Suppression of Zebrafish (Danio rerio) FSH and LH Gene Expression by in vivo Transcriptional shRNA

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ABSTRACT

Gonadotropins (GtHs), follicle stimulating hormone (FSH) and luteinizing hormone (LH) play a major regulatory role in reproductive process. They both contain a common glycoprotein α subunit (GtH α , common α) and a hormone-specific β subunit, such as FSH β and LH β , respectively. Zebrafish (Danio rerio) was used as a model fish and the technique of RNA interference (RNAi) was employed to knockdown these three glycoprotein subunits. Three short-hairpin RNA (shRNA) expression vectors and 3 miss-matched shRNA expression vectors as control for each subunit gene was constructed, and the depression efficiency was tested in vivo by microinjection and the RNA levels of GtHs gene were monitored by RT-PCR. The data revealed that the expression of GtH α and FSH β mRNA was obviously depressed. The GtHs-GFP expression vectors for each subunit gene was further constructed, and changes of these three subunits at the protein level were stimulated by co-injection of GtHs-GFP expression vector and GtHs-shRNA expression vector in vivo. The data indicated that the shRNA knockdown efficiency is highly related to the forms of vector construction, and the best construction can knockdown GtH α subunit expression as much as 89.53%.This study also suppressed the GtH α gene expression by morpholino, the data indicated that GtH α morpholino led to the suppression of embryonic development and also embryonic mutant as the result of the injection of GtH α -shRNA. In the result of co-injection morpholino can knockdown GtH α subunit expression as much as 94.32%. However, attainment of fish infertility by shRNA expression vectors with considerations of tissue-specific promoters, remains to be further investigated.

Keywords : gonadotropin ; RNA interference ; transgenic ; expression vector ; infertility

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