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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 till 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 now 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), and has been an influential cytology journal for over 60 years, publishing research articles on functional cytology and histology in animals and plants.

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Coverage extends to:
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- biology of cell development and senescence;
- nerve and muscle cell biology;
- cellular basis of diseases.

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MAIN LECTURES

COMBINED CELL AND GENE THERAPY FOR EPIDERMOLYSIS BULLOSA

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LAMB3-dependent generalized Junctional Epidermolysis Bullosa (JEB) was targeted by transplantation of epidermal cultures originated from transgenic epidermal stem cells. We report life-saving regeneration of the entire epidermis on a seven-year-old JEB child suffering from a devastating form of JEB. The regenerated transgenic epidermis remained stable throughout the entire follow-up period and did not form blisters, even upon shear force. The proviral integration pattern was maintained *in vivo* and epidermal renewal did not cause any clonal selection. Clonal tracing showed that the human epidermis is sustained by a limited number of long-lived stem cells, detected as holoclones that can extensively self-renew and produce short-lived progenitors that replenish terminally differentiated keratinocytes. In studying the different behaviour of JEB and *COL7A1*-dependent generalized Dystrophic EB (RDEB) cultures we discovered a pivotal role of YAP in sustaining human epidermal stem cells, which explains the progressive stem cell loss observed in JEB. Epidermal stem cell depletion of primary JEB keratinocytes is due to perturbation of the YAP/TAZ pathway. YAP/TAZ expression is significantly decreased in JEB keratinocytes, which do not contain nuclear YAP but only phosphorylated, inactive YAP. The JEB phenotype is recapitulaled by Laminin 5 ablation and consequent YAP/TAZ down-regulation in normal cells. Restoration of adhesion properties by Laminin 5-gene therapy rescues normal nuclear levels of YAP/TAZ and clonogenic potential. Enforced YAP recapitulates Laminin 5-gene therapy in JEB cells, thus uncoupling adhesion from proliferation in epidermal stem cells. This work has important clinical implication for an efficient *ex vivo* gene therapy of JEB.

CORRELATIVE ELECTRON MICROSCOPY IN MODERN BIO-MEDICAL RESEARCH

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Correlative light–electron microscopy (CLEM) is a very effective technique that combines live-cell imaging and electron microscopy for ultrastructural morphological characterization of dynamic intracellular organelles[1,2]. The use of fluorescent protein (FP)-tagged chimeras allows the user to follow the movements and/or behaviour of intracellular structures in a live cell and to fix it at the moment of interest. The subsequent immuno-electron microscopy processing can then reveal the three-dimensional architecture of the same structure, together with precise recognition of the FP-labelled protein[1-3]. The process resembles the taking of a high-resolution snapshot of an interesting live scene. The power of this approach opens new avenues for understanding of complex cellular processes that operate in health and disease.

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EVOLUTION OF RETINOIC ACID SIGNALING FUNCTIONS IN DEVELOPMENT

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Retinoic acid (RA) is a vitamin A-derived morphogen controlling important developmental processes in vertebrates, and more generally in chordates, including axial patterning, organogenesis, and cell differentiation. In the chordate embryo, endogenous RA levels are controlled by RA synthesizing and degrading enzymes, and the RA signal is transduced by two retinoid receptors: the retinoic acid receptor (RAR) and the retinoid X receptor (RXR). Both RAR and RXR are members of the nuclear receptor superfamily of ligand-activated transcription factors and act as heterodimers to activate the transcription of target genes in the presence of their ligand, all-*trans* RA. RA signalling was long thought to be a chordate innovation. However, the identification, in a number of different non-chordate taxa, of homologs of genes encoding the main components of chordate RA signalling challenged this view and suggested that the RA signalling system might have a more ancient evolutionary origin. Furthermore, recent experimental evidence indicates that RA signalling might be active during development of animals other than chordates. Here, I will present some of our work on the characterization of RA signalling functions in different animal lineages, non-chordate and chordate. In an attempt to retrace the evolutionary history of RA signalling, I will discuss the obtained results in a comparative context. Taken together, the presented data will reveal novel insights into the origin of the RA signalling pathway as well as into the evolutionary history of retinoid receptors.

ABSTRACTS

BEHAVIOURAL RESPONSE TO THE INCREASE OF ENVIRONMENTAL TEMPERATURE IN ADULT ZEBRAFISH (*Danio Rerio*)

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Anthropic activities are causing severe damages on the natural environment from climate change to habitat destruction. The animal's first response to natural and anthropic environmental changes is behavioural and therefore behaviour can be considered a link between physiological and ecological processes[1]. Water temperature is an important environmental parameter influencing the distribution and the health of fish and it plays a central role in ectothermic animals affecting their physiology and behaviour[2]. Zebrafish (*Danio rerio*) is a poikiloterm and euriterm cyprinid that in the natural environment is subject to seasonal and daily thermal fluctuations and it could be a good model to study the effects of the water temperature change on the central nervous system and animal behaviour. Our group demonstrated that housing adult zebrafish in cold (18°C) and warm (34°C) water for 21 days strongly affects the brain proteome and fish behaviour[3]. This work aims to further investigate the effect of increasing water temperature in adult zebrafish maintained for 21 days at 26°C (control) and 34°C (treatment) on anxiety, social preference and aggressive behaviour. The results confirm that heat treatment alters the behaviour of the zebrafish that spends more time in potentially risky environment such as the top area, the uncovered and bright area and zones far from the social group. This behavioural alteration in the wild could expose the animal to an increase in predatory attacks and reduce its survival rate.

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MORFOLOGICAL AND MOLECULAR SIGNATURES OF CHORDATE DEVELOPMENT: INSIGHTS FROM THE TUNICATE *Botryllus schlosseri*

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Although studies have identified conserved aspects of organogenesis across and within phyla, it is unknown whether and how organogenesis differs during sexual and asexual development in the same species and if a convergent morphology implies convergent molecular mechanisms. Thanks to its ability to reproduce both sexually and asexually, *Botryllus schlosseri,* a colonial tunicate, provides a key for answering these questions. Sexual and asexual reproductions are characterized by different starting points (a zygote in embryogenesis and a bud from stem cells in blastogenesis), but resulting in almost identical individuals having the same body plan and producing the same organs and tissues. By combining transcriptome sequencing with confocal, two-photons, and electron microscopy, and digital 3D reconstructions from histological serial sections of major embryonic and blastogenic developmental stages, we characterized the molecular and morphological signatures along both developmental pathways. Moreover, with tissue and cell-type specific molecular signatures, we identified the developmental origin of nervous system, endostyle, blood cells, and germ cells, and followed their morphogenesis, cytodifferentiation, and gene expression along development. This study outlines the molecular and morphological landscape of the two developmental modes and demonstrates that different molecular paths can lead to the same outcome.

PPARβ/δ δ ANTAGONISM COUNTERACTS 6-OHDA NEUROTOXICITY IN DOPAMINERGIC-LIKE NEUROBLASTOMA CELLULAR MODEL

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Among the proposed mechanisms underlying Parkinson disease (PD), free radical damage is believed to play a pivotal role in the development and/or progression of the disease. Recently, PPARs, a class of transcription factors, have been linked by us and others to neurodegeneration. The detrimental role of PPARβ/δ together with the down-regulation of mBDNF/TrkB pathway was proposed in Alzheimer and Parkinson[1, 2]. These pathological conditions are closely related to the abnormal increase of 4-HNE protein adducts. Since the 4-HNE is recognized as intracellular PPARβ/δ agonist, blocking the PPARβ/δ-4-HNE interaction under neurotoxic conditions could be useful for cellular health. On these bases, in this work we used differentiated SH-SY5Y cells exposed to 6-OHDA to obtain a valuable PD *in vitro* model. This cellular model was exposed to a specific PPARβ/δ antagonist (GSK0660). The 6-OHDA expositions determined a significant increase of cellular death, while in combination with the GSK0660 cell viability was restored. In this regard we observed an up-regulation of the mBDNF/TrkB/CREB survival pathway, contrariwise the protein levels of the cleaved caspase 3 and 9 were significantly decreased. These events were associated with a decrease of 4-HNE protein adduct levels in cells exposed to 6-OHDA and GSK0660 compared to cells treated only with 6-OHDA. In addition, the expression of 47 genes directly or potentially involved in PD etiology was assayed by TaqMan gene expression array cards. GSK0660 in 6-OHDA treated cells was able to up-regulate genes related to mitochondria functions, ubiquitin proteasome system and BDNF pathway. Moreover, PPARβ/δ silencing by siRNA reduced 6-OHDA neurotoxic effects in our *in vitro* model. In summary, PPARβ/δ antagonist displays neuroprotective activity against 6-OHDA toxicity.

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HUMAN NEUROEPITHELIAL STEM CELLS TO UNCOVER TORCH-RELATED MICROCEPHALY

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After the recent Zika virus (ZIKV) outbreak in Latin and Central America, there is an urgent need to investigate the molecular mechanisms of microcephaly-inducing pathogens of the TORCH (Toxoplasma, Others, Rubella virus, Cytomegalovirus and Herpes simplex virus) group. Remarkably, TORCH-infection results in severe perinatal cortical malformations, including microcephaly, and in up to 50% of abortion cases around the world. In the present research, we aim to investigate the molecular and cellular events during the infection from TORCH viruses, by employing an innovative population of human neural stem cells, called neuroepithelial stem (NES) cells[1], and their neuronal progenies. NES cells are long-term, self-renewing human neuropotent stem cells forming neural rosettes reminiscent of the radial arrangement and apico-basal polarization established by neuroepithelial cells in the native neural tube. NES cells can be derived from both early developing nervous system and induced pluripotent stem (iPS) cells. Select TORCH viruses (*e.g.*, Herpes simplex Virus 2, Coxsackie B virus, Human Cytomegalovirus) were tested for their ability to infect NES cells and their neuronal progenies. We investigated cytopathic effects, cell cycle dysregulation and cell death induction at different times from infection. We also focused our attention on centrosomes and pTBK1, a kinase involved in several physiological cellular pathways, including antiviral innate immune response and cell cycle progression. We previously found that ZIKV infection produces supernumerary centrosomes and disrupts pTBK1 localization from centrosomes to mitochondria thus impairing cell cycle progression and inducing cell death[1]. Thus, NES cells provide a platform to explore neurodevelopmental human conditions and may help in deciphering the pathogenetic mechanisms of cell damage and death resulting from viral infection.

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ROLE OF PACAP CHARGED gH-625 LIPOSOME AS DELIVERY IN CENTRAL NERVOUS SYSTEM AND ITS NEUROPROTECTION EFFECTS

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a peptide of secretin/glucagon hormones. The members of this family have numerous functions thanks to the interaction with its receptors. The widespread distribution of PACAP and its receptors indicates that it could exert physiological actions. It acts as a neurotransmitter and neuromodulator in central nervous system. The disadvantage of PACAP is short half-life in the bloodstream and its passage to the cerebral parenchyma is prevented by the presence of the blood-brain barrier (BBB). Thanks to restrictive action and high selectivity of BBB, many drugs fail to cross it and are pumped out. For this reason, it has been developed a nanodelivery system, which increases PACAP half-life and involves a liposome, conjugated to peptide gH625. This peptide derives from the glycoprotein H of the *Herpes simplex* virus type 1 and is able to perturb the double layer of the membrane without break it. Moreover, it has been seen to be able for the transport of drugs. In this study we evaluated the efficiency of gH625-liposome loaded with PACAP when crossing a dynamic BBB model *in vitro* in order to highlight the difference with liposome loaded with PACAP. We used a bioreactor, LiveBox2, consisting of a double chamber with double independent flows and separated by a porous membrane; bEnd3 cells endothelial cells of the mouse brain were used to form the cerebral endothelial barrier on the top of the membrane in the upper chamber. After 30 minutes of treatment we have seen an increase of gH625 PACAP in the outlet of the lower chamber compared to lipoPACAP. Furthermore, we evaluated the effect of PACAP in SH-SY5Y differentiated cells treated with different concentrations of 1-metil-4phenilpyridine (MPP+) to induce a neurodegeneration effect. After 24h of exposure to MPP+ and PACAP, we observed a neuroprotection effect of PACAP at a concentration of 10^{-7} M. Our results shown that gH625-lipoPACAP is more efficient to cross BBB compared to lipoPACAP and PACAP can exert a neuroprotective effect on SHSY-5Y cells.

EXTRACELLULAR VESICLES RELEASED BY MOUSE MESOANGIOBLAST STEM CELLS ARE ABLE TO INFLUENCE BOTH MACROPHAGE AND T LYMPHOCYTES BEHAVIOUR

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Stem cells release large amount of EV that are involved in immune cell regulation. The aim of our work was to determine the effect (in terms of cell proliferation, migration ability and phagocytic capability) of EV released by mesoangioblast stem cells (A6) on T lymphocytes (Jurkat cells) and macrophages (RAW 264.7), as these two cell types could influence tissue regeneration. By incubating A6-EV with Raw264.7 cells we found that EV inhibited macrophage proliferation. Indeed, cell proliferation curve showed that cells treated with A6-EV have a proliferation index significantly reduced than untreated cells. We have also evaluated the possible EV involvement on macrophage migratory phenotypes. By performing wound healing assays we observed that Raw264.7 treated cells showed increased migration when compared to non-treated cells. In the tissue microenvironment, macrophages migrate to carry out their function of recognising and eliminating dead cells, debris, and foreign particles via phagocytosis. These functions can be modulated by several stimuli. As we found that EV effects macrophage migration, we next evaluated their action on macrophage phagocytosis. *In vitro* phagocytosis index calculation highlighted that EV increase Raw264.7 phagocytic capacity. In our previous paper we have already demonstrated that A6-EV contain Hsp70 as a transmembrane protein[1] and it is well known from literature data that this protein is able to stimulate macrophagic activity. To elucidate the possible Hsp70 involvement on phagocytosis, we performed phagocytosis assays in the presence of neutralizing antibodies (*i.e.*, anti-Hsp70, anti-TLR2, anti-TLR4 antibodies), or in the presence of exogenous Hsp70. The obtained results demonstrated that A6-EV increase phagocytosis through Hsp70 and its surface receptors. Preliminary results also demonstrated that A6-EV also influenced Jurkat cell behaviour. It seems that A6-EV induce Jurkat cells to increase the expression of the surface marker CD193, a protein which is also expressed on Th2 cells. In conclusion, our data indicate that A6-EV influences immune system cells.

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PRIORITIZATION OF CHEMICAL COMPOUNDS WITH THE ABILITY TO DISRUPT REPRODUCTION BY COMPUTATIONAL TRANSCRIPTOMICS: A TWO CASE STUDIES

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Thousands of chemicals used worldwide have the potential to harm reproduction and toxicity. information for most of these compounds are currently lacking. However, due to experimental costs and time and in agreement with the requirement of the 3Rs framework, it is not practical to evaluate toxicity for all the compounds to which humans and ecosystems are potentially exposed. Developing alternative approaches with the ability to prioritize harmful compounds is of paramount importance. Here, system biology approaches were employed to characterize the dynamics underlying ovarian development in two non-model fish species, the swordfish (*Xiphias gladius*) and the Largemouth bass (*Micropterus salmoides*). By leveraging the power of advanced computational approaches, we identified genes likely to play a key role towards maturation that were used to interrogating the Comparative Toxicogenomic Database (CTD) in order to identify compounds with the potential to affect ovary maturation. Chloropicrin and Cyclosporine in the swordfish and Quercetin and Tretinoin in the Largemouth bass were identified as the best candidate compounds with the ability to disrupt ovarian maturation. In Largemouth bass, predictions were validated by qPCR in both ovary and liver confirming the harmful effect of these compounds on reproduction. These studies demonstrate that by utilizing computational approaches and online knowledge bases to understand the underlying molecular response of organisms, it is possible to identify putative chemical candidates that may impact reproductive health. This approach is highly relevant for classifying chemicals prior to conducting risk assessments, and we propose that this is a viable approach for chemical prioritization, reducing animal numbers, and developing safer chemicals in the public domain.

DIFFERENT MORPHOLOGICAL APPROACHES TO COMPARE HEALTHY AND PATHOLOGICAL HUMAN KNEE JOINT STRUCTURES

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Human meniscus plays a crucial role in knee load transmission and distribution, shock absorption, joint stability, lubrication and congruity[1], while cartilage is a smooth tissue promoting articular strands sliding. The aim of this work is to compare healthy (control) and pathological joint structures. We evaluated the ECM component, the appearance and distribution of calcification areas, and the modifications in the different experimental condition by means of histological and ultrastructural observation. Control menisci and cartilage cells occasionally show small condensed chromatin masses and well preserved organelles. Both in trauma and in osteoarthritis (OA), increasing chromatin condensation, organelle degeneration and cytoplasmic vacuolization can be observed[2]. In pathological conditions, particularly in OA, autophagic vacuoles, also appear. In this case a high chromatin condensation, a diffuse cytoplasmic vacuolization with degeneration of organelles and several necrotic cells can be observed, especially in elderly subjects. Calcification areas occur both in traumatic and menisci and cartilage. In particular, traumatic menisci appear similar to osteoarthritic ones, especially in adult subject. In both, disorganization and loss of collagen fibers and reduction of collagen fibers size can be also observed, if compared to control condition. Both in traumatic and OA subjects, we observed apoptotic-like features[3]. We can conclude that trauma might induces an increasing meniscal and cartilage degeneration, comparable to physiological aging, and also to development or OA progression.

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A COMPARATIVE VIEW ON SEXUAL DEVELOPMENT GENES IN BASAL SARCOPTERYGIANS

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The process of sexual development involves a high variety and fine-tuning of complex mechanisms, regulating gametogenesis, molecular sex determination and differentiation. The collection of information from basal sarcopterygians, coelacanths and lungfishes, given their phylogenetic position, is crucial to improve our understanding of the molecular evolution of pathways involved in reproductive functions. Despite the generality of the phenomenon itself the mechanisms how sex is determined are very different among various taxa, have evolved repeatedly and independently across metazoans and the underlying molecular pathways can change quickly during evolution. We analyzed the transcriptomes of *Latimeria menadoensis* and *Protopterus annectens* to characterize fifty genes related to sex differentiation and gametogenesis and we evaluated their expression in gonads and several other organs. We find that the intermediate position of lungfish between actinopterygians and tetrapods is also reflected on the level of gene expression profiles related to sexual development. However the phylogenetic position and the presence/absence patterns often reveal a closer affinity to the tetrapod orthologs.

INFLUENCE OF 3D NANOSTRUCTURED SCAFFOLD ON hASC BEHAVIOR

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In the field of stem cell-based therapies, human Adipose derived Stem Cells (hASC) are a very interesting model for their relative abundance in adipose tissue, easy isolation, high self-renewal potential and low immunogenicity. It is know that hASCs, if associated with a biocompatible scaffold, play an important role in induce new vascularization. In 2016, Cherubino et al.[1] demonstrated that hASCs, associated with a 3D collagen scaffold, INTEGRA® Flowable Wound Matrix (FWM), and implanted in nude mice, were able to promote the formation of new vessels although, after scaffold removal, no hASCs were found. Based on these results, the purpose of this study was to evaluate the effect

of FWM on hASCs by *in vitro* experiments. Our findings indicated that, despite the hASCs established interaction with the FWM through the formation of pseudopodia-like structures, in the 3D scaffold cells survival was limited. qPCR and ELISA evaluation showed that hASCs grown in absence of the scaffold maintained their stemness and the expression of angiogenic markers was higher compared to the cells grown in the scaffold. Besides, the interaction with FWM seemed to lead hASC toward adipogenic differentiation. In conclusion, our study suggest that, *in vitro*, hASCs are less efficient to induce angiogenesis when are associated with the FWM. However, *in vivo,* the presence of 3D scaffold is fundamental to guarantee a suitable cell niche. Hence, a possible solution may be to use the FWM together with the hASCs conditioned medium, which contain the growth factor able to induce angiogenesis.

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HIGH-SPEED TWO-PHOTON FLUORESCENCE FUNCTIONAL IMAGING OF CORTICAL AND SUBCORTICAL REGIONS

S. Bovetti[1,3], C. Moretti[1], S. Zucca[1], M. Dal Maschio[1], P. Bonifazi[2], T. Fellin[1]

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Two-photon laser scanning fluorescence microscopy allows imaging the activity of extended cellular networks at high spatial resolution within largely scattering tissue, such as the mammalian brain[1-3]. However, scanning microscopy is intrinsically limited in its time resolution by the sequential illumination scheme. In this talk, I will present a scanless two-photon microscope allowing the detection of the suprathreshold activity of neurons monitored using the genetically encoded calcium indicator GCaMP6, with subcellular spatial resolution and high temporal resolution (up to 1 kHz). When coupled to the use of GRIN lens-based endoscopes, we demonstrate the scanless approach allows performing deep brain imaging at high temporal resolution. Moreover, the scanless approach can be efficiently combined with single-photon optogenetic manipulation for all-optical manipulation and fast readout of neural activity. As proof-of principle, we validate this approach in anesthetized mice *in vivo* investigating the role of a specific class of interneurons, the PV-positive cells, in the modulation of the spatial and temporal profile of network dynamics in superficial cortical layers.

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MOLECULAR MARKERS FOR THE STUDY OF NEUROGLIA IN THE DEVELOPING AMPHIOXUS

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Neuroglia is an important cell type in the nervous tissue of bilaterians but neglected in comparative studies. Amphioxus, the most basal split in the chordate tree, is a promising reference point to determine how the glial organization of vertebrates came to be. Previous work[1] shows that amphioxus has different glial cells but these are not clearly homologous in morphology to specific vertebrate cell types. Therefore, molecular characterisation of glial cells in the developing amphioxus is necessary to study neuroglia evolution in the chordate lineage. We cloned orthologs of the intermediate filament protein *GFAP*, the amino acid transporters *EAAT*, the glycine transporters *GlyT*, the enzyme *glutamine synthetase*, and the oligodendrocyte transcription factors *olig*, which are markers for different vertebrate glial cells. Then, we characterised their expression patterns by single and double in situ hybridisation, to test co-expression of putative glial and established neuronal markers. Amphioxus-specific gene duplication events complicate the attribution of orthology with vertebrate genes. Two *GFAP-like* genes are expressed in distinct dorsoventral domains along the neural tube of the late neurula. In particular, one of them is detected dorsally in cells negative for neuronal markers and might be either glial cells or neural progenitors, while the other is found in the somites and some ventrolateral cells of the neural tube expressing the neuronal marker *synapsin*. In the CNS, *EAAT* is only detectable in a few cells that also co-express *synapsin*, while *glutamine synthetase* is highly expressed in the anterior cerebral vesicle. In contrast with vertebrates, the amphioxus *GlyT1.2* is widely expressed in glycinergic neurons, while *GlyT2.1* seems to be expressed by some glial cells, besides somites and glycinergic neurons. Finally, *olig* genes are expressed in undifferentiated cells of the posterior neural tube and discrete anterior cell clusters. Double staining with the motor neuron marker *VAChT* suggests that *oligs* are implicated in motor neuron differentiation, like in vertebrates, but it is unclear whether they are also implied in glia specification.

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ALBEDO AND FLAVEDO FROM "*LIMONCELLA OF MATTINATA*": A NEW POTENTIAL SOURCE OF ANTIOXIDANTS

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Limoncella is the common name of a fruit tree of Rutaceae family, a rare and ancient Mediterranean variety of *Citrus* genus. The fruit is composed of flavedo (10-20%) and pulp (35-40%). Albedo is the spongy white layer underlying flavedo, a source of flavonoids[1,2] and usually food waste for the industries. Albedo, together with septum and core, constitutes 30-40% of the fruit weight. In this study, albedo, particularly thick and sweet, and flavedo, were morphologically and chemically characterized. Preliminary observations of fresh, dry and fixed albedo and flavedo samples, were carried out by means of a variety of microscopy approaches: light, fluorescence, transmission, scanning and environmental scanning electron microscopy[3]. Semi-thin sections were stained with safranin, methylene blue, Azur II and basic fuchsin, to highlight and compare characteristic elements of the albedo and flavedo of this ancient fruit. All morphological approaches allowed us to observe parenchymal cells with a considerable central vacuole. Elements of secondary lignified wall characteristic of the xylem were observed such us spiral tracheids, with regular pitting walls. Microanalytical analyses showed that carbon, oxygen, chloride, potassium and calcium were associated to albedo and flavedo structures. Further studies are necessary for a better biodiversity and eco-sustainability valorization of the *Limoncella* biovar. In particular, its albedo, useful for recycling of organic waste in agriculture and food industries, and important source of flavonoids, deserves a new attention and highlighting.

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NATURAL ANTIOXIDANTS AS PREVENTION OF ANTI-INFLAMMATORY DRUG-INDUCED MUSCLE ATROPHY *IN VITRO*

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Muscle atrophy and its complications can be related to sedentariness, serious injury, aging, hormone diseases and cancer[1]. Furthermore, oxidative stress increasing, such as that due to prolonged anti-inflammatory drug administration, is one of the major causes of skeletal muscle mass reduction[2]. Many studies have revealed that some natural antioxidants are able to counteract it, thus preventing or limiting muscle damage[3]. Here we have evaluated, by means of morpho-functional analysis, in particular by ESEM observations, the effect of a virgin oil flavonoid in C2C12 myotubes treated with a glucocorticoid drug generally used to simulate muscle wasting *in vitro*. Control myotubes appear long and confluent, with preserved mitochondria. Differently, glucocorticoid-treated cells show altered and smaller fibers, loss of mitochondrial membrane potential and disorganized mitochondria cristae. Flavonoid administration before glucocorticoid treatment seems to preserve myotube size, which appears comparable to the control one. These preliminary results could promote the use of this natural antioxidant in glucocorticoid-induced muscle wasting prevention. Further studies are in progress to identify molecular patterns implicated in skeletal muscle mass preservation.

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POTENTIALLY USEFUL DIAGNOSTIC AND PROGNOSTIC MARKERS IN COLON CANCER: A PROTEOMIC-BASED INVESTIGATION

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Colorectal cancer (CRC) is a leading cause of cancer death worldwide. Although the molecular changes that occur during CRC tumorigenesis and progression has been elucidated, tumors with similar histopathological appearances often manifest significantly different clinical behavior. In this scenario it is crucial to identify new signatures that are able to predict patient's prognosis. In the present study, we firstly performed a comparative proteomic profile of pooled colon cancer tissues paired with adjacent non-tumoral mucosa, to investigate potential target proteins correlated with carcinogenesis. After that, we used a three-step approach to compare normal-colon cancer and liver metastasis from the same patient, in order to identify putative proteomic signatures for CRC occurrence and metastasis. We selected unique and common proteins involved in tumorigenesis (normal versus tumoral) and metastasis (tumoral versus metastasis). The differentially expressed proteins, functionally classified, have been suggested to act at multiple tumor progression steps, affecting cell proliferation, apoptosis, metabolic pathways, oxidative stress, cell motility and invasion. Interestingly, in the present study we identified Transgelin (four different isoforms), a 22 kDa actin-binding protein, as a possible tumor suppressor and biomarker for CRC, and cathepsin D (two different isoforms), a lysosomal aspartyl endopeptidase, differentially expressed between tumor and metastatic tissue.

AN INTRIGUING RELATIONSHIP BETWEEN TELEOST Rex3 RETROELEMENT AND ENVIRONMENTAL TEMPERATURE

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Transposable elements (TEs) are known to be responsive and susceptible to environmental changes[1-3]. However, the correlation between environmental conditions and TE sequence evolution still remains an unexplored field of research. Among vertebrates, teleosts represent a successful group of animals adapted to a wide range of different environments and their genome is constituted of a rich repertoire of TEs[4]. The Rex3 retro-element is a lineage specific non-LTR retrotransposon[5] and thus represents a valid candidate for performing comparative sequence analyses between species adapted to diverse temperature conditions. In this study, partial reverse transcriptase sequences of the Rex3 retroelement belonging to 39 species of teleosts were investigated through phylogenetic analysis. Our findings highlighted an intriguing behavior of the analyzed sequences showing a clusterization of Rex3 sequences isolated in species living in cold waters (Arctic and Antarctic regions and cold waters of temperate regions) compared to those isolated in species living in warm waters. This is the first evidence of a correlation between environmental temperature and Rex3 retroelement evolution.

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COCAINE-INDUCED LIVER DISEASE IN THE EUROPEAN EEL, *Anguilla anguilla*

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Cocaine is a potent psychostimulant and highly addicting drug, causing hepatotoxicity in humans, laboratory animals and fish[1]. Due to the worldwide use of cocaine, it is detected in surface waters[2]; despite its low concentrations, cocaine induces toxic effects in the European eel[3,4]. In this study, the effect of cocaine on the liver of the European eel was evaluated. A stock solution of 0.006 mg/mL cocaine free-base in ethanol was prepared. Male silver eels were exposed for fifty days to 20 ng/ L of cocaine; untreated control, vehicle control and two post-exposure recovery groups (exposed to cocaine and then deprived of it and only exposed to tap

water, for three and twenty days, respectively) were also made. The following parameters were evaluated: liver morphology; cytochrome oxidase (COX) activity, as a marker of oxidative metabolism[5]; caspase-3 activity, as a marker of apoptosis activation[6]; GRP78 expression, as a marker of the unfolded protein response[7]; blood glucose level, as a marker of stress[8]; serum levels of alanine aminotransferase (ALT) and C-reactive protein (CRP), markers of liver injury[8,9]. In the exposed eels, loss of parenchymal cells and lipid content, and necrotic areas were observed. COX and caspase-3 activities, GRP78 expression, blood glucose level, ALT and CRP levels increased. In the post-exposure recovery eels COX returned to control values, caspase-3 activity was lower whereas GRP78 expression, blood glucose level, ALT and CRP levels were higher than controls. These results show that even low cocaine concentrations affect the eel liver, suggesting potential impact on the survival of this species.

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A COMBINED *IN VIVO* AND *EX VIVO* STUDY OF SOLID LIPID NANOPARTICLES BIODISTRIBUTION

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Solid Lipid Nanoparticles (SLN) are well known drug delivery systems due to their versatility, safety and ability to protect molecules from degradation upon administration[1]. In this study we investigated the biodistribution of SLN functionalized with polysorbate80 (P80), a non-toxic surfactant that increases the NPs half-life[2] lowering their uptake by the reticuloendothelial system[3]. SLN were labelled with two fluorophores, Indocyanine green and Rhodamine, to assess both *in vivo* and *ex vivo* biodistribution. Mice were treated with SLN by intraperitoneal injection, and fluorescent emission kinetic data obtained *in vivo* by an optical imager demonstrated a preferential uptake in the anatomical area of liver. Excised liver, kidney and spleen were also analysed for fluorescent emission. To evaluate SLN accumulation in the tissues, some treated mice were perfused and the organs were processed to allow analysis with light (LM), fluorescent (FM) and transmission electron (TEM) microscopy. SLN were observed in the hepatic tissue, while no evident fluorescent signal was detected in kidney and spleen. LM and FM images of livers revealed no histological alteration after treatment: SLN entered the hepatocytes, localizing in the cytoplasm without causing evident structural damage, but a marked increase in lipid content was found, especially in centrilobular vein area. A cell stress was noticed after TEM analysis: the nuclei were almost completely euchromatic, there was a rarefaction of glycogen and the rough endoplasmic reticulum lost its classical arrangement. However, no relevant organelle damage was found. These results provide a solid methodology for a combined study of *in vivo* and *ex vivo* SLN biodistribution, that should be taken into consideration in planning the utilization of these NPs for therapeutic purposes.

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EFFECTS OF COPPER ON *Xenopus laevis* LIVER

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Copper is an essential element to living organisms for which it is involved as a co-factor in multiple physiological processes included carbohydrate and lipid metabolism, red blood cell formation and iron absorption and transport[1]. However, at high concentrations this metal is toxic because induces reactive oxygen species formation (ROS) that lead to oxidative stress and so cause damage to biological structures[2]. In this study, we evaluated the effects of copper on liver of *Xenopus laevis* after three weeks of exposure at 1mg/L of CuCl, the maximum concentration accepted for human use (ARPA, DPR236/88). *X. laevis*, like amphibian is directly exposed to the aquatic environment and sensitive to pollutants. For this reason, *X. laevis* is an important biomarker and so as a good model organism for ecotoxicological studies[3]. The effects of copper were analysed at light microscope on the livers of control and treated frogs and by Hematoxylin-Eosin and Mallory stainings to study the general histology, and by PAS and Perls stainings to evaluate the changes on glycogen metabolism and increase of hemosiderin presence, the iron storage complex, respectively. The tissue of treated livers showed evident damages and an increase in size and number of melanomacrophages, a clear indirect marker of inflammation and degenerative processes[4]. Instead, by Real-time PCR, we detected also mRNA expression levels of ATP7B, an important copper-transporting that appeared down-regulated in the treated frogs. We revealed that this decrease was also for the specific protein by Western Blotting and confirmed by immunohistochemistry technique using a polyclonal antibody anti-ATP7B. In conclusion, the data are indicative that a continuous exposition at 1mg/L of CuCl, a low concentration, damages the *X. laevis* liver suggesting the need for further experiments to elucidate better the toxicity of this metal and its environmental impact and on human health.

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$ALCL_3$ INDUCES NEUROBEHAVIOURAL ALTERATIONS IN *Danio rerio* LARVAE

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Aluminium occurs naturally in the environment, but a variety of its compounds are produced and used in different activities of human daily life increasing so the release of this metal in the environment, particularly in water bodies. Our previous studies showed that aluminium chloride ($AlCl_3$) induced phenotypic alterations and damages of the nervous system with decrease of the glial fibrillary acidic protein (GFAP) expression in *Danio*

rerio fish[1], optimal model organism for ecotoxicological analysis and for the study of neurodegenerative and neurobehavioral diseases[2]. In order to assess the toxic effects of this metal, *Danio rerio* embryos at shield stage were exposed to $AlCl_3$ at the concentrations of 50, 100 and 200 μM respectively for 72 h. We compared the swimming performances of treated larvae with those of the control larvae, assessing different parameters like Distance moved, Velocity mean, Cumulative movement, Meander and Heading using the DanioVision instrument. Collected data showed that $AlCl_3$ significantly affected the behavioural parameters with a trend inversely proportional to the concentrations, in fact the performances worsen at low concentrations compared to higher doses[3]. In this light, we analysed mRNA expression level by qPCR of different marker genes of neural development and function, including *c-fos*, *app*a and *app*b. *C-fos* is an immediate-early gene often used as indirect marker of neuronal activity[4], while *app*a and *app*b are the homolog genes of the mammalian amyloid precursor protein (APP), an essential gene for normal **brain development** and a key player for the Alzheimer's disease pathogenesis[5]. We observed that the expression of these genes was affected by $AlCl_3$. The results confirmed toxic effect of $AlCl_3$ on *D. rerio* larvae, suggesting the need for further experiments to uncover the mechanisms by which the aluminium exposure affects the normal developmental processes and might be at basis of neurological and behavioural disorders.

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NOVEL APPLICATIONS OF LONG-ESTABLISHED HISTOCHEMICAL TECHNIQUES TO STUDY NANOPARTICLE-CELL INTERACTIONS AT TRANSMISSION ELECTRON MICROSCOPY

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Transmission electron microscopy (TEM) is the technique of choice to explore the effects of nanocomposites on biological systems. The high resolution of TEM allows the location and dynamic tracking of nanoparticles (NPs) inside cells and tissues, providing crucial information on their actual interactions. It is thus mandatory that NPs are unequivocally detected in the inter- and intracellular space. This is easily obtained for NPs containing electron dense components such as metal ions, but it may be difficult when they are made of organic components (*e.g.*, polymers or lipids) whose moderate electron density makes them hardly discernible in the cytosolic milieu. We faced this situation with various types of NPs and solved the problem by setting up novel applications for long-established histochemical techniques. Chitosan-based and phospholipidic NPs were made clearly visible at TEM by labelling them with fluorochromes during their synthesis, and subsequently applying diaminobenzidine (DAB) photo-oxidation, that gives rise to a finely granular electron dense product thanks to the reactive oxygen species originating upon fluorochrome irradiation[1,2]. By this method, not only the uptake mechanisms and intracellular distribution of these NPs were revealed, but also their degradation pathways, thanks to the presence of DAB precipitates on the NPs remnants inside secondary lysosomes and residual bodies. However, in the absence of fluorochrome labelling DAB photo-oxidation cannot be applied: as an alternative approach, specific histochemical methods giving rise to electron dense products may be used, such as Alcian blue staining[3]. Hyaluronic acid-based NPs were thus visualized at TEM, and we were able to describe the very early step of their uptake as well as their degradation, which were impossible to get by conventional morphology.

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EFFECTS OF MOLECULES WITH NEUROTROPHIC ACTIVITY IN AN *IN VITRO* MODEL OF PARKINSON'S DISEASE

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Oxidative stress results from an in balance between oxidative species and scavenging antioxidant systems. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) may be either harmful or beneficial to the cells, depending on their concentrations. Methionine acts as amino acid precursor for glutathione that protects the cells from oxidative damage and plays pivotal role in cell detoxification from oxidative stress[1]. The aim of this research was to study the neurotrophic and antioxidant activities of methionine and taurine on an *in vitro* model of PD. The methionine and taurine effects were first evaluated on an oxidative model based on H_2O_2 treatment, then followed by the set-up of a PD *in vitro* model by treating dopaminergic neurons with 6-OHDA[2]. The effects of methionine and taurine were evaluated by MTS assay, Western Blotting analysis and Immunofluorescence. Methionine and taurine were both able to counteract the pro-oxidative death effects of H_2O_2 and 6-OHDA by decreasing the apoptotic markers (caspase 9, Bcl-2), by modulating oxidative stress markers (Mn-sod, catalase) and the oxidative stress index (ratio Mn-sod/catalase); by increasing the PI3K/AKT survival pathway and antioxidant markers like a Nrf2. The results so far obtained confirmed the potential neuroprotective activities of methionine and taurine in the PD *in vitro* model. The study will continue by evaluating the possible protective activities of these molecules on mitochondrial dysfunctions using an oxidative phosphorylation uncoupling like a TMRM in live cell, JC-1 and the activation of pro-survival pathways depending on neurotrophine modulation in the PD *in vitro* model.

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A NEW INSIGHT IN THE AXON AND DENDRITIC DEVELOPMENT: THE FMRP-RACK1 PARTY

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The development of axons and dendrites depends on the external cues that stimulate the translation of specific mRNAs localized to the growth cones. The mRNAs are transported to edge of neurites by RNA binding proteins which interacting with translational machinery promote the translation of these mRNAs[1]. FMRP is an RNA-binding protein which represses the translation of many mRNAs. In neurons, its depletion determines the exaggerated translation of these specific mRNAs leading to dendritic and axonal aberrant development, two peculiar features of Fragile X syndrome patients[2]. Here, we show that FMRP forms a complex with the ribosomal Receptor of Active C Kinase 1 (RACK1) on polyribosomes[3], thus mediating its localization on translational machinery. Moreover, the up-regulation of RACK1 affects the translational repressor activity of FMRP as results by the stimulation of PSD-95 mRNA translation, one specific targets of FMRP, and by the reduced level of FMRP phosphorylation. Finally, we observed that the morphological abnormalities induced by Fmr1 siRNA in cortical neurons were rescued by the overexpression of a mutant form of RACK1 that cannot bind ribosomes. Thus, these results provide a new mechanism underlying neuronal and FXS development.

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POSSIBLE ROLE OF THE SYNOVIAL MICROENVIRONMENT AND ITS INVOLVEMENT IN JOIN PATHOLOGIES

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Osteoarthritis (OA) is a degenerative joint disease that is the major cause of disability in the elderly[1]. Synovial inflammation plays a key role in symptoms and pathophysiology of OA. The interaction between the articular cartilage and the synovium causes a positive feedback cycle leading to matrix remodelling, cartilage disintegration and increase in osteoclastogenesis. To understand the pathogenetic mechanisms, the researchers focused on the characterization of the synovial membrane (SM) and on the cells isolated from it. Macrophage and mast cells (MCs) infiltrate in the OA-SM is related with disease progression and pain[2]. Mesenchymal stromal cells, called Synovial-Derived Stem Cells (SDSCs) are involved in the initial phases of osteoarticular disease and in the capacity of regeneration[3]. Recently, stromal cell type with a peculiar ultrastructure called telocytes (TCs) has been demonstrated in SM, but their role is far to be clarified[4]. The aim of our research is to analyse the morphological changes that involve the SM (peculiar attention in TCs presence was paid) and to evaluate how SDSCs from OA-SMs were able to activate Peripheral Blood Mononuclear Cells (PBMCs) in comparison with cells isolated from healthy subjects. Histological and ultrastructural analyses demonstrate the presence of TCs in SM of both normal and OA subjects; however, the massive presence of MCs was identified only in OA-SMs. Finally, using *in vitro* co-culture tests, it was found that only SDSCs from the OA context were able to induce the differentiation of PBMCs into functional Osteoclasts. The pathological microenvironment generated in OA influences different cellular populations and further studies are still required to understand how cells participate in damage induction and in manifestation of pain.

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DEVELOPMENT AND OPTIMIZATION OF A NON-ENZYMATIC METHODS OF NEURON ISOLATION FROM ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES

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Conventional methods of cell isolation from various tissues involve a multistep enzymatic process for tissue digestion. This approach is the most conventional, especially for neuronal tissue, but enzyme administration can negatively affect the expression of cell surface receptors. Here we tested a non-enzymatic technique of tissue disaggregation employing Medimachine II on organotypic hippocampal slice cultures (OHSC). The use of Medimachine-based method of disaggregation is already known in literature for many types of tissue but not in the particularly sensitive nervous one. OHSC, prepared from 7-days-old Wistar rats using an interface method, were both enzymatically digested and mechanically disaggregated. In particular, some slices were treated with trypsin/EDTA (0.125% with 5% BSA and 0.005% DNAse) for 10 min at 37°C; other slices were disaggregated using Medimachine II, adding 1ml PBS into the Medicons chamber's and processed for 10s of working. The cell suspensions obtained were filtered using a 100 µm FILCONS filter, labelled with different cellular markers (TMRE, PI, LTG) and different mAbs and analysed by flow cytometry and confocal microscopy. Both enzymatically and mechanically disaggregated samples showed similar physical characteristics (in both flow cytometric and confocal microscopic analyses) and comparable cellular parameters, measured by means of different probes. In particular, PI staining showed analogous frequencies of apoptotic/ necrotic cells. Instead, mechanical method allowed the rapid obtainment of enriched cell suspension with good viability. We can affirm that Medimachine II is a simple, fast and standardised alternative for tissue processing: it requires fewer reagents and less time compared with the enzymatic method. Furthermore, the apparent minimal impact of non-enzymatic processing on cell functions seems to qualify this procedure as an optimal alternative for flow cytometric and microscopic analyses.

EXPLORING THE POTENTIAL OF MILD OZONISATION IN ADIPOSE TISSUE REGENERATION AND DIFFERENTIATION

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In the last decades, the medical use of gaseous ozone (O_3) has been progressively increasing as an alternative/adjuvant treatment for several diseases. In particular, low O_3 concentrations proved to induce the so-called eustress[1] by activating anti-oxidative response pathways[2] and stimulating cellular metabolism. In order to explore the potential of mild ozonisation in tissue regeneration and differentiation, we investigated the effects of low O_3 concentrations (10 and 20 µg O_3/mL O_2) on human adipose-

derived adult stem (hADAS) cells derived from the stromal-vascular fraction of subcutaneous adipose tissue harvested by liposuction. By combining histochemical and morphometric analyses at light microscopy (LM) with ultrastructural morphology at transmission electron microscopy (TEM), we monitored the adipogenic process at early (6 days), intermediate (16 days) and late (20 days) differentiation steps, and demonstrated that O_3 treatment promotes lipid accumulation in hADAS without inducing deleterious effects (*e.g.*, cell death, organelle damage, delipidation). We also investigated the effect of low 03 concentrations on whole adipose tissue by treating murine fat explants maintained under *in vitro* conditions[3]. Lactate dehydrogenase evaluation revealed that mild ozonisation did not induce any stress; this result was confirmed by observations at LM and TEM, which demonstrated morphological features similar to controls. Again, no delipidation was found following the mild oxidative stress induced by O_3 exposure, and biochemical analyses demonstrated the activation of cellular antioxidant response. The results of our pilot experiments pave the way to future studies aimed at elucidating the effect of mild ozonisation on adipose tissue for tissue regeneration and engineering.

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EFFECTS OF TRIBUTYLTIN ON RETINOID X RECEPTOR GENE EXPRESSION AND GLOBAL DNA METHYLATION DURING INTRACAPSULAR LARVAL STAGES OF THE GASTROPOD *Nassarius mutabilis*

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Organotin-based antifouling compounds, such as tributyltin (TBT), are potent and well-known inducers of imposex in marine gastropods. Actual evidence in this regard suggests that nuclear Retinoid X Receptor (RXR) might contribute to the development of TBT-induced imposex though multiple mechanisms that include, among others, epigenetic regulation. Here, we aimed to assess the effects of TBT on RXR gene expression and global DNA methylation during intracapsular larval stages of the gastropod *Nassarius mutabilis*, a widely distributed species along the Mediterranean coasts, particularly those of the Adriatic Basin. Thus, an egg capsule culture was set up as alternative *in vitro* model to investigate the effects of environmental concentrations of TBT during key stages of *N. mutabilis* development. In our study, we first observed that exposure of egg capsules to TBT results in a significant impact on the morphology of fertilized eggs, as indicated by a consistent modification in their shape and size. In addition, following the partial cloning of *N. mutabilis* retinoid receptor (*Nm*RXR), we found using an optimized qPCR assay that the *Nm*RXR is present in the developing embryos and that their treatment with TBT significantly impact embryogenesis also modulating the global methylation status. Our findings support a role for RXR also in *N. mutabilis* development providing more information on retinoid-xenobiotic interactions in developing gastropod embryos.

REVERSING EPITHELIAL-TO-MESENCHYMAL TRANSITION THROUGH AUTOPHAGY INDUCTION IN GLIOBLASTOMA CELLS

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Cell migration and invasion are highly regulated processes involved in both physiological and pathological conditions. We demonstrated that autophagy modulation regulates the migration and invasion capabilities of glioblastoma (GBM) cells by downregulating Epithelial-to-Mesenchymal Transition (EMT) master genes and up-regulating cadherin transcription[1]. We also observed that autophagy negatively modulates Wnt β catenin signalling and promotes β catenin relocalization whitin the cell, thus promoting the formation of cell-cell adhesion complexes[2]. Experiments performed in primary GBM cells, from patients, confirmed the results obtained in established cell cultures. It is known that Wnt β catenin and ERK pathways co-operate to promote tumorigenesis in several cancer types[3], and that β catenin association to cadherin complexes via α catenin depends on EGFR-mediated ERK1/2 signalling[4]. In order to investigate if ERK pathway is a target of autophagy activation in GBM cells, we analyzed ERK1/2 phosphorylation and found that it strictly depends on autophagic status. Moreover, as ERK signalling is activated downstream to EGFR activation, we are investigating the effect of autophagy modulation on EGFR expression and localization. Notably, EGFR receptor, similarly to Wnt receptor, is often up-regulated and constitutively activated in GBM, such as in other cancer types, thus representing a promising target for immunotherapy. Overall, our results indicate that autophagy modulation triggers a molecular switch from a mesenchymal to an epithelial-like phenotype in GBM cellular models, likely due to the inactivation of at least two signal transduction pathways, that are crucial for tumor proliferation, invasiveness and stemness maintanance. Since the agressiveness and lethality of GBM is defined by local invasion and resistance to chemotherapy, our evidence provides a further rationale for in-depth dissecting the autophagy impact on the gliomagenic process.

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CELL AND ORGAN MODELS TO TEST NANOCARRIERS FOR THE TREATMENT OF SKELETAL MUSCLE

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Neuromuscular diseases such as myotonic dystrophies are still incurable; some therapeutic molecules proved to be efficient in experimental models but their clinical application is prevented by their high toxicity and/or degradability. Nanocarriers has a great potential as drug-delivery systems for controlled and targeted drug release[1], and the scope of this work was to explore the suitability of some biocompatible nanoparticles (NPs) for skeletal muscle. The uptake and intracellular fate of liposomes and of Poly Lactic-co-Glycolic Acid (PLGA) NPs were investigated in C2C12 cells by confocal fluorescence microscopy (CFM) and transmission electron microscopy (TEM). C2C12 cells are

immortalized murine myoblasts that spontaneously differentiate into myotubes after serum reduction, thus providing an *in vitro* model of proliferating and terminally differentiated non-cycling muscle cells. The NPs were labelled with either fluorescein isothiocyanate (liposomes) or Nile red (PLGA NPs) to make them detectable at CFM. Liposomes entered the cells by fusion with the plasma membrane and underwent rapid cytosolic degradation, while PLGA NPs entered the cells *via* endocytosis, underwent endosomal escape but re-entered the endo-lysosomal pathway, being rapidly degraded. Both NPs showed good biocompatibility, since no cell damage was found in C2C12 myoblasts and myotubes following their uptake[2]. As a second step, we investigated the biodistribution of liposomes and PLGA NPs in explanted mouse skeletal muscles maintained in a bioreactor under fluid dynamic conditions[3]. The intramuscular administration of the NPs in the whole organ revealed that, despite the good results obtained in cultured cells, both nanocarriers were unable to enter muscle fibres, mostly accumulating in the perimysium and endomysium. Thus, NPs functionalization studies are presently in progress to improve cell muscle targeting.

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IMMUNOHISTOCHEMICAL STUDY OF HUMAN TEMPOROMANDIBULAR JOINT DISC

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The articular disc is described as a biconcave lens with two faces, superior and inferior, two margins, medial and lateral, and two extremities, anterior an posterior. The articular disc can be also divided into three different functional portions: posterior band, intermediate zone and anterior band. The extracellular matrix of the disc is composed mainly of collagen I and elastin and their distribution is different in the three functional zone[1]. The collagen I is the predominant ECM component forming a network very important for resisting tensile forces. The elastin is also present in entire disc, but its distribution is different depending on the region; this protein is associated with resistance and elasticity and it is responsible to maintain the shape after deformation. Some authors have demonstrated that collagen I is more present in the posterior and lateral zones in respect to elastin[2]. Here we studied the localization of collagen I and elastin in normal human temporomandibular joint disc and in retrodiscal tissue by confocal laser scanning microscopy. Our results demonstrated that both proteins are present in entire disc and they run parallel to each other. In particular, collagen I and elastin, in intermediate zone, have an antero-posterior orientation, with longitudinal direction in condylar surface, while oblique orientation in temporal surface. In medial margin, the tested proteins have a similar staining pattern, and they cross each other with an oblique orientation. In lateral zone, the staining pattern of collagen I is more represented than to elastin and they form a thick network. Our present results suggest that, during temporomandibular joint movements, the lateral margin of disc is submitted to a major compression forces due to a major presence of collagen I; however, the medial margin, corresponding to attachment of lateral pterygoid muscle, is submitted mainly to elastic forces because we observed similar staining patterns for both tested proteins.

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SARCOGLYCANS AND DYSTROGLYCANS PRESENCE IN EPITHELIAL TISSUES: AN IMMUNOHISTOCHEMISTRY STUDY

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Sarcoglycan is an independent sub-complex within the larger dystrophin-glycoprotein complex and is composed of at least four sarcoglycan subunits α-sarcoglycan, a type I transmembrane protein, and β-, γ- and δ-sarcoglycans, which are type II transmembrane proteins; all sarcoglycans play a structural role with signalling functions on the muscle cells. It was demonstrated that α- and -sarcoglycan are expressed exclusively in skeletal and cardiac muscle, whereas β- and δ-sarcoglycans are more widely expressed. A fifth sarcoglycan, ε–sarcoglycan, was , was identified and it is highly homologous to α-sarcoglycan, but like β- and δ-sarcoglycans it is widely expressed in various tissues. About this subunit, levels were highest in lung, moderate in brain, heart, and low but detectable in kidney and liver whereas α-sarcoglycan was not detected in lung or in other non-muscle tissues. Another mammalian sarcoglycan, ζ-sarcoglycan, has been identified by an antibody specific to ζ-sarcoglycan; this protein, highly related to γ- and δ-sarcoglycan, was found as a component of the vascular smooth muscle sarcoglycan complex. In order to verify the presence of sarcoglycans in nasal, bronchial, intestinal and urinary biopsies from 10 patients and their interaction with dystroglycans, we performed immunofluorescence reactions on these biopsies who underwent for other pathological reasons. Moreover, in the same samples, also we carried-out immunofluorescence reactions testing mucins, a superfamily of highly glycosylated protein, they are part of mucus. For the first time, our immunofluorescence results, confirmed also by molecular analysis, show that: (i) sarcoglycans are expressed in the basal, lateral and apical cell sides; (ii) sarcoglycans co-localize in the apical region with mucins and dystroglycans; (iii) dystroglycans co-localize with mucin in the cellular apical region. Our results suggest that an interaction between these sarcoglycans and mucus exists and, in our opinion, dystroglycans can play a key role in this interaction. On the basis of our results, we hypothesize that dystroglycans and sarcoglycans may have a role in the determination of the cell's polarity, supported by the co-localization of mucins and dystroglycans in the apical area.

MUTANT NEUROSERPIN INDUCES MITOCHONDRIAL ALTERATIONS IN A NEURONAL MODEL OF THE NEURODEGENERATION FENIB

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The neurodegenerative condition FENIB (familiar encephalopathy with neuroserpin inclusion bodies) is caused by polymerization and deposition of the neuronal serpin (neuroserpin, NS) within the endoplasmic reticulum of neurons[1]. With the aim of understanding the toxicity due to intracellular accumulation of NS polymers, we have generated transgenic neural progenitor stem cells from mouse fetal cerebral cortex, stably expressing the control green fluorescent protein (GFP), or human wild type, polymerogenic G392E or truncated (delta) NS. In this cellular model, we have described the upregulation of several genes involved in the defense against oxidative stress in G392E NS cells[2]. We are currently investigating the involvement of mitochondria in NS polymer toxicity. Our analysis of mitochondrial distribution shows a higher percentage of perinuclear localiza-

tion in G392E NS cells, in contrast with a higher proportion of filamentous mitochondria in cells expressing wild type NS or GFP. Analysis of cellular morphology by simultaneous staining of the actin cytoskeleton and the mitochondrial network shows that cells with altered mitochondrial distribution lack well-developed neurites. The mitochondrial phenotype is aggravated to mitochondrial fragmentation in the presence of the pro-oxidant glutathione chelator diethyl maleate, particularly in G392E NS cells, and this phenotype can be rescued by treatment with antioxidant molecules (alpha-tocopherol and melatonin). We have also evaluated the potential of the inner mitochondrial membrane in G392E NS compared with wild type NS cells, and we found that G392E NS cells have a lower membrane potential, which could take part in the chronic oxidative stress state we have described for G392E NS cells[2].

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AAV-MEDIATED *IN VIVO* FUNCTIONAL SELECTION OF TISSUE-PROTECTIVE FACTORS IN A 6OHDA MOUSE MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is a progressive neurodegenerative disorder. The prevalence is estimated to be about 1.3% to 1.5% for persons above the age of 60 years in Europe[1]. The number of individuals with PD will have doubled by the year 2030. Currently, there is no cure for PD[2]. For this reason, in order to identify possible neuroprotective treatments, an innovative screening (FunSel –Functional Selection) of different murine secretoma factors was performed in a 6OHDA mouse model. Funsel is an innovative screening already used in a previous work to identified a protective factor in acute ischemia[3]. This screening is based on *in vivo* transduction of an AAV vector library coding for different secretoma factors, followed by the induction of a selective stimulus[4]. The library of AAV are injected *in vivo* to transduce neurons; after two weeks the 6-OHDA was inoculated in C57BL6 mice striatum. Then, vector cDNA inserts were recovered from the injected tissue in order to identify neuroprotective factors. At present, a first pool containing 50 genes was assayed. In these experiments, four genes were identified, named A, B, C and D. Presently, only the first two were studied in the PD animal model. Gene A appears significantly protective in the behavioural tests as well as in the maintenance of TH-neurons viability. It exerts also protective effects in counteracting apoptosis, as demonstrated by TUNEL assay. The gene B, even if enriched by the FunSel procedure, did not display any protective activity. These initial findings are of relevant importance for a disease that, to date has only symptomatic cures that, however, do not prevent the progression of the disease[2].

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HUMAN iPSCS TO UNDERSTAND WDR62-ASSOCIATED MICROCEPHALY

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Microcephaly is a heterogeneous and incurable disorder that results from the depletion of neural stem cells due to genetic lesions or congenital infections. Distinct genetic mutations are implicated in microcephaly; usually with a recessive inheritance pattern. Some of the genes that cause microcephaly control crucial aspects of neural development and may also be evolutionary involved in the expansion of the cerebral cortex characteristic of primates. 18 loci have been associated with autosomal recessive primary microcephaly (MCPH); mutations in *WDR62* cause the second most common form of MCPH underlain by a constellation of structural cortical malformations; including simplified gyral pattern and callosal abnormalities; suggesting that it acts as a critical hub of human cerebral development[1]. WDR62 is a centrosome-associated protein; involved in symmetric versus asymmetric cell division choice from neural stem cells during corticogenesis. We recently derived iPSCs from a microcephalic patient carrying a novel *WDR62* mutation[2]. To define the functional consequences of this mutation; we applied a protocol for hiPSC-directed differentiation into cerebrocortical neurons; allowing us to recapitulate the main corticoneurogenetic events *in vitro*. Furthermore; we obtained a stable and self-renewing population of neuroepithelial stem (NES) cells with cortical identity from WDR62-iPSCs; setting an optimal model to study early neurogenetic events and the impact this mutation has on human early neural stem cells. We found that mutant WDR62 protein does not correctly localize to centrosomes in mitotic NES cells; showing instead a diffused pattern. Mutant WDR62-iPSCs and their neural progeny may represent a unique platform to better understand the molecular and cellular events underlying MCPH.

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TESTING THE BIOCOMPATIBILITY OF INNOVATIVE BIOMATERIALS ON A CO-CULTURE OF DENTAL PULP STEM CELLS AND ENDHOTELIAL CELLS

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Dental pulp stem cells (DPSCs), a population of stem cells able to differentiate into mature osteoblasts, are part of a niche, in which their crosstalk with endothelial cells is important in the cellular response to biomaterials used in restorative practice. Endothelial cells are equally important in bone regeneration, given their role in producing modulators of bone development and maintaining vascular homeostasis. DPSCs were co-cultured with the endothelial cell line EA.hy926 in presence of two new kinds of biomaterial for both dentistry and orthopedic applications: Chitlac-coated BisGMA/TEGDMA thermosets[1] and alginate/hydroxyapatite-based (Alg/Hap) nanocomposite scaffolds[2]. Cytotoxicity and cell proliferation were investigated by MTT assay and LDH release; the osteogenic differentiation was evaluated by measuring alkaline phosphatase (ALP) activity, performing alizarin red staining and quantifying the expression of procollagen and bone sialoprotein II (BSPII). The formation of new vessels was monitored by optical

microscopy. When cultured together, the proliferation of both cell types is increased, as well as DPSC osteogenic differentiation and EA.hy926 vessel formation. Chitlac-coated thermosets show low cytotoxicity and their presence appears to further increase ALP activity and alizarin red staining, and to lead to the formation of thicker vessels by EA.hy926 cells. Alg/HAp scaffolds are also able to enhance the osteogenic potential of the co-culture system, as demonstrated by the increase in ALP activity and in procollagen and BSPII expression. In conclusion, both biomaterials seem to have a potential role in restorative dentistry and the DPSCs/endothelial cells co-culture could be considered a useful tool to investigate the biocompatibility and the engraftment potential of novel biomaterials.

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NONYLPHENOL EFFECTS ON HUMAN PROSTATE CELLS

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In recent years, a large body of evidence suggest a strong relationship between prostate diseases and human exposure to endocrine disruptor chemicals (EDCs) with estrogens mimicking actions, such as alkylphenols (AP). Among AP, Nonylphenol (NP) is widely used in plastics formulations as non- ionic surfactants, in agricultural and personal care products and therefore is commonly found as contaminants in rivers, lakes, seas and sediments. Hence, the aim of this study was to evaluate the effects of NP, a well-known xenoestrogen, on human adenocarcinoma prostate cells (LNCaP) and we compare its effects with 17-β-estradiol (E2), the most estrogen circulating in humans. First we assessed the effects of NP and E2 on LNCaP proliferation after 48h of exposure. Both NP and E2 enhanced LNCaP proliferation and cell progression in S phase of cell cycle, at $1x10^{-10}$ M and $1x10^{-9}$ M, respectively. In order to understand the molecular events involved in the cell proliferation increase, through RT-qPCR, we studied changing in gene expression of genes involved in proliferation and inflammation pathway such as Ki67, Cyclin D1, c-myc, IL-8 and IL-1β. Then, to highlight a possible involvement of estrogen receptors, we studied their expression and cellular localization. Differently from E2, NP induced a strong increase in ER expression but it did not interfere with ERβ expression. Moreover, using immunofluorescence we have observed ER translocation from cytoplasm to nucleus suggesting its activation. Interestingly, both NP and E2 act only on ER translocation, although after different times of exposure. Data obtained, suggest the injurious effects of nonylphenol on prostate cells and highlight some aspects of molecular pathways involved in prostate responses to xenoestrogens.

DEHP EFFECTS ON RAT TESTIS AFTER EARLY LIFE EXPOSURE

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In recent years, growing number of incidences of male reproductive disorders have been associated with exposure to Endocrine Disruptors Chemicals (EDCs) during perinatal and neonatal life. Among EDCs, phthalates are widely used as plasticizers and solvents across a large number of products and are now considered ubiquitous in the environment. Diethylhexyl phthalate (DEHP) is the most frequently detected phthalate and its effects on reproductive system depend on doses and developmental stage of organism at the time of exposure. Hence, the aim of this study is the examination of the effects of early life exposure to different doses of DEHP (GD7-PND6) on neonatal rat testes at PND6. First, testis were stained with haematoxylin and eosin and were evaluated for histopathological effects. To better characterize DEHP effects on germ cells, Sertoli cells and Leydig cells immunohistochemistry for ki67 and TUNEL assay have been performed. In order to study testis structural changes, cord diameters have been measured by measuring the diameter of tubular cross sections perpendicular to the tubular length direction. To understand the potential mechanism for phthalate impairing testis development and steroidogenesis, we also performed AR, PPARγ and P450scc immunostaining. We also analysed whether DEHP was able to induce alteration in the expression of as gap junctions (GJ) such as connexin 43 (Cx43) which is important for maintaining spermatogenesis and establish the blood-testis barrier (BTB). Results obtained, showed that after *in utero* exposure, DEHP has profound effects on Leydig cells during the neonatal period and this could affect Leydig cell function at later stage and it may be associated with TDS post-natally.

POSSIBLE ROLE OF CD93 IN PLACENTAL VILLOUS TREE DEVELOPMENT: A MORPHOLOGICAL STUDY

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During the first stage of placentation, the villous placental trees are characterized by the most superficial layer called syncytiotrophoblast, by the underlying cytotrophoblastic cells layer and a central extra-embryonic mesoderm[1]. From the pluripotent mesenchymal compartment takes origin an important process that allows the *de novo* formation of fetal capillaries, *i.e.* the vascularization. Vascularization is composed by two sequential steps: vasculogenesis that is characterized by the proliferation, differentiation and migration of hemangiogenic cells guided by paracrine stimuli from cytotrophoblastic cells; and the angiogenesis that is characterized by the formation of new vessels from a pre-vascular network[2]. CD93, also known as complement component C1q receptor, is a transmembrane glycoprotein of 652 amino acids. CD93 is expressed on the surface of different cellular types such as monocytes, neutrophils and endothelial cells[3]. The aim of this study was to investigate the expression of CD93 in placental tissues and its possible role during placental development by immunohistochemistry and cell culture techniques. CD93 was expressed in the cytotrophoblastic cells layer and in endothelial cells of the foetal vessels while the syncytiotrophoblast appeared negative in first trimester placentas. In the third trimester placentas, CD93 was present only in few cytotrophoblastic cells and in the endothelial cells of the foetal vessels. In addition, CD93 expression was significantly decreased from first to third trimester placentas. In conclusion, the localization of CD93 suggest a pivotal role of this receptor in placental villi development, in particular in the formation of foetal vessels.

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NEW STRATEGIES TO TREAT GLIOBLASTOMA MULTIFORME: DIFFERENT *IN VITRO* APPROACHES TO UNDERSTAND ANTICANCER TARGETS

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Glioblastoma Multiforme (GBM) is the most common tumor of the Central Nervous System. Current therapies are associated with side effects and are unable to contrast the relapsing GBM forms due to high proliferation cells rate, tending to form metastases. Several studies highlighted the role of ion channels and intracellular calcium levels in cells proliferation and migration processes. To overcome limits of classical oncotherapy, platinum(IV) prodrugs, characterized by low effective dose, have been synthesized. Among these, the new prodrug Pt(IV)Ac-POA represents a promising complex, able to generate a synergistic action in the hypoxic tumour cell microenvironment. Compared to cisplatin, this prodrug bearing as axial ligand (2-propynyl)octanoic acid (POA), which is an histone deacetylase inhibitor, has a higher activity, increasing exposure of nuclear DNA to higher platination and promoting cells death. The present study addressed the effects induced by Pt(IV)Ac-POA on human U251 glioblastoma cells, evaluating morphological and functional alterations, also focusing on ion channels characterization. In parallel, we tested the response of U251 to different concentrations of myco-phytotherapic supplement called "Ganostile" (Miconet s.r.l.), containing several extract of medicinal mushrooms, to evaluate its antitumor activity, in combination with chemoterapeutics. Results demonstrated that (i) Pt(IV)Ac-POA induced cell death through different pathways at concentration lower than those tested for other platinum analogues, (ii) the combination with the Ganostile exposure caused an effect on cell cycle blockade and cell death. These data suggest that cell cycle arrest induction using natural compounds could be a promising strategy to make cancer cells more susceptible to treatment with innovative anticancer drugs.

NEW IMMUNOHISTOCHEMICAL DATA ON THE NON-TRADITIONAL LARGE NEURON TYPES OF THE GRANULAR LAYER OF THE HUMAN CEREBELLAR CORTEX

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The microcircuitry of the cerebellar cortex is commonly composed by five neuron types. Studies evidenced the presence of large neuron types in the granular layer: candelabrum neuron, neuron of Lugaro, unipolar brush neuron, globular neuron, synarmotic neuron and perivascular neuron distributed in three different zones of the granular layer[1-4]. Though, the large neuron types play a considerable role in the circuitries of the cerebellum and experimental evidences demonstrated the presence of different subpopulations of large neuron types positive to GABA, peptides and cholinergic markers[1,2,4,5]; they continue to be neglected and still now called *non-traditional large neurons*. The aim of this study was to ascertain through immunohistochemical methods the existence of monoaminergic and neurotensiergic subpopulations of non-traditional large neuron types in the human cerebellar cortex. Sections of human *post-mortem* cerebellar cortex were tested with different polyclonal antibodies against serotonin (5-HT), dopamine transporter (DAT), dopamine receptor type 2 (DRD_2), neurotensin (NT) and neurotensin receptor type 1 (NTR_1). Immunoreactions were revealed by streptavidin-biotin technique and 3,3'-diaminobenzidine (DAB). The results demonstrate a strong positivity for all the antigens in different non-traditional large neuron types, in particular, perivascular neurons positive to 5-HT, DRD_2, NT and NTR_1 in the three zones of the granular layer were detected. This study, demonstrate the presence of serotonergic, dopaminergic and neurotensiergic subpopulations of non-traditional large neuron types of the human cerebellar cortex. Moreover, these results confirm the existence in the human cerebellar cortex of at least 11 different neuron types must be considered[2,4], which may play a considerable role in the motor and non-motor functions of the cerebellum and in its disorders.

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IMMUNOHISTOCHEMICAL AND TRACTOGRAPHIC APPROACHES ON THE HUMAN CEREBELLAR DOPAMINERGIC SYSTEM

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Studies reported a role of the cerebellum in dopaminergic functions such as motor activity and reward-motivational behavior[1], as well as in dopamine related disease[2,3]. Rodents show mainly the presence of dopaminergic afferent fibres to the cerebellum. Moreover, it has been demonstrated cerebellum-basal ganglia interconnections[4]; instead, the existence of direct links among the cerebellum and the midbrain dopaminergic nuclei as well as the presence of cerebellar dopaminergic neurons is still unclear. The aim of this study was to evaluate in the human cerebellum using immunohistochemical and neuroimaging-tractographic approaches the presence of cerebellar neuronal subpopulations and of dentate-midbrain links. The study was conducted on two different samples of subjects, which the same features: age, sex and absence of brain diseases. Fragments of autoptic human cerebellum were fixed in an aldehyde-picric acid solution, embedded in paraffin, cut into 5μm sections and submitted to immunohistochemistry with polyclonal antisera for dopamine transporter (DAT) or dopamine receptor type 2 (DRD2). T1 weighted neuroimaging data were acquired using a 3T Achieva Philips scanner. Diffusion signal modelling was performed using constrained spherical deconvolution technique. The immunoreactions revealed DAT and DRD2 positivity in the cerebellar cortex in Purkinje neurons, granules and synarmotic neurons; in the dentate nucleus, the positivity was found in large and small neurons. Moreover, the tractographic existence of direct links between the dentate nucleus and the dopaminergic cell groups (A9 and A10) were demonstrated. This study suggests a role of the dopaminergic cerebellar system in Parkinson's disease and Schizophrenia and may also lead to new transcranial magnetic stimulation therapeutic protocols.

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LANTHANIDES EFFECTS ACROSS *Daphnia magna* GENERATIONS

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The applications in technologies of lanthanides, as Cerium and Erbium, have made them as emergent contaminants with a toxic impact on environment. Recent literature, highlights their ecotoxicity, bioaccumulation and action in order to predict the possible environmental risks. Few studies focused on the toxicity effects linked to their intracellular concentration on *Daphnia magna*[1-3] wich is an excellent biomonitoring aquatic species[4]. To date, ecotoxicological experiments based on multi-generational field exposures are still little considered. In this study, chronic multigenerational effects on *D. magna* were assessed using various exposure times (3, 7, 14, and 21 days) in three generations (F0, F1 and F2). Each generation was exposed to environmental concentrations of Ce (0.54 μg/L) and Er (0.43 μg/L) and the effects included: organisms' size, reproduction, survival, ROS determination, activity of SOD, CAT and GST, expression of ABC transporter[5], and uptake. Results evidenced that chronic multi-generational exposure of daphnids reduced survival, growth and reproduction, increasing ROS, SOD and CAT from F0 to F2. Ce reduced the number of offsprings after each generation, while Er delayed the time of offspring emergence, but not their number. ROS, SOD, CAT and GST evidenced that Er is slightly more toxic than Ce. Up- and downregulation of genes was limited, but Ce and Er activated the ABC transporters. Their uptake decreased through exposure time and generations.

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INTERPLAY BETWEEN AUTOPHAGY AND REPRODUCTION: WHAT Ambra1 AND Epg5 ZEBRAFISH MUTANT LINES REVEAL

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Autophagy, a conserved and essential pathway to maintain cellular homeostasis, has been implicated in several physiological processes, including reproduction. In this field, autophagy plays critical roles in many steps, such as primordial germ cells survival, differentiation and degradation of maternal material during the first stages of development. Today, the use of knockout models is increasing our knowledge in this field. Ambra1 is a positive regulator of the Beclin1-dependent autophagic pathway, also involved in cell proliferation and apoptosis. The zebrafish genome contains two *ambra1* paralogous genes, *ambra1a* and *1b*. Differently from knockdown results, *ambra1a*$^{-/-}$ and *1b*$^{-/-}$ mutant embryos do not display overt developmental defects, due to compensatory effects of the paralogous gene remaining active. Silencing of zebrafish *ambra1b* gene leads to all-male individuals as demonstrated by visual analysis of secondary sexual features and reproductive behavioral of *ambra1b*$^{-/-}$. However, this particular phenotype is not due to physiological oocyte's apoptosis leading to testis differentiation in zebrafish but to a statistically significant reduction of PGC's number in 10-h post fertilization *ambra1b*$^{-/-}$ embryos, thus suggesting a direct involvement of this protein in primordial germ cell survival. Silencing of the Epg5 protein, a Rab7 effector involved in fusion specificity between autophagosomes and lysosomes, determines a premature reduction of both male and female reproductive capabilities or complete infertility starting from 8-10 months of age when zebrafish reproductive potential is generally at the highest level. The in-depth analyses in progress will contribute to unveil the possible role of Ambra1 infertility issues related to modifications in PGCs survival and to draw a more comprehensive picture of connections between specific autophagic proteins and reproduction.

A HUMAN NEUROMUSCULAR JUNCTION MODEL SYSTEM: AN ORGAN-ON-A-CHIP APPROACH.

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The development of a stable and functional synapse at the neuromuscular junction (NMJ) requires the reciprocal communication between spinal motor neurons and skeletal muscle fibers. The complexity of this highly specialized structure makes the *in vitro* modelling of the NMJ a challenging task. Nevertheless, in the past two decades co-culture systems to recreate the NMJ *in vitro* were developed to address concerns about animal models[1]. A further improvement in this issue was recently provided by microfluidics that, unlike in mass co-cultures, allows spatial and temporal control over different microenvironment by manipulating either neural cells or muscle cells populations independently[2]. Hence, exploiting an *organ-on-a-chip* approach, our aim is to obtain a reliable and predictive *in vitro* human model of NMJ in physiological and pathological conditions, to investigate the occurrence of NMJ detriment in disease. For this purpose, motor neurons derived from human iPSCs[3] and human skeletal muscle cells derived from perivascular muscle progenitors, namely pericytes[4], are seeded in two separated chambers of a microfluidic device. The two side of the device are linked together through microchannels that enable the axonal outgrowth to the muscle side, but not cell bodies migration, allowing the compartmentalization of the two cell population without interrupting the cell-cell communication. While being designed as a reliable platform to investigate the molecular actors of NMJ processes, the setup is versatile enough to host patient-specific cells and perform functional and molecular analysis.

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EFFECTS OF CPTH6, A HISTONE ACETYLTRANSFERASE INHIBITOR, ON ZEBRAFISH DEVELOPMENT

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The thiazole derivative CPTH6 (3-methyl-cyclopentylidene-[4-(4′-chlorophenyl)thiazol-2yl]hydrazone) is a pCAF and Gcn5 histone acetyltransferase inhibitor able to specifically reduce acetylation of histone H3 and H4, as well as acetylation of α-tubulin. This activity leads to growth inhibition, apoptosis and autophagy in cancer cell lines with different histotypes. Recent preliminary data indicate that CPTH6 inhibits tumor progression *in vivo* by repressing tumor-induced angiogenesis. Prompted by the observation that the knockouts of pCAF and Gcn5 were shown to affect the development of the cardio-vascular system in zebrafish, we decided to explore the effects of CPTH6 in the same experimental model system. Treatment of zebrafish embryos with CPTH6 doses lower than LC50 significantly impaired cardiac looping, as determined by the expression domain of the myocardial marker cmlc2, resulting in bradycardic larvae. The effects of CPTH6 on vascular development, analyzed in both wild type and in Casper g(kdrl:GFP;gata1:dsRed) zebrafish lines, revealed a remarkable activity of this molecule in altering sub-intestinal vein and intersegmental vessel formation. These vascular phenotypes were strictly associated to an altered blood flow. We also observed a delay in hatching of the larvae, which could be due to an impaired nervous system development, as suggested by a reduced expression of anterior neural markers and by a decreased locomotor activity. Overall, these data indicate that zebrafish is a convenient model system to study CPTH6 activities *in vivo*, paving the way to future experiments aimed at identifying molecular pathway controlled by CPTH6 targets during development and conserved in human oncogenesis.

DOES OBESITY AFFECT THE MUCIN SECRETION OF THE RAT INTESTINAL MUCOSA?

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The gastrointestinal tract plays a key role in obesity through its contributions to satiety, production of gut hormones, absorption of nutrients, changes in microbiota and their metabolic products. There are many gastrointestinal diseases for which obesity is a significant risk factor. Several data demonstrated that high-fat diet (HFD) induced obesity causes gut hyper-permeability and subclinical mucosal inflammation. In addition, nutritional factors are suggested to induce neuroplastic changes in the enteric nervous system (ENS). In our study we used diet-induced obesity (DIO) rats as a model to explore possible effects of HFD on the gastrointestinal system. The research interest was mainly addressed to point out modulations of the mucous secretion by the secreting goblet cells (GC) of the intestinal mucosa. The investigation was performed applying both lectins as marker for sialic acid recognition and antibodies useful to characterize the GC secretory products and secretion regulatory factors. Plasticity within myenteric and submucosal plexus were also investigated by an immunochemical approach. The analysis of the epithelium glucidic profile revealed a higher occurrence of sialoglycoconjugates, characterized by Sia-D-Gal(ß1,3)-D-GalNAc terminal sequences, in the duodenum and jejunum of DIO rats compared with the controls, while a general weaker expression of sialic acid 2,3-linked to D-Gal was found in the proximal colon of rats fed a HFD, than in controls. A distinctive features of such a decrease is the quite complete loss of staining in the GCs. A modulation of the parameters relative to the mucous secretion was also observed: in DIO rats, a higher production of Muc2, the major secretory gel-forming mucin, was observed in GCs of the proximal colon respect to the control rats. Likewise, only preliminary data have been obtained on modulation of the ENS in DIO rats. The hypothesis that leptin may contribute to membrane-associated and secreted mucin production via the activation of leptin receptors may be suggested as a perspective for further investigation.

GERMLINE DEVELOPMENT WITH TWO MITOCHONDRIAL GENOMES TO COPE WITH

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Germline cells are usually homoplasmic, carrying and transmitting a single mitochondrial variant. Heteroplasmy (the presence of more than one mitochondrial haplotype in an individual) is usually reported as an unfavourable condition affecting mitonuclear interactions[1,2], and having implications for embryo development and organism fitness. Despite that, there are no studies investigating heteroplasmy if not at mtDNA level, mostly due to technical limitations. By using the only known animal system showing stable and natural heteroplasmy, we investigated the presence of divergent mitochondrial variants at the protein level, and their localization within tissues, cells, and organelles. About a hundred of species of bivalve molluscs show two mitochondrial lineages: one transmitted through eggs (F-type) and the other through sperm (M-type). The amino acid sequence divergence between two conspecific F and M lineages reaches 52%, allowing the production of antibodies against the two variants of a same protein[3]. We immunolocalized the F- and M-type variants of three mitochondrial proteins in the germline and somatic tissues of both sexes at different developmental stages. We found heteroplasmy at the organelle level in undifferentiated germ cells of both sexes, and in male soma. During spermatogenesis, germ cells carrying the M-type might gain advantage due to faster proliferation or through selfish genetic elements that can spread by destroying competitors[4], as in the case of cytoplasmic male sterility in plants[5]. A specific phenotype - such as membrane potential or membrane tag[6] - could also participate in the process.

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SATELLITE DNA SUPPORTS THE MONOPHYLY OF LACERTIBAENIA (LACERTIDAE PLUS AMPHISBAENIA) IN SQUAMATE PHYLOGENY

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In phylogenetic inference, convergent evolution may be a critical problem for evaluating relationships among organisms adapted to the same niche. This is the case of squamate reptile possessing elongated and limbless bodies (snakes, amphisbaenians and dibamids) as an adaptation to burrowing[1]. Molecular markers can override this obstacle as is the case with satellite DNAs that, owing to their evolutionary dynamics, can be useful to discriminate taxa, not only at the level of genera and species but also at higher-rank taxon level. IMO-TaqI is a slow-evolving satellite DNA family described in lacertid lizards[2], which are regarded as the sister group to amphisbaenians[3]. In fact, with the use of degenerate primers, this repetitive element was PCR-amplified from the genome of the amphisbaenian *Trogonophis elegans* as well as from *Gallotia galloti*, a primitive member of the family Lacertidae[4]. This satellite DNA was found in the genomes of neither Teiiod species nor other squamate representatives. This suggests that IMO-TaqI represents a molecular synapomorphy of the Lacertibaenia clade, including Amphisbaenia and Lacertidae.

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THE EXPRESSION OF RAF KINASE PROTEIN INHIBITOR (RKIP) ON DIFFERENT HISTOLOGICAL TYPES OF LEIOMYOMA

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Uterine leiomyomas are smooth muscle tumors of the uterus and represent the most common benign tumors of female reproductive tract. They are the leading indication for hysterectomy[1]. A classification can be made according to histological characteristics of leiomyomas. Although, usual leiomyoma is the most common, other histological types exist. We can distinguish into atypical leiomyoma that is characterized by polymorphic nuclei, with bizarre nuclei and it is also called bizarre leiomyoma; leiomyoma with high mitotic activity and cellular leiomyoma and lipoleiomyoma. Finally, Leiomyosarcomas are the rare malignant counterparts[2]. Raf Kinase Protein Inhibitor (RKIP) is considered primarily as a suppressor of the metastasis gene. We have recently demonstrated that is expressed in human myometrial and leiomyoma tissue and we showed that RKIP inhibition by locostatin, reduces the ECM components, the proliferation and the migration in both leiomyoma and myometrial cells[3]. The aim of study was to test the expression of RKIP in the different histological types of leiomyoma tissues. The expression of RKIP, evaluated by immunohistochemistry, is high in leiomyomas bizarre, while is low in cellular leiomyomas, and is completely absent in leiomyosarcomas.

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STUDY OF 3,5-DIIODO-L-THYRONINE INFLAMMATORY ROLE IN OBESE ZEBRAFISH MODEL

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The 3,5-diiodo-L-thyronine (3,5-T_2) is an endogenous metabolite of thyroid hormones which has been reported to improve adiposity and associated disorders. Studies on the administration of 3,5-T_2 to high-fat diet (HFD) induced obese rodents have shown that this endogenous metabolite of thyroid hormones is able to prevent the body weight increase and reverts the HFD-associated proinflammatory pattern. Given that several brain and gut functions and obesity pathophysiological pathways are conserved between zebrafish (*Danio rerio*) and mammals, the obese adult zebrafish has been recently used as an experimental model to investigate fundamental processes underlying central and peripheral obesity driven inflammation. Thyroid hormones are highly conserved in zebrafish and the administration of 3,5-T_2 stimulates thyroid-sensitive tissues. In this study, we aim to determine, for the first time, whether 3,5-T_2 regulates central and peripheral inflammation in Diet Induced Obesity (DIO) zebrafish model. For this purpose, we supplemented the zebrafish water, during or after the DIO, with 3,5-T_2. The DIO zebrafish showed altered intestinal morphology with damaged villi accompanied by overexpression of several inflammatory markers. The 3,5-T_2 treatment enhanced the alteration of intestine morphology and the expression of the proinflammatory markers if given both after and during the DIO. Moreover, inflammatory status observed in the intestine was accompanied by brain inflammation, as indicated by the increase in microglia activation. Our results reveal that the effect of 3,5-T_2 in fish intestine and brain deviates from the typical one shown in mammals and opens new avenues to investigate new effects of this thyroid metabolite using zebrafish animal model.

EPIGENETIC CONTROL OF GENE EXPRESSION IN HUMAN MESENCHYMAL STEM CELLS DURING OSTEOGENIC DIFFERENTIATION

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Adult stem cells are widely used in cellular therapy not only because of their intrinsic poten-tial but also because their use does not raise ethical issues. Dental pulp is an interesting source of postnatal progenitor cells/stem cells[1]. Epigenetic regulation has been considered an important mechanism for influencing stem cell differentiation. In particular, histone deacetylases (HDACs) have been shown to play a role in the osteoblast differentiation of mesenchymal stem cells (MSCs)[2,3]. In this study, the ef-fect of the HDAC inhibitor, valproic acid (VPA), on bone formation *in vivo* by MSCs was determined. Surprisingly, VPA treatment, unlike other HDAC inhibitors, produced a well-organized lamellar bone tissue when MSCs-collagen sponge constructs were implanted sub-cutaneously into nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice, although a decrease of osteocalcin (OC) expression was observed. Consequently, we decided to investigate the molecular mechanisms by which VPA exerts such effects on MSCs. We identified the glucocorticoid receptor (GR) as being responsible for that downregulation, and suggested a correlation between GR and HDAC2 inhibition after VPA treatment, as evi-denced by HDAC2 knockdown. Furthermore, using co-immunoprecipitation analysis, we showed for the first time in the cytoplasm, binding between GR and HDAC2. Additionally, chromatin immunoprecipitation (ChIP) assays confirmed the role of GR in OC downregula-tion, showing recruitment of GR to the nGRE element in the OC promoter. In conclusion, our results highlight the existence of a cross-talk between GR and HDAC2, providing a mecha-nistic explanation for the influence of the HDAC inhibitor (namely VPA) on osteogenic dif-ferentiation in MSCs. Our findings open new directions in targeted therapies, and offer new insights into the regulation of MSC fate determination.

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X-RAY IRRADIATED MOUSE NEURAL STEM/PROGENITOR CELLS RECOVER NORMAL VIABILITY AND CELL CYCLE WITH DOSE-DEPENDENT KINETICS

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Most of the neuronal cells of the mammalian central nervous system are generated by neural stem/progenitor cells (NSPCs) located in the ventricular and subventricular zones of the embryonic neural tube. The capacity NSPCs for self-renewal and neuronal differentiation is affected by pathological conditions and environmental agents, leading to decreased neurogenesis and cognitive impairment. Ionizing radiation (IR) is a genotoxic agent that has been shown to negatively affect neurogenesis, but its effects on NSPC survival, self-renewal and differentiation are only partially understood. We have studied the response of NSPCs derived from the mouse embryonic cerebral cortex to low (0.2 Gy), moderate (1 Gy) and high (10 Gy) doses of X-rays IR at different time points after treatment (4, 24 and 48 h or 8 days). During the first 24-48h post-irradiation, we found that 10 Gy IR caused partial NSPCs mortality, with roughly 30% of irradiated cells undergoing apoptosis at each time point. Decreased survival was accompanied by delayed cell cycle progression and upregulation of cell cycle inhibitors, apoptotic markers and glial differentiation markers in irradiated cells. Surprisingly, these effects were largely recovered within a week of irradiation, leading to the recovery of viability, cell cycle and gene expression profiles similar to those of untreated cultures. In agreement with this functional recovery, NSPCs were able to efficiently repair the double-strand breaks induced by 10 Gy irradiation, as detected by the nuclear levels of phosphorylated histone H2AX. NSPC cultures treated with lower IR doses showed lesser effects that were recovered by 24h (0.2 Gy IR) or 48 h (1 Gy IR) post-irradiation. These results suggest that NSPCs from the developing cerebral cortex contain a IR-resistant subpopulation, which is able to successfully repair IR-induced DNA damage, escape IR-dependent cell cycle arrest and resume pre-irradiation growth rates, avoiding apoptosis, senescence or differentiation. Future work will investigate the selective survival of NSPCs to IR, the capacity of surviving NSPCs for neuronal differentiation and the long-term epigenetic changes induced by IR.

ANASTOMOTIC HEALING IN A RAT MODEL OF PERITONITIS: EVALUATION OF THE EFFECT OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ADMINISTRATION

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Anastomotic leakage is a harmful complication after colorectal surgery. The use of non-steroidal anti-inflammatory drugs is a cornerstone in the post-operative management but could be a risk factor for dehiscence[1] since the inflammatory reaction constitutes the first, essential phase of the wound repair sequence. The non-steroidal anti-inflammatory drugs are used to prevent adhesion at the anastomosis site during the stage of inflammation but may lead to deterioration or retardation of wound healing. The anastomosis strength depends by collagen deposition[2], regulated by cyclooxygenase enzymes through metalloproteinase modulation[3], in particular MMP9 is known to be associated with inflammation and anastomotic leak. Sepsis is characterized by a reduction of submucosal collagen synthesis, thus non-steroidal anti-inflammatory drugs could even more threaten the anastomotic healing when used in patients with peritonitis. Our aim was to study the morpho-functional effects of postoperative administration of two commonly used non-steroidal anti-inflammatory drugs, Diclofenac and Ketorolac, on the healing process of colocolic anastomoses constructed under condition of fecal peritonitis in a rat model. We assessed the anastomosis strength and morphological features of tissue wound healing, as well as immunohistochemical metalloproteinase 9 expression and collagen deposition. We found no significant difference between the treated and control groups, except a significant decrease of inflammatory cells and metalloproteinase 9 expression in the treated rats. Our findings showed that Diclofenac and Ketorolac administration did not affect post-surgical healing and did not increase the leakage risk of colo-colic anastomoses during peritonitis.

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EMBRYOTOXIC EFFECTS OF POLYSTYRENE MICROPLASTICS IN ZEBRAFISH (*Danio rerio*)

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Aware of the serious threat that plastic represents to the environment and to living organisms, the international scientific community has, over the last decade, focused the attention on the toxicological effects that micro and nanoplastics exert mainly on aquatic organisms. The aim of this study is to investigate the effects of polystyrene (PS) microplastics (MPs) on embryos and early life stages of zebrafish *Danio rerio* by performing a fish embryo toxicity test (FET). Zebrafish embryos were exposed to 200 particles/mL of 10 µm PS-MPs for 144 h post fertilization (hpf). The concentration was chosen in accordance with previous laboratory studies using zebafish adult and embryos. After specific extraction of samples, according to a patent for industrial invention to the Italian Ministry of Economic Development number no. 102018000003337, Microscopic (Zeiss) and Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer) identification methods were applied for MP detection in zebrafish larvae. The results obtained showed that PS-MPs were accumulated on the surface of egg chorions and were able to cause hatching retardation, morphological abnormalities including tail and spinal column deformity, oedema and blood pooling. Moreover, at 144 hpf, the molecular analysis of gene expression of the genes involved in the anti-oxidant detoxification systems (cytochrome P450-CYP and Glutathione-*S*-transferase-GST) and oxidative stress response (catalase-CAT and superoxide dismutase-SOD) highlighted a clear trend of transcription up-regulation for all the selected genes in response to PS-MPs exposure. Therefore, according to the results obtained and previous literature, it is possible to affirm that the environmentally relevant concentrations of microplastic particles affect larval fish ecology and that embryo-larval stages are more sensitive than adult forms, thus representing a direct threat to fish populations and marine biodiversity.

A NOVEL CLASS OF L1 CHROMATIN ENRICHED TRANSCRIPTS MAINTAINS T LYMPHOCYTES' CELL IDENTITY AND REGULATES THEIR DIFFERENTIATION

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Transposable Elements (TEs) are emerging as key epigenetic molecules involved in cell plasticity and tissue-specific regulation; they represent a prolific source of transcription factor binding sites, noncoding RNAs expression, pluripotency gene regulation and 3D genome organizers. However, their specific function in regulating the epigenome in adult cell commitment and differentiation is unexplored yet. Therefore, we are dissecting their dynamics in primary T lymphocytes. We found that T cells subset show a specific and dynamic pattern of TEs expression, in particular LINE1 (L1), which are stably chromatin associated transcripts and hierarchically organized in Naïve CD4+ T cells; interestingly, upon T cell activation and differentiation, L1 RNAs is quickly downregulated in a TCR dependent and cell proliferation independent manner; mTORC-1 signaling pathway is regulating these dynamics. Furthermore, L1 RNAs localize in specific chromatin compartments actively transcribed (H3K4me3 and H3K36me3 enriched regions), avoiding heterochromatin (H3K9me3 depleted regions), foreseeing a possible ncRNAs regulatory function. L1 chromatin RNA knock down in CD4+ Naïve cells showed an increase ability to differentiate in effector subsets, suggesting that these transcripts have the ability to govern T cell identity. Through the generation of chromatin enriched RNA seq datasets and the *de novo* transcripts reconstruction we have identified that L1 are contained within the introns of other transcriptional units, foreseeing a regulatory function in increasing transcriptome complexity. We further investigated L1 dynamics in tumor context, as nothing is known regarding the epigenetic features of Tumor Infiltrating Lymphocytes (TILs). Interestingly, we discover an aberrant L1 re-expression in exhausted TILs, foreseeing their possible role in immunosuppression and their eventual usage as molecular biomarkers or adjuvants in therapy.

A NEW PROTECTIVE AGENT AGAINST X-RAY DAMAGE OF HEMATOPOIETIC STEM CELLS

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Aim of radiotherapy is the cancer cell damage destroying their ability to divide and grow, by using high-energy ionizing radiation (IR), although causing side effects on healthy cells. A limiting factor in the use of radiotherapy is the acute and long-term bone marrow injury: IR induces hematopoietic stem cell apoptosis and senescence. The direct ionization of cellular macromolecules generates the formation of reactive oxygen species (ROS), which lead to functional cell alterations[1]. A new isoform of human MnSOD, isolated from liposarcoma cells and obtained in a synthetic recombinant form (rMnSOD), exerts the radiosensitizing effect for tumor cells and meanwhile a radioprotective effect on healthy cells[2]. In our study, we analyzed the rMnSOD radioprotective effect on human umbilical cord hematopoietic stem cell (CB-HSCs). Mononuclear cells were isolated from cord blood after informed consent of healthy donors, received by the Cord Blood Bank (Ba.S.C.O) of "Santobono Pausilipon" hospital in Naples, by using Ficoll/Hypaque gradient. Cultured CB-HSCs were incubated with 0.5 µM rMnSOD for 24h and then irradiated (2Gy) by using Mevatron (Siemens, Italia). After irradiation, an aliquot of cells was immediately stained with 0.4% Trypan Blue and cell viability and senescence was evaluated after 24h from irradiation, by using Guava Easy Cyte Flow Cytometer. Our preliminary results showed a viability increase of rMnSOD treated cells after IR exposure, compared to the only irradiated cells. Senescence evaluation confirmed the protective action of rMnSOD. HSC protection from IR should be a primary goal in the development of new medical countermeasures against radiation damages.

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CENTRAL AND PERIPHERAL CONTROL OF REPRODUCTION IN ZEBRAFISH GR $^{-/-}$ MUTANT LINE

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Glucocorticoids (GC) modulate diverse aspects of physiology and behavior, including energy homeostasis, inflammatory and stress response, through activation of its cognate receptor (GR). In the zebrafish model, morpholino knockdown of maternally derived gr mRNA triggers developmental defects that limit larval stage survival[1]. Homozygous gr$^{-/-}$ larvae, obtained by CRISPR-Cas9 approach, are viable through adulthood, although with reduced fitness and early life survival[2]. gr$^{-/-}$ fish are fertile, but with reduced reproductive capabilities. Starting from this last evidence, this study investigates how the *gr* mutation can influence the reproduction affecting the hypothalamic-pituitary-gonad axis signaling. On this regard, the expression of the key genes orchestrating the reproductive event were analyzed both at the central and peripheral level. At central level the expression of *kiss* isoforms and *gnrh3*, were analyzed and *kiss1* resulted significantly impaired in gr $^{-/-}$ mutants. At peripheral level, attention focused on class III b and IV follicles, which in wild type fish reach the competence and are ready for final maturation and subsequent ovulation. The histological analysis did not show any significant variation between wild type and gr$^{-/-}$ ovaries. On the contrary, real time PCR analyses showed a downregulation of *kiss* isoforms, *mprs, activins* and *lhcgr* mRNAs in class IV follicles isolated from *gr*$^{-/-}$ mutants respect to wild type, suggesting that *gr*$^{-/-}$ mutation mainly affects ovulation.

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PUBERTY ONSET OF MEDITERRANEAN SWORDFISH: A MULTIDISCIPLINARY APPROACH TO UNVEIL THE DYNAMIC OF OVARY MATURATION

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The Mediterranean swordfish *Xiphias gladius* is a highly migratory and important commercial fish species recently put through a recovery plan by the International Commission for the Conservation of Atlantic Tunas (ICCAT). In order to support such conservation effort, RNA-sequencing represents a suitable approach to discover molecular pathways involved in metabolism and reproduction. Here, by means of Illumina sequencing, ovary mRNA from mature and immature females were analysed in order to gain insights into puberty onset. Moreover, a thorough histological investigation of the ovaries and extensive analysis of biometrical data were performed to integrate the available molecular information of this wild caught fish species. The final swordfish transcriptome assembly was composed by 100.869 sequences, of which 30.398 with a Gene Ontology (GO) annotation and 25.151 unigenes. Moreover, differential expression analysis (DEA) followed by GO and KEGG pathway analyses revealed that most of the genes involved into key biological functions underlying reproductive maturation such as ovarian steroidogenesis, RNA/DNA processing and lysosome formation/maturation, in addition to transport and lipid metabolism, were up-regulated in mature ovaries. The size at first maturity (L50) was established as well as the GSI-cut off value, corresponding to a GSI threshold that differentiated spawning from immature individuals. The present study provides the first *de novo* assembly of the swordfish transcriptome in addition to deliver a wealth of information regarding GSI pattern, reproductive season and L50. The integration of these data provides a better and comprehensive understanding of the reproductive biology of the swordfish from molecular to macroscopic scale with remarkable improvements towards a sustainable stock management throughout the next years.

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ALTERATIONS IN THE GLYCOSYLATION PATTERNS OF MURINE DUODENAL MUCINS INDUCED BY HIGH-FAT DIET

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The high-fat diet (HFD), typical to Western-style eating habits, predispose to alterations in intestinal glycosylation and consequent inflammatory disease states, cancer/tumorigenesis and increase of susceptibility to pathogens. Here we investigate the effects of HFD on the mucins secreted by the duodenum of the mouse by classic and lectin-binding histochemical methods. Mucins in this tract are secreted by both goblet cells in the villi and Brunner's glands. Samples of duodenal mucosa were obtained from twelve adult mice of both genders divided into a control group fed a standard diet and a group fed a HFD for 25 weeks. Samples of duodenum were removed and processed for histochemistry. Classic histochemical staining included Periodic acid-Schiff (PAS), Alcian Blue (AB) at pH 2.5 and High Iron Diamine (HID). Lectin-binding experiments were performed with FITC -labelled lectins (SBA, PNA, MAA-II, SNA, WGA, ConA, Paradoxical ConA, UEA-I, LTA, AAA), also with sialylation and desulfation pretreatments. Intensity of Optical Density (OD, for classical histochemistry) or corrected total cell fluorescence (CTFC, for lectin-binding) was scored by image analysis and compared by statistical methods between control and HFD groups by Student's t-tests. In Brunner's glands HFD showed significantly lower intensity or staining/lectin-binding for PAS and most of lectins, except for LTA and WGA in which the opposite was observed. In particular, a neat decrease of binding intensity in HFD was observed with Paradoxical ConA. In villar goblet cells a significant decrease was observed for HID and lectin-binding, except for WGA in which to opposite was found. In conclusion, HFD diet results in a general reduction of mucin glycosylation, in particular of sulfated residuals in the villar goblet cells and 1,4 N-acetylglycosylated residuals in Brunner's glands (as detected by Paradoxical ConA). Sulfation affects bacterial mobility, whereas 1,4 GalNac have been proposed has having cytostatic and antibiotic functions. Thus, the reduction of the cited residuals could have an important role on gastrointestinal functions and health conditions.

EFFECTS MEDIATED BY α7 NICOTINIC RECEPTOR IN RAT SCHWANN CELLS: IMPLICATIONS IN PERIPHERAL NERVE REGENERATION

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Peripheral nerve fibers are able to regenerate. During nerve regeneration, Schwann cells (SCs) assume a phenotype known as *Repair Schwann Cells*, relevant for promoting an anti-inflammatory environment and axonal regeneration. SCs are cholinoceptive; in fact they express functional muscarinic cholinergic receptors favoring SCs differentiation towards myelinating phenotype[1-3]. Recently we have also characterized the expression of 7 nicotinic receptor. This receptor is faintly expressed in sciatic nerve fibers and in SCs *in vitro*. Its expression significantly increases both *in vivo* and *in vitro*, after nerve injury or in presence of Bradykinin (Bk), a neuropeptide known for its pro-inflammatory effects. In fact we observed that sciatic nerve dissected and maintained *in vitro* for 24 h both in absence and in presence of BK, showed a significant increase of α7 receptors. Moreover the selective activation of this receptor with (R)-ICH3 caused a modulation of uPA and MMPs responsible of the microenvironment modifications favoring Wallerian degeneration and promoting nerve regeneration. Similarly, the treatment of cultured SCs with BK appears to promote the *repair Schwann cells* phenotype, favoring the changing in cell morphology and up-regulating the expression of GFAP and c-jun. The activation of α7 receptor by selective agonist (R)-ICH3 after BK treatment, appears further promote this phenotype modulating inflammatory environment in terms of cytokines, growth factors and proteases production. These results suggest that α7 nicotinic receptor may be a cholinergic receptor expressed only by repair Schwann cells. Considering its anti-inflammatory role, α7 receptors may contribute to generate a microenvironment improving peripheral nerve regeneration.

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PROTECTIVE AND REPARATIVE EFFECTS OF THE ANTI-FUNGAL DRUG FLUCONAZOLE ON SEROTONIN-INDUCED ALTERED NEURONAL DIFFERENTIATION: RESULTS ON MIDBRAIN MICROMASS CULTURES

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Multiple sclerosis (the second cause of neurological disability in European young adults) i*s* an inflammatory autoimmune disease characterized by disruption of myelin ensheathing axons and axonal damage. Recent data showed the antifungal azole miconazole to induce prodifferentiating effect in oligodendrocyte precursors through the inhibition of retinoic acid (RA) catabolic enzymes. The aim is to evaluate the protective and reparative effects of azoles on serotonin (5HT)-induced alterations of neuronal differentiation, using the micromass test. Rat embryo (E13) midbrain cells cultured at high density normally move together and form distinct foci (micromasses, tridimensional aggregation of cell bodies) interconnected by bundles (aggregation of neuronal processes). Micromasses were incubated during the whole culture period with a known promoter of differentiation (RA), with a known differentiation inhibitor (5HT) or with azoles in clinical use (fluconazole, miconazole and itraconazole) alone or in mixture. 5HT inhibitory effects at 50-100 μM was confirmed. Among RA and the selected azoles, the most promising molecule in our model was fluconazole, tested at 5-100 μM. In order to test protective effects of fluconazole on 5HT inhibition, we co-exposed micromasses to both molecules during the whole culture period. Results show that co-exposed groups displayed parameters comparable to controls, suggesting a protective effect of fluconazole. A second set of experiments were devoted to the evaluation of a fluconazole-related reparative effect. Cultures were exposed during the first day to 5HT alone and during the remaining culture days to fluconazole alone. The one-day 5HT exposure affected development while after the post-exposure to fluconazole a reparative effect was evident. The data of the present work suggest both a protective and reparative effect of fluconazole in a micromass model of neurodegeneration, suggesting this drug as a good candidate for pharmacological repurposing.

CDK5 INVOLVEMENT IN ASCIDIAN NEUROGENESIS

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Cyclin-dependent kinases (CDKs) are a family of serine-threonine kinases whose activity requires interaction with cyclins[1]. Although the majority of CDKs plays a key role in controlling cell division cycle, there is an important exception: Cyclin-dependent kinase 5 (CDK5). CDK5 gets activated by its neural specific activators, CDK5R1 and CDK5R2[2], and its proper activity is critical during vertebrate neurogenesis. During brain development, CDK5 complex is implicated in neural survival, migration as well as dendritic outgrowth and synapse formation[3]. Most research on CDK5 has focused on vertebrates, while only few studies have been performed in other animal groups[4]. In the genome of the ascidian *Ciona robusta*, homologs of CDK5 and its regulators are present and, based on our analysis, their expression patterns are comparable with those reported in vertebrates. We started exploring CDK5 involvement in neural development of *C. intestinalis*, specifically inhibiting CDK5 activity by drug treatments. Even if the overall morphology was not affected, the central nervous system of larvae exposed to low doses of the inhibitor showed specific malformations. Overall, our results suggested that CDK5 functions are highly conserved between ascidians and vertebrates, setting the stage for further research about its regulation during ascidian development.

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EFFECTS OF PROPYLPARABEN ON *Danio rerio* LARVAE DEVELOPMENT

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Parabens are widely used in cosmetics, toiletries, food, and pharmaceuticals. The research on the effects of parabens on health is limited, and the effects of parabens on development have not been systematically investigated. The present study used zebrafish *(Danio rerio)* larvae to determine the acute toxicity of propylparaben (PP) at early developmental stages. The fertilized eggs were exposed for 96h at 5 different concentrations of PP and daily observed with inverted optical microscope recording four apical observations as indicators of lethality: coagulation of fertilized eggs, lack of somite formation, lack of detachment of the tail-bud from the yolk sac, lack of heartbeat. On this basis, the calculated LC50 value was 3.98 mg/L. The most common sublethal alteration in all experimental groups was the enlarged and misshaped yolk sac. Particularly, sublethal PP concentrations led to a decrase of lipids contents in the whole body and an increase in the yolk sac of 96h larvae. Our study shows that yolk sac abnormalities, induced by PP, result in an impairment of lipid metabolism that may lead to the observed developmental delay and other sublethal alterations in exposed embryos, such as hyperexcitability.

HEMOCYTE RESERVOIRS IN THE MOLLUSC *Pomacea canaliculata*: EVIDENCE FROM MORHPOLOGICAL AND IMAGE-BASED FLOW CYTOMETRY ANALYSES

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Invertebrates do not possess adaptive immunity, still they display an outstanding capability of colonizing new environments. Through cell-based and humoral innate immune responses, invertebrates manage stressing conditions and immunological stimuli, and can distinguish commensal from pathogenic bacteria. Scanty information is available about how circulating and immune-related cells, *i.e.*, hemocytes, can differentiate, cross-talk and finely diversify specific responses against the vast array of antigens they are subjected to. In this perspective, the combination of post-genomic and high-throughput approaches can answer to numerous questions still pending about invertebrate hemocyte maturation and differentiation. The research organism *Pomacea canaliculata*, an invasive freshwater snail distributed worldwide, offers the uncommon possibility to perform multiple hemolymph withdrawals from the same animal, simulating repeated bleedings and allowing the analysis of a forced hemocyte turnover. Morphological and functional analyses on hemolymph samples collected 4 times at intervals of 24 h each identified Group I and Group II circulating hemocytes, but did not evidence significant alterations between the time points. The high-throughput analysis of hemocyte phenotypic features performed with ImageStream® X Mark II Imaging Flow Cytometer, followed by the cell clustering on a morphological base, confirmed in control animals the hemocyte groups previously described and did not reveal significant changes in the relative abundance of the hemocyte groups after 4 hemolymph withdrawals. Our observations suggest the existence in *P. canaliculata* of hemocyte reservoirs that store hemocytes and mobilize them following hemorrhages or immune stimulation. Further investigations will contribute to identify the location of these hemocyte reservoirs and determine if in *P. canaliculata* they also play a role in hemocyte maturation and selective activation.

WHICH IS THE POTENTIAL DIFFERENTIATION PATTERN OF HUMAN DENTAL PULP STEM CELLS AND FIBROBLASTIC CELL POPULATIONS?

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This study aims to investigate the *in vitro* differentiation pattern of human dental stem cells and two populations of human fibroblasts towards the osteogenic, odontogenic and adipogenic differentiation[1]. Human dental pulp stem cells (hDPSCs), gingival fibroblasts (hGFs) and foreskin fibroblasts (hFFs) were cultured in both osteogenic and adipogenic media for 7, 14 and 21 days. RNA extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) were performed to assess the expression of specific markers for osteogenic (*RUNX2, ALP, SP7/OSX*), odontogenic (DSPP) and adipogenic (*PPAR-γ2, LPL*) differentiation. In osteogenic culture conditions, both hDPSCs and hFFs showed expression of *RUNX2*, during all stages of culture. *SP7/OSX* expression exhibited a moderate peak at day 14, while *ALP* showed a progressive upregulation during the period of culture. In contrast to hDPSCs and hFFs, *RUNX2* expression was downregulated in hGFs, while the expression of *ALP* has not increased during the culture period. However, hGFs showed a striking peak in *SP7/OSX* expression at day 14. Interestingly, *DSPP* expression was increased in cultured hDPSCs, but not in hFFs and hGFs. In adipogenic culture conditions, although a significant upregulation of *PPAR-γ2* and *LPL* expression was observed in all experimental groups at early time points, upregulation was most prominent in hFFs. The present findings show that hDPSCs represent the most appropriate cell population for regenerative purposes involving bone and dental tissues and that hGFs and hFFs have also a certain capability to form bone hard tissue. However, these two cell populations are more prone towards adipogenic differentiation than hDPSCs.

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EFFECTS OF HORMONAL STIMULATIONS ON METABOLIC STATE, ENDOCANNABINOID SYSTEM AND STEROIDOGENIC ENZYMES IN HUMAN CUMULUS CELLS

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The choice of controlled ovarian stimulation (COS) protocols, together with parental age and etiology of infertility, is crucial for the success of Assisted Reproduction Technology (ART)[1]. In COS, hormones trigger an ovarian hyperstimulation, inducing the development of follicles and the maturation of several oocytes. FSH and LH, which act on follicular somatic cells, regulating the maturation of the oocyte, are widely used in ART, but it is still under debate which approach is to be preferred. Different COS protocols induce differential gene expression and protein patterns of cumulus cells (CCs) and granulosa cells. Given the known dependency of oocytes from CCs in terms of hormone production and lipid/carbohydrate metabolism, our study aimed to highlight the differences induced by COS protocols based on (i) urinary FSH (uFSH), (ii) recombinant FSH (rFSH) and (iii) FSH+LH administration on: CCs steroidogenesis, metabolism

and endocannabinoid system. Fourier Transform Infrared Microspectroscopy highlighted changes in the amount and characteristics of CCs macromolecules. The three groups of patients were similar for age, BMI, FSH and AMH levels. We found differential expressions, due to COS protocols, in the steroidogenesis pathway investigated through *star*, *cyp11a1*, *hsd3b*, *cyp17a1* and *cyp19a1*; in the endocannabinoid system, evaluated by the analysis of the four main receptors (*cnr1*, *cnr2*, *gpr55* and *trpv1*) and the enzymes involved in the metabolisms of anandamide (*nape-pld* and *faah*) and 2-acylglycerol (*dagl* and *magl*); the lipid (*ppara*, *pparg*, *fasn* and *srebp*) and carbohydrate (*glut1* and *glut9*) metabolisms, which were associated to the macromolecules composition of CCs and the ART outcome. Our findings provide new insights on the effects of different COS protocols on ovarian functions. The information obtained on CCs provides evidence about the best protocol to obtain good quality oocytes.

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THERAPEUTIC EFFECT OF POLYMERIC NANOMICELLES FORMULATION OF GALUNISERTIB (LY2157299) ON CCL4-INDUCED LIVER FIBROSIS IN RATS

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Hepatic fibrosis (HF) is a major cause of liver-related mortality and, so far, no effective antifibrotic drug is available. Advanced HF, and its end-stage condition, cirrhosis, is characterized by extracellular matrix (ECM) components aberrant and excessive accumulation, that cause architectural and vascular distortion leading to functional liver failure, variceal bleeding and emerging hepatocellular carcinoma (HCC). Among the spectrum of mediators possessing profibrotic properties, TGF-β1 is a key profibrogenic cytokine in HF by affecting various liver-specific cells, including hepatic stellate cells (HSC) and portal fibroblasts, towards excessive ECM producing myofibroblasts, the major effector cells of fibrosis. Literature data report that LY2157299 (LY-Galunisertib), a TGF-β1 receptor inhibitor, is very effective in the HF treatment and it is currently under clinical investigation in HCC patients. As LY availability in the blood could be negatively affected, a carrier (micelles) based on polygalacturonic acid and polyacrylic acid has been designed and the antifibrotic potency of encapsulated LY formulation *vs* free LY was studied in a CCl_4–induced HF rat model (weighing 180-200 g Sprague-Dawley rats). The HF was induced by intraperitoneally injection of CCl_4 (40% in olive oil) weekly thrice for 2 w. After, the rats were injected with encapsulated LY formulation or free LY weekly thrice for 2 w. Histopathological changes in the liver were assessed using hematoxylin and eosin staining for general observation. HF was evaluated from specimens stained with Masson's trichrome and quantified by computer image analysis. Moreover, the HSC activation was evaluated by immunohistochemistry analysis of alpha-smooth muscle actin. The results showed a prominent antifibrotic potency of encapsulated LY formulation respect free drug. In particular, it improves liver histology and attenuates collagen deposition and HSC activation.

ENHANCERS ARE INVOLVED IN THE ACTIVATION OF ANAEROBIC GLYCOLYSIS DURING CARDIAC AGING

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The cardiac aging is often associated with an impairment of mitochondrial oxidative phosphorylation and an increase of anaerobic glycolysis[1]. This metabolic remodeling causes an "energy deficit" that contributes to impairment of cardiac function in the elderly[2]. Gene expression analysis has revealed that cardiac aging is accompanied by down-regulation of genes involved in energy metabolism, including genes associated with mitochondrial function (*e.g.*, fatty-acid metabolism) and turnover[3]. This finding suggests that aging-related metabolic remodelling is caused by changes in the expression of metabolic genes. Despite this, the molecular mechanisms causing these changes are not completely known[3]. Enhancers play a key role in defining the gene expression program of heart development and cardiac hypertrophy[4,5]. Therefore, alteration of the activity of enhancers could contribute in defining the gene expression changes responsible for metabolic remodelling in old cardiomyocytes. To test this hypothesis, we investigated the activity state of enhancers in relationship to gene expression and metabolic changes occurring in cardiac aging. To this end, we integrated metabolic data with RNA-seq and ChIP-seq data for H3K27ac and H3K27me3 (two histone markers that define active and repressed enhancers, respectively) obtained from cardiomyocytes purified from the heart of mice at different ages (8 weeks old, and 6 and 18 months old, corresponding to young, adult and old-age onset mice). We found that a large fraction of enhancers undergoes a change in activity and that this is associated with a variation in the transcription level of neighbouring genes: a set of enhancers passed from a state of inactivity or of little activity to an active state, which included those associated with genes involved in glycolysis. By comparing the enhancer dataset with metabolic profiles, we found that the activation of enhancers neighbouring glycolytic genes was associated with an increase of metabolites of anaerobic glycolysis. Together, these results demonstrate that enhancers are involved in promoting the gene expression changes - in particular, activation of anaerobic glycolysis - occurring in the cardiomyocyte during aging.

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APREMILAST: A NEW DRUG TO RESTORE THE PATHOLOGICAL TH_1-TH_{17}/TH_2 IMBALANCE IN CUTANEOUS MESENCHYMAL STEM CELLS OF PSORIATIC PATIENTS

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Psoriasis (PsO) is a multifactorial immune-mediated inflammatory disease, which involves skin, joints, or both. It is associated with several comorbidities, including metabolic and other chronic inflammatory diseases[1]. Several studies showed that psoriasis is characterized by an imbalance between Th_1-Th_{17} and Th_2 infiammatory axes that influences the immunosuppressive ability of cutaneous mesenchymal stem cells (MSCs) that in turn secrete

higher amount of associated cytokines, inducible nitric oxide synthase (iNOS) and vascular endothelial growth factor (VEGF)[2]. The purpose of our study was to evaluate the effects of Apremilast drug, an oral phosphodiesterase 4 inhibitor, on the expression of VEGF, iNOS and the tryptophan metabolism enzyme indoleamine 2,3-Dioxygenase (IDO)[3] by MSCs isolated from skin of healthy subjects and psoriatic- patients. MSCs from skin of control (C-MSC) and psoriatic (PsO-MSCs) subjects were isolated at baseline (T_0) and after 12 weeks of treatment (T_{12}) with Apremilast. MSCs were characterized according to the Dominici's criteria, and the expression of VEGF, iNOS and IDO was analized by immunocytochemistry and immunohistochemistry. Our results show that both PsO-MSCs and healthy MSCs attain the minimum criteria for MSCs definition; PsO-MSCs at T_0 express higher level of iNOS and VEGF than C- and Pso-MSCs at T_{12}, whereas IDO expression was lower. In conclusion, Apremilast may affect the physiopathological pathway of psoriasis; after 12 weeks of treatment, PsO-MSCs display properties nearer to the phisyological profile of C-MSCs. The drug is therefore able to drive psoriatic cells towards controls.

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IDENTIFICATION AND EXPRESSION STUDIES OF PUTATIVE STEM/PROGENITOR CELL MARKERS IN THE UROCHORDATE *Botryllus schlosseri*

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In the colonial ascidian *Botryllus schlosseri*, a cyclical generation change guarantees the recurrent (weekly at 20°C) renewal of the zooids. During the blastogenetic cycle (*i.e.*, the interval of time between a generation change and the following one), buds progressively grow to the adult size before replacing the old zooids. With the aim of better elucidating the process stem cell differentiation, with particular reference to the genesis of haemocytes during the of the colonial ascidian, we screened the *B. schlosseri* genome and transcriptome, looking for transcripts/genes showing similarity to vertebrate molecular markers of haematopoietic stem/progenitor cells. On these sequences, after an in silico translation, we performed the phylogenetic reconstruction that, always, returned us the tunicate relevant position, within the protochordates cluster, of vertebrate sister group. The four mammalian orthologous genes, used as markers for the recognition of haematopoietic stem/progenitor cells, identified in *B. schlosseri*, are *bsabcg2*, *bscd133*, *bsgata1/2/3* and *bsgata4/5/6*. The ISH assay, performed by antisense specific riboprobes, on haemocyte monolayers and colony sections, resulted in a labelling of the sub-endostylar haemolymph lacunae. This results matches previously morphological data that identified the endostyle as a stem cell niche, strengthening our idea to use *bsabcg2, bscd133, bsgata1/2/3 and bsgata4/5/6* genes for the identification of haematopoietic stem/progenitor cells in *B. schlosseri*. Quantitative real time PCR (qRT-PCR) highlighted the overexpression of the considered genes in the mid-cycle phase of the blastogenetic cycle. During this phase, there is the formation of new secondary buds emerging from the primary buds. The higher transcription levels of *bsabcg2*, *bscd133*, *bsgata1/2/3* and *bsgata4/5/6* in the mid-cycle phase reflect the presence of undifferentiated cells involved in proliferative and differentiation events required for the formation of the new blastogenetic generation.

FAS/FASL PATHWAY IN IMMUNO-ESCAPE AND PROMOTION OF CHONDROGENIC DIFFERENTIATION OF HUMAN DENTAL PULP STEM CELLS

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Human dental pulp stem cells (hDPSCs) represent a suitable source of stem cells due to high proliferation rate, pluri/multipotency and ability to modulate inflammatory processes through different pathways. As previously demonstrated, after direct coculture with PBMCs, hDPSCs induced apoptosis in $CD4^+$ and $CD8^+$ T-cells through Fas/FasL pathway. However, cell death did not occur in Fas bearing hDPSCs[1,2]. This study aimed 1) to evaluate how Fas and FasL are modulated following the activation of extrinsic apoptotic pathway and 2) to investigate the role of Fas and FasL in inducing chondrogenic differentiation. c-Kit^+/$STRO\text{-}1^+$ hDPSCs were co-cultured with activated PBMCs and exposed to different concentrations of human recombinant FasL (rc FasL) protein. The expression of extrinsic apoptosis-related markers (c-FLIP, FADD, caspase 8 and 3) and chondrogenic markers (Sox9, Collagen I and Collagen II) was evaluated. After rc FasL-stimulation hDPSCs displayed a higher proliferation rate and no expression of cleaved caspases 8 and 3. In addition, increased FasL and c-FLIP in hDPSCs were detected after stimulation. Conversely, the Fas expression was reduced after rc FasL treatment. These effects were partially reverted by the use of FasL-inhibitor. On the other hand, as long as chondrogenic induction occurred, hDPSCs exhibited an increase of both Fas and FasL at day 7, besides an increased expression of Sox9, which was even up-regulated after rc FasL stimulation. The chondrogenic commitment of hDPSCs was then confirmed by augmented expression of Collagen I and Collagen II. Our data indicate that hDPSCs are able to modulate the activation of extrinsic apoptotic pathways through c-FLIP and that Fas/FasL pathway plays a key role both in immuno-escape and in favoring chondrogenic induction.

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REGENERATIVE POTENTIAL OF HUMAN DENTAL PULP STEM CELLS IN AN ANIMAL MODEL OF STRESS URINARY INCONTINENCE

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Stress urinary incontinence (SUI), the most common type of urinary incontinence, is defined as an involuntary leakage of urine due to physical stress involving an increase in bladder pressure. It is associated with life quality issues, depressive symptoms and social discomfort. The pathophysiology consists in a damage of the external urethral sphincter affecting both muscle and nerve tissue components. The current conventional therapies are mainly represented by rehabilitating methods, pharmacological and/or surgical treatments. However, these therapies are not able to revert the primary cause of incontinence, in fact only symptoms can take relief by those treatments[1]. Regenerative medicine with

the use of mesenchymal stem cells might provide an alternative tool for the treatment of SUI. The aim of this study was to evaluate the regenerative potential of human dental pulp stem cells (hDPSCs) in an animal model of SUI. Human DPSCs can be easily isolated during routine tooth extraction procedures, own a wide differentiation potential, immunomodulatory properties and do not present ethical issues[2-4]. In the first phase of the study hDPSCs were induced towards the myogenic commitment *in vitro* following a 24 h pre-conditioning with a demethylating agent 5-aza-2′-deoxycytidine (5-Aza). The differentiation was demonstrated by immunofluorescence, Western blot and real time PCR analyses against the myogenic markers, *i.e.* Pax7, myogenin, desmin and myosin, at different experimental time points. In the second phase, surgical transection of the pudendal nerve was performed in female rats, thus inducing the onset of stress urinary incontinence. One week after surgery, hDPSCs pre-differentiated with 5-Aza were injected in the urethral sphincter. Four weeks later, histological and immunohistochemical analyses showed that the sphincter thickness was almost recovered, hDPSCs engrafted in the external urethral sphincter, committed towards myogenic lineage *in vivo* and promoted vascularization. An appreciable recovery of the continence was reported in hDPSCs-treated rats. Moreover, human DPSCs were also detected within the nerve, thus suggesting their participation in reinnervating the formerly injured nerve. Our data, in association with further investigations on paracrine and immunomodulatory abilities of hDPSCs, might allow to propose them as a promising tool for future alternative therapies in the treatment of SUI.

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ENGINEERED EXTRACELLULAR VESICLES FROM HUMAN PERIODONTAL LIGAMENT STEM CELLS: A PROMISING TOOL IN BONE REGENERATION.

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Bone regeneration is a complex physiological process of bone formation, and it is involved in continuous remodelling during life[1]. Bone regeneration represents still a challenge[2]. Currently, there is a plethora of different strategies to ameliorate bone-regeneration process[3]. Recently, the development of biomaterials loaded with stem cells provided a promising treatment for bone defects[4]. It was demonstrated that scaffolds enriched with Mesenchymal Stem Cells (MSCs) and/or their derivatives, as conditioned medium (CM) and extracellular vesicles (EVs), may improve bone regeneration *in vivo*[4,5]. In this work we evaluated the bone regeneration capacity of a membrane (3D-COL) enriched with human Periodontal-Ligament Stem Cells (hPDLSCs) and CM or EVs or EVs engineered with polyethylenimine (PEI-EVs). The performance *in vitro* and *in vivo* to induce angiogenic factors necessary to starting the vascularization procession and to promote the bone regeneration has been evaluated. *In vitro* results showed an increased expression of osteogenic markers in hPDLSCs cultured with 3D-COL/PEI-EVs, associated with the increased levels of Vascular Endothelial Growth Factor (VEGF) and VEGF receptor 2 (VEGFR2). The constructs have been grafted in bone calvaria defects. After 6 weeks of implantation the samples were processed for histological examination. Histological analyses evidenced the activation of bone regeneration and the presence vascularization process in rats grafted with 3D-COL/hPDLSCs/PEI-EVs. MicroCT confirmed the histological results. This tool was capable to accelerate the overall regeneration process producing new bone with biomechanical properties that are identical to normal bone. The use of cell derivates could open a new strategic possibility in the field of bone regeneration.

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HYPOXIA AS A STIMULUS UPON NEONATAL SWINE MENISCUS CELLS: HIGHWAY TO PHENOTYPIC MATURATION OF MENISCAL FIBRO-CHONDROCYTES?

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Menisci are essential structures in the knee joint where they cover fundamental biomechanical and protective roles[1-3]. Menisci are characterized by a peculiar structure that, on one hand, allow them to perform their particular role in the stifle joint, but simultaneously make them a very challenging structure to deal with[2]. Immature menisci are featured by numerously elongated cells (fibrocytes-like) in a disorganized matrix composed almost completely of collagen type I and few glycosaminoglycans (GAGs) and have a rich vascularization, on the other hand, mature and functional menisci are characterized by few round-shaped cells, a matrix rich of well ordinated collagen fibres (above all collagen type II) and GAGs, and preserve vascularization only in the outer zone (aka *red zone*)[1]. Great interest, in both human and veterinary medicines, is reserved to the treatment of the injuries of the inner and avascular zone (aka *white zone*) of the meniscus: until now, there are no perfect solutions for the regeneration or the replacement of this tissue once injured[3]. This work is focused on the utilization of an environmental factor like hypoxia in meniscal tissue culture, in order to evaluate if it could be utilized to improve meniscal culture with a view to tissue engineering. Ninety menisci from neonatal pigs (day 0) were harvested and cultured under two different atmospheric conditions (hypoxia with 1% O_2 and normoxia) until 14 days. Samples were analysed at 0, 7 and 14 days through histochemical (Safranin-O staining), immunofluorescence and RT-PCR (Sox-9, Hif-1a, Hif-2, Collagen I and II, both methods) and biochemical (DNA, GAGs, DNA/GAGs ratio) techniques to record any possible differences in maturation of meniscal cells. Safranin-O staining allowed to show an increment in matrix deposition and round-shape "fibro-chondrocytic" cells quantity of hypoxia-cultured menisci respect to controls under normal atmospheric conditions. The same maturation shifting was observed by means of immunofluorescence and RT-PCR analysis, characterized by an increment of Sox-9 and collagen II, moving from day zero to 14-days under hypoxic environment, and by biochemical analysis, with an increment of DNA/GAGs ratio typical of mature meniscal tissue (characterized by few cells and much GAGs). This study shows that hypoxia can be considered as a booster to achieve meniscal cells maturation and opens considerably opportunities in the field of meniscus tissue engineering.

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EFFECT OF HIGH-CARBOHYDRATES DIET IN LIVER INJURY IN A MOUSE MODEL OF NAFLD/NASH

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Nonalcoholic Fatty Liver Disease (NAFLD) has become the most common liver disease of current times and represents a multifactorial disorder involving a complex network of factors (*i.e.*, diet). NAFLD is characterized by a benign accumulation of triglycerides in the liver but may degenerate in a more severe form associated with inflammation and fibrosis known as Nonalcoholic Steatohepatitis (NASH)[1]. Currently, no pharmacological therapies are available for NAFLD/NASH and the only clinical strategy is linked to diet modifications. Despite it is increasingly emerging that an abuse of carbohydrates could be involved in the progression of liver injury, the comprehension of sugar damages is still far to be completed[2]. Aim of this study is to evaluate the impact of a high-carbohydrates/Low Fat Diet (LFD) comparing to the High-Fat Diet (HFD) and Standard Diet (SD) in a nutritional mouse model of NAFLD/NASH at eighteen months of induction. Histological, Real Time PCR (assessing *tgfb1*, *ccn2* and *lepr*) and immunohistochemical (using perilipin, CD68, TGF-β1, CTGF, leptin, leptin receptor and α-SMA antibodies) analyses were performed. Interestingly, our preliminary results showed that the abuse of prolonged unbalanced HF or LF diets lead to comparable liver damages (signs of steatosis, inflammation and fibrosis). Accordingly, a significant increase of all tested molecules was detected in treated mice respect to control animals. Our study highlighted that the simple substitution of fats with carbohydrates is not sufficient to prevent or mitigate the progression of NAFLD to NASH. Further studies will be necessary to define the impact of diets to liver damage and the most appropriate alimentary management able to slow down liver injury and reduce the risk of diet-related disease such as metabolic syndrome.

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POSTNATAL DEVELOPMENT OF PROLIDASE DEFICIENT MICE (*DAL*) CEREBELLUM: OXIDATIVE AND INFLAMMATORY PATHWAYS EVALUATION

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Prolidase enzyme, a member of matrix metalloproteinase family, regulating extracellular matrix (ECM) turnover and brain development during pre and postnatal period, is the only enzyme able to divide dipeptides containing proline or hydroxyproline residues at the C-terminal end of an amino acid chain. Mutations in the prolidase gene are responsible for Prolidase Deficiency (PD), an extremely rare human autosomal recessive disorder. PD patients present variable disease onset and a broad spectrum of phenotypes including severe skin lesions, vascular anomalies and various degrees of mental alterations. Our previous studies showed an altered morphology of the cerebral and cerebellar cortex *i.e.*, lobulation anomalies and ectopic cells presence. Immunohistochemistry was performed on PD and control mice to evaluate expression and changes of oxidative stress and inflammation markers (*i.e.*, iNOS, SOD1, COX2, TGFβ and IL6), 10, 21 and 60 days after birth. Qualitative and quantitative results in PD mice demonstrated: (i) increase of NOS1 and COX2 immunopositivity in Purkinje cells at P21 and P60; (ii) SOD1, TGFβ and IL6 immunoreactivity enhancement, detected in the granular cell layer and Purkinje cells, respectively, at both P21 and P60, with the strongest labelling at the latest timepoint. These findings suggest an imbalance of ROS production/elimination followed by the activation of inflammatory pathway, worsening during postnatal development. In conclusion, the absence of a fully functional prolidase enzyme seemed to affect the expression of oxidative and inflammatory proteins in cerebellar cortex as possible consequence of the induced cytoarchitectural alterations and ECM changes.

THE AUTISM RISK GENE SETD5 IS REQUIRED FOR CHROMATIN METHYLATION, NEUROTRANSMISSION ASSOCIATED GENE EXPRESSION AND NORMAL SOCIAL INTERACTIONS IN ZEBRAFISH

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SETD5 loss-of-function (LoF) mutations in humans have been recently associated to intellectual disability (ID) and autistic spectrum disorders (ASD). SETD5 gene encodes for a putative histone H3 methyltransferase highly expressed in the brain. and it falls within the critical interval deleted in the "3p25.3 microdeletion syndrome", characterized by ID, microcephaly and congenital heart defects. The aim of this study is to characterize *setd5* LoF zebrafish models generated by morpholino injection and CRISPR-Cas9 gene editing technique. *setd5* expression is localized in the developing central nervous system of zebrafish larvae and in specific brain areas of adult zebrafish. *setd5* morphants show a reduction of Histone 3 methylation status of Lysine 36 (H3K36) when compared to control morphants, supporting the possible catalytic activity of *setd5* as H3K36 methyltransferase. Moreover, *setd5* LoF zebrafish display reduced expression of synaptic proteins and enzymes responsible of neurotransmitter metabolism, associated to microcephaly, a significant reduction of body length and locomotor activity in both larvae and adults. In addition, *setd5* LoF adults are characterized by a reduced social interaction when compared to wild type siblings. Such autism-like altered behavioral traits triggered by *setd5* LoF are ameliorated by risperidone, an antipsychotic drug commonly used to treat behavioral traits in ASD patients. The validation of the zebrafish *setd5* LoF mutants as reliable models for ASD/ID might have an important therapeutic impact for people affected by these neurodevelopmental syndromes. Indeed, the characterization of the molecular pathways altered by *setd5* LoF may support the screening for targeted compounds able to rescue

the developmental and behavioral defects observed in these zebrafish mutants, to identify novel promising compounds for ameliorating behavioral alterations in human individuals affected by ASD/ID due to SETD5 haploinsufficiency.

EXPOSURE OF ZEBRAFISH LARVAE TO LOW CONCENTRATIONS OF CADMIUM AND ZINC AND EVALUATION OF THE HAIR CELL REGENERATION BY A VISUAL AND MOLECULAR APPROACH

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Deafness caused by the loss of inner ear hair cells is one of the most common sensory diseases in mammals. Fish exhibit hair cells in the lateral-line neuromasts which are composed of a cluster of central sensory hair cells surrounded by supporting cells structurally and functionally similar to mammalian inner ear hair cells. Molecular characteristics are also shared. Zebrafish, is regularly used as a powerful animal model to analyse *in vivo* ototoxicity, since, similarly to other non-mammalian animals, is able to regenerate damaged hair cells. Among the factors leading to hair cells disruption, heavy metals are of particular concern, since they are important environmental pollutants. In this study, zebrafish larvae were exposed to different increasing concentrations of cadmium and zinc. The disruption and the regeneration of neuromast hair cells, were monitored *in vivo,* during the experiment by mean of a fluorescent vital dye DASPEI [2-(4-(dimethylamino)styryl)-*N*-ethylpyridinium iodide]. In addition, molecular markers of metal toxicity such as metallotionein-2 (*mt2*) and metal regulatory element (MRE)-binding transcription factor-1 (*MTF-1*) were analysed by RT-PCR. Gene expression of claudin b (*cldnb*) and phoenix (*pho*) were analysed as well, since they are expressed in the supporting cells which are suspected to play a primary role in hair cells regeneration. Heavy metal concentrations corresponding to0.5 mg/L for Cd and 1.0 mg/L for Zn lead minor mortality, caused hair cells disruption and did not compromise the regenerative process. On the contrary, higher concentrations of Cd and Zn were not tolerated by the fish. Finally, while the molecular markers involved in metal toxicity response resulted overexpressed during the whole exposure period, an increasing *cldnb* and *pho* gene expression trend suggested that the functionality of supporting cells was not compromised by metal exposure, making these cells important in regenerative processes of neuromast hair cells.

LIPID SIGNALLING IN AUTOSOMAL DOMINANT LEUKODYSTROPHY: MORPHOFUNCTIONAL ASPECTS

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Autosomal dominant leukodystrophy with autonomic disease (ADLD) is an extremely rare and late onset lethal progressive neurological disorder. It is characterized genetically by alterations in the expression of the nuclear protein Lamin B1, mainly due to LMNB1 gene duplication, and clinically by autonomic abnormalities and age associated demyelination in the central nervous system (CNS), without any effective treatment up to date[1]. Myelin preserves the integrity of nerve fibers and influences the transmission of impulses in both peripheral nervous system (PNS) and CNS. Phosphoinositides are highly expressed in the brain, they mediate both cytoplasmic and nuclear signaling associated with brain function[2]. Given that lipids play active roles in myelination, and aberrant expression of lipids are evident in various neurological disorders such as Alzheimer's and Huntington's disease, we hypothesize that the alteration of lipid pathways might represent an important event underlying the disease phenotype[3]. We have created two ADLD experimental models by overexpressing Lamin B1 in the oligodendrocytic cell line MO3.13 and in the astrocytic cell line U87-MG. Both cell types are typically involved in CNS myelination processes, being oligodendrocytes the myelin producing cells in CNS. Cells were transduced with lentiviral vectors and puromycin selected. After selection, cells were tested for the expression of target molecules at mRNA and protein levels. In addition, ADLD patient fibroblasts were cultured and compared with healthy donor fibroblasts. In both the experimental models and the patient cells, Lamin B1 overexpression was associated with the down-regulation of the Leukemic inhibition factor (LIF) pathway (LIF, STAT3, p21, PI3K, mTOR, ps6k ribosomal protein) responsible for the physiological myelination process and involved in many inflammation processes. Moreover, cells overexpressing Lamin B1 showed an increase in p53 and protein 14.3.3 expression, indicating an activation of the apoptosis pathway, and a morphological alteration of the cell nucleus membrane observed with electron microscopy. These results indicate that overexpression of Lamin B1 results in down-regulation of lipid signaling pathways that could explain the disease phenotype.

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HYALURONIC ACID-BASED NANOCOMPLEXES AS NOVEL DRUG-NANOCARRIERS TO TREAT MYOTONIC DYSTROPHY

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Myotonic dystrophies (DMs) are genetic disorders characterized by progressive myopathy, myotonia and multiorgan involvement. Skeletal muscles are especially affected, and no therapy is currently available, the conventional treatments being only aimed at mitigating symptoms. Nevertheless, some molecules (*e.g.*, pentamidine or anti-sense oligonucleotides) proved to treat the pathogenic causes of DMs in experimental models, although they cannot be applied in therapy due to toxicity/degradability[1]. To overcome these limitations, novel polymeric hyaluronic-acid-based nanoparticles (HA-NPs) were synthesized by ionic gelation technique[2]. These NPs (size ~200 nm) have a Z potential of -30 mV, and may be loaded with pentamidine isethionate (encapsulation efficiency ~80%). The biocompatibility of HA-NPs and their interactions with muscle cells were evaluated *in vitro* using C2C12 murine muscle cells as a model system, as they may grow in culture as myoblasts or differentiate into myotubes. The trypan-blue exclusion test and MTT assay showed that HA-NPs are non-toxic. Fluorescence confocal microscopy demonstrated a rapid, efficient and time-dependent uptake of FITC-labelled HA-

NPs by both myoblasts and myotubes. Transmission electron microscopy showed that HA-NPs enter the cell by endocytosis, and after 24 h incubation they may be found in the cytoplasm both inside membrane-bounded vesicles and free in the cytosol as a consequence of endosomal escape. NPs were never found in the nucleus and no organelle damage was ever observed in both myoblasts and myotubes. At 48 h, many residual bodies were found inside the cells, which suggests that HA-NPs are degraded *via* the endo-lysosomal pathway. All these data demonstrate that of HA-NPs are highly biocompatible for muscle cells and promise to be suitable for efficiently carrying pentamidine inside muscle cells.

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EVALUATION OF OOCYTE QUALITY IN GRANULOSA AND CUMULUS CELLS OF PATIENTS UNDERGOING PMA

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We investigate the apoptosis rate of individual granulosa cell-oocyte and cumulus cell-oocyte (COC), associated with the levels of molecules playing a critical role in the regulation of cell death or survival. These molecular analyses have been done to verify the difference of competence between oocytes producing embryos able to reach the blastocyst stage compared with embryos arrested during the *in vitro* culture. From each single follicle: granulosa cells were processed for Western blotting analyses, using the following antibodies: pAKT, ERK 1/2, pERK 1/2; cumulus cells were used for *in situ* immunofluorescence with the same antibodies. DNA fragmentation rate was measured by TUNEL assay. We have involved 58 patients and recovered 255 MII oocytes, of which 197 were fertilized and the derived embryos had the following evolution: 117 transferred, 57 vitrified and 23 arrested; 58 oocytes failed the fertilization or were in GV or MI stages. In the cumulus cells: we found a significant inverse correlation between oocytes resulting in transferred and arrested embryos in the ratio pAKT/TUNEL; nuclear localization of pERK1/2 showed a significant inverse correlation pERK1/2/TUNEL and a significant direct correlation with the intracellular accumulation of pERK1/2/pAKT. In granulosa cells: oocytes able to produce blastocysts, ERK1/2 /TUNEL ratio was higher than in cells of arrested embryos. Cumulus and granulosa cells showed different levels of expression of the investigated molecules. We found that in the cumulus cells of the oocytes able to produce blastocysts, the pAKT/TUNEL ratio is higher than in cumulus cells of arrested embryos, indicating that pAKT is involved in survival pathways. Moreover, pERK1/2 has an anti-apoptotic effect, when translocated into the nucleus. In granulosa cells: ERK1/2 indicates that it is involved in survival pathways. Briefly, we demonstrated that DNA fragmentation rate related to specific molecular levels could be considered a molecular marker of oocyte competence, for the evaluation of a prognostic pattern of blastocyst formation.

MicroRNAs CONTROL IN ZEBRAFISH CARDIAC HYPERTROPHY: A MODEL OF STUDY IN TRANSLATIONAL MEDICINE

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Zebrafish is an emerging model to study cardiac diseases since is still lack studies about hypertrophy. In this study, for the first time in zebrafish, it was induced a cardiac hyertrophy by using phenylephrine treatment in hearts cultured in ex-vivo with the aim to have a translational model to use in the study of human disease. The effect of the treatment has been valued for dose and timing by histology and immunohistochemistry. Moreover, due to the similarities between fish and mammalian genomes, using qRT-PCR experiments, it was analyzed the expression of some microRNAs (miR-1, miR-133a), already known to be involved in cardiac regeneration and in inducing hypertrophy hearts in mice and humans. The experiments showed down-regulation of miRNAs, especially miR-133a, demonstrating the importance of that miR in the hypertrophy conditions in zebrafish as well as in mouse and human. To confirm their role in cardiac hypertrophy, the *in vivo* inoculation of sequences of complementary miRs have demonstrated their key role in control the cardiac hypertrophy also in zebrafish. The hypertrophic increase of myocytes, has been more evident by the treatment with the anti-miR 133a. The results suggest the possibility to activate the FGF-receptor pathway, necessary to start the epicardial and myocardial hypertrophy process. This experimental system, using different and easier model of study, should provide clues to understand human pathophysiology.

STEROIDOGENIC ENZYME PROTEIN EXPRESSIONS IN *Coturnix coturnix* TESTIS DURING THE REPRODUCTIVE CYCLE

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Sex steroid hormones are mainly synthesized within reproductive organs, and once secreted they regulate different physiological events in target tissues. In the testis, somatic cells as well as germ cells, synthesize sex hormones start a common precursor, the cholesterol, via a series of enzyme-catalyzed reactions[1,2]. The quail, *Coturnix coturnix* is a seasonal breeder with a physiological switch on/off of gonadic activity. To more thoroughly comprehend the steroidogenic pathways that govern the seasonal reproductive cycle, we have investigated the localization of StAR protein and steroidogenic enzymes (3β-HSD, 17β-HSD, P450 aromatase and 5 -Red) as well as androgen and estrogen levels, in the testis of reproductive and no-reproductive quails. We demonstrated that StAR, 3β-HSD, 17β-HSD, P450 aromatase and 5 -Red were always present in the somatic (Leydig and Sertoli cells) and germ cells (spermatogonia, spermatocytes I and II, spermatids and spermatozoa). In addition, by Western blotting analysis we demonstrated that 17β-HSD, P450 aromatase and 5 -Red showed the highest expression levels during the reproductive testis compared to non-reproductive one. Accordingly, we also found that during the reproductive phase the highest titres of testosterone, 17β-estradiol and 5 -dihydrotestosterone are recorded. In conclusion, our findings demonstrated that in *C. coturnix*: 1) both somatic and germ cells are involved in local synthesis of sex hormones; 2) 17β-HSD, P450 aromatase and 5 -Red expressions as well as testicular androgens and estrogens

increased in reproductive quail testis. This study strongly indicates that the steroidogenic process in quail testis exhibits seasonal changes with the promotion of both androgenic and estrogenic pathways in the reproductive period, suggesting their synergic mechanism in the spermatogenesis regulation.

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ROLE FOR RETICULON-1C IN SKELETAL MUSCLE DIFFERENTIATION

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Skeletal muscle is one of the most dynamic and highly plastic tissues and it is essential for posture, locomotion, and breathing. Muscle atrophy or genetic muscle disorders, such as muscular dystrophy, are characterized by degeneration of myofibers and replacement with fibrotic tissue. Skeletal muscle contains an extensive network of endoplasmic reticulum (ER), called sarcoplasmic reticulum, which plays an important role in the regulation of proteostasis and calcium homeostasis. Environmental and genetic factors that disrupt the endoplasmic reticulum (ER) function cause an accumulation of misfolded proteins in the ER lumen leading to ER stress. To alleviate the stress and restore homeostasis, the ER activates a signaling network called the unfolded protein response (UPR). Interestingly, recent studies suggest that UPR pathways play pivotal roles in muscle stem cell homeostasis, myogenic differentiation, and regeneration of injured skeletal muscle. Accumulating evidence also suggests that ER stress may have important roles in the pathogenesis of inflammatory myopathies and genetic muscle disorders[1]. Among the known proteins that regulate ER structure and function there is RTN-1C, a member of the reticulon proteins family localized on the ER membrane. We have previously demonstrated that RTN-1C expression modulates cytosolic calcium concentration and ER stress pathway[2,3]. In this study we demonstrated that differentiation of C2C12 myoblast, a well-established model to study muscle biology, positively correlates with RTN-1C expression and UPR pathway up-regulation. To better characterize the role of the reticulon protein on muscle cell differentiation and regeneration, we performed *in vivo* experiments using either a model of muscle injury such as cold injury, or mdx mice, a photogenic model of Duchenne muscular dystrophy. Interestingly, we found that RTN-1C is up-regulated in mice undergoing active regeneration and localized in the injured skeletal muscle. Our results strongly suggest that RTN-1C may become a new target to enhance muscle regeneration and repair following injury.

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MONITORING CELL BEHAVIOR UNDER VARIOUS CULTURE CONDITIONS THROUGH A NEW HOLOTOMOGRAPHIC MICROSCOPE

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The study of three-dimensional (3D) morphology changes of a single mammalian cell is useful to understand its response to various stimuli. In particular, outer membrane changes can be an evidence of cellular functions ones, such as those involved in tumor formation, death, cell differentiation and interactions with the extracellular space. The conventional techniques used to evaluate surface changes show some disadvantages, such as sample coating for scanning electron microscopy[1], fluorescent labeling for confocal microscopy[2] and other limits related to the timing for obtaining morphological data. Recently, a novel imaging method based on Holotomographic Microscopy (HM), which utilizes optical diffraction tomography (ODT) to quantitatively and non-invasively investigate biological systems, has been developed. ODT reconstructs the 3D refractive index distributions of live cells by providing structural and chemical information about the cell, including dry mass, morphology, and dynamics of the cellular membrane. Here, hematopoietic, muscle and epithelial cells, exposed to different culture conditions or pharmacological treatments have been analyzed *in vivo* with HM. The images obtained demonstrate that this instrument can be considered a powerful new tool for the rapid evaluation of *in vivo* cell response to various stimuli without any labeling, staining or coating.

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EFFECTS OF *Curcuma longa* ON KERATINOCYTES FROM HUMAN PTERYGIUM

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Pterygium has been defined as a triangular shaped degenerative and hyperplastic process, occurring medially and laterally in the rhyme eyelid, in which the bulbar conjunctiva encroaches on the cornea[1]. Moreover, pterygium implies a strong inflammatory condition and currently requires surgical treatment that often results in the recurrence of a lesion more clinically aggressive than the original one[2]. *Curcuma longa* has been used for centuries to aid common human disease for its properties including anti-inflammatory, antioxidant, antineoplastic, pro- and anti-apoptotic, anti-angiogenic, cytotoxic, immune-modulatory, and antimicrobial effects[3]. To identify an alternative strategy to currently available surgical procedures, in this study we investigated the effects of *in vitro* treatment with *C. longa* of keratinocytes derived from explants of human pterygium. Explants were put into culture and derived keratinocytes were treated with an alcoholic extract of 1.3% *C. longa* in 0.001% Benzalkonium Chloride for 3, 6, and 24 h. Cultured cells were examined for CAM5.2 (anti-cytokeratin antibody) and CD140 (anti-fibroblast transmembrane glycoprotein antibody) expression between 3th and 16th passage to assess cell homogeneity. TUNEL technique

and Annexin-V/PI staining in flow cytometry were used to detect keratinocyte apoptosis. We showed that *C. longa* exerts a pro-apoptotic effect on pterygium-derived keratinocytes already after 3 h treatment. Moreover, after 24 h treatment, *C. longa* induces a significant increase in TUNEL as well as Annexin-V/PI positive cells in comparison to untreated samples. Our study confirms previous observations highlighting the expression, in pterygium keratinocytes, of nuclear VEGF and gives evidence for the first time to the expression of nuclear and cytoplasmic VEGF-R1. Our results suggest that *C. longa* could be an effective tool to avoid surgical procedures or, at least, to prevent pterygium recurrences after surgery.

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EFFECTS OF D-ASPARTIC ACID TREATMENT ON PREP EXPRESSION AND AMPA RECEPTOR/ERK–AKT PATHWAY ACTIVATION IN RAT TESTIS

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Steroidogenesis and spermatogenesis are processes that involve a complex interaction of a wide range of hormones and factors among which a considerable interest in the past two decades has been directed to the amino acid D-Aspartic acid (D-Asp). Although it has been demonstrated that D-Asp plays a key role in spermatogenesis through the activation of testosterone synthesis, the underlying molecular pathways responsible for the action of D-Asp in reproductive processes are little known. Recent studies have demonstrated that propyl endopeptidase (PREP), an enzyme belonging to the serine protease family, is required for a normal reproductive function; indeed, the lack of functional activity of this enzyme may lead to marked alterations of the gonads and, ultimately, gametes. Particularly, PREP seems to play a role in the morphological remodeling, occurring in the first wave of rat spermatogenesis, a function attributed to its co-localization with tubulin in the cytoplasm of germ cells. In this study, we conducted *in vivo* experiments consisting of acute (intraperitoneal injection of 2 μmol/g body weight) and chronic (15 days drinking solution) administration of D-Asp to adult rats to evaluate the effects of this amino acid on PREP protein expression and AMPA receptor/ERK-AKT pathway activation. By Western blotting we found that D-Asp upregulated the expression of PREP in rat testis. Immunofluorescence analysis revealed PREP overexpression in Leydig cells, Sertoli cells, and spermatogonia. In addition, PREP was found to co-localize with GluA2/3, an AMPA receptor subunit, whose protein expression also increased after D-Asp treatments. We also found a significant increase in ERK and Akt activities in D-Asp-treated rat testes. Therefore, the results strongly suggest that D-Asp induces spermatogenesis through activation of PREP and AMPA receptor/ERK-Akt pathway.

A BLACK PEPPER EXTRACT INDUCES ANTIOBESOGENIC AND ANTIDIABETIC EFFECTS IN *IN VITRO* CELL MODELS

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Plants are a relevant source of health-promoting compounds and bioactive molecules and in the last decades there has been a growing interest toward plant-derived pharmaceuticals, dietary supplements and functional foods. Recently, β-caryophyllene (BCP), a sesquiterpene present in various plant essential oils, has attracted increasing attention because of its several biological activities. Among them, analgesic, anti-inflammatory, anti-oxidative and neuroprotective properties[1] are possibly due to the selective interaction with the peripherally expressed cannabinoid receptor 2 (CB2), whose activation is notably devoid of the cannabinoids psychotropic effects mediated by the CB1 receptor. Importantly, BCP has also been demonstrated to directly activate peroxisome proliferator-activated receptor-α (PPARα)[2], involved in liver lipid metabolism, and to trigger the activation of PPAR [3], involved in adipogenesis. Giving the growing scientific interest in BCP, the aim of our study was to investigate the metabolic effects of a black pepper extract (PipeNig®), containing 80% β-caryophyllene. In particular, we focused on its potential antiobesogenic and antidiabetic activities in three *in vitro* cell models: 3T3-L1 preadipocytes, C2C12 myotubes and HepG2 hepatocytes. Our preliminary results show that PipeNig® reduces 3T3-L1 adipocyte differentiation and lipid accumulation. Moreover, acute exposure of C2C12 myotubes with different concentrations of PipeNig® improves glucose uptake activity. The HepG2 hepatocyte steatosis model allowed us to evaluate the ability of PipeNig® to interfere with free fatty acids-induced steatosis. Altogether, our study indicates that 3T3-L1, C2C12 and HepG2 can be useful *in vitro* models to study adipogenesis and glucose uptake, in physiological conditions, and obesity, diabetes and steatosis in pathological contexts. Moreover, it highlights novel and interesting properties of β-caryophyllene, suggesting potential applications in the prevention of metabolic diseases.

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NANOPARTICLES TREATMENTS AFFECT THE GLYCOSYLATION PATTERNS OF MUCINS SECRETED BY THE CEMENT GLANDS OF *Pelophylax* kl. *esculentus*

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The cement gland is a transient organ of embryonic and larval Anura that disappears soon after hatching. Its secretion is used for adhesion to substrate avoiding swimming until the larva starts to move and feed autonomously. It is considered as an experimental model for vertebrate axial patterning and its development can be influenced by both stimulatory and inhibitory cell interactions. We previously showed that engineered nanoparticles (NP) treatments can affect embryonic development in the frog *Pelophylax* kl. *esculentus*. Here we investigate their effects on the development of the cement gland, in particular how they can affect the glycopatterns of the secreted mucins. Embryos at the developmental stage 10 (earliest involution of blastopore dorsal lip) were treated with iron, nickel or cobalt NP. A control group

and one treatment per NP at concentrations of $2xLC_{50}$ were considered, for a total of four groups. Each group included about 20 individuals. Groups were monitored for ten days and then sacrificed, fixed in a 4% paraformaldehyde solution in 0.1 M phosphate buffered-saline pH 7.4 at 4°C, and embedded in a Technovit 8100 kit. Classic histochemical staining included Periodic acid-Schiff (PAS), Alcian Blue (AB) at pH 2.5 and High Iron Diamine (HID). Lectin-binding experiments were performed with FITC -labelled lectins (SBA, PNA, WGA, ConA, Paradoxical ConA, UEA-I, LTA, AAA). *Pelophylax* kl. *esculentus* presents paired cement glands whose secretion was intensely PAS positive and negative to AB pH 2.5 and HID. Lectin-binding was intense with WGA, moderate with SBA and weak with PNA and UEA. PNA and SBA binding were more intense in the treatments. UEA binding decreased in the iron treatment. It is concluded that the secretion is made by neutral mucins with several glycosaminylated residuals and small amounts of galactosyl/galactosaminylated and fucosylated residuals. Galactosyl/galactosaminylated residuals increase in treatments and in the iron treatment only fucosylation is reduced. Thus, nanoparticles treatments affect the glycosylation patterns of cement gland secretion with possible consequences on the adhesion properties of mucus, which need further investigations.

HISTOCHEMISTRY AS SUPPORTING TOOL IN GRASSLAND ECOSYSTEM MANAGEMENT: APELIN SYSTEM DETECTION IN EWE REPRODUCTIVE APPARATUS

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Adipokines are molecules involved in energy metabolism and represent important links between nutritional status, neuro-endocrine axis and healthy pregnancy[1]. We analysed the presence of apelin (APLN) and its receptor (APLNR) in the reproductive apparatus of the sheep, into a research project aimed to achieve a sustainable grassland productive ecosystem management. 15 adult female ewes (Comisana x Appenninica) in dry stage were fed with fresh hay from June to the pasture maximum flowering (MxF). From this period to maximum dryness, the control group (Cnt) was fed with fresh hay while, the experimental group (Exp) was also supplemented with 600 g/day/head of barely and corn (1:1). Ovary, ampulla and uterus samples were collected at each time and processed to perform RT-PCR, morphological and immunohistochemical analysis. Samples for molecular biology were frozen in liquid nitrogen. Samples for histochemical procedures were fixed in 10% neutral buffered formalin, included in paraffin wax and treated with polyclonal rabbit anti-APLN and anti-APLNR antibodies[2]. Positive staining for APLN and APLNR were observed in the ovary corpus luteum. RT-PCR evidenced both transcripts in the examined organs. As the genital tract concerns, the highest levels were detected in the Cnt group ewes in the luteal phase compared to the MxF group in the anoestrous phase. APLN was detected in the epithelium lining the ampulla and uterus and in the uterine glands. APLNR was showed in the ampulla secreting cells, in the epithelium lining the uterus and uterine glands. APLN showed a high expression in the Cnt group compared to Exp one. The distribution and expression of the apelin system in the reproductive apparatus suggest its involvement in the ewe reproductive functions. Differences evidenced could be mainly related to the cyclic activity of organs, that seems to be affected also by the diet. Data suggest that a more energetic diet can anticipate the beginning of the oestrous cycle.

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METHYLATION AND DEMETHYLATION, AN ULTRASTRUCTURAL STUDY ON THE CELL NUCLEUS

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Methylation and demethylation are two epigenetic processes of a big relevance for different biological pathways. The two events happen on the carbon in position five of the cytosine belonging to the so called CpG island. The methylation implies the addition of a methyl group on the cytosine, forming the 5-methylcytosine (5mC) thanks to enzymes called DNMT (DNA-Methyltransferase). After, when required, the methyl group is oxidized or demethylated by a family of enzyme called TET, forming the 5-hydroxymethylcytosine (5hmC). The role of the 5mC is generally correlated with gene expression repression, while the 5hmC function must be clarified. In this context, in order to elucidate the hypothetic role of these markers we decide to investigate at ultrastructural level, by looking at the distribution of two epigenetic modifications putting our attention on different areas of the cell nucleus. Our study where carried out by using transmission electron microscope, light microscope and molecular biology techniques. We observed that in condensed regions of the nucleus the DNA is always highly methylated rather than hydroxymethylated, but in the so called perichromatin region the pattern changes. Indeed, in this region it was possible to notice an abundancy of demethylation underlined both by the presence of the 5hmC and of the enzymes involved in the processes: TET2. This result could allow to hypothesize a sort of activating role for the oxidized modification respect to its reduced form and underline how the perichromatin region is a dynamic region where DNA status changes.

EFFECT OF MENAQUINONE PRODUCING PROBIOTICS ON THE FIN REGENERATION OF ZEBRAFISH

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Probiotics are microorganisms (usually live) known to exert many beneficial effects on the host when administered in appropriate quantities. They have proven their positive impact on a wide range of model organisms like fish, chicken etc. and form a sustainable replacement against the usage of harmful drugs, chemical and antibiotics. Previous studies have demonstrated the positive effect of probiotics in improving skeletal health by using zebrafish as a model organism[1]. *Bacillus subtilis* is a known producer of menaquinone (vitamin K2), a fat soluble vitamin considered to have a role in bone health[2]. The current study aims at exploring the positive impact of probiotic mixture containing *B. subtilis* in aiding fin regeneration studies. Zebrafish is proven to be an ideal model to study fin regeneration owing to its short fin regeneration period[3]. Zebrafish with their caudal fin amputated are maintained individually and being treated with probiotics until the fin is completely regenerated. Impact of probiotics in aiding improved regeneration over the control group was constantly monitored throughout the experiment. Finally, the extent of skeletal development and bone mineralisation was compared across individuals using Alcian blue - Alizarin red double staining. The current approach of using regenerative caudal fin proves highly valuable and efficient for testing the efficiency of probiotics. The study also eliminates the need of sacrificing multiple individuals over the course of study. Zebrafish is considered a good model to translate the current findings into industrial and scientific significance.

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DEVELOPMENT OF A BIOREACTOR FOR BLOOD VESSELS TISSUE ENGINEERING

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Cardiovascular disease (CVD) is considered the leading cause of mortality and morbidity in America and worldwide, causing 31.5% of all global deaths[1]. To date, the golden standard procedure to treat CVD is to bypass the blocked vessels with an autologous vein harvested from the patient through coronary bypass graft surgery[2]. However, this treatment presents limitations due to native blood vessels availability and long-term failure. Hence, human tissue engineered blood vessels (TEBV) could represent a valid therapeutic alternative thanks to the possibility to produce patient specific vessels starting from autologous stem cells. Moreover, TEBV represents an excellent platform for drug screening of pharmacological candidates prior to pre-clinical animal studies[3]. Here we report the design and development of a bioreactor for TEBV generation allowing different parameters setting such as duty and rest cycles, pressure and frequency of stimulation. Perivascular progenitor cells, from both mouse and human, are embedded in a gelatin methacrylate (GELMA) scaffold, a denatured collagen-based matrix that thanks to its natural origin drive cell adhesion and differentiation[4]. This cell-GELMA solution is poured into an opposite designed mold and, after UV polymerization, a blood vessel like structure of about 3 mm of diameter and 20 mm of length is obtained. These artificial vessels are cultured for at least 10 days to ensure proper cellular differentiation, with or without perfusion stimulation, and then analyzed by histological staining and immunofluorescence assay.

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Hoxb1 FUNCTION IN THE DEVELOPING MOUSE AUDITORY SYSTEM RHOMBOMERE 4-DERIVED

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Hoxb1 gene is essential for the specification of rhombomere 4 (r4)-derived auditory sensory and motor neurons contributing to the formation of specific auditory subcircuits. As we previously showed, R4 largely contributes to the motor cochlear efferent neurons and Hoxb1 loss strongly prevents the proper development of the auditory system. As a matter of fact, Hoxb1 mutants display an increased auditory threshold that leads to severe hearing impairments. It is known that medial olivo-cochlear motoneurons (MOCs) which synapse with outer hair cells (OHCs) are involved in the cochlear amplification mechanism. Indeed, we found a strong morphological damage of the OHCs and the total absence of MOCs when Hoxb1 function was abolished in r4[1]. A hypothesis is that MOC neuron endings could play a trophic function on OHCs and that the physical interaction between MOCs and OHCs is essential for proper maturation and functioning of OHCs[2]. In order to assess if the degeneration of OHCs and consequently altered hearing thresholds might be caused by the absence of synaptic/trophic stimulation of OHCs from the MOC fibers, we analyzed Hoxb1 mutant for the dorsal (sensory) and the ventral (motor) domain respectively. The sensory cochlear populations were affected in Hoxb1flox Atoh1-Cre and Hoxb1flox Ptf1a-Cre mice, whereas the olivocochlear motoneurons were deleted by using Hoxb1flox Nkx2.2-Cre mutants. The transmission and scanning electron microscopy's study showed that the absence of Hoxb1 in sensory domain of r4 does not impair the proper development of OHCs, which maintain a regular morphology and fail to reproduce the severe phenotype observed in Hoxb1null mutants. On the other hand, our preliminary data on Hoxb1flox Nkx2.2-Cre mutants seem to highlight a key role for MOCs, which origin from this domain, on OHC survival and sound amplification.

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PERIPHERAL NEUROPATHY RELATED TO OBESITY: A MICROANATOMICAL STUDY OF SCIATIC NERVE IN OBESE RATS

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Obesity and to a greater extent type-2 diabetes are associated with impaired glucose utilization and peripheral neuropathy. Different studies demonstrated that diet-induced obese rats develop whole-body insulin resistance and sensory neuropathy associated with reduced sensory nerve conduction velocity, thermal hypoalgesia and decreased intraepidermal nerve fiber density in the skin. The prediabetic stage is a leading cause of peripheral neuropathy, accounting for approximately 35.5% of undiagnosed cases. Moreover, dyslipidemia may contribute to the development of peripheral neuropathy. The aim of this study was to evaluate the effects of a high-fat diet (HFD) on the sciatic nerve in the rodent model of diet-induced obesity (DIO). DIO rats exposed to high-fat diet *ad libitum*, provide a useful animal model sharing several common features with human obesity. DIO rats were studied after 5 weeks when the obese phenotype appeared and after 17 weeks of the HFD. They were compared to the control rats with no fat diet (CHOW). Histochemical, immunohistochemical and immunochemical analysis were performed to evaluate nerve fiber changes of the sciatic nerve. Systolic blood pressure, glycaemia and insulin levels were higher in DIO rats only after 17 weeks of the HFD. No changes in total cholesterol and triglycerides were found. An increase of thiobarbituric reactive substances and oxidated proteins was observed in the serum of DIO rats compared to CHOW. Axon area and myelin thickness did not change in large and small nerve fibers in DIO rats. A decrease of 200-kDa neurofilament immunoreactivity and a reduced expression of myelin basic like-protein were observed in obese rats compared to the control. An inflammatory condition, with an increase of interleukin-β and oxidative stress were detected in the sciatic nerve of the obese rat. Our findings support the hypothesis that obesity, characterized by hyperglycaemia and adipose tissue accumulation, may represent a risk factor for neuropathy.

KIDNEY CHANGES IN OBESE RATS: EVIDENCE FOR NEPHROPATHY RELATED TO DIET.

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Increased food intake, reduced physical activity, and altered metabolic processes are the variables affecting energy balance inducing obesity. Obesity, in association with diabetes and hypertension, can contribute to the increased incidence of chronic kidney diseases (CKD). On the other hand, kidney has an important role in the complex inter-organ communication occurring with the development of inflammation and fibrosis during obesity.
Down-regulation of the dopamine D2 receptor results in increased renal expression of injury markers and proinflammatory factors independent from blood pressure increase. In obese Zucker rats, defective D1-like and D2-like receptors expression is not inherited but contributes to hyperinsulinemia associated with obesity. This study was done to clarify in rats with Diet-Induced Obesity (DIO) the possible relationships between high-fat diet, kidney damage and possible inflammatory processes. Histochemical and immunohistochemical techniques were used. Rats of 7 weeks of age exposed to high-fat diet were used. After 5 weeks, when rat body weight was increased significantly compared to the control group (CHOW), were designated as DIO rats. Rats were followed for other 12 weeks. After 17 weeks of a high-fat diet, systolic blood pressure, glycaemia and insulin levels were higher in DIO rats compared to CHOW. The kidney of DIO rats showed glomeruli partially collapsed with increased thickness of the glomerular basement membrane. Analysis for IL-1β revealed in DIO rats an increased expression at the level of glomeruli and in basal portion of proximal and convoluted tubules cells. Changes in the expression of the D1-like receptors, in particular of the D5 receptor, and D2-like dopamine receptors was evident in the DIO rats, compared to control animals. These findings indicate that in DIO rats, nephropathy is a complex phenomenon, and could be related to inflammation induced by body weight gain or related risk factors such as hypertension and/or hyperglycemia.

RESCUE STRATEGIES FOR IMPROVING DEFECTICE PROTEIN TRAFFICKING OF THE AUTISM-LINKED MUTATION R451C IN NEUROLIGIN-3

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Autism Spectrum Disorders (ASDs) are neurodevelopmental syndromes, characterized by behavioral deficits and altered neurotransmission. The etiology of these disorders is complex due to mutations arising in hundreds of genes, along with environmental-derived factors. Among the genetic risk factors, the R451C substitution in the synaptic protein Neuroligin-3 (NLGN3) has been highly characterized. It is known from *in vitro* studies, that the mutation affects folding of the extracellular domain of the protein, causing its retention in the Endoplasmic Reticulum (ER) with only ~10% of the mutant protein reaching the synapse. We have shown that the accumulation of the mutant protein in the ER causes a stress condition and the activation of the UPR *in vitro*[1] and in a mouse model expressing R451C NLGN3 as the endogenous protein. Specifically, *in vivo*[2], UPR activation is uniquely detected in the cerebellum and is responsible for the alterations in synaptic neurotransmission that we have detected in this brain area. More recently, we have generated a new cell-based model system allowing us to study the altered trafficking of R451C NLGN3. The result of the screening of an FDA-approved library of compounds identified candidates for improving impaired protein trafficking and correct membrane localization of the mutant protein. The most effective compounds belong to the glucocorticoid family. Collectively, our data show a possible strategy to rescue NLGN3 folding, which could potentially be applied to other protein misfolding disorders.

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THE COLONIAL ASCIDIAN *Botryllus schlosseri* FOR THE STUDY OF STEM CELLS AND THEIR INVOLVEMENT IN HOMEOSTASIS AND REGENERATION

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The colonial ascidian *Botryllus schlosseri* emerged in1950s as an important model organism for the study of developmental biology and comparative immunology. *B. schlosseri* is a colonial tunicate that can reproduce both sexually and asexually. The asexual cycle is characterized by the initial formation of a thickened disc of somatic stem cells in the lateral body wall of the parental zooid. This disk gradually evaginates and becomes a vesicle where organogenesis takes place, developing an adult blastozooid. Stem cells are, therefore, essential for asexual development. In this work, we review the potentiality of this species for studying stem cells and regeneration. Several experiments of zooid removal demonstrated the remarkable regenerative capabilities of this species, highlighting the cell fate plasticity and homeostasis of the species. When all the blastozooids and buds are surgically removed from a colony, leaving only the tunic with its vasculature, the circulating stem cells aggregate and form a vesicle which undergoes morphogenesis regenerating a new individual. Moreover, isolated buds in a colony where their parental adult zooids were removed, and the connection between the remaining buds and the colonial common vasculature was interrupted, are able to survive. Buds were initially seen to regress, but resumed development only after the regeneration of connection with the colonial vasculature. New zooids can also develop by budding even from fragments of buds. Exploiting such alternative developmental pathways, which have never been studied at molecular and cellular level, it will be possible to follow the fate of stem cells and the mechanisms of tissue and whole body regeneration.

EFFECT OF CARBON NANOPARTICLES ON THE EMBRYONIC DEVELOPMENT OF SEA URCHIN (*Paracentrotus lividus*)

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Due to its limited water turnover, the Mediterranean Sea is extremely sensitive to the accumulation of polluting substances, which can lead to a progressive degradation of the marine

ecosystem. As part of polluting substances entering the environment through human activities, there are the carbon nanoparticles (CNPs) that find applications in many consumer products like cosmetics and personal care products (hair coloring), textiles (by conferring conductive properties to natural fibers and fabrics), diagnostic materials, paints, foods (bread, caramelized sugar, corn flakes, biscuits, etc.), medicines (human cancer treatment), electronics (by improving electrical conductive properties of polymer nanocomposites) and more. Among aquatic organisms, sea urchin (*Paracentrotus lividus*) is widely used as laboratory animal model to perform nanoecotoxicology studies by assessing the effects of various nanomaterials on its embryonic development, especially those that occur during the early stages. Light microscopy-based embryotoxicity investigations performed on sea urchin embryos, bearing increasing amounts of CNPs (0.5, 2.5 and 25×10^{13} CNPs/500 cm^3 of Adriatic sea water collected along the Salento cost, Southern Italy) from fertilization until the larva stage (up to 72 h), revealed that these NPs cause malformations and alteration of the normal progression through the development stages. In addition, as detected by Real Time PCR, relevant alterations were found for some genes involved in the regionalization of the embryo along the D/V axis up to 24, belonging to the gene regulatory network (nodal, lefty, bmp2/4, tbx2/3, wnt5, wnt8 and univin). The degree of these malformations/alterations always depended on the increasing amount of CNPs. The dynamic light scattering (DLS) measurements showed that the chemico-physical properties of native CNPs changed as consequence of NPs concentration. The NPs dispersing medium was also important. Depending on increasing amounts of NPs and whether the dispersing medium was mQ water or sea water, higher values of size and lower values of -potential were observed, respectively. As a whole, these results once again validate *P. lividus* as an excellent laboratory animal model to conduct nanoecotoxicology studies, and also raise concerns about CNPs released into the environment as a result of degradation or dispersion. It worth noting that changes in marine systems could involve the loss of historical ecosystem services for human populations with dreadful consequences.

CHARACTERIZATION OF A NEW ZEBRAFISH (*Danio rerio*) KNOCK-OUT MODEL TO STUDY *IN VIVO* THE ROLE OF CERS1 GENE IN EPILEPSY PREDISPOSITION

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Epilepsy, one of the most common neurological conditions, is a pathology characterized by recurrent spontaneous seizures often associated with specific behavioral alterations. Recently, the identification of mutations in ceramide synthase 1 (Cers1) occurring in a family with autosomal recessive myoclonus epilepsy, displayed that impairment of one of the core enzymes in sphingolipid metabolism leads to a neurodegenerative seizure disorder. To understand the role of Cers1 in the pathogenesis of this new variant of epilepsy, we perform *in vivo* functional studies using zebrafish as disease model. In particular, we generated a new zebrafish Cers1 knock-out mutant to analyze the phenotype associated with Cers1 depletion during development. In Cers1-null mutants, we identified the presence of seizure-like swimming alterations and the upregulation of neuronal markers like BDNF and pERK. In addition, the measurement of brain activity in-vivo revealed the presence of abnormal Ca^{++} spikes in neurons exclusively in mutants. In particular, exposure to epileptogenic molecules prompted cers1 mutants to exhibit behavioral changes as well as anomalies in electrical activity associated with neuronal hyperexcitability. This novel model provides valuable insights into the Cers1 associated epilepsy providing new opportunities for the identification of pharmacological compounds able to control seizure activity and improve neurodevelopmental outcomes in epileptic patients.

IDENTIFICATION AND CHARACTERIZATION OF A NEW MURINE MUSCLE-DERIVED VASCULAR PROGENITOR (MVP)

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Skeletal muscle is one of the most abundant tissue in the human body. It is composed mostly by differentiated structures, like myofibers and blood vessels, supported by a heterogeneous mononuclear cell fraction necessary to maintain and restore muscle homeostasis[1]. This interstitial compartment counts different cell populations, including stem cell precursors and immune system cells, some of which are not well characterized[2]. Here we describe a novel skeletal muscle resident cell population, characterized by the expression of cell surface antigens SCA1 and PDGFRB and the transcription factors GLI1 and RUNX2. We refined an isolation protocol based on magnetic beads sorting and *in vitro* selection, starting from the mononuclear fraction of murine skeletal muscle. We studied their *in vitro* properties in different cell culture media, establishing that these cells are capable to acquire both cobblestone and tube-forming morphology, a characteristic of endothelial cells. Indeed, we confirmed that these cells express von Willebrand factor (vWF) while they lack myogenic or adipogenic differentiation markers. After cryopreservation these cells can be expanded for many passages *in vitro*, maintaining their distinctive phenotype. Furthermore, preliminary data suggest that this new population is also able to improve the differentiation of satellite cells *in vitro*. These results encourage us to better characterize the role of this population i) as a stem cell with endothelial differentiation potential, ii) as a cell type supporting differentiation of myogenic precursors.

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NANO-STRUCTURES WITH HYPERTHERMIC PROPERTIES FOR BIOMEDICAL APPLICATIONS

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In recent years, our group focused on magnetic nanoparticles (NPs), which are able to induce hyperthermia due to molecular vibration in an alternating magnetic field. Hyperthermia was applied *in vitro* with different aims, and the effects on cells were analyzed by applying vitality tests, and light and electron microscopy techniques. We used superparamagnetic iron oxide NPs to induce delipidation in 3T3 adipocytes and human adipose-derived adult stem cells. Immediately after hyperthermia, we observed a drastic lipid loss that persisted for at least 24 h in the absence of cell death, damage or dedifferentiation, thus opening interesting perspectives for the application of hyperthermia

to treat obesity[1]. We also applied hyperthermic treatment to cancer cells, known to be more sensitive to heat shock than healthy cells, in order to induce apoptosis[2]. A glioblastoma cell line (U87MG) was treated with either biomimetic magnetic NPs (BMNPs) or S1 NPs. BMNPs, synthetized with the protein MamC from magnetotactic bacteria, may act as both drug carriers and hyperthermic agents, being promising tools for the treatment of many types of tumors[3]. S1 are amphiphilic polymer, dodecyl grafted poly(isobutylene-alt-maleic anhydride) coated zinc-doped iron oxide (Fe_3O_4) NPs of 15±2 nm size, and show a high thermal capacity. These NPs proved to be efficient in increasing both culture medium temperature and cell death rate, thus suggesting their suitability for the treatment of various cancers, especially the non-operable ones such as glioblastoma, by means of magnetic hyperthermia.

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GRAPHENE OXIDE FOILS AS OSTEOINDUCTIVE DENTAL PULP STEM CELLS SUBSTRATE

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Graphene oxide (GO), a hydrophilic graphene derivative, has received increasing attention due to the ability to disclose exceptional features of pristine graphene as well as the capacity to be well dispersed in both water and organic solvents. GO is used to synthesize free-standing GO foils characterized by cross-linked GO sheets with enhanced mechanical properties and no tendency to release GO flakes in aqueous solution[1]. Several studies on GO demonstrated great osteoconductive and osteoinductive abilities for regulating osteoblastic differentiation[2]. The aim of this work was to synthesize GO foils and to evaluate their capability to improve Dental Pulp Stem Cells (DPSCs) differentiation towards the osteogenic/odontogenic lineage. GO foils do not evidence cytotoxic effects towards DPSCs, rather, DPSCs viability is significantly increased for cells grown on GO foil and SEM analysis evidences the deposition of consistent extracellular matrix by DPSCs with respect to cells grown on polystyrene at the earliest time of culture (*i.e.*, 3 and 14 days). Gene expression of osteogenic markers and alkaline phosphatase (ALP) activity assay demonstrate DPSCs differentiation towards the osteoblastic lineage. Indeed RUNX2, a key transcriptor factor associated with osteogenic differentiation, as well as SP7, responsible for triggering bone matrix mineralization, are significantly augmented after 7 and 14 days of culture on GO foil with respect to the control, respectively. These results demonstrate that GO foils are able to guarantee cell growth and adhesion, good biocompatibility, even better than polystyrene, moreover they are able to promote a faster and stronger differentiation of DPSCs towards the osteogenic phenotype.

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THE EFFECTS OF NEW AND SUSTAINABLE AQUAFEED INGREDIENTS ON ZEBRAFISH REPRODUCTION

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For many years aquafeed formulation have been based on fish meal and fish oil as main ingredients because they represent the ideal feed components for fish. However, due to ecological and economic implications, alternative nutritious and sustainable ingredients must be identified and tested. At this regard, insects represent a very promising example. Several studies have been published over the last years about insect inclusion in aquafeeds, but results are still controversial and exclusively focused on a short part of fish life cycle and information about the effects of insect-based diets on fish reproduction are presently missing. For this reason, the present study investigated for the first time the effects of insect-based diets (25 and 50% fish meal substitution) administration on zebrafish (*Danio rerio*) reproduction. Compared to conventional aquaculture species like trout, gilthead seabream and European seabass, zebrafish has the advantage to have a short life cycle (from embryo to adult in about six months) and to provide abundant biological information from genomic sequencing. Three experimental diets, including increasing levels of Black Soldier Fly (*Hermetia illucens*) full-fat prepupae, were provided over the whole zebrafish life cycle, from larvae to 12-months-old adults. The biological and physiological effects of the tested diets on mature females, ovary development and egg quality were evaluated through a multidisciplinary approach integrating histological, molecular, gas chromatographic and spectroscopic analyses.

PHYSIOLOGICAL EFFECTS OF INSECT-BASED DIETS DURING *Danio rerio* LARVAL DEVELOPMENT

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A key challenge for future feed and food security is meeting demand for sustainable, domestic grown, alternative protein/lipid sources. These alternative sources are expected to enter the European feed market as replacers for animal-derived proteins. As regards aquaculture, for many years it relied on the use of fishmeal (FM) and fish oil (FO) as main ingredients in aquafeeds. However, for its further development alternative, nutritious and sustainable ingredients must be identified and tested. The introduction of new ingredients in aquafeeds must be carefully analysed, since it is known that modulatory effects of different feed ingredients on fish physiological responses, development and the gut microbiota, exist. Interconnecting land and ocean may be a valuable strategy to promote circularity in the aquaculture sector through the conversion of the great amount of organic waste produced on land in valuable macromolecules to be used in the aquatic environment. On this regard, insects represent a very promising example. Most insect species are cultured on land-produced by-products and, in addition, insect culture is sustainable in terms of both land use and water consumption because of their low environmental requirements. The aim of the present study was to test the biological effects of diets including increasing levels of Black soldier fly (*Hermetia illu-*

cens) prepupae meal during *Danio rerio* larval development. A multidisciplinary approach integrating biometric, histological, molecular, gas chromatographic, microbiological and spectroscopic analyses was applied in order to obtain a comprehensive overview about the effects on fish development. Results showed that insect meal can be included in zebrafish diet to replace up to 50% of the fish meal without significantly affecting fish physiology and development.

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100 MHZ ELECTROMAGNETIC FIELD RADIATION EFFECTS ON ZEBRAFISH *Danio rerio* EMBRYONIC DEVELOPMENT: A MULTIDISCIPLINARY STUDY

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Over the last decades, the population exposure to electromagnetic fields (EMFs) has progressively increased. Various effects have been described on cell biology, mainly related to DNA damages, but, to date, there are few evidences of an association between EMF and embryonic development[1]. To provide reliable data, the present study used a multidisciplinary approach to evaluate stress response, oxidative stress, cholesterol metabolism and apoptotic/autophagic processes during zebrafish (ZF) *D. rerio* embryo development exposed to EMF. The EMF here adopted was a 100 MHz radio-broadcasting frequency and was produced by a TranverseElectroMagnetic (TEM) cell. New fertilized ZF embryos (3000 ± 30) 0 h post fertilization (hpf) were assigned in triplicate to a control group and a 100 MHz EMF-exposed group, the latter contained in the TEM cell. The embryos were cultured in petri dishes inside heated tanks to exclude thermal outcomes. Samplings were performed at 24, 48 ant 72 h post fertilization (hpf). The results showed that a 100 MHz EMF, affected ZF embryonic development, from 24 to 72 hpf. Particularly, the EMF 48 hpf stage showed a growth reduction respect to control, an increased transcription of oxidative stress genes, the onset of apoptotic/autophagic processes and a modification in cholesterol metabolism. Data here obtained showed unequivocally the *in vivo* effects of EMF on an animal model.

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INTERFERENCE WITH THE CANNABINOID RECEPTOR CB1 AFFECTS GNRH AND AGRP1 NEURONAL DEVELOPMENT IN ZEBRAFISH EMBRYOS

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Cannabinoid receptors 1 (CB1) are widely expressed in the central nervous system and have been recognized as regulators of adult plasticity and brain development, including wiring of neuronal connections. CB1 receptors are targets of both endocannabinoids and phytocannabinoids (*e.g.*, Cannabis-derived D^9-THC) and it has been reported that children exposed in utero to cannabis present neurobehavioral and cognitive impairments[1]. In the zebrafish embryo, previous data showed that CB1 receptor knockdown causes abnormal axonal growth in forebrain areas, characterized by failure in fasciculation of axonal tracts[2]. Since this area is rich in Gonadotropin Releasing Hormone (GnRH) and Agouti-related peptide (AgRP) expressing fibers, the aim of this study was to assess whether pharmacological modulation of CB1 could modify GnRH and AgRP axonal pathfinding during zebrafish early neurodevelopment. Immunofluorescence analysis suggested that, during early developmental stages, CB1 is expressed in forebrain areas where both GnRH3 and AgRP1 fibers are present and co-localize with GnRH3-expressing axons. Transgenic GnRH3:EGFP and AgRP1:mCherry zebrafish embryos treated with different CB1 ligands (agonist, antagonist and reverse agonist) showed axon misrouting and abnormal pathfinding of both GnRH3 and AgRP1 fibers in the anterior commissure. Similar phenotypes were obtained by morpholino-mediated CB1 knockdown. CB1 pharmacological modulation influenced CB1, GnRH3 and AgRP1 expression levels, as well as the expression of some genes involved in axonal growth and cell migration, such as Stmn2a/b, Negr1 and Sez6a/b. Overall, these results indicate that CB1 act during development as regulators of axonal pathfinding on GnRH and AgRP neurons. Moving from the animal model to humans, the implications of these results are important in the contest of prenatal exposure to Cannabis.

References

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TIME-LAPSE IMAGING COMBINED WITH ARTIFICIAL NEURAL-NETWORK ANALYSIS PREDICTS OOCYTES AND PREIMPLANTATION EMBRYOS DEVELOPMENTAL COMPETENCE

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Infertility is a major pathology that afflicts about 20% of couples worldwide and, in addition, it affects a third of female patients that undergo oncological treatment (~56,000/year in Italy) and, as a consequence, are at risk of premature ovarian failure. Key to all the ART (artificial reproductive technologies) strategies implemented to rescue or preserve women fertility is the maintenance of the oocyte developmental capacity, as this has an impact on rates of preimplantation, implantation and clinical pregnancy. To this end, oocytes and preimplantation embryos have been observed during *in vitro* culture with the aim of identifying non-invasive and objective quality markers. Here, we propose the combination of time-lapse imaging with artificial neural network (ANN) analysis on mouse oocytes during the germinal vesicle-to-metaphase II transition (15 h culture) and throughout human preimplantation development (120 h culture). Our classification platform entails three main steps: 1) collection of time-lapse images; 2) evaluation of time-lapse sequence images of each oocyte/embryo by a particle image velocimetry software that detects cytoplasmic movements; 3) analysis of cytoplasmic movement profiles through an ANN that predicts developmental competence. Specifically, cytoplasmic movements of single oocytes/embryos were measured as time series and used to train and test a Long-Short Term Memory (LSTM) neural network. Following a ten-fold cross validation of the training set, the LSTM was trained with 90% of data and tested on the remaining. Our platform was capable of identifying developmental competent or incompetent oocytes with an accuracy of 91%[1,2] and, based on the analysis of the cytoplasmic movement occurring

during the first two cell divisions of single embryos, the trained LSTM reached an 82% classification accuracy in the prediction of development to the blastocyst stage. This classification method is expected to improve assisted reproduction treatments.

References

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