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OR 02-4

OCCURRENCE OF TWO PARALOGOUS AMBRA1 TRANSCRIPTS AND KNOCKDOWN EFFECTS ON ZEBRAFISH DEVELOPMENT

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Ambra1 is a positive regulator of the Beclin 1-dependent programme of autophagy recently identified in mouse. In this study, we cloned the full-length cDNAs of ambra1a and ambra1b zebrafish paralogous genes. As in mouse, both Ambra1 proteins coded by these two genes contain the characteristic WD-40 repeat region. Expression analysis showed that transcripts of both genes are present as maternal RNAs in the ovulated eggs and display a gradual decline until 8 hpf, being replaced by zygotic mRNAs from 12 hpf onwards. After 24 hpf, the transcripts are mainly localized in the head, suggesting a possible role for these proteins in the development of the central nervous system. To check the functional significance and ontogenetic roles of ambra1a and b mRNAs, we adopted a reverse genetic approach by using morpholino knockdown to block translation (ATGMO). This analysis showed that treatment with ATGMOs causes severe embryonic malformations, as the affected prelarvae could survive for only 3 and 4 days in ambra1a and -b morphants, respectively. Immunoblot analysis at 72 hpf evidenced a reduction of LC3-II level, a marker of autophagic activity thus suggesting a deficiency of the autophagic process. Although some defects, such as body growth delay, curved shape and haemorrhagic pericardial cavity, were present in both morphants, the occurrence of specific phenotypes, such as major abnormalities of brain development in ambra1a morphants, suggests the possible acquisition of specific functions by the two paralogous genes that are both required during development and do not compensate each other following knockdown. Preliminary experiments performed with the use of missplicing MOs to block post-transcriptionally zygotic transcripts confirm the involvement of ambra1 in brain development. However, larvae could survive for 12 days after fertilization and this will allow us to study effects of the two paralogous genes knockdown after the completion of the organogenesis process.

OR 02-5

ANALYSIS OF MYOKINE GENE EXPRESSION IN ZEBRAFISH SKELETAL MUSCLE IN RESPONSE TO SWIMMING-INDUCED ACTIVITY

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In mammals, skeletal muscle is known to produce cytokines ("myokines") in response to muscle contraction. Myokines, such as interleukin (IL)-6, IL-15 and IL-8, are believed to play an important role in skeletal muscle function and in the beneficial effects of exercise. In fish, little is known regarding the expression of myokines in skeletal muscle and the regulation of their expression by swimming. We have previously established the optimal swimming speed for adult zebrafish and demonstrated that zebrafish subjected to a 20-day training protocol at this speed causes a significant increase in growth. In the present study, we aimed at investigating the possible involvement of myokines in the response of skeletal muscle to swimming-induced exercise. First, we identified a number of myokines and myokine receptors in public zebrafish sequence databases, including IL-4, IL-4R, IL-6, IL-6Ra, gp130, IL-8, IL-13, IL-13R, IL-15, IL-15R, FSTL1a, LIF, LIFR, BDNF, FGF21, EPO and EPOR. We designed specific primers to each of these sequences to analyze their expression in the zebrafish skeletal muscle by qPCR. For all the genes analyzed, the expression levels in the zebrafish skeletal muscle were very low or, in some cases, not detectable. Furthermore, we found no significant

differences in the mRNA expression levels of this set of genes in skeletal muscle following the 20-day training protocol. In view of these results, despite a significant potentiation of growth that evidences a functional activation of the skeletal muscle under the present swimming conditions, the lack of changes in myokine expression at the termination of the training protocol prevents us from concluding that myokines might be involved in the physiological adaptation to exercise in zebrafish. Therefore, we believe that it will be essential to perform time course swimming experiments in order to analyze the temporal expression patterns of myokines and their receptors throughout swimming-induced exercise.

OR 02-6

VISUALIZATION OF GLUCOCORTICOID RECEPTOR ACTIVITY IN VIVO

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Glucocorticoids (GCs) are steroid hormones, which are released from the adrenal glands cortex to regulate the vertebrate physiology. GCs are important regulators of development, metabolism, bone differentiation and function, response to stress, apoptosis and circadian cell cycle rhythmicity. Synthetic analogues of these hormones are widely prescribed as anti-inflammatory drugs. GCs activate glucocorticoid receptor (GR) ligand-dependent transcription factors that regulate gene expression by binding to a conserved DNA motif, the glucocorticoid response element (GRE). Using the Tol2 transposon system, we generated independent, stable transgenic lines containing 9 tandem GREs upstream of a thymidine kinase (tk) minimal promoter and the enhanced green fluorescent protein gene [Tg(9xGRE:EGFP)]. In these transgenic lines, glucocorticoids-responding cell populations can be detected in a simple way in both living and fixed zebrafish, by means of fluorescent microscopy and in situ hybridization respectively. Transgenic embryos exhibit ubiquitous fluorescence from early somitogenesis onwards. By 24 hpf the EGFP is mainly localized in brain and somites. From 4 dpf the fluorescence appears to be localized in the gut and liver. Application of the synthetic corticosteroid dexamethasone increases EGFP fluorescence in a dose-dependent manner, thus validating the specificity of the transgenic GRE reporter lines. The Tg(9xGRE:EGFP) animals represent a new model to analyse in vivo GCs target tissues and cells. Furthermore, these lines are powerful tools for the identification of compounds that act as selective GCs pathway modulators.