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European Journal of Histochemistry
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The *European Journal of Histochemistry* was founded in 1954 by Maffo Vialli and published until 1979 under the title of *Rivista di Istochimica Normale e Patologica*, from 1980 to 1990 as *Basic and Applied Histochemistry* and in 1991 as *European Journal of Basic and Applied Histochemistry*. It is published under the auspices of the University of Pavia, Italy.
The *European Journal of Histochemistry* is the official organ of the Italian Society of Histochemistry and a member of the journal subcommittee of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC).

The Journal publishes Original Papers, Technical Reports, Reviews, Brief Reports, Letters to the Editor, Book Reviews, Views and Comments, concerning investigations performed with the aid of biophysical, biochemical, molecular-biological, enzymatic, immunohistochemical, cytometric, and image analysis techniques.
Areas of particular interest to the *European Journal of Histochemistry* include:
- functional cell and tissue biology in animals and plants;
- cell differentiation and death;
- cell-cell interaction and molecular trafficking;
- biology of cell development and senescence;
- nerve and muscle cell biology;
- cellular basis of diseases.

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37TH NATIONAL CONGRESS OF THE ITALIAN SOCIETY OF HISTOCHEMISTRY

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Opening Lecture

FROM THE STUDY OF ENAMEL TO NEW INSIGHTS ON EPITHELIAL CANCERS

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In order to better understand the process by which enamel becomes the hardest extracellular calcified matrix of the body, our group has carried out transcriptomic screens of the rat incisor enamel organ. This has led to the discovery of three genes encoding novel proteins named amelotin (AMTN), odontogenic ameloblast-associated (ODAM), and secretory calcium-binding phosphoprotein proline-glutamine rich 1 (SCPPPQ1). These genes belong to the secretory calcium-binding phosphoprotein (SCPPP) cluster whose members are implicated in stabilization of calcium and phosphate ions in tissue fluids and regulation of their deposition in extracellular matrices. The three proteins are expressed in maturation stage ameloblasts as well as in the junctional epithelium (JE), the part of the gingiva that seals away the periodontal tissues from the aggressive oral environment. In both cases, they localize to a specialized basal lamina (sBL) that attaches epithelial cells to the mineralized tooth surface. The knockout of ODAM exhibits a JE phenotype, and its expression is downregulated during periodontal disease. Furthermore, the major pathogens implicated in periodontal disease can actively degrade ODAM. Strikingly, ODAM, but not the other two proteins, is also expressed by stem cell clusters in the periodontium when periodontal integrity is affected, and by various epithelial neoplasias whose tissues of origin normally do not express it. Altogether, these findings suggest that ODAM behaves as a multifunctional matricellular protein that participates both in structuring the aBL and in regulating cell status.

Supported by the CIHR, CRC program, FCI and RSBO-FRQ.

Oral Presentations

TEMPERATURE EFFECT ON MUSCLE GROWTH IN *ACIPENSER BAERII* YOLK-SAC LARVAE

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In intensive aquaculture, fish species are constantly exposed to environmental stressful conditions, among which, temperature and stocking density are considered the major ones that may have an impact on the physiology, health, welfare and behaviour of cultures fish.[1] The aim of the current study was to investigate the stress impact and muscle growth in precocious stages of *Acipenser baerii* subjected to three rearing temperatures. After hatching, larvae were subjected to three different rearing temperatures (16°C, 19°C and 22°C) until the yolk-sac was completely absorbed. Larvae were sampled at hatching, schooling and complete yolk-sac absorption. Histological, histometrical, histochemical and immunohistochemical analyses were executed, with the scope of evaluating muscle growth and development (total fibers area, fast fibers area, slow fibers area; anti proliferating cell nuclear antigen; anti-caspase) and stress biomarkers (heat shock protein 70 and 90) were assessed. SAS software (v. 9.3, Cary Inc., NC) was used to perform statistical analysis. Total Fibers Area (TFA) and Fast Fibers Area (FFA) were higher in the schooling stage at 19°C; no differences were found regarding the Slow Fibers Area (SFA) at the tested temperatures. A significantly higher number of proliferating cells was observed in the schooling phase at 22°C than at 16°C, which is in accordance with another study on Siberian sturgeon.[2] The anti-caspase immunohistochemistry was never observed in the larvae at any temperature. HPS70-immunopositivity was not observed in the muscle and HSP90 immunopositivity was evident at 19°C, which appears in full accordance with a part of a study on HSP's.[3] This study indicates that a lower rearing temperature would appear more appropriate for Siberian sturgeon larvae rearing, but further studies are necessary to deepen the effect of temperature on a mid-long term basis.

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IMMUNOHISTOCHEMICAL EXPRESSION OF AQUAPORIN 1 IN PLEURAL EFFUSIONS FROM MALIGNANT MESOTHELIOMA: AN APPROACH BY CELL-BLOCK PROCEDURE

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Some diagnostic biomarkers have been proposed in pleural malignant mesothelioma (PMM), such as calretinin, CK5/6 and podoplanin, but no prognostic predictors are still available. Recently, Aquaporin 1 (AQP1) has been highlighted in PMM bioptic samples and its high levels (>50% of neoplastic cells) have been suggested to be an independent prognostic predictor. However, pleural effusions (PE) represented an alternative way to achieve the diagnosis in PMM but to increase the diagnostic accuracy, the cell block procedure (CBP) allows better cell architecture preservation, hematoxylin-eosin staining as well as immunohistochemistry or molecular analyses.[1-3] Herein we investigated a series of PE cytological samples from patients affected by PMM, utilizing CBP to verify the hypothesized prognostic role of AQP1. 47 PE taken from an equal number of PMM patients were retrospectively analyzed from files of our department; from 37 patients thoracoscopic tissue fragments were also available. After thoracentesis, the PE cytological content was centrifuged to create pellets, that were immediately fixed in 4% neutral buffered formalin for 45 min at room temperature. The corresponding paraffin-embedded blocks were cut; on 3-μm thick sections, hematoxylin and eosin (H&E) was applied. Silane-coated serial sections were immunohistochemically stained with the following antibodies: AQP1 (B-11, 1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA), calretinin, cytokeratin 7; cytokeratin 5/6 and TTF-1. The cohort was composed by 36 men and 11 women (mean age: 65 years; age range: 56-87 years). At H&E from CBP atypical mesothelial cells were organized in cohesive groups, three-dimensional clusters or as single cells; cytological details revealed mild atypia in round nuclei, some prominent nucleoli and dense cytoplasm with a pale rim. Immunohistochemical expression of AQP1 documented the linear and circumferential membranous staining, not exclusively lining the apical cellular portion. The percentage score ranged from 0% to 100% and a score >50% was considered as clinically significant, as elsewhere suggested.[4] 28 PMM cases showed AQP1 expression >50% with a significant association with increased survival, being the median progression free survival (21 months) higher that for patients with <50% AQP1 (8 months). All cases showed positive immunostains for calretinin and CK 5/6, confirming thus the mesothelial histogenesis, while no immunoexpression was recorded for cytokeratin 7 and TTF1.

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CYCLIN D1 AS IMMUNOHISTOCHEMICAL AID IN THE DIAGNOSIS OF RENAL TUMORS WITH EOSINOPHILIC APPEARANCE

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Chromophobe cell carcinoma (CCC) and oncocytoma of the kidney (RO) are two distinct neoplasms, accounting for 10% of renal tumors, with a common cell of origin (intercalated cell of the collecting duct), but characterized by different biologic behavior and clinical outcome. Moreover, an eosinophilic variant of CCC shows overlapping features with RO; on the other hand, histochemical stain (Hale colloidal iron) as well as immunohistochemical markers (CK7, CD117, vimentin and E-cadherin) do not always allow a reliable clear-cut distinction between them. However, the immunohistochemical expression of cyclin D1, a G1-specific cyclin crucial in regulating cell cycle progression in G1/S transition, has been documented in RO, while conflicting results emerged in CCC.[1,2] In the present study, we evaluated the diagnostic utility of cyclin D1 in a series of eosinophilic CCC and RO neoplasms. From files of our institutions, 17 cases of eosinophilic variant of CCC and 15 cases of RO have been retrospectively retrieved; patients were 20 males and 12 females (age range 36-78 years, mean age 54 years). After a confirm of morphological diagnosis on the basis of H&E as well as the above mentioned immunohistochemical stainings, 5 μm thick sections taken from one representative formalin-fixed paraffin-embedded neoplastic tissue blocks were cut and incubated with anti-cyclin D1 (SP4, Neomarkers, Fremont, CA, prediluted antibody) for 30 min at room temperature. The percentage of positively stained cells was assessed by semi-quantitative optical analysis according to a four-tiered system (<1% positive cells = negative staining; 1–10% positive cells = focal staining; 11–50% positive cells = heterogeneous staining; >50% positive cells = diffuse staining), as elsewhere reported.[3] 8/17 CCC cases showed a weak positivity in <5% of neoplastic cells and only one case demonstrated a diffuse expression, while 14/15 RO cases were positive for cyclin D1, with a diffuse staining in 8 cases and focal or heterogeneous staining in 6 cases. Therefore, our findings strongly suggest that a diffuse immunopositive reaction in >50% of tumor cells, supports the diagnosis of RO with high specificity (94%). Finally, taking also into consideration the more uniform and diffuse Hale colloidal iron stain as well as CK7 immunoreactivity mainly observed in CCC, the integration with cyclin D1 may help to better differentiate these two renal tumors with eosinophilic appearance.

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THE LINK BETWEEN PROGENITOR CELLS AND INFLAMMATION IN UTERINE LEIOMYOMAS ONSET

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Uterine leiomyomas are considered the main benign tumors originating from the myometrium and involving the female reproductive system.[1] It is still unclear the question about their pathophysiology;[2] it is known that the uterus is endowed with high plasticity and that property suggests the existence of a progenitor/undifferentiated cell population inside that, if dysregulated, may play a role in the onset of uterine pathologies. To date, several groups with different approaches investigated the existence of undifferentiated cells in the myometrium and leiomyomas,[3] and great attention has been paid to the role of inflammation in leiomyomas development .[4] The correlation that links the two aspects, inflammation and undifferentiated cells, in the occurrence of leiomyomas has never been investigated. Here progenitor cells were isolated from healthy myometrium (MPCs) and from leiomyomas (LPCs); cells were strictly characterized, looking to the morphology and to the expression of selected proteins by indirect immunofluorescence (IIF) and immunocytochemistry (ICC); furthermore, the presence of selected cytokines related to inflammation involved in fibrosis growth, such as IL6, TNF-α, IFN-γ, GM-CSF and TGF-β, was checked. Our results confirm the existence of progenitor cells in uterine tissues; isolated cells from leiomyomas express and secrete much more cytokines related both to inflammation and to leiomyoma onset than from myometrium. In particular, the expression of TGF-β, the main player in the progression of the fibrosis, is higher in LPCs than MPCs, meaning that undifferentiated cells may sustain inflammation and in turn exert profibrotic effects.

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HEPATIC PROGENITOR CELLS AND HISTOLOGICAL PATTERN IN NON-ALCOHOLIC FATTY LIVER DISEASE: A POSSIBLE ROLE FOR PNPLA3 VARIANT AND OXIDATIVE STRESS

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Non-alcoholic fatty liver disease (NAFLD) is the result of intricate interactions between resident and recruited cells.[1] Patatin-

like phospholipase domain containing 3 gene (PNPLA3); I148M variant has been associated to NAFLD,[2] but its relationship with specific histopathological patterns is not well characterized. We included 40 adult patients with biopsy-proven NAFLD. Histology was evaluated by routine histomorphological stains. Hepatic stem/progenitor cell (HPC) compartment, hepatic stellate cells (HSC) activation and macrophage pool were evaluated by immunohistochemistry. Our results demonstrated that HPC activation was significantly correlated with NAFLD activity score (NAS) and fibrosis score. PNPLA3 variant carriers showed higher steatosis, HPC activation, number of α Smooth Muscle actin (αSMA) + HSC and portal S100A9+ macrophages compared to wild-type patients. To investigate a potential underlying mechanism, we measured the activity of Nox2, a key enzyme producing reactive oxidant species, and serum 8-iso-prostaglandin (PG)F2α. Serum 8-iso-PGF2α levels were significantly higher in I148M variant carriers compared to non-carriers and were correlated with HPC activation and portal inflammation. Nox2 was correlated with NAS, the degree of steatosis, lobular inflammation, HPC activation, and with the number of αSMA+ lobular and periportal HSC. In conclusions, NAFLD patients carrying PNPLA3 I148M variant showed a specific predominant periportal histological pattern characterized by high activation of HPC compartment, HSC and macrophage pools and increased oxidative stress.

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THE USE OF SIRIUS RED IN ASSESSING DERMAL COLLAGEN REORGANISATION IN MICE LACKING 11 1 INTEGRINS

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Elucidation of a potential role in vivo of α11β1-integrins for the control of collagen fibril arrangement would increase the understanding on the mechanisms of fibrillogenesis and may reveal a possible therapeutic target in the management of fibrotic diseases.[1,2] In order to answer this question, here, we analyzed the overall organization of the dermal collagen network in samples of back skin of α11β1-integrin-deficient mice (KO). Collagen remodeling was assessed light microscopically (n=4) on paraffin sections after Sirius red staining. Traditionally, the use of Sirius Red staining is associated to study of collagen fiber bundle thickness using circularly polarized light. Here Sirius red stained sections were imaged at the structure light microscope and analyzed fluorescently. Following acquiring at the structured light microscope images were evaluated by Image J for fractal dimension and lacunarity, which express the level of complexity of the network and network heterogeneity respectively. The results showed increase in fractal dimension (1.40±0.06 in α11 KO mice *vs* 1.24±0.05 of control mice, p=0.009), and reduction in lacunarity (0.78±0.06 in α11 KO mice 0.97±0.02 of control mice p=0.002) in KO mice. These differences indicate an overall re-organization of dermal collagen network in skin devoid of integrins α11β1. This study show that sirius red staining is a suitable method to evaluate the overall organization of demal collagen and that α11β1-integrins are involved in the control of skin biomechanics by influencing the overall organization of the dermal collagen network.

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MILD OZONISATION INDUCES LIPID ACCUMULATION IN HUMAN ADIPOSE-DERIVED ADULT STEM CELLS

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Oxygen-ozone (O_2-O_3) therapy is a modestly invasive procedure based on the regeneration capabilities of low ozone concentrations, and used in medicine as an adjuvant treatment for several diseases.[1] In this work, we investigated the effects of mild ozonisation on human adipose-derived adult stem (hADAS) cells.[2] Adipose tissue is used in regenerative medicine and tissue engineering and we investigated the potential of low O_3 concentrations in stimulating hADAS cell differentiation. The cells were grown in appropriate medium to obtain differentiation into the adipoblastic lineage. According to,[3] hADAS cells were seeded on glass coverslips and exposed to O_2-O_3 gas mixtures (5 and 10 μg O_3/mL O_2) using an OZ02 FUTURA apparatus (Alnitec); cells exposed to pure O_2 or air served as controls. Cells were treated at early (6 days), intermediate (16 days) and late (20 days) differentiation steps and the effects were evaluated 2 and 24 h after gas exposure. Trypan blue test excluded increased cell death following treatment. After gas exposure, cells were fixed with aldehydes and processed for either light or transmission electron microscopy. For brightfield microscopy, cells were stained with Red oil to visualize lipid droplets. Cell and lipid droplet areas were measured (Image J), and statistical comparisons revealed that exposure to 10 μg O_3 significantly increased lipid content after 24 hours at all the differentiation steps considered, whereas 5 μg O_3 exerted a limited effect. Ultrastructural analysis demonstrated no alteration of cell organelles in any samples at any treatment times. It is known that an appropriate level of reactive oxygen species is required to stimulate adipogenesis. It is therefore likely that the lipogenic effect observed in hADAS cells is related to the mild oxidative stress caused by low O_3 concentrations.

This work was supported by University of Verona, Italy (Joint Projects 2015).

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BEHAVIOR OF PERIODONTAL LIGAMENT AFTER APPLICATION OF PRECALIBRATED FORCE: AN IMMUNOFLUORESCENCE STUDY

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The periodontal ligament (PDL) is a membrane-like connective tissue highly vascularized surrounding the root of a tooth. It lies between the hard tissues of alveolar bone and cementum of teeth and serves to anchor the tooth to the alveolus and it provides several functions as physical, sensory, and trophic functions. This tissue is continuously exposed to mechanical stress during the phases of mastication. The application of orthodontic force creates a change in parodontal structures and the PDL tissue could be injured and then occlusal trauma could occur.[1]
The aim of this study was to evaluate PDL modifications induced by application of a precalibrated and constant orthodontic strength, with coil spring NiTi 50 gr, at different stages of treatment: 7, 14, 21 and 30 days from strength application, respectively.
For the study, we analyzed the expression of collagen I, IV, fibronectin and VEGF by immunofluorescence techniques and confocal microscopy.
Results have shown that in the early stages of strength application the protein pattern for all tested proteins is particularly subject to change. The most important data is that in the late stages of strength application the expression of the proteins begins to return to normal values and at day 30 the recovery of the expression of collagen I, collagen IV, fibronectin and VEGF is achieved.
We can assume that the mechanical stress induced by strength application of coil spring NiTi 50 gr causes, in the early stages of strength application, an extracellular matrix proteins remodeling and neoangiogenesis processes; at day 30 we observe the recovery of structure and functions of PDL. This is the most important data because we have the proof that, at day 30 (when the applied force is not more operative), no damage occurs at PDL level.

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ERG AND KPNA2 EXPRESSION IN ADVANCED PROSTATIC CARCINOMA AND LYMPH NODE METASTASES

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Prostate carcinoma (PC) is the most common malignant tumor in men in developed countries; however, despite the high occurrence, its molecular basis is still not fully understood. Most prostatic cancers overexpress the ERG oncogene and Karyopherin 2 (KPNA2). These genes play a role in prostatic carcinogenesis, but their prognostic significance is controversial. The aim of this study is to determine the prognostic significance of ERG and KPNA2 expression and their association to early PSA biochemical recurrence in advanced prostate cancers with lymph node metastases. A series of 65 consecutive advanced pN1 prostate cancer samples obtained by radical prostatectomy with lymphadenectomy has been analyzed for ERG and KPNA2 expression by immunohistochemistry. For each case, the following clinical data were collected: age, pre-operative serum PSA levels, Gleason score, TNM stage, and follow-up. PC recurrence was investigated by serum PSA assay and defined by a PSA concentration >0.2 ng/mL after a nadir of <0.1 ng/mL following radical prostatectomy. ERG positive staining was found in 25/65 cases (38%), and KPNA2 in 56/65 cases (86%) and were not detected in normal prostatic tissue. Immunohistochemical concordance was found between primary tumor and lymph node metastases in 62/65 (95%) of cases. The follow-up was known in all cases and PSA recurrence occurred in 25/65 cases (38%). ERG positivity, either alone or in conjunction with KPNA2 positivity, was strongly associated with early PSA recurrence (ERG+/KPNA+ phenotype: OR 22.2 [6.0-82.3]; ERG+ alone: OR 17.9 [5.1-63.5]; p<0.0001 for both). The ERG+ phenotype seemed to be selected in metastasis-initiating clones. KPNA2 expression is significantly associated with the tumor stage (p<0.00001). These results suggest that ERG and KPNA2 may have a prognostic value and their positivity in PC might warrant more aggressive treatments.

DIETHYLHEXYL PHTHALATE EFFECTS ON TESTICULAR HISTOPATHOLOGY

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Phthalates are synthetic chemicals widely used as plasticizers since they provide flexibility in plastics. Diethylhexyl phthalate (DEHP) is commonly used in a wide range of consumer products. Several studies have shown that DEHP exposure can lead to serious anomalies for male reproductive tract like cryptorchidism, hypospadias, impaired spermatogenesis and reduced fertility. Thus, in this study we examined the effects of DEHP on neonatal testicular histopathology. Pregnant Wistar rats were gavaged from gestation day (GD) 7 to GD 21 and from postnatal day (PND) 1 to 6 with vehicle, 10, 30, 300, 600, or 900 mg/kg bw/day DEHP. At PND 6 rats were anesthetized, decapitated and testes were removed. After Bouin fixation and paraffin embedding, testes were stained with hematoxylin and eosin and were evaluated for histopathological effects. At the highest DEHP doses, gonocytes appeared to be enlarged and multinucleated and diameter of the seminiferous tubules were reduced. Leydig cells tended to group together dose dependently from DEHP 30 mg/kg. To better understand if DEHP induces gonocyte proliferation and if Leydig cell clusters are due to hyperplasia, proliferation markers were evaluated by immunohistochemistry. In order to study structural changes, we performed double staining for 3β-hydroxysteroid dehydrogenase (3β-HSD, specific

for Leydig cells) and α-smooth muscle actin (SMA, specific for peritubular and perivascular cells). SMA staining was strong in peritubular myiod cells and perivascular cells in both control and treated groups. At the high dose DEHP, Leydig cells positively stained for 3β HSD were found also inside the tubules.
Results show that after in utero exposure DEHP effects on Leydig cells were seen at lower doses than effects on gonocytes and tubule diameters. These results were compared to results from a similar study on a mixture of 13 chemicals including DEHP.

EDCs EFFECTS ON HUMAN PROSTATE CELLS LNCaP.

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Endocrine disrupting chemicals (EDCs) comprise a group of compounds that have been examined extensively due to the potential harmful effects in the health of human populations. Many epidemiological and *in vitro* studies have corroborated the association between EDCs exposure and male and female reproduction disorders, but less is known about their effects on prostate gland. Thus, in this study we investigated the effects of two different EDCs: the estrogenic nonylphenol (NP) and the antiandrogenic dibutylphthalate (DBP) on adenocarcinoma prostate cells (LNCaP). Moreover, we compared their effects with 17β-estradiol (E2) action in order to investigate possible mimetical behavior. After 24h of exposure NP stimulated cell proliferation at low concentration of 10^{-10} M, on the contrary DBP induced a cell proliferation decrease at 10^{-8} M. NP and DBP had a different activity on gene expression: NP increased mRNA levels of ki67, cyclin D1 while it decreased p53 gene expression; instead DBP didn't interfere on mRNA levels of MCT4 and strongly decreased ki67 and cyclin D1 expression. To assess estrogen (ERs) and androgen (AR) receptors involvement, we evaluated the expression of ERs and AR with immunofluorescence and western blot techniques. NP induced and early ERα cytoplasm-nucleus translocation respect to DBP; both of them didn't affect ERβ and AR localization and expression.
Results obtained suggest that NP and DBP have different activities on LNCaP cells. NP stimulates cell proliferation and is involved in the activation of some prostate cell cycle key regulators similarly to E2. These effects place the attention of the dangerous risks of exposure to estrogens mimicking compounds. On the contrary, DBP doesn't mimick E2 effects and it influences cell viability probably activating molecular pathways involved in the programmed cell death, so it may be considered a potential risk for male fertility.

HISTOCHEMICAL DETECTION OF MUCUS SECRETING CELLS IN A STABILIZED *IN VITRO* CACO2/HT-29 CO-CULTURE MODEL

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The interactions between nutrients and intestine have been studied up to now by assays performed on *in vitro* human cell cultures. The main limitations of this approach are represented by both the difficulty to handle and the differences/complications compared to the physiological condition. Recently, we optimized an innovative model in which two human intestinal cell lines, *i.e.* Caco2 and HT-29, were co-cultured. Caco2 cell line is mostly absorptive and do not secrete mucus, while HT-29 are a heterogeneous population, comprising scattered enterocyte elements and mucus secreting cells. To mimic the human intestinal epithelium, the co-culture was set up with a ratio of 70/30 Caco2 / HT-29 respectively, starting from the parental cell populations differentiated as previously described.[1,2] The present work was designed to study the time course of the mucus secretion in two different cell growth conditions: i) in a standard culture conditions and ii) in presence of an excess of nutrients. In both conditions, co-culture was harvested at confluence (T0) and at 3, 7, and 15 days post-confluence (T3, T7, and T15, respectively). In the standard group, the culture medium was changed every four days, while in the excess group on alternate days from T0. Cells were seeded in 24 well plate inserts and fixed in 4% paraformaldehyde. The membranes were removed from the inserts by means of forceps and placed in embedding cassettes. Samples were dehydrated through an ascending series of ethanol, embedded in paraffin, cut with a microtome, and PAS stained. In standard conditions, together with the non-mucus-secreting cell type, which are always present, the mucus-producing cells increased since T7, while at T15 these cells were scattered. These observations are compared with both the excess group and previously obtained ultrastructural results.
As the mucus presence is relevant not only for the barrier function but also for the nutrient permeability, the present co-culture model enlighted the morpho-functional changes induced by the modifications of the cell growth conditions.

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LIPOSOMES AS A PUTATIVE TOOL TO INVESTIGATE NAADP SIGNALING IN VASCULOGENESIS

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Nicotinic acid adenine dinucleotide phosphate (NAADP) is the

newest discovered intracellular second messengers, which is able to release Ca^{2+} stored within endolysosomal (EL) vesicles. NAADP-induced Ca^{2+} signals mediate a growing number of cellular functions, ranging from proliferation to muscle contraction and differentiation.[1] Recently, NAADP has recently been shown to regulate angiogenesis by promoting endothelial cell growth.[2] It is, however, still unknown whether NAADP stimulates proliferation also in endothelial progenitor cells, which are mobilized in circulation after an ischemic insult to induce tissue revascularization. Herein, we described a novel approach to prepare NAADP-containing liposomes, which are highly cell membrane permeable and are therefore amenable for stimulating cell activity. Accordingly, NAADP-containing liposomes evoked an increase in intracellular Ca^{2+} concentration, which was inhibited by NED-19, a selective inhibitor of NAADP-induced Ca^{2+} release. Furthermore, NAADP-containing liposomes promoted EPC proliferation, a process which was inhibited by NED-19 and BAPTA, a membrane permeable intracellular Ca^{2+} buffer. Therefore, NAADP-containing liposomes stand out as a promising tool to promote revascularization of hypoxic/ischemic tissues by favoring EPC proliferation.[3]

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EXPRESSION OF MATRIX METALLOPROTEINASES AND THEIR TISSUE INHIBITORS IN NASAL POLYPOSIS

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In nasal polyposis, a chronic multifactorial disease of the sinonasal mucosa, anatomic morpho-structural alterations, allergic and syndromic diseases are considered comorbidities. We investigated matrix metalloproteinases (MMPs 2-7-9) and their tissue inhibitors (TIMPs 1-2) concentrations in different etiopathogenetical groups of patients with nasal polyposis in relation to recurrence after sinonasal surgery. The study group consisted of 45 patients with nasal polyposis (subjects with allergic rhinitis, syndromic diseases or dysventilations of osteomeatal complex due to morphostructural alterations) who underwent endonasal sinus surgery. We also collected 10 patients who underwent septoplasty as control. Immunohistochemistry of nasal mucosa fragments, western blotting and polymerase chain reaction analysis showed increased matrix metalloproteinases levels (-9 more than -2 and -7) and decreased tissue inhibitors of matrix metalloproteinases levels (-1 less than -2), in patients with nasal polyposis compared with control group and in particular in syndromic patients compared to allergic and morphostructural patients. We observed higher risk of recurrence in syndromic patients than in allergic and morphostructural ones after 36 months from surgery.

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ISOPRENALINE-INDUCED EXOCYTOSIS AND MELATONIN SECRETION IN THE RAT PAROTID GLAND

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Our previous works showed, by TEM, melatonin to be associated with the secretory granules in human salivary glands[1] and further, in the rat parotid gland, melatonin receptors to be attached to the secretory granules.[2] Currently, we exposed Equitesin-anaesthetized rats to the β-adrenoceptor agonist isoprenaline, in a dose causing marked acinar degranulation (5 mg/kg i.p.), with the hypothesis that melatonin would accompany the secretion of the granules into the lumina. Pre-administration of isoprenaline, the right gland was removed to serve as control. The left gland was removed after 60 min. Samples were fixed, dehydrated, embedded in Epon Resin and processed to demonstrate melatonin reactivity by the immunogold staining method. The labelling density (expressed as number of gold particles per μm^2/granule) and the density of melatonin-positive granules (per 100 μm^2/cell) were estimated. In pre-stimulated samples melatonin immunoreactivity was located in the secretory granules, about half the number of granules displayed labelling for melatonin. As expected, isoprenaline provoked a massive exocytosis of granules. Both melatonin labelled and non-labelled granules were reduced to the same extent. Melatonin labelled gold particles appeared in the lumina. The findings support the view that serous cells of salivary glands are able to store and secrete melatonin by regulated pathways upon stimulus.

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APOPTOTIC DEATH OF M2 ACUTE MYELOID LEUKEMIA CELLS BY ROS PRODUCTION

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The main treatment of Acute Myeloid Leukemia in children is chemotherapy, which can lead to side effects. Recently, a new isoform of human MnSOD was isolated and obtained in a synthetic recombinant form (rMnSOD). This isoform can kill T-ALL cells, without cytotoxic effects on healthy cells.[1] The rMnSOD is characterized by the presence of a leader peptide, which allows the protein to enter cells through estrogen receptors unlike the wild type form. The selective oncotoxic activity of rMnSOD is due to an increase in the level of H_2O_2 in cancer cells, which contain lower levels of catalase than healthy cells.[2] In this study we analyzed the rMnSOD effects on Kasumi-1 cell line. Detection of estrogen receptors (ER1 and ER2) was obtained by qPCR assay. Cell viability and apoptosis were analyzed by flow cytometry through Annexin V assay after 1 μM, 1.5 μM and 2 μM rMnSOD treatments. Apoptotic fragmentations were demonstrated by confocal imaging. Mitochondria were detected by MitoTracker® Red CMXRos. A very high amount of both ER1 and ER2 was detected in Kasumi-1 cells. Annexin V analysis showed that 2 μM rMnSOD treatment induced apoptosis in cells (about 50%). Confocal microscopy showed typical alterations of apoptosis, following 24h treatment of 1.5 μM and mainly 2 μM rMnSOD, such as nuclear fragmentations and apoptotic-bodies. The internalization of rMnSOD in mitochondria was demonstrated by its colocalization with MitoTracker, following 6h incubation. In conclusion, rMnSOD exerts toxic activity against leukemia cell line. Further observations of rMnSOD effects on AML M2 pediatric patient cells are in progress.

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NATURAL SESQUITERPENES AS POTENTIAL MODULATOR OF INTRACELLULAR PATHWAYS IN CHOLANGIOCARCINOMA PROLIFERATION

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Many natural compounds are able to control apoptotic signaling pathways particularly by modulating the NFkB and STAT3 cascades.[1] The natural sesquiterpenes β-caryophyllene (CRY) and beta-caryophyllene oxide (CRYO) exhibited *in vitro* chemosensitizing properties[2] in different human cancer cell lines, among which liver cancer cells. Cholangiocarcinoma (CCA), that originates at the level of the intrahepatic or extrahepatic bile ducts, is one the most aggressive type of cancer for its chemotherapy resistance and invasiveness. Increasing efforts are directed to identify new cellular targets for cancer cell survival and metastatization[3] and some alternative approaches. In this context, our previous studies highlighted that a combined treatment of low-dose doxorubicin (DOXO) and a nontoxic concentration of CRY (10 μg/mL) and CRYO (10 μg/mL) increased the effectiveness of the anticancer drug. Present study was aimed at identifying the possible interference of the combined treatment of low-dose DOXO and CRY or CRYO on the STAT3 activation pathway in CCA cells. To this end, cholangiocytes were exposed to the test substances for 24h and 72h, before to evaluate the apoptosis, through the Annexin V staining, and the nuclear translocation of STAT3, through immunofluorescence. We found that DOXO in association with CRY, but not with CRYO, suppresses the STAT3 activation in CCA cells with a time-dependent effect. Accordingly, an increased apoptosis was found in cholangiocytes treated with DOXO + CRY, compared to DOXO alone. Our results show for the first time that the combination DOXO and CRY is able to modulate the STAT3 pathway and could be considered a blocker of the same signaling cascade.

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PROLONGED DARKNESS DECREASED LIVER FIBROSIS IN AN EXPERIMENTAL MODEL OF PRIMARY SCLEROSING CHOLANGITIS THROUGH miR-200b DOWNREGULATION

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Cholangiocytes are the target cells in cholestatic human liver diseases including primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) that are characterized by biliary damage and liver fibrosis.[1] We tested the effects of melatonin therapy or prolonged exposure to dark reduce liver fibrosis and biliary proliferation in the model of bile duct ligated rat (BDL).[2] No data exist regarding the role of melatonin in primary sclerosing cholangitis (PSC). Thus, we aimed to determine the therapeutic effects of prolonged dark therapy or melatonin administration on hepatic fibrosis in the $Mdr2^{-/-}$ mouse model of PSC through the evaluation of biliary mass, liver fibrosis, angiogenesis and miR-200b expression. After administration of melatonin or exposure to darkness, $Mdr2^{-/-}$ mice show elevated melatonin levels in serum and inhibition of biliary mass, together with a decrease in liver fibrosis and angiogenesis. In addition, miR-200b expression is increased in $Mdr2^{-/-}$ mice and PSC patient samples compared to controls and decreased in $Mdr2^{-/-}$ mice exposed to dark or to melatonin treatment. The inhibition of

miR-200b in mouse model decreased biliary proliferation, liver fibrosis and angiogenesis. *In vitro,* overexpression of AANAT or inhibition of miR-200b in cholangiocytes reduced the expression of miR-200b, angiogenesis and fibrosis genes. Dark therapy or targeting melatonin/miR-200b axis may be considered an important target in the management of biliary damage and liver fibrosis in cholangiopathies including PSC.

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CHARACTERISATION OF SYNOVIAL MEMBRANE DERIVED MESENCHYMAL STEM CELLS: A COMPARATIVE ANALYSIS OF DIFFERENTLY AGED POPULATIONS

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Cartilage lesions due to trauma or degenerative diseases are the most common sources of articular pain and disability in an ageing population and their healing continues to represent a challenge to scientists and clinicians. Several standard procedures have been routinely utilized to address chondral damage demonstrating favorable short to medium outcomes. Mesenchymal stem cells (MSCs) are interesting candidates to treat cartilage lesions as they can be isolated from several musculoskeletal reservoirs (*i.e.* bone marrow, adipose tissue, bone tissues, periosteum) as well as from synovial tissues.[1,2]. Despite a widespread use of these cells, some parameters such as tissue source and donor specificity are often overlooked, even if there are clear evidences indicating that these features influence MSC behaviour and regenerative potential. The purpose of our study was to isolate and define the characteristics of synovium-derived MSCs from differently aged subjects and clarify discrepancies between "young" and "old" MSCs in term of phenotype, morphology and differentiation potential. No significant differences were detected in term of the minimum characterisation criteria of MSCs,[3] but cells from older subjects showed changes related to their possible involvement in the onset/maintenance of cartilage damage. Further investigations will seek the relevance of these changes, in order to deep our knowledge on MSC active role in cartilage repair (*e.g.* dampening down inflammatory processes, producing repair cartilage themselves or encouraging reparative cellular recruitment).

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UCP2 AND *SOD2*: ADAPTIVE AND PROTECTIVE ROLE DURING MITOCHONDRIAL ROS PRODUCTION IN RAT LIVER

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Uncoupling proteins (UCPs) are a family of mitochondrial inner membrane proteins with a pore shaping structure that determines the dissipation of electrochemical proton gradient formed during mitochondrial respiration. Their main role is to generate decoupling between mitochondrial respiration and oxidative phosphorilation, releasing energy in the form of heat. The UCP2 isoform, up-regulated in stress condition, is involved in the control of ROS production during the mitochondrial metabolism. Likewise, the mitochondrial superoxide dismutase 2 (SOD2) is particularly expressed in stress condition, to deplete mitochondrial superoxide anion. In this study, we decided to investigate, in presence of saturated fatty acids and *p,p*-DDE, the main metabolite of the hydrophobic pesticide DDT, the involvement of these proteins in the adaptive mechanisms activated in rat liver for the control of ROS production and for the attenuation of damages caused by them. Male Wistar rats were exposed to DDE (10 mg/kg body weight) and saturated fats (45%) daily for 4 weeks, either individually or in combination. 4 experimental groups were performed: 1, control; 2, saturated fats; 3, saturated fats in combination to DDE; 4, DDE-treated). First of all, our results show the increase of mitochondrial H_2O_2 in all groups vs control; lipid peroxidation was also observed in presence of DDE, either alone or in combination with the saturated fats, thus indicating a role of DDE in the oxidative stress. In these stress conditions, data obtained by immunohistochemistry and Western blot analysis demonstrate the up-regulation of both UCP2 and SOD2: the first causing protonic dissipation, to limit the anion superoxide formation; the latter, as a critical component involved in O_2^- reduction. In this way it is verified the conversion of O_2^- in H_2O_2 which will be partially eliminated by peroxidase enzymes (GPXs). In conclusion, our data demonstrate that both UCP2 and SOD2 functions are indirectly and directly involved in the control of mitochondrial ROS formation, taking part to the preservation of cellular function in liver.

NUCLEAR TRANSLOCATION OF THE METALLOTHIONEIN UNDER STRESS CONDITIONS: EFFECTS OF HIGH FAT DIET AND p,p-DDE IN RAT TISSUES

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Metallothioneins (MTs) are a small group of proteins involved in different cellular functions. Primarily engaged in the transport, binding and recycling of both essential and toxic heavy metals, they also exhibit a powerful free radical scavenging activity. MT functions are strictly correlated with its sequence: MT shows 20 cysteine residues (30% of total amino acids)

involved in the metal coordination and/or in electron-donor during oxidation reactions with pro-oxidant species. Firstly characterized as a cytoplasmic protein, MT can be localized in the nucleus, in response to cellular events such as proliferation or intracellular oxidation. It has been proposed that MT protects the nuclei from the oxidation occurring with progression of the cell cycle.

In this study, we investigated MT expression and synthesis in liver and kidney of Wistar rats fed for 4 weeks with DDE in combination with standard or high fat diet (HFD), that promotes DDE accumulation in lipid deposits and increases oxidative stress.

Animals were divided into 4 groups: N (standard diet); D (HFD); D+DDE (HFD + DDE 10 mg/Kg body weight); N+DDE (standard diet + DDE 10 mg/Kg b.w.). Real-Time PCR analysis indicated, in liver, a down regulation of MT gene in all groups *vs* N, in particular in D and D+DDE groups. On the contrary, in the kidney a down regulation was observed only in D group, whereas in both DDE-treated groups MT expression was up-regulated. These data were confirmed by IHC and western blot analysis. IHC analysis also suggested an increase in nuclear positivity in the presence of DDE in both tissues, subsequently confirmed by western blot on cytosolic and nuclear extracts. In conclusion, we demonstrate that, independently to transcriptional regulation, in presence of pro-oxidant substances the MTs translocate into the nucleus. These data indicate a possible protective effect of MT towards oxidative DNA damage or an ancillary role in the transfer of metals from the cytosol to the nucleus, to ensure the functioning of the transcription apparatus and the correct protein turnover.

THE ROLE OF N-CADHERIN AND NOTCH1 EXPRESSION IN THE PROGRESSION OF CUTANEOUS MELANOMA

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Epithelial-mesenchymal transition (EMT) has been suggested to have a driving role in the acquisition of a metastatic potential by melanoma cells.[1] Important hallmarks of EMT include both E-cadherin downregulation and increased expression of N-cadherin. This switch in distinct classes of adhesion molecules leads melanoma cells to lose contact with adjacent keratinocytes and interact instead with stromal fibroblasts and endothelial cells, thus promoting dermal and vascular melanoma invasion. This allows tumor cells to migrate to distant host tissues and establish metastases.[2] A key regulator in the induction of EMT in melanoma is the Notch1 signaling pathway that, when activated, is prompt to upregulate N-cadherin expression. Consequently, melanoma cells gain enhanced survival, proliferation and invasion properties, driving the tumor toward a more aggressive phenotype.[3] In the present study, we investigated the expression of N-cadherin by immunohistochemical analysis in tissue samples from primary cutaneous melanomas and lymph node metastases and found a significant association between N-cadherin and Notch1 presence in the same tumor samples. Moreover, we demonstrated that a high expression of N-cadherin and Notch1, both in primary lesions and in lymph node metastases, predicts an adverse clinical outcome in melanoma patients, suggesting N-cadherin and Notch1 co-presence as a predictive factor in early and advanced stage melanomas.

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NUCLEAR LOCALIZATION OF PKCα IS ASSOCIATED WITH CELL CYCLE ARREST AND ERYTHROID DIFFERENTIATION IN DEL(5Q) CELLS DURING LENALIDOMIDE TREATMENT

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PI-PLCβ1 is involved in cell proliferation, differentiation and MDS pathogenesis. Moreover, the increased activity of PI-PLCbeta1 reduces the expression of PKCα that, in turn, delays the cell proliferation and is linked to erythropoiesis.[1,2] Lenalidomide is currently used in del(5q) low-risk MDS patients, where it can suppress the del(5q) clone and restore a normal erythropoiesis.[3] Here we studied the effect of Lenalidomide on 16 low-risk del(5q) MDS patients, del(5q) and non-del(5q) cell lines, mainly focusing on erythropoiesis, cell cycle and PI-PLCβ1/PKCα signalling. Overall, 11 subjects were clinically evaluable: ten cases (90%) showed a favorable response (5 CR, 3 HI-E and 2 HI-E with cytogenetic response), and the remaining case had a stable disease. At a molecular level, both responder patients and del(5q) cells showed a specific induction of erythropoiesis and a nuclear translocation of PKCα. Moreover, Lenalidomide could induce a selective G0/G1 arrest of cell cycle in del(5q) cells, slowing the rate proliferation of this cell clone. Altogether, our results could not only better explain the role of inositide-dependent signalling in erythropoiesis, but it could also lead to a better comprehension of the Lenalidomide effect on del(5q) MDS and pave the way to innovative targeted therapies.

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SARCOGLYCAN COMPLEX IN HUMAN TRACHEAL TISSUE: AN IMMUNOFLUORESCENCE STUDY

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The sarcoglycan complex (SGC) in made up by transmembrane glycoproteins, α-, β-, γ-, δ-sarcoglicans, and as a part, by dystrophin-glycoprotein complex (DGC). The DGC also includes dystrophin, dystroglycan (α- and β-) and syntrophins; the interaction of the DGC with components of the extracellular matrix may have an important role in force transmission and sarcolemma protection. Moreover, α-dystroglycan appears to have a core protein consisting of two roughly globular domains connected by a segment, which most likely corresponds to a mucin-like central region.[1] Several studies also demonstrated that sarcoglycans, which are involved in stabilization of sarcolemma during muscle contraction, are expressed in some epithelial tissues as gingival, breast and prostatic one.[2] The aim of our study is to demonstrate the expression of sarcoglycans in tracheobronchial epithelial tissue, related with the presence of mucins. Mucins, which are a superfamily of highly glycosylated proteins, are the main constituent of mucus. In the conducting airways mucus is produced by the tracheobronchial glands and it plays a key role in maintaining the health of epithelial tissues. Biochemical studies shows that human tracheobronchial gland mucous (HTMG) cells secreted typical airway mucins, and immunohistochemical studies showed that these cells expressed different isoforms of MUC, especially those of our interest: MUC4 and MUC16.[3] We performed an immunofluorescence reaction, using antibodies against β-sarcoglycan and against MUC4. To avoid any possible fluorescence interference between the primary antibody and the mucus gel the sample was pre-treated following a mucolytic protocol (N-acetylcystein 10% in Phosphate buffer 0.2 M).

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MESENCHYMAL STEM CELLS IN HIDRADENITIS SUPPURATIVA: A NEW PIECE OF THE DISEASE MOSAIC

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Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease. It generally involves the apocrine gland-bearing area of the body and induces deep painful lesions, often associated with other physical and psychosocial comorbidities. Onset of HS is caused by an early occlusion of follicle, followed by dilatation of pilosebaceous unit, rupture and release of follicular contents into the dermis. Then, an inflammatory state is established, as demonstrated by the various immunological abnormalities found in skin lesions. Mesenchymal stem cells (MSCs) were successfully isolated from different tissues, included skin. Researches support the hypothesis of their early involvement in various skin diseases, such as psoriasis and atopic dermatitis. However, the role of MSCs in HS has never been evaluated. In this study, MSCs were isolated from lesions of patients affected by HS (HS-MSCs) and characterized. Their immunological profile was examined by assessing the levels of twelve cytokines related to Th1, Th2 and Th17 pathways on conditioned medium by ELISA array. Inflammatory activity of HS-MSCs was also studied by immunocytochemistry. MSCs isolated from healthy subjects were used as controls. HS-MSCs revealed an over-expression of most of the molecules analyzed, demonstrating the activation of MSCs in the disease towards a pro-inflammatory state. Even more, the fact that these same cytokines play a role in HS, as demonstrated by previous studies, strengthens the hypothesis of MSCs contribution in disease onset and progression.

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PROGESTERONE RECEPTOR MEMBRANE COMPONENT 1 (PGRMC1) EXPRESSION IN CANINE MAMMARY TUMORS

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Canine mammary tumors (CMT) are the most common neoplasms in female dogs and histopathological examination is the gold standard for their diagnosis and classification.[1] Recently, PGRMC1 has been considered a putative biomarker for diagnosis and prognosis in human breast cancer, which shares some common features with canine counterpart.[2] This study represents the first description of PGRMC1 expression in CMT.
First, we evaluated PGRMC1 expression by immunohistochemistry in three major histopathological types: normal/hyperplastic tissue, simple adenomas and simple adenocarcinomas. Epithelial cells showed positivity to PGRMC1 and we applied a scoring system considering the percentage of positive cells and the intensity of expression defined as A (weak), B (moderate) and C (strong). Normal/hyperplastic sample showed almost 100% of positive cells and a strong intensity of PGRMC1 expression. The same features were present in adenomas but with a more variable intensity. In adenocarcinomas, the percentage of positive cells was lower (30-60%) and the intensity was weak in tubular parts, while both features became progressively negative in the solid parts of the tumor. Western blot analysis in healthy and neoplastic mammary tissue biopsies of dogs undergoing surgery revealed the presence of the 25 kD PGRMC1 band in both types of tissue. Further investigations are in progress to determine differences in protein level according to the 3 different major types of tumors. Moreover, PGRMC1 will be assessed in blood samples of dogs affected by different types

of CMT to evaluate if it could represent a prognostic serum biomarker as previously demonstrated in human lung and kidney cancer.[3]

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CONTRIBUTION OF miR-145-5p/Ago2 COMPLEX TO THE REGULATION OF EPITHELIAL-MESENCHYMAL TRANSITION

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The epithelial-mesenchymal transition (EMT) is essential for cell fate determination during development but it is involved in pathological processes like cancer as well, being one of the first steps in the mechanisms leading to metastasis. miR-145-5p is one of the most widely recognized tumor-suppressor miRNAs, able to regulate cell migration and EMT through the contribution of the RISC complex in which Argonaute (Ago) proteins are required for target recognition and gene silencing.[1] Ago2 is an important member of the Ago family and its overexpression correlates with a transformed phenotype in breast cancer cells.[2] With the aim to unravel miR-145-5p/Ago2 contribution to the suppression of cancer progression in epithelial tumors, here we show that: i) miR-145-5p and Ago2 are down-regulated in breast tumor *vs* normal tissues; ii) the restored expression of miR-145-5p in breast cancer cell lines results in the reduction of tumor phenotype; iii) Ago2 expression is positively and specifically regulated by miR-145-5p; iv) miR-145-5p-dependent Ago2 induction is necessary for the inhibition of cell migration; v) when Ago2 is depleted, the formation of an alternative miR-145-5p/Ago1 active complex redirects miR-145-5p tumor suppressor function and correlates with a more invasive phenotype in breast cancer cells. These results open to the identification of miR-145-5p/Ago2-dependent molecular networks involved in the maintenance and progression of cancer phenotype.

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SARCOGLYCAN SUB-COMPLEX IN CARDIAC MUSCLE OF PATIENTS DECEASED FOR SEPSI

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The sarcoglycan sub-complex (SGC), member of the DGC, is made up of six subunits of transmembrane glycoproteins named α-, β-, γ-, δ-, ε-, and ζ-sarcoglycan (SG). This complex is localized in the sarcolemma of skeletal muscle fibers and its function is to connect the extracellular matrix to the cytoskeleton playing a key role in stabilizing muscles and their sarcolemma during contraction and relaxation. Clinical studies have shown that myocardial contractility is reduced in severe sepsis which is a life-threatening organ dysfunction which prevalence has increased significantly in recent decades. It has been shown that in 40% of patients with sepsis and cardiac dysfunction the range of mortality is of 70-90% if compared to the 20% mortality in septic patients without cardiovascular involvement. Recent studies support that an increased plasma membrane permeability in cardiac fibers, could be responsible for sepsis-induced myocardial dysfunction; they have also shown a decreased expression of some DGC proteins, suggesting a role of DGC in the pathogenesis of sepsis induced cardiomiophaty. The critical importance of the SGC in maintaining sarcolemmal stability led us to hypothesize that even these proteins could be involved in sepsi-induced cardiomyopathy. The aim of the present work was to analyze the expression of sarcoglycans in human cardiac muscle samples from deceased patients because of sepsis, with cardiovascular involvement. By histological and immunofluorescence techniques we obtained results which show critical structural alteration of cardiac fibers sarcolemma and that the expression of β-, γ- and ε-sarcoglycans, but not the α-sarcoglycans, is reduced. We hypothesize that members of the sarcoglycans sub-complex could be involved in structural alteration of sarcolemma, leading to sepsis induced cardiomiophaty, even if the pathogenetic mechanism are still unclear.

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EPITHELIAL-TO-MESENCHYMAL TRANSITION: ROLE OF GLYCOGEN SYNTHASE KINASE-3β AND PPAR-γ IN DSS-INDUCED COLORECTAL FIBROSIS

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Intestinal fibrosis is a common complication of Inflammatory Bowel Disease characterized by an excessive deposition of extracellular matrix proteins producted by activated myofibroblasts[1] that are derived not only from resident fibroblasts but also from dedifferentiation of epithelial cells as a result of epithelial to mesenchymal transition (EMT).[2] Aim of this study was to evaluate the in vivo expression of regulatory molecules like TGF-β, Smad3, E-cadherin, Snail, ZEB1, β-catenin, and GSK-3β after the administration of new PPARγ modulator in an experimental mice model of intestinal fibrosis induced by administration of DSS. Chronic colitis and fibrosis were induced by oral administration of 2.5% DSS (w/v) for 6 weeks. GW9662 (GW), a selective PPAR-γ inhibitor, was also administered by intraperitoneal injection at the dose of 1 mg/kg/day combined with GED treatment. 65 mice were randomly divided into five groups (H2O as controls n=10, H2O+GED n=10, DSS n=15, DSS+GED n=15, DSS+GED+GW n=15). Histological, morphometric, immunohistochemical, western blot and Real Time PCR analyses, using TGF-β, Smad3, E-cadherin, Snail, ZEB1, β-catenin, GSK-3β and PPAR-γ antibodies, were performed. Daily oral administration of GED significantly reduced the main markers of fibrosis, represented by α-SMA, collagen I-III, and fibronectin, as well as the pro-fibrotic IL-13, TGF-β and Smad3, while increased PPAR-γ. Ged restored the EMT markers, such as E-cadherin and β-catenin, reduced the levels of nuclear Snail and ZEB1, and upregulated GSK-3β. These effects, induced by GED treatment, were reverted by the concomitant administration of the irreversible PPAR-γ inhibitor GW. These results suggest that GED was able to regulate the expression of molecules involved in the development of intestinal fibrosis and in EMT, modulating the levels of E-cadherin and β-catenin and upregulating GSK-3β.

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