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