斑馬魚發育初期促性腺素受體基因失活之研究

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ABSTRACT

Biosafety has been a new concept while the biotechnology making continuous progress and lots of transgenic and cloned animals are produced. Sterility is a necessary adjunct to the exploitation of transgenic animals unless completely secure and locked facility is available. The development, growth, reproduction and physiological metabolism of animals are tightly connected with endocrine system. The hypothalamus-pituitary-gonads axis and the expression of related genes in the axis play an important role in controlling the reproduction. The gonadotropin receptors, LH receptor (LHR) and FSH receptor (FSHR), play a crucial role in mediating the action of gonadotropins on reproductive functions in gonads. Although many mutations in FSHR and LHR have been discovered in males and females with various types of hypogonadism, their roles at early developmental stages are still not clear. The present studies were conducted to investigate the timing and localization of LHR and FSHR expression and their effects on the expression of reproduction related genes at early developmental stages of zebrafish by reverse transcriptase-ploymerase chain reaction (RT-PCR), PCR hybridization, Northern blot and whole mount in situ hybridization. One dominant negative form of LHR with mature peptide of 662 residues was cloned and two splicing forms of LHR were expressed in the eggs at 12 hpf, 24 hpf, 48 hpf and 72 hpf. The expression of LHR, FSHR, VASA, SDF1 and CXCR4b could be detected at stages of 1-2-cell, 512-cell, germ ring, 12 hpf, 24 hpf, 48 hpf and 72 hpf. Knockdown of LHR and FSHR by morpholino or siRNA affected the expression of LHR, FSHR, LH, FSH, CEBP, STAR, VASA, NANOS1, SDF1 and CXCR4b in zebrafish in different manners. This is the first evidence that genes knockdown by could be performed vector-based siRNAs in fish. The migration of primordial germ cells (PGCs) was affected from 12 to 72 hpf after LHR and FSHR morpholinos were injected. Additionally, knockdown of LHR resulted in death of PGCs. The above data indicate that the expression of gonadotropin receptor genes and related genes, which may participate in steroidogenesis and PGCs migration, and can be inhibited or affected by morpholino oligomers and siRNAs. This could be an alternative for fertility control.

Keywords : follicle stimulating hormone receptor (FSHR) ; luteinizing hormone receptor (LHR) ; primordial germ cells (PGCs) ; RNA interference (RNAi) ; reproductive endocrine ; biosafety

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