapy on PI-PLCβ1 promoter and the changes in PI-PLCβ1 expression in MDS patients.

Symposium II: Mechanisms of signal transduction

THE MET ONCOGENE: A NEW TARGET FOR CANCER THERAPY

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Tumor progression requires the accumulation of many genetic and epigenetic lesions. However, cancer cells may rely on only one of them to maintain their malignant properties (*oncogene addicition*). MET and HER family members are receptors independently involved in several human cancers; tumors addicted to either MET or EGFR have been identified. Anti-EGFR drugs are currently used in the treatment of metastatic lung and colon cancer. Several anti-MET inhibitors are in clinical trials, few of them being in phase III. However, as for other targeted therapies, only a fraction of patients responds to these drugs (*primary resistance*). Moreover, almost invariably responsive patients develop pharmacological (*acquired*) resistance and undergo relapse.

Several studies have shown the presence of a biochemical and functional interplay between MET and EGFR; notably, MET gene amplification was recently identified as a mechanism of acquired resistance to the EGFR inhibitors Erlotinib and Gefitinib in NSCLCs.

Aim of this study was to deeply analyze the reciprocal role of these receptors in mediating resistance to their specific inhibitors. We found that, in lung and gastric cancer cell lines *addicted* to MET, activation of HER family members through ligand stimulation or mutational activation contributes to overcome MET inhibition. In both cases, resistance to target therapies was mediated by the reactivation of AKT and ERK1/2 pathways. In a mirrow study, we found that MET overexpression or paracrine/autocrine MET activation, through its ligand HGF, confer resistance to the anti-EGFR antibodies Cetuximab and Panitumumab in colorectal cancer. When we moved from preclinical to clinical setting, we found that *MET* amplification is associated with primary and acquired resistance to anti-EGFR therapies in patients. Notably, in patient-derived colon xenografts, EGFR blockade could be overcome by MET kinase inhibitors. These last results highlight the role of MET in mediating primary and secondary resistance to anti-EGFR therapies in colon cancer and encourage the use of MET inhibitors in patients displaying resistance as a result of *MET* activation.

Overall, our results demonstrate that, as a consequence of the partial overlap of their downstream signaling pathways, MET and HER family members can mediate resistance to the respective targeted therapies. Our findings provide a rationale for combined inhibition of MET and HER family members in different clinical settings.

RET ONCOGENE IN HUMAN CANCER

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RET (Rearranged during Transfection) codes for the functional tyrosine kinase receptor for neurotrophic growth factors of the GDNF family. Through the autophosphorylation of tyrosine residue 1062 in the C-tail of the receptor, RET triggers activation of RAS/MAPK and PI3K/AKT signaling pathways, thereby sustaining cell growth and invasion. Genetic alterations of RET are associated to human cancer. In a fraction of papillary thyroid carcinoma (PTC), RET is targeted by chromosome translocations or inversions, causing the recombination of its tyrosine kinase encoding domain to the N-ter of heterologous proteins, thus generating chimeric RET/PTC oncogenes. Secondary to their constitutive dimerization mediated by coiled-coil motifs in the RET fusion partners, RET/PTC oncoproteins feature constitutively active kinase and transforming properties. In another type of thyroid cancer, medullary thyroid carcinoma (MTC), RET is targeted by point mutations that also lead to the constitutive activation of its kinase function. These mutations are present in virtually 100% of familial MTC (MEN2 syndromes) and half of sporadic MTC cases. There is no effective systemic therapeutic option for patients with locally invasive or metastatic MTC. Thus, we looked for ATP competitive small molecule kinase inhibitors (TKI) able to inhibit RET at clinically achievable doses. We identified vandetanib (ZD6474), a type I TKI, as a potent RET inhibitor able to obstruct kinase activity with a 100 nM IC50. Vandetanib blunted proliferation and tumorigenesis induced by oncogenic R *et al.* leles and in a phase III clinical trial (ZETA trial) reduced approximately by half the risk of MTC patients to undergo progression; based on these data, vandetanib has been approved for treatment of MTC in USA and Europe. Cancer cells may develop resistance to TKIs and second generation drugs featuring increased affinity for the target or ability to bind target in an alternative conformation may be useful to overcome these limitations. We found that a new compound, ponatinib, a type II TKI, is a low nM RET kinase inhibitor, able to kill MTC cells expressing oncogenic R *et al.* leles and shrink tumors induced by MTC cells in xenografted mice. Importantly, ponatinib is active also against RET mutants (V804) refractory to vandetanib. Very recently, oncogenic conversion of RET has been proved also in cancers other than thyroid ones, such as lung adenocarcinoma and chronic myelomonocitic leukemia (CMML). It is tempting to speculate that RET TKIs may prove useful not only in thyroid cancer patients but also in patients affected by these additional cancer types.

ENDOCYTOSIS: A ROLE IN ERBB2 TARGETED THERAPY

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The ErbB family of receptors comprises four closely related members: EGFR (ErbB1 or HER1), ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4). These receptors play important roles in cell proliferation, survival, migration and differentiation, and also in development and progression of cancer. In particular, ErbB2 is over-expressed in approximately 30% of invasive breast cancers, which often display a highly invasive and metastatic phenotype, resistance to conventional chemotherapy and hormone-therapy. The anti-proliferative effect of the ErbB2 specific humanized antibody Trastuzumab in cells over-expressing ErbB2 has lead to its effective use in current therapeutic protocols. However, notwithstanding the extensive use, the mechanisms of action of this antibody have not been defined yet and some controversial data have been reported. We suggest that some of these

controversial results can be reconciled by showing that Trastuzumab treatment induces two levels of activity, depending on the length of the treatment. Furthermore, differences in the level of ErbB1 expression might modulate the cellular response to Trastuzumab. In short term treatment, Trastuzumab induces activation and phosphorylation of ErbB2, and its heterodimerization with ErbB1. This activation of ErbB2 generates a signaling cascade that leads first to phosphorilation of ErkERK1/2 and subsequently to the dephosphorylation of AKT. Moreover, at early time points, Trastuzumab promotes the endocytosis and recycling of the receptor back to the plasma membrane. Silencing of ErbB1, by RNA interfering, leads to early degradation of the activated ErbB2, probably rerouting it from recycling endosomes to a degradative compartment.

It has been extensively shown that long term treatment with Trastuzumab leads to the block in G1 of the treated cells, and that this effect is more effective in cells expressing low levels ErbB1. In this work we provide a model of the Trastuzumab mechanism of action in ErbB2 over-expressing cells: low levels of ErbB1 lead to a more efficient down-regulation of ErbB2, determining a consistent reduction of the receptor from the PM and attenuation of its specific proliferation signals.

PARAFIBROMIN IS DOWNREGULATED IN HUMAN ADRENOCORTICAL ADENOMAS AND CARCINOMAS

A. Porzionato[1], G. Masi[1], M. Iacobone[2], V. Macchi[1], C. Stecco[1], E. Lavezzo[1], G. Viel[2], G. Favia[2], G. Palù[1], L. Barzon[1], R. De Caro[1]

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Germline inactivating mutations of the HRPT2 (CDC73) gene cause the hyperparathyroidism-jaw tumour syndrome, while somatic HRPT2 mutations and down-regulation of the encoded protein parafibromin have been reported in sporadic parathyroid, renal, gastric, and breast tumors. Following our finding that parafibromin is highly expressed in mouse and human adrenal cortex, this study aimed at investigating whether parafibromin is involved in adrenal tumorigenesis. Analysis of HRPT2 expression, mutations, and promoter methylation in normal adrenal glands (n=5), adrenocortical adenomas (n=63), adrenocortical carcinomas (n=13), and pheochromocytomas (n=10) from patients evaluated at Padova University Hospital. *In vitro* evaluation of the effect of parafibromin on proliferation and apoptosis of adrenocortical carcinoma cells was also assessed. Parafibromin was absent or poorly expressed in adrenal medulla and pheochromocytomas, highly expressed in the nucleus of most normal adrenocortical cells, and significantly downregulated in adrenocortical adenomas and carcinomas. Parafibromin overexpression in the NCI-H295R adrenocortical carcinoma cell line decreased cell viability and induced apoptosis. The mechanism of parafibromin downregulation remains unknown, since no HRPT2 mutations nor methylation of the promoter were detected in tumors with low parafibromin expression and a full-length protein was detected at western blot analysis. Downregulation of parafibromin expression in human adrenocortical tumours, and its antiproliferative and proapototic activity in adrenocortical carcinoma cells, suggest a role for parafibromin in adrenocortical tumorigenesis.

INTEGRATED TRANSCRIPTOME/MIRNOME ANALYSIS REVEALS STEP-BY-STEP DIFFERENCES IN AN ANIMAL MODEL OF LIVER CARCINOGENESIS RECAPITULATING HUMAN HEPATOCELLULAR CARCINOMA

A. Petrelli,[1] A. Perra,[2] D. Cora',[1] P. Sulas,[2] S. Menegon,[1] C. Manca,[2] C. Migliore,[1] G.M. Ledda-Columbano,[2] S. Giordano,[1] A. Columbano[2]

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Expression profiles are a powerful tool for unclosing cancer biomarkers and therapeutic targets. We performed an integrated analysis of genome-wide mRNA and microRNA (miR) expression profiles to characterize the molecular events involved in the step-by-step progression (preneoplastic nodules-adenoma-early HCC, advanced HCC) of hepatocellular carcinoma (HCC) in the rat Resistant-Hepatocyte (R-H) model. Interestingly, while clustering analysis of the transcriptome grouped together preneoplastic lesions and advanced HCC, suggesting that the majority of the genes dysregulated in HCC are already aberrantly expressed in early lesions, miRNome analysis did not co-cluster the two populations but, very interestingly, stratified the lesions according to their stage of progression to HCC. The results also unveiled specific genes/miRs, altered in the very early steps of the carcinogenic process, in the transition from adenoma to early HCC or in the progression to advanced HCC. By assessing the correlation between the expression of each miRNA and its targets, we determined that distinct pathways are aberrantly activated in different stages of the carcinogenic process. This integrated approach was also able to identify molecular events discriminating the preneoplastic lesions that will progress to HCC from those that spontaneously regress. Finally, 110 orthologous genes were almost super imposable between rat and human HCC signatures, supporting the value of the R-H model in recapitulating human liver cancer.

This systematic analysis deciphered the molecular phenotypes of the several steps involved in the onset and progression of HCC and investigated their variations at mRNA and miR levels. In view of the striking similarity between mRNA and miRs commonly dysregulated in rat and human HCC, our results provide a valuable source for future studies and highlight promising genes, miRNAs, pathways and processes which may be useful for diagnostic or therapeutic applications.

THYROID HORMONE RECEPTORS DOWNREGULATION IS AN EARLY EVENT AND IS MAINTAINED ALLACROSS THE STEP-BY-STEP PROCESS OF HEPATOCARCINOGENESIS

C. Frau, V.P. Leoni, R. Loi, S. Pinna, A. Perra, G.M.Ledda-Columbano, A. Columbano

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Thyroid hormone receptors (THRs) mediate the pleiotropic activities of the hormone Triiodothyronine (T3) in many biological functions.These receptors are transcription factors expressed as different isoforms encoded by THRα and THRβ genes. Recently,several studies showed altered expression of THRs at mRNA and protein levels and identified somatic mutations of THR genes in several human cancers. Hepatocellular carcinoma (HCC) is the third most common cause of cancer mortality. Despite progress in understanding the molecular mechanism leading to hepatocarcinogenesis, HCC prognosis remains very poor.Toinvestigate the molecular basis of hepatocarcinogenesis we employeda rat model of chemically-induced HCC (R-H model)in which it is possible to generate and analyze discrete lesions at different stages of progression. These lesions can be classified according to their positivity to two markers, glutathione S-transferase (GSTP) and Cytokeratin 19 (CK-19).In contrast with what has been described in human HCC,our studies have shown the complete absence of mutations in the coding regions of THRα and THRβ genes in rat HCCs. In these tumors, instead, we demonstrated a significant downregulation of the expression of the isoforms THRα1 and THR 1. Moreover, downregulation of THR 1 was present in very early preneoplastic lesions detected 10 weeks after initiation, suggesting that its downregulation is a very early event in the hepatocarcinogenesis process. Remarkably, THRβ1downregulation was observed in preneoplastic lesions positive for CK-19, a marker of stem/progenitor cells,characterized by a more aggressive behaviour. Interestingly, we found an inverse relationship between degree of differentiation and THRβ1 expression in 5 human hepatoma cell lines.

In order to analyze the possible mechanism responsible for the decreased expression of THRβ1,we performed epigeneticstudies. The results obtained, however, did not show a significant methylation of CpG islands in THRβ promoter. Since miRNAs are important regulators of gene expression, weare currently performing analysis aimed at assessing the role of this miRNAs in the aberrant expression of TH*R*β1. In particular, 5 identified microRNAs predicted by in silico analysis to bind to THRβ gene will be analyzed.

Symposium III
Stem cells, development and regenerative medicine

HUMAN INDUCED PLURIPOTENT STEM CELLS AS A PATIENT-SPECIFIC TOOL TO OPTIMIZE TREATMENT STRATEGIES FOR CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA

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Human induced pluripotent stem cells (hiPSCs) generated from fully differentiated somatic cells offer a unique opportunity for the study of cardiovascular biology and the development of personalized medicine approaches. The effectiveness of hiPSCs has recently been tested in several congenital cardiovascular disorders, confirming their ability to give rise to differentiated cardiomyocytes (CMs) that possess the main functional and morphological characteristics of the disease *in vivo*. The aim of our study was to create an *in vitro* model *patient-specific cell-based system* that could facilitate the screening of new therapeutic molecules for the treatment of Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT), an inherited life threatening form of arrhythmia elicited by adrenergic stimulation caused by mutations in the cardiac ryanodine receptor gene (*RyR2*).

We developed a cardiac model of CPVT through the generation of iPSC from skin fibroblasts of a CPVT patient carrying a heterozygous mutation in the cardiac ryanodine receptor gene (*RyR2)* and their subsequent differentiation into cardiomyocytes (CMs). A comprehensive electrophysiological analysis through whole-cell patch-clamp and intracellular electrical recordings of spontaneously beating cells revealed the presence of delayed afterdepolarizations (DADs) in CPVT-CMs, both in resting conditions and after -adrenergic stimulation, resembling the cardiac phenotype of the patients. Furthermore, in accordance with previous data on CPVT-*RyR2* knock-in mouse model, treatment with KN-93, an inhibitor of the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), drastically reduced the presence of DADs in CVPT-CMs, rescuing the arrhythmic phenotype induced by catecholaminergic stress.

We have developed a cell system able to reproduce the CPVT phenotype and to respond to specific chemical treatments *in vitro*. Our result also confirmed the anti-arrhythmic effect of KN-93 we previously shown in mice RyR2 knock-in models in a human specific system, strengthening the potentiality of hiPSCs in drug screening applications and development of customized therapies.

BRAIN, STEM CELLS AND MENINGES

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We have characterized adult brain and spinal cord leptomeninges and demonstrated that they contain nestin-positive cells, endowed with self-renewal and proliferative properties. We named these cells LeSCs. LeSCs can be expanded as neurospheres and induced to specifically differentiate *in vitro* into either functional neurons or mature oligodendrocytes. When injected in the hippocampus, some of the cells appear to integrate in the existing neuronal network.

LeSC were 30.6% of leptomeningeal cells at E20 and gradually decreased in adult brains. LeSCs express other NSC-related markers (vimentin, DCX, SOX2, Tuj1, GFAP) as well as the neural crest marker p75. Proliferating LeSCs were found in all developmental stages. At the level of the spinal cord, LeSCs proliferate and increase in number following traumatic injury. By an *in vivo* transduction approach using a lentiviral GFP vector to label meningeal cells, we found that 7 days post-injury GFP-labelled cells were in the fibrotic scar and in the parenchyma nearest to the lesion; of these, approx 80% were LeSCs. One month post injury, GFP-positive cells persisted in the superficial laminas of the dorsal horn and expressed neuronal-related markers (Tuj1, NeuN). Apparently, none of the meningeal migrating cells expressed markers for oligodendrocytes or astrocytes.

In conclusion, we have identified a population of leptomeningeal cells with stemness properties that participate to parenchymal reaction to injury of spinal cord. LeSCs are present in and can be extracted from adult brains and spinal cords thus opening new perspectives for regenerative medicine-based therapies of neurodegenerative diseases.

IDENTIFYING HUMAN CORNEAL EPITHELIAL STEM CELLS TO TREAT BLINDNESS

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Stem cell therapy is the main treatment modality to restore vision in patients blinded by a disease known as limbal stem cell deficiency. If corneal stem cells can be more readily identified, isolated and maintained *ex vivo*, patients could be treated with better quality grafts. With prior knowledge that the extracellular matrix prote*in vitro*nectin (VN) is present within the corneal stem cell (SC) niche and that it supports SC *in vitro*, we postulated that the VN receptor (integrin $\alpha v\beta 5$) is expressed by, and can be used to identify and isolate SC within the cornea. Cadaveric human corneas were used to isolate and expand epithelial cells from the corneal SC niche, and $\alpha v\beta 5$ expression determined by flow cytometry and immunofluorescence. Integrin expressing cells were isolated by magnetic-activated cell sorting then assessed by immunocytology, colony forming efficiency, RT-PCR and microarray analysis. Integrin $\alpha v\beta 5^+$ cells co-localized to N-cadherin$^+$/CK-15$^+$ putative corneal SC. $\alpha v\beta 5$ was restricted to 3% of the total epithelial cells, which expressed more SC markers and formed more colonies compared to $\alpha v\beta 5^-$ cells. Transcriptional profiling of $\alpha v\beta 5^{+/-}$ cells by microarray identified several highly expressed interferon-inducible genes, including the chemokine IP-10, which localized to corneal SC. IP-10 may have an immunosuppressive role in the limbus and may attract mesenchymal SC which have been identified in the cornea and are themselves immunosuppressive. $\alpha v\beta 5$ is a candidate corneal SC marker since its expression is restricted and $\alpha v\beta 5^+$ limbal epithelial cells have properties of corneal SC. Knowledge of the niche's composition and the genes expressed by its SC will facilitate isolation and maintenance of these cells for therapeutic purposes.

MIR-9 REGULATES PNS DIFFERENTIATION DURING DEVELOPMENT OF ASCIDIANS (TUNICATA)

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MicroRNAs are non-coding transcripts of approximately 21 nucleotides highly conserved in different metazoan and acting as post- transcriptional regulators of gene expression. Anti-miR Peptide Nucleic Acids (PNA) are pseudopeptide chains bearing the four nucleobases mimicking the antisense oligonucleotide base-matching properties. PNAs exhibit stronger and more selective binding affinity for complementary nucleic acid strands than natural nucleic acids. Ascidians are marine sessile, filter-feeding animals, which develop through a swimming larva that shows the basic chordate features, comprising a notochord, which runs the length of the tail, and a dorsal tubular central nervous system (CNS). Moreover, ascidian larvae possess a peripheral nervous system (PNS), mainly composed of multiple series of epidermal sensory neurons. We identified and experimental validated the spatio-temporal expression of some miRNAs, known to be expressed preferentially in the nervous system of vertebrates, in two species of ascidians, *Ciona intestinalis* and *Phallusia mammillata*. Our results show that in both species, miR-124 is expressed in all the nervous system; miR-7 is expressed in peripheral sensory cells of the larva and the juveniles; miR-184 expression is limited to the developing nervous system of the larva and extends to the endostyle (an endoderm derivative) of the adult. Mir-9 is expressed, only in *C. intestinalis,* in few cells of the CNS directly connected to the otolith, a gravity sensory organ. Inhibition of miR-9 by PNA caused a negative regulation of the differentiation of peripheral epidermal sensory neurons. These results give insights into the evolutionary history of miRNA in relation with the emergence of chordate nervous system novelties.

EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND RELATED RECEPTORS BY HEPATIC PROGENITOR CELLS IN HUMAN LIVER DISEASES

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Hepatic stem/progenitor cells (HPCs) are stem cells residing in the peripheral branches of the biliary tree; these cells are able to differentiate towards hepatocytes and cholangiocytes. HPC activation is involved in the progression of chronic parenchymal diseases (*e.g.* chronic viral hepatitis) and

chronic biliary diseases (*e.g.* Primary Biliary Cirrhosis: PBC). HPCs participate in the repair of liver damage either through the replacement of dead cells or by driving fundamental repair processes, including fibrosis and angiogenesis.[2] Few information exists regarding the expression of VEGFs by HPC in the course of liver pathologies. In this study we evaluate: (i) the presence of HPCs in PBC and HCV-related Cirrhosis (HCV-C) samples, and (ii) the expression of VEGFs and VEGF-Rs in PBC and HCV-C samples. Our results show (i) PBC samples present a more extensive expansion of HPC population in comparison with HCV-C samples; (ii) PBC samples show a more extensive angiogenesis if compared to HCV-C; (iii) PBC samples are characterized by an increased expression of VEGF-A and VEGF-C if compared to HCV-C; (iiii) the number of HPCs expressing VEGFs is correlated with the extension of ductular reaction and angiogenesis. The role of VEGFs in the expansion of HPC niche plays an important role in human liver chronic diseases.

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NEUROPEPTIDE Y INHIBITS BILIARY PROLIFERATION OF CHOLESTATIC RATS BY PARACRINE AND AUTOCRINE MECHANISMS

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The neurotransmitter neuropeptide Y (NPY) exerts its functions through six subtypes of receptors (NPYR). Biliary homeostasis is regulated by several factors by autocrine/paracrine signaling(1). NPY inhibits cholangiocarcinoma growth (2), however, no information exists regarding the autocrine/paracrine role of NPY on biliary hyperplasia during cholestasis. The aims of this study were to determine: (i) the expression of NPY and NPYR in cholangiocytes; and (ii) the paracrine/autocrine effects of NPY on cholangiocyte proliferation. Methods: Normal or BDL rats were treated with NPY, anti-NPY antibody or vehicle for 7 days. NPY and NPYR expression was assessed in liver sections and isolated cholangiocytes. NPY secretion was assessed in serum and bile from normal and BDL rats, as well as supernatants from normal and BDL cholangiocytes and normal rat cholangiocyte cell line (NRICC). We evaluated intrahepatic bile ductal mass (IBDM) in liver sections. Using NRICC, the effects of NPY or anti-NPY on cholangiocyte proliferation were determined. NPY and all NPYR expression are increased after BDL. NPY levels are lower in serum and cholangiocyte supernatant from BDL compared to normal rats. Chronic NPY treatment decreases PCNA expression and IBDM both in BDL rats and in NRICC. Administration of anti-NPY antibody increase cholangiocyte proliferation and IBDM to BDL rats and in NRCC. Conclusion: Therapies targeting NPY-mediated signaling may be a target for the treatment of cholangiopathies.

MICRORNA REGULATORY NETWORKS ARE INVOLVED IN OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS FROM BONE MARROW

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Human Mesenchymal Stem Cells (hMSCs) obtained from adult bone marrow are capable of differentiating in several cell types, including condrocytes, osteoblasts and adipocytes. This feature could be better exploited through a deeper knowledge of molecular mechanisms of differentiation, specifically toward microRNA cell fate control. hMSCs from bone marrow were isolated and analyzed by flow cytometry for the surface markers.

Cell culture at the first passage were induced to osteoblasts commitment by differentiation medium.

Cells were harvested at 7, 14, 28 days after induction and total RNA were extracted. Samples were enriched for microRNA and small RNA library has been prepared and processed on SOLiD sequencing system. The surface antigen profile matched with expected markers. Data analysis showed that clusters of microRNA were differentially expressed and several isoform were detected. Three novel microRNA candidate were inferred from alignment of the reads to the entire genome. Computational methods allowed development of microRNA network models specifically related to differentiation stages and their mRNA targets predictions.

Symposium IV
Cell stress: cell death and survival

FROM ATP TO PTP: MITOCHONDRIA AT THE INTERFACE BETWEEN CELL SURVIVAL AND DEATH

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The permeability transition denotes an increase of the mitochondrial inner membrane permeability to solutes with molecular masses up to about 1,500 Da, resulting in mitochondrial dysfunction that may lead to cell death. It is presumed to be mediated by opening of a channel, the permeability transition pore (PTP), whose molecular nature remained a mystery. By following the interactions of matrix cyclophilin D (CyPD) with mitochondrial proteins (CyPD facilitates PTP opening in mammalian mitochondria)we were able to define the molecular nature of the PTP.We show that CyPD binds the OSCP subunit of the F_0F_1 ATP synthase in the same region as the ATP synthase inhibitor benzodiazepine (Bz) 423; that Bz-423 sensitizes the PTP to Ca^{2+} like CyPD itself; and that decreasing OSCP expression by RNA interference increases the sensitivity of the PTP to Ca^{2+}. Purified dimers of the ATP synthase, which did not contain VDAC or adenine nucleotide translocator, were reconstituted into lipid bilayers. In the presence of Ca^{2+}, addition of Bz-423 triggered opening of a channel whose currents were typical of the mitochondrial megachannel, the PTP electrophysiological equivalent. Channel openings were inhibited by the ATP synthase inhibitor AMP-PNP (g-imino ATP, a non hydrolyzable ATP analog) and by Mg^{2+}/ADP. These results indicate that the PTP forms from dimers of the ATP synthase. Our findings provide a novel and stimulating working hypothesis that should help address outstanding issues including species-specific features of the PTP of rats, yeast and *Drosophila melanogaster*; and the mystery of *Artemia franciscana*, an anoxia and salt-tolerant brine shrimp whose mitochondria are refractory to PTP opening. Together our results suggest a dual function for complex V, ATP synthesis and PTP formation. The key enzyme of life appears therefore to be also the molecular switch that signals the presence of fully depolarized, dysfunctional mitochondria to promote cell death and/or mitophagy.

TARGETING PI3K/AKT/MTOR SIGNALING IN ACUTE LEUKEMIAS

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Despite considerable advances in designing new polychemotherapyprotocols, the prognosis of adult patients with acute leukemias is poor, especially in the elderly. Therefore, there is a need for novel targeted and less toxic therapies, particularly for patients who develop resistance to traditional chemotherapeutic drugs. The PI3K/Akt/mTOR signaling pathway regulates a wide range of physiological cell processes, that include differentiation, proliferation, apoptosis, autophagy, metabolism, motility, and exocytosis. However, constitutively active PI3K/Akt/mTOR signaling characterizes many types of tumors where it negatively influences response to therapeutic treatments. Hence, targeting PI3K/Akt/mTOR signaling with small molecule inhibitors may improve cancer patient outcome. The PI3K/Akt/mTOR signaling cascade is overactive in acute leukemiasof both myelogenous (AML) and lymphoid (ALL) lineage, where it correlates with enhanced drug-resistance and poor prognosis. Data emerging from preclinical settingsdocumentedthat small inhibitor molecules targeting PI3K/Akt/mTOR signaling induced cell cycle arrest, apoptosis, and decreased drug-resistance in acute leukemia cells, both *in vitro* and *in vivo*. Moreover, PI3K/Akt/mTOR inhibitors were capable of targeting leukemic stem cells, the most relevant target for leukemia eradication, whereas they tended to spare healthy hematopoietic stem cells. Several clinical trials employing inhibitors of this signaling cascade are now underway in patients with acute leukemias and some encouraging results have been reported. Thus, we propose that novel drugs targetingthe PI3K/Akt/mTOR pathway may offer a novel and less toxic treatment option for AML and ALL patients, most likely in combination with a lower dosage of traditional chemotherapeutic agents.

SKIN MELANOCYTES: A PROMISING TOOL TO INVESTIGATE THE COLLAGEN VI-RELATED MITOCHONDRIAL DYSFUNCTIONS

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Muscle biopsy represents the conventional approach for diagnosis and pharmacological survey of congenital muscular dystrophies. We recently found that normal melanocytes express several sarcolemmal components, including muscle dystrophin isoform. Moreover dystrophin-deficient melanocytes from Duchenne Muscular Dystrophy (DMD) patients displayed morphological and functional alterations of mitochondria similar to those detected in muscle cells. Ullrich congenital muscular dystrophy (UCMD), caused by mutations in collagen VI genes, is characterized by mitochondrial dysfunction due to deregulation of the permeability transition pore. We performed a comparative study on melanocytes and myoblasts obtained from one UCMD patient. UCMD skin melanocytes, as well as myoblasts, showed morphological alterations of mitochondria: increased size, reduced matrix density and disrupted cristae. Furthermore UCMD melanocytes showed a latent mitochondrial functional defect unmasked by inhibition of the ATP synthase. These results prove that melanocytes could be a promising low invasive tool to investigate mitochondrial defects and to evaluate drug efficacy in collagen VI related myopathies.

POSTER SESSION

AN IMMUNOHISTOCHEMICAL STUDY OF COLLAGEN I, IV, FIBRONECTIN AND VEGF ON HUMAN PERIODONTAL LIGAMENT AFTER APPLICATION OF ORTHODONTIC FORCES

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Orthodontic tooth movement (OTM) is the result of a biological response to interference in the equilibrium of dental complex by externally applied force; it is characterized by remodeling changes in dental and periodontal tissues. The periodontal ligament, which lies between the hard tissues of alveolar bone and root surface, serves to anchor the tooth to the alveolus and functions as a cushion between these hard tissues to migrate occlusal force during mastication. Prolonged application of external mechanical force, exceeding bio-elastic limits of tooth supporting structures, induces an increase in remodeling of PDL and alveolar bone and induces tooth movement. On this basis, we have studied the different modification of periodontal ligament (PDL) during OTM, both in pressure and tension side, evaluating the expression of different proteins as collagen I, collagen IV, fibronectin and VEGF, by immunohistochemical techniques. Our results have shown that the staining pattern level of all tested proteins depends on the time of forces application and on the pressure and tension side. In general, it is possible to observe two different phases: a response phase to the applied forces, which determines major modification in proteins staining pattern; finally, we observe a remodeling phase where the proteins staining pattern return to be similar to the protein pattern of ligament in normal condition. All these results suggest that the periodontal ligament is a tissues characterized by plasticity which easily adapt to application of external forces.

SARCOGLYCAN SUB-COMPLEX IN GINGIVAL TISSUE

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The sarcoglycan sub-complex, made up of α-, β-, γ-, δ-, ε- and ζ-sarcoglycans, is a multimember transmembrane system which provide a mechanosignaling connection from the cytoskeleton to extracellular matrix. Sarcoglycans have been found in many kind of tissues as epithelial tissue where they seem to be involved in cell-cell adhesion using their cadherin-like domain; by that, it was supported that sarcoglycans are involved in different pathological condition of epithelial tissues. So, we performed an immunofluorescence study of the sarcoglycan sub-complex in normal gingival tissues and in gingival tissue of patients affected by periodontitis and scleroderma, two different pathological condition where it is possible to observe an inflammation and alteration of the gingival epithelium. Results obtained from normal samples have shown the presence of a staining pattern for each sarcoglycan in gingival epithelium; pathological samples results, instead, have shown that the entire sarcoglycan sub-complex changes in staining pattern level in dependence of the inflammation degree. All these data suggest a key role of sarcoglycans in maintenance of epithelia architecture by their mechanosignaling functions and they also suggest an involvement of this protein system in inflammation pathways.

SARCOGLYCANS AND INTEGRINS IN MASSETER MUSCLE OF BABOONS

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Sarcoglycans and integrins are transmembrane proteins which play, in muscle tissues, signaling and mechanical functions which are important for the development and the integrity of muscle. Since it was supported the existence of a bidirectional signaling between sarcoglycans and integrins, it become important to investigate these protein systems together. In fact, our previous study on smooth and skeletal muscle have shown that sarcoglycans and integrins seem to cooperate regulating muscle metabolic feature and frequency of contraction; moreover, our results on masseter muscle of human and chimpanzee have suggested that these proteins could be also involved in regulation of the intensity of force. So, in the present study we continued the phylogenetic study investigating, by immunofluorescence and molecular techniques, sarcoglycans and integrins in masseter muscle of baboons of high and low dominance, social groups with different degree of aggressiveness. Results have shown the presence of all tested proteins in the masseter muscle of baboons of high dominance; instead, in low dominance baboons, we found positive fibers and negative fibers both for sarcoglycans and integrins in the same microscopic field. Results, showing for the first time the existence of normal muscular fibers which are negative for the entire sarcoglycan sub-complex and integrins, suggest the existence of alternative and unknown protein systems which seem to be correlated with the aggressiveness degree, intensity of force and phylogenesis.

NEUROPROTECTIVE ACTIVITY OF IBUPROFEN AND LIPOIC ACID CONJUGATE IN AN ALZHEIMER'S DISEASE RAT MODEL

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Alzheimer's disease (AD) is a frequent form of senile dementia. Neuroglobin (Ngb) has a neuroprotective role, decreases the levels of Aβ peptide and, promoting Akt phosphorylation, activates cell survival involving cyclic-nucleotide response element-binding protein (CREB). A new molecule, (IBU-LA), has been synthetized and administered to AD rat model to counteract AD progression. The aim of this study has been to investigate the IBU-LA-mediated induction of Ngbneuroprotective and anti-apoptotic activities.

Brain morphology has been analyzed through Bielschowsky staining, Aβ(1-40) and Ngb expression by immunohistochemistry; Akt, p-Akt, CREB and p-CREB expression by western blot; apoptosis through cytochrome c/Apaf 1 immunocomplex formation and TUNEL analysis.

Bielschowsky staining and Aβ(1-40) expression show few nerve connections and Aβ(1-40) expression in Aβ sample, preserved neuronal cells and Aβ(1-40) expression lowering in IBU sample, mostly in IBU-LA one. Ngb level decreases in Aβ

samples, compared to control and IBU-LA samples. p-Akt /Akt and p-CREB/CREB ratios reveal a reduction in Aβ sample, going back to basal level in control and IBU-LA samples. Cytc/Apaf 1 co-immunoprecipitate occurs and TUNEL positive nuclei percentage decreases in Aβ sample. Probe test performance shows an increased spatial reference memory in IBU-LA sample compared to Aβ sample.

These evidences reveal IBU-LA administration capability to maintain a high Ngb level allowing Ngb to realize a neuroprotective and antiapoptotic role, representing a valid tool in therapeutic strategy in AD progression.

IMMUNOHISTOCHEMICAL EXPRESSION OF ANGIOGENESIS MARKERS IN HUMAN PTERYGIUM

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Human pterygium is a common ocular surface disorder that is associated with chronic UV exposure. Histopathologically, this lesion is characterized by proliferation, inflammation, fibrosis, extracellular matrix remodelling and excessive angiogenesis, a process of new blood vessels formation from preexisting vasculature that underlies a large number of physiological processes, such as growth and differentiation, wound healing, and abnormal conditions, such as neoplasia.

Nestin is a intermediate filament (IF) protein originally described as a neuronal stem cell marker. Nestin is also expressed in newborn vascular endothelial and therefore recognized as a specific marker for newly formed blood vessels in normal and various tumor tissues.

Notch1, a type 1 transmembrane protein, is associated whit a variety of cellular events, including cell fate determination, cellular differentiation, proliferation and apoptosis. This protein has also a important role in vascular development, including proliferation and migration of endothelial cells.

The aim of this study is to evaluate, by immunohistochemistry on formalin–fixed and paraffin embedded sections, nestin and notch1 expression in the epithelial and endothelial cells of primary pterygium compared to normal conjunctival tissue, in order to determine whether these factors may participate in the development of pterygium via angiogenesis process.

The results will be discussed.

IMMUNOHISTOCHEMICAL STUDY OF SEVERAL COMPONENTS OF RENIN-ANGIOTENSIN SYSTEM IN SARDINIAN PTERYGIUM

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Pterygium is a common ocular surface disorder characterized by excessive cell proliferation, inflammation, fibrosis, angiogenesis and extracellular matrix remodelling.

Angiotensin II (Ang II) is the principal effector peptide of the renin-angiotensin system, the most powerful system for regulating blood pressure and plasma volume. It has a central role in cardiovascular homeostasis and is implicated in the development of cardiac, vascular renal pathologies, regulation of the angiogenic process and cell proliferation. Ang II mediates its effects through at least two types of receptor, AT_1 and AT_2, which are both seven-trasmembrane G proteins-coupled receptors. The AT_1 receptor accounts for the majority of the known functions of Ang II in various tissues, while little information is available regarding the physiological roles of AT_2 and its signal-transduction pathway. From recent studies, one emerging function of the AT_2 receptor concerns its pro-apoptotic role. In fact, AT_2 seems to promote apoptosis in a wide variety of cell type. However, recent reports indicate that AT_2 in cardiovascular tissues may be growth-promoting in synergic action with the AT_1 receptor. The purpose of the present study is to investigate, by immunohistochemical (IHC) analysis, the presence and the possible role of Ang II, AT_1 and AT_2 in the formation of pterygium.

NOTCH1 EXPRESSION AND ANGIOGENESIS IN CUTANEOUS MELANOMA: AN IMMUHISTOCHEMICAL STUDY

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Notch signaling is involved in cell differentiation, proliferation and apoptosis in fetal and adult tissues. Moreover, increasing evidence suggests the involvement of Notch in tumorigenesis. An up-regulation of Notch1 has been found in melanoma lesions and cell lines compared to normal melanocytes. In particular, Notch1 confers a more aggressive phenotype and a metastatic potential to melanoma cells.

A recent theory suggests that tumor initiation and growth are driven by cancer stem cells (CSCs). It seems that alterations in signaling pathways governing stem cells would allow them to resist antigrowth signals and undergo uncontrolled proliferation and tumorigenesis. Notch signaling, indeed, plays a role in the maintenance of melanoblasts (Mbs), including melanocyte stem cells (MSCs), by preventing initiation of apoptosis. Moreover, several studies have identified the importance of Notch1 signaling in tumor neovascularization.

Nestin has been widely used as a marker for stem/progenitor cells as well as being reported to be a superior angiogenic marker to evaluate neovascularity in tumor endothelial cells.

In this study, we investigate by immunohistochemistry the expression of activated Notch1 and its association with nestin expression in a series of primary melanomas and lymph node metastases to determine the prognostic impact of Notch1 signaling, through the maintenance of MSCs and the involvement in angiogenesis, in cutaneous melanoma. The results will be discussed.

EXPRESSION OF GLUCOCORTICOID RECEPTOR IN PRIMARY HUMAN BREAST CANCER

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Breast cancer remains the most commonly diagnosed malignancy among females. Several progresses were made in understanding the underlying mechanisms of cancer development and several drugs and therapy strategies were approved for the preventive approach of this disease.

Breast cancer can be classified according to estrogen (ER), progesteron (PR), and HER2 receptor expression. Recent evidence suggests that activation of the glucocorticoid receptor (GR) contributes to breast cell survival, although the incidence of GR expression in primary human breast tumours is not well established. In cancer therapy, glucocorticoids are widely used and they have cell type-specific pro-apoptotic or anti-apoptotic effects. GR is a nuclear receptor that, when activated by its specific ligand, can act as a transcription factor that binds to glucocorticoid response elements (GREs) or negative GREs. It affects inflammatory responses, differentiation, and cell proliferation. The ligand-activated GR induces G1 cell cycle arrest or apoptosis in immature thymocytes, impairs the proliferation and differentiation of neural progenitor cells in vivo and *in vitro* impairs proliferation of fibroblasts and of undifferentiated mammary epithelial cells. There is no reported study addressing GR expression in T4 breast cancer patients. Thus, the aim of the present study was to investigate, through immunohistochemistry, the expression and distribution of GR in T4 breast cancer, in order to determine its association with clinical and pathological parameters as well as with patients' outcome. The results will be discussed.

PACAP AND VIP AFFECT NESTIN EXPRESSION IN SERUM DEPRIVED GLIOMA CELLS

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Studies on the proliferative activity of pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) in cancer cells have shown that both peptides can affect cell growth depending on a model system, time of incubation or the peptides concentration used (Sokolowska and NowaK 2008). Changes in cellular microenvironment caused by trophic factor deprivation interfere with cancer progression, acting on cell-to-cell interactions between cancer cells and their ability to proliferate (Longo *et al.*, 2010). However, it is not known whether trophic factor removal may have an impact on PACAP and VIP affect malignancy of glioma cells. In the present study, using an *in vitro* model mimicking microenvironmental changes related to trophic factor deprivation, we explored the effects of both PACAP or VIP on C6 glioma cells grown either in normal growth medium (10%FBS) or in serum-free medium. Cell proliferation and expression of proteins related to cell malignancy (GFAP and nestin) were assessed by MTT assay, immunoblot and confocal microscopy analysis. Results demonstrated that serum deprivation and, to a greater extent, the treatment with PACAP or VIP decreased cellular proliferation and nestin protein expression. In conclusion, these results suggest that both peptides enhance the effects of serum deprivation on cell growth and malignancy. These data also confirm previous studies demonstrating that Nestin expression might change in cancer cells as a result of environmental signaling.

NAP MODULATES THE EXPRESSION AND DISTRIBUTION OF CLEAVED CASPASE-3 IN RETINA OF STREPTOZOTOCIN-INJECTED RATS

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NAP (davunetide), is a neuroprotective peptide derived from activity dependent neuroprotective protein with highly potent neurotrophic and neuroprotective effects in nervous system including the retinal ganglion layers (Idan-Feldman A. *et al.*, 2011; Jehle T. *et al.*, 2008). In the present study we investigated the effect of NAP on diabetes-induced retinal degeneration in streptozotocin-injected rats. A single intraocular injection of 100 µg/mL NAP or vehicle was administered 1 week after intraperitoneal injection of streptozotocin (STZ) (60 mg/kg). Retinal expression and distribution of cellular apoptosis marker, Cleaved Caspase-3, was performed 3 weeks after diabetes induction, using Western blot and confocal microscopy analysis. To test whether NAP treatment induced the activation of MAPK/ERK and/or PI-3K/Akt pathways we measured p-ERK and p-AKT proteins expression. Cleaved Caspase-3 expression level was increased in the retina of STZ-injected rats. NAP treatment counteract this effect. We observed an elevated number of cleaved Casp-3 positive cells in all retinal layers of STZ treated rats by using confocal microscopy. NAP treatment reduced its expression in the retina. The retinoprotective activity of NAP seemed to be mediated through the activation of MAPK/ERK pathway. Furthermore NAP increased the expression of p-ERK in the inner plexiform layer and in the outer plexiform layer.

NONYLPHENOL PROMOTES PROSTATE CELL PROLIFERATION THROUGH INTERACTION WITH ER

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Nonylphenol (NP) is widely used as surfactants in industrial and agricultural applications and in plastic formulations. NP belongs to Endocrine Disrupting Chemicals (EDC) with xenoestrogenic activity, called xenoestrogen, present in the environment as pollutants. Its xenoestrogenic activity was demonstrated both *in vitro* and *in vivo*. However, there are only few studies on the NP effects on prostate cell lines. Estrogens play an important role in development and growth of the prostate and may cause some pathologies, including cancer. NP, mimicking endogenous estrogen, could have a negative influence on prostate physiology. In this study we examined the effects of NP and 17β-estradiol (E2) on the proliferation of non tumorigenic prostate epithelial cell line (PNT1A) and their interaction with estrogen receptors. These effects were also studied in presence of selective estrogen receptor antagonist ICI182,780. We found that NP and E2 stimulate PNT1A proliferation in a dose-dependent manner, but the NP effect was lower than E2. Immunofluorescence and western blot analyses revealed that both NP and E2 induce cytoplasm-nucleus translocation of ERα. The nuclear localization of ERα

by E2 was already shown after 2h of treatment and only after 6h by NP. The inhibition of these effects by adding ICI182,780 was shown. Surprisingly, NP and E2 didn't affect the localization of ERβ. These results suggest that NP stimulates PNT1A proliferation probably through the interaction with ERα that in turn is involved in the activation of some prostate cell cycle key regulators.

SMALL LEUCINE RICH PROTEOGLYCANS ARE DIFFERENTLY EXPRESSED IN NORMAL AND PATHOLOGICAL ENDOMETRIUM

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During the woman's fertile period, the non pregnant uterus is subject to constant cyclic changes, involving mainly the endometrial and myometrial tissue. The cell cycle modification induced the switching from a high proliferative state to a differentiation state, typical of the secretory phase. The complex mechanisms that control the balance between proliferation, differentiation, cell death and the structural remodeling of the extracellular matrix can contribute to the benign or malignant endometrial pathological state. The small leucine-rich proteoglycans (SLRPs) are important components of cell surface and extracellular matrices. In the present paper we describe the distribution of SLRPs in the physiological endometrium and in the pathological endometrium. Through immunohistochemistry we have shown that the distribution patterns of SLRPs were completely modified in the pathological endometrium as compared to normal endometrium.

The expression of SLRPs was low/absent in all the endometrial pathologies examined as compared to normal endometrium. We observed an increase of lumican from proliferative to secretory phase of the endometrium and a decrease of fibromodulin, biglycan, and decorin. In menopause endometrial tissue the level of expression of fibromodulin, biglycan, decorin and lumican dramatically decreased.

The results reveal the prominence and importance of proteoglycans in the tissue architecture and extracellular matrix organization.

NEUROCHEMISTRY OF SCALP ARTERIES INNERVATION IN PATIENTS SUFFERING FROM CHRONIC MIGRAINE

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Several data suggest that scalp arteries and the vanilloid receptor TRPV1, calcitonin gene related peptide (CGRP) and substance P (SP) may be involved in chronic migraine (CM). We examined the aspect and density of TRPV1-, CGRP-, and SP-like immunoreactive (LI) innervation in surgical samples of human scalp arteries from treatment-resistant CM patients and control subjects. Patients gave informed consent. The length of immunoreactive nerve fibres in vessel cross sections was quantified by computerised image analysis. Density of innervation was evaluated as ratio of the total fibre length to the vessel section area and in relation to the total innervation revealed by protein gene product 9.5 (PGP9.5).

Average of innervation in CM and control samples showed statistically significant differences for TRPV1 (P=.009), CGRP (P=.001) and SP (P=.037), and not for PGP9.5 (p=.31) (t-test). Analysis of the ratio of TRPV1-, CGRP- and SP-LI fibers to PGP9.5-LI ones for each artery gave a statistically significant higher amount of TRPV1-LI fibres in CM compared to control samples (P<.02, U-test). Peptide-LI fibers, though more abundant in CM tissue, did not significantly differ between the two groups.

This is the first demonstration for a TRPV1-LI innervation of human scalp arteries. Further, an increase of TRPV1-LI innervation is shown in subjects affected by CM compared to controls. Peptides show a trend to increase. This supports the viewpoint of a role of scalp arteries and the involvement of TRPV1 and possibly CGRP and SP in CM.

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CALCITONIN GENE-RELATED PEPTIDE IN DORSAL ROOT GANGLIA AND SKIN OF A RAT MODEL OF BORTEZOMIB-INDUCED PERIPHERAL NEUROPATHY

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Bortezomib (BTZ), a selective proteasome inhibitor, is an anticancer drug to treat multiple myeloma. In clinical practice, a painful peripheral sensory neuropathy (PN), involving impairment of Aδ and C type primary afferent fibers, is the major side effect of its administration.

To explore neurochemical changes possibly involved in BTZ-induced PN, we used a well-established rat model of BTZ-induced PN to study the expression of the neuropeptide calcitonin gene-related peptide (CGRP) in L4-L5 dorsal root ganglia (DRGs) and intraepidermal innervation of hindlimb footpad skin. In the DRGs, CGRP mRNA relative levels decrease after chronic BTZ treatment. Immunohistochemistry showed that CGRP-labelled neurons were mostly small- and medium-sized and that their percent frequency increased after treatment. In agreement with the sensory peripheral symptoms of BTZ-induced PN, quantitative analysis of the CGRP- and pan-neuronal marker PGP9.5-labelled intraepidermal nerve fibers revealed that the BTZ treatment induced a statistically significative decrease of the linear density of the sensory innervation.

The results obtained show that BTZ treatment selectively affects subsets of DRG neurons likely involved in the processing of nociceptive stimuli. Decrease in linear density of intraepidermal sensory innervation further supports the concept that BTZ-induced neurochemical changes may contribute to the persistence of pain in BTZ-induced PN.

MELATONIN ROLE IN PREVENTING U937 HEMOPOIETIC CELL DEATH

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Melatonin is a powerful anti-oxidant with a fundamental role in ameliorating homeostasis in a number of specific pathologies.It acts both as a direct radical scavenger and by stimulating production/activity of intracellular anti-oxidant enzymes.

A survey of literature shows thatin leukocytes melatonin mainly exerts an anti-apoptotic role, as also demonstrated in our previous studies reporting melatonin prevention of apoptosis induced by UV-B irradiation in hemopoietic U937 cells(Luchetti *et al*., 2006).

In this work melatonin activity *vs* hydrogen peroxide, etoposide and staurosporine, apoptotic triggers able to increase radical oxygen species levels, has been evaluated. (Salucci *et al*.,2013). U937 cells were melatonin pre-treated and then exposed to chemical agents.

Potential melatonin anti-apoptotic effects were evaluated through morphological approaches, further analysed by means of Tali Image-Based Cytometer, able to monitor cell viability as well as apoptosis presence.

After all chemical treatments, typical apoptotic features appeared at morphological level. A cell death decrease was evidenced in all experimental conditions pre-treated with melatonin,in which, on the other hand, the presenceof autophagic vacuoles was also observed.

Moreover, quantitative analysesusingsupravitalpropidium iodide to evaluate cell viability,evidenced that melatonin administration significantly prevents cell death.

These preliminary results confirm melatonin ability to act as an antioxidant and anti-apoptotic molecule in U937 cellsexposed to chemical agents.Furthermore,a protective autophagy could play a rolein preventing apoptotic cell death.

PRESENCE OF COLLAGEN TYPE VI IN DENTIN

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Collagen type VI is an ubiquitous extracellular matrix (ECM) protein expressed in the stroma and it also forms a microfibrillar network associated with the basal membrane of most tissues. Collagen type VI microfibrillar network appears to anchor the basement membrane to the underlying connective tissue, interacting with several ECM constituents.[1]

To clarify the functions of collagen type VI in human dentin,[2] we performed a biochemical and immunohistochemical assay on sound dentin and primary cell cultures obtained from dental pulp of healthy donors using a polyclonal antibody anti-collagen type VI. Fluorescence, scanning and transmission Electron Microscopy (SEM and TEM) analysis were also performed on dentin specimens labeled with anti-collagen type VI antibodies to assay its distribution and three-dimensional organization in sound primary and permanent human molars.

Collagen type VI was found within the matrix produced by primary cultures of pulp fragments and further assessed by immunofluorescence on cell cultured on thick slices of permanent teeth. Moreover, the confocal analysis showed a gradient in the expression of collagen type VI from the pulp decreasing toward the outer dentin and enamel junction.

SEM and TEM confirmed the collagen type VI localization within the peritubular dentin.

Further studies should clarify the role of collagen type VI in dentin and its role in the formation of secondary and terziary dentin.

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CANONICAL TRANSIENT RECEPTOR POTENTIAL CHANNEL 3 AS A NOVEL TARGET FOR CELL-BASED THERAPY IN ENDOTHELIAL COLONY FORMING CELLS

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Endothelial colony forming cells (ECFCs) are endothelial progenitor cells capable of acquiring a mature endothelial phenotype. ECFCs are mobilized from bone-marrow to promote vascularization and represent a promising tool for cell-based therapy of ischemic diseases. We have shown that VEGF stimulates peripheral blood-derived ECFC (PB-ECFCs) proliferation and tubulogenesis by causing oscillations in intracellular Ca^{2+} concentration that are driven by the interplay between $InsP_3$-dependent Ca^{2+} release and store-operated Ca^{2+} entry (SOCE). The therapeutic potential of umbilical cord blood-derived ECFCs (UCB-ECFCs) has been shown in different studies. Furthermore VEGF-induced proliferation of UCB-ECFCs is faster than their peripheral counterpart. Unlike PB-ECFCs, UCB-ECFCs express the usual transient receptor potential channel-3 (TRPC3), that mediated diacylglycerol (DAG)-dependent Ca^{2+} entry and promotes angiogenesis in endothelial cells. This study aimed at investigating whether the higher proliferative potential of UCB-ECFCs was associated to any difference in the molecular underpinnings of their Ca^{2+} response to VEGF.

Our data demonstrated that VEGF induced asynchronous Ca^{2+} oscillations in UCB-ECFCs that are shaped by the interplay between the $InsP_3$-dependent Ca^{2+} release and SOCE. Unlike PB-ECFCs, the Ca^{2+} transients do not arise in the absence of extracellular Ca^{2+} entry and after pharmacological and genetic suppression of TRPC3.

CARDIAC FIBROBLAST-DERIVED EXTRACELLULAR MATRIX AS A MODEL FOR THE STUDIES OF CARDIAC PRIMITIVE CELLS IN NORMAL AND PATHOLOGICAL ADULT HUMAN HEART

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Cardiac tissue regeneration is guided by stem/primitive cells and their microenvironment. The aim of the study was to characterize the interactions between extracellular matrix and cardiac primitive cells (CPCs) and to evaluate the influence of cardiac ECM typical of normal (ECM-N) and pathological (ECM-P) conditions (ischemic heart disease) on biological properties of CPCs.

To this aim, ECM deposited *in vitro* by cardiac fibroblasts isolated from normal and pathological adult human hearts was used for the culture of CPCs. The receptors mediating ECM-CPC interactions and their influence on biological properties of CPCs were examined by immunofluorescence, immunoblotting and PCR-based array (integrin expression), BrdU incorporation (proliferation), TdT assay (apoptosis), scratch wound assay (speed of migration).

The ECM-N was composed mainly of fibronectin, laminin α_2 and collagen I, while ECM-P contained also laminin α_1 and tenascin X. Expression of integrin $\alpha1$, $\alpha3$, $\beta4$ in CPCs was lower, while that of $\alpha7$ was higher in the presence of ECM-P. Proliferation of pathological CPCs peaked in the presence of ECM-P, while migration was fastest on ECM-N. No statistically significant advantage of specific ECM type was evident in protection from apoptosis.

These results indicate that the activity of cardiac fibroblasts and its modification in chronic ischemic heart disease determines biological properties of CPCs. Such influence should be taken into consideration when attempting ischemic cardiac tissue stem cell-based regeneration.

SUBCELLULAR DISTRIBUTION OF MELATONIN RECEPTORS IN HUMAN PAROTID GLAND

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The purpose of this study was to reveal, in human parotid gland, the ultrastructural localization of melatonin receptors MT1 and MT2.

Fragments of parotid glands obtained from 8 patients, following informed consensus, were fixed for immunocytochemical evaluation by Transmission Electron Microscopy (TEM). After embedding in Epon Resin, ultrathin sections were cut and incubated overnight at 4°C with a rabbit polyclonal antibody specific for MT1, and MT2. Sections were then incubated for 1 hr with a secondary antiserum conjugated to 15 nm gold particles. Finally, sections were contrasted, and observed in a JEOL 100S TEM.

Reactivity for MT1, and, with less intensity, for MT2 appeared in secretory granules of parotid acinar cells and in cytoplasmic vesicles of both acinar and ductal cells. Plasma membranes were also stained, although slightly.

The peculiar intracytoplasmic distribution of these receptors suggests that they may act as an uptake system for melatonin inside the cells.

CYTOCHEMICAL METHODS FOR TRACKING NANOPARTICLES AND MONITORING DRUG DELIVERY INSIDE THE CELL

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Chitosan nanoparticles (ChiNPs) have been attracting increasing interest for their potential in biomedical applications as drug delivery systems since they are able to protect the encapsulated drugs and/or improve their efficacy by making them able to cross biological barriers and reach their intracellular targets. Detecting the intracellular location of ChiNPs and monitoring the release of the loaded molecules is crucial for designing drug delivery strategies. By using fluorescently-labelled ChiNPs and diaminobenzidine photoconversion to correlate fluorescence and transmission electron microscopy we precisely described the intracellular fate of ChiNPs in neuronal cells *in vitro*. We demonstrated that ChiNPs enter neuronal cells by endocytosis; in the cytoplasm they occur both inside membrane-bounded vesicles and free in the cytosol, and accumulate around the cell nucleus. The efficacy of ChiNPs in delivering D-Ala2-D-Leu5-enkephalin (DADLE) to the same neuronal cell line was then tested. DADLE is a hypometabolising synthetic opiod potentially useful for biomedical applications, but its short half-life makes a systemic administration inefficient. We demonstrate by immunoelectron microscopy that ChiNPs are effective opioid delivery carriers to neuronal cells, protecting incorporated molecules from enzymatic degradation and prolonging their intracellular effects.

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ULTRASTRUCTURAL AND IMMUNOCYTOCHEMICAL STUDY OF SKELETAL MUSCLES IN A MURINE MODEL OF DOWN SYNDROME

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Down syndrome (DS) is a genetically-based disease which, in humans, affects about 1 over 700 newborns and is due to the presence of all or part of an extra chromosome 21. Among their several pathological traits, DS subjects suffer from an altered motor coordination but, although these difficulties in motility represent a serious problem in daily life, scarce data exist in the literature on skeletal muscles in DS. This is likely due to the obvious difficulties in obtaining bioptic material from patients with DS; however, this limitation may be partly

overcome using suitable animal models. The Ts65Dn mouse bearing a trisomy for a segment of chromosome 16 (i.e. the homologue of human chromosome 21) is the most extensively studied murine model of DS since displays a remarkable number of phenotypic traits expressed in the human condition, including motor dysfunctions. By combining morphometry and immunocytochemistry at transmission electron microscopy, we examined the fine structure of skeletal myofibres, with particular attention to myonuclei, in adult and late adult Ts65Dn mice and their age-matched euploid controls, with the aim to evaluate the combined effect of DS and age on skeletal muscle. Our observations demonstrated in Ts65Dn mice an irregular arrangement of myofibrils and structural alterations of mitochondria, which often occurred in large clusters instead of being lined between myofibrils. In addition, myonuclei showed morphological modifications and changes in the amount of factors involved in RNA processing.

PHYSICAL EXERCISE POSITIVELY AFFECTS ACTIVATION AND DIFFERENTIATION *IN VITRO* OF SATELLITE CELLS FROM SKELETAL MUSCLES OF SARCOPENIC MICE

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Ageing is associated with a progressive loss of muscle mass, strength and function: this condition is known as sarcopenia and represents an important risk factor for physical disability in elderly. The mechanisms leading to sarcopenia are still largely unknown and no specific therapy is presently available to counteract its onset or progress. Many studies have stressed the importance of physical exercise as an effective approach to prevent/limit the age-related muscle mass loss. We evaluated the effects of physical exercise on the activation and differentiation potential of cultured satellite-cell-derived myoblasts obtained from quadriceps muscles of old exercised, old sedentary and adult sedentary (control) mice. Cytochemical and immunocytochemical techniques were applied at light and transmission electron microscopy. Our results demonstrated that: a) physical exercise induces an increase in number of activated satellite cells; b) myoblasts from exercised muscles show morphological features quite similar to myoblasts from adult subjects, whereas myoblasts from non exercised muscles exhibit nuclear and cytoplasmic alterations suggestive of a reduced metabolic activity; c) myotubes differentiated from myoblasts of exercised muscles resemble the myotubes from adult myoblasts, whereas myotubes from non exercised muscles show marked structural alterations, especially in the cytoskeletal apparatus.

FLUORESCENCE PHOTOCONVERSION OF DIAMINOBENZIDINE AS A TOOL FOR DETECTING PHOTOSENSITIZING MOLECULES AT TRANSMISSION ELECTRON MICROSCOPY

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Photosensitizers (PSs) are chemical compounds able to absorb light and dissipate energy through photochemical processes producing highly unstable chemical species (i.e., singlet oxygen, free radicals or reactive oxygen species) that can damage the cell structures eventually inducing cell death. PSs may be modified by addition of suitable chemical groups (such as acetate or phosphate) to increase their intracellular accumulation: the photophysical and photochemical properties of the native PSs are affected, but the modified compounds behave as fluorogenic substrates (FSs) since once inside the cell the added groups are removed by cellular esterases and the native PS characteristics are restored.

In the attempt to localize photoactive molecules at transmission electron microscopy, HeLa cells were loaded with two different FSs, Rose Bengal acetate or Hypocrellin B acetate, and the photophysical properties of the intracellularly restored PS molecules were exploited to photoconvert diaminobenzidine into an electrondense product.

By this approach we demonstrated that these FSs enter the cells by endocytosis, being rapidly converted into the native PSs at the cell surface. PS molecules were also found in endosomes, lysosomes and multivescicular bodies, as well as free in the cytosol. This ultrastructural localization of the photoactive molecules accounts for the multiorganelle photodamage induced by irradiation of FS-loaded cells.

NUCLEAR LOCALIZATION AND PHOSPHORYLATION OF PHOSPHOINOSIIDE-PHOSPHOLIPASE C 1 CORRELATE WITH BREAST CANCER METASTASIS

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Activation of the enzyme phosphoinositide-phospholipase C γ1 (PLCγ1) is thought to play a critical role in both cytoskeletal changes and migration associated with the metastatic process. Activation of PLCγ1 by phosphorylation can occur downstream of many tyrosine kinase receptors including epidermal growth factor receptor, vascular endothelial growth factor receptor-2, c-MET, platelet-derived growth factor receptor, and also certain integrins. Activation induces hydrolysis of phosphatidylinositol 4,5-biphosphate to form the second messengers diacylglycerol and inositol-1,4,5-triphosphate, which in turn activate a number of signalling pathways. PLCγ1 is highly expressed in several tumours, including breast carcinomas in which the enzyme has been shown to be required for epidermal

growth factor induced migration of breast cancer cells. In order to establish the significance of PLCγ1 subcellular localization and phosphorylation (PLCγ1-pY783 and PLCγ1-pY1253) in breast cancer , we compared, through the use of different methods, two different breast cancer models: the low-tumorigenic BT-474 cell line and MDA-MB-231 cell line which represents a more aggressiveness de-differentiated cell type, obtained from a pleural effusion from a patient.

PROTECTIVE ACTIVITY OF CIS9,TRANS11 CONJUGATED LINOLEIC ACID ISOMER IN A MOUSE MODEL OF GLUTEN SENSITIVITY: AN HISTOMORPHOLOGICAL STUDY

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COX inhibition by indomethacin and gliadin administration to sensitized transgenic mice expressing the HLA-DQ8 heterodimer (DQ8 mice), was recently proposed as specific animal model of gluten sensitivity. Conjugated Linoleic Acid, a collective term used to describe a mixture of c9t11, and t10c12 isomer of linoleic acid, exhibited protective effects against gluten-mediated toxic effects on DQ8 mice intestine, however the role of individual isomer is still unclear.

Three groups of 6-12 weeks-old DQ8 mice (n=7 each) were examined. One group received indomethacin (1.5 mg/100 mL in drinking water) and it wasintra-gastrically administered with 500 g of a chymotryptic digest of gliadin (days 0, 3, 5 and 7) before sacrifice (day 10) (indo+gliadin, T). Another group was pre-treated by oral administration of c9,t11 isomer for 14 days (15 mg/day) before indo+gliadin (C9+T); the lastuntreated group was used as control (Ct). A morphometric analysis was performed on paraffin sections (5 m) of small intestinesamples to evaluate the protective effects produced by the c9,t11 pre-treatment.In particular, the sections were stainedwithhematoxylin-eosin,to observe the general morphology and to evaluate the villus/crypts ratio, orwith PAS to determine the occurrence and distribution of globet cells. Intestinal villi atrophy and decreased number of PAS+ globet cells occurred in T micewhereas intestinal histomorphology of C9+T animals was comparable with that of Ct. Presented findings demonstrates a clear protective effect of c9,t11 isomer against the harmful effects triggered by indo+gliadin treatment.

TOXIC EFFECTS OF ALUMINUMON ZEBRAFISH LARVAE

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Aluminum is the most abundant metal in the Earth's crust. Alzheimer'sdiseaseand Parkinson'sdiseasehave been previously associated with the presence of this metal (*Bondy S.C.,Neurotoxicology, 31, 575-581; 2010*). In this work the toxicity of aluminum was evaluated on the developmentof the zebrafishnervous system.Zebrafishlarvae at protruding mouthstage were exposed to different concentrations of aluminum chloride ($AlCl_3$) for 72 hours to test the lethality index.The mortality and the phenotypic analysis were determined after 24, 48 and 72 hours of treatment.A reduction of the response to stimuli in live larvae was observed from 100 uM of $AlCl_3$ just after 24 hours of the exposition and the severity of this abnormality was directly proportionate to the increase of the concentration used. Pericardial edema and impaired cardiac functionwere present in 50% of these larvae after 48 hours. In the light of these results sections of zebrafishlarvae, after 48 hours of the exposure at 100 uM of $AlCl_3$,were enclosedin paraffin and processed by ABC technique and TUNEL assay. The experiments of immunohistochemistry performed with a polyclonal antibody anti-GFAP, marker of glial cells, at a dilution of 1/300 showed a reduction of signal intensity in allareas of larvaebrainwith largest decreases in the hindbrain. An increase of the apoptotic cellswas also observed in the brain through TUNEL test.These data show a toxic effect exerted by aluminum on glial cells.Since the glia plays an important role on the functionality and protection of nerve cells, this effect could be the basis of thedevelopment ofneurological disordersinduced by this metal.

ISOLATION OF MESENCHYMAL STEM CELL POSSIBILITY FROM DIFFERENT INTRA-ORAL TISSUES: ADVANTAGES FOR THE CLINICAL PRACTICE

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Mesenchymal stem cells (MSCs) are of great interest for tissues and organs regeneration. Bone marrow has been the first source of MSCs and at present several other tissues are investigated for the isolation of these pluripotent cells. Recently, the oral tissues, which are easily accessible for dentists, have been also indicated as an interesting source of stem cells. In this study, we investigated the characteristics of MSCs isolated from different oral regions in order to evaluate their potential application in the regeneration of damaged maxillofacial tissues. Samples from human periodontal ligament, dental pulp, maxillary periosteum as well as bone marrow were collected in order to obtain and compare different stem cell populations. Cells were morphologically and immunophenotipically characterized. Their proliferation potential and ability to differentiate into mesenchymal lineage were also assessed. We showed that all tested cell populations displayed a similar fibroblast-like morphology and superimposable immunophenotype. Slight differences were instead observed in their proliferation and differentiation potentials. Considering these peculiar features they can be considered interesting cell sources in stem cell-based bone/periodontal tissue regeneration approaches, allowing the surgeon to reduce tissue loss, trauma, complications and discomfort for the patients in view of clinical practice.

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A NEW PERSPECTIVE FOR THE TREATMENT OF HUMAN ENDOMETRIAL CANCER

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The endometrial cancer is one of the principal causes of death in women (Siegel *et al.*, 2012). The main treatment for this cancer is hysterectomy that involves loss of fertility (Holland, 2008). If the tumor is not well defined and metastasizes, radio/chemo therapy are needed. The survival after chemotherapy is about 5-10 years, because it cannot kill all tumor cells. So, an isoform of manganese superoxide dismutase isolated from a human liposarcoma cell line, obtained as recombinant molecule (rMnSOD), which displayed oncotoxic action on cultured breast cancer cells (Mancini *et al.*,2006), was used to treat human endometrial cancer cells. This enzyme catalizes the dismutation of O_2 – free radicals in H_2O_2, preventing the accumulation of ROS. H_2O_2 can be further converted into H_2O and O_2 by catalase and/or glutathione peroxidase. Once rMnSOD penetrates cancer cells, it transforms free radicals into H_2O_2. This, in neoplastic cells, may lead to an high accumulation of H_2O_2 that causes apoptosis of them, because of their lower level of catalase (Mancini *et al.*, 2008). We evaluated the effects of the rMnSOD treatment on cultured human endometrial adenocarcinoma cell line HTB-112. We demonstrated the oncotoxic effect of this protein, which lead HTB/112 cells to apoptotic death, by immunocytochemistry at light and electron microscopy and by comet assay test, which shows early DNA damage induced by the accumulation of H_2O_2.

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BRAIN MORPHOLOGICAL ANALYSIS OF OBESE ZUCKER RAT: MODEL OF METABOLIC SYNDROME

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Metabolic syndrome (MetS) is a disorder characterized by the development of insulin resistance, with subsequent hyperinsulinemia, that increases the risk of cerebrovascular and cardiovascular diseases. Obesity is probably a risk factor for Alzheimer's disease and vascular dementia and is associated with impaired cognitive function.

The obese Zucker rat (OZR) represents a model of type 2 diabetes exhibiting a moderate degree of arterial hypertension and hyperlipidemia. To clarify the possible relationships between MetS and brain damage, the present study has investigated brain microanatomy of OZRs compared with their littermate controls lean Zucker rats (LZRs).

Male OZRs and LZRs of 12 weeks of age were used. Their brain was processed for analysis of nerve cell number by neuronal specific nuclear protein (NeuN) immunohistochemistry and phosphorylated neurofilament (NFP) immunoreactive axons analysis. The possible occurrence of astrogliosis was investigated by processing brains for immunohistochemical analysis of glial fibrillary acidic protein (GFAP).

In frontal cortex and hippocampus of OZRs reduced number of neurons was related to a decrease of Neu-N expression compared to LZRs. A significant increase in the size and number of GFAP immunoreactive astrocytes was also observed.

These findings suggest that OZRs developed as an animal model of type 2 diabetes, may also represent a model for assessing the influence of MetS on brain. This could clarify the pathophysiology of neurological injury reported in obese individuals and/or affected by MetS.

ANALYSIS OF P2X7 PURINERGIC RECEPTOR, CCL-20 AND IL-8 IN HUMAN PERIODONTAL LIGAMENT STEM CELLS

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Periodontal disease is the most common oral inflammatory condition of bacterial etiology, representing the main cause of dental loss in developed countries. We have isolated and characterized human mesenchymal stem cells from PDL (hPDLMSCs). In hPDLMSCs ATP is released under mechanical stress and induces the expression of osteopontin via P2Y1/Rho kinase pathway. In this study, we showed by reverse transcriptase-PCR, western blot analysis and confocal immunofluorescence that hPDLMSCs express P2X7R.3'-0-(4-benzoyl)benzoyl-ATP (BzATP), the most potent P2X7R analog, triggered an increase in intracellular Ca2+ and in ethidium bromide uptake, both effects were markedly reduced by the P2X7R irreversible antagonist, oxidized ATP (oATP), suggesting the expression of functional P2X7R. Our data indicate that when BzATP was added to the hPDLMSCs culture medium for 24h a marked increase in the secretion of IL-8 and CCL20 occur. This effect was counteracted by cell pretreatment with either oATP or the highly selective and potent P2X7R competitive antagonist A-740003 suggesting that in these cells extracellular ATP mediate a pro-inflammatory response via P2X7R. This work shows that hPDLMSCs express functional P2X7R whose activation is linked to the production of pro-inflammatory/angiogenic factors indicating that: a)hPDL-MSCs may play a role in the inflammation processes underlining periodontal diseases, b)once used in a tissue repair therapy they can contribute to the control of the environment in which they engraft.

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