Analysis of the cAMP Production in Human 293T Cells Transfected with Zebrafish (Danio rerio) Mutated Gonadotropin Recept

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ABSTRACT

Biosafety has been a new conflict as the biotechnology making continuous progress and many transgenic and cloned animals have been produced. Sterility is a necessary adjunct to the exploitation of transgenic animals unless completely locked facility is available. The hypothalamus-pituitary-gonads axis and the expression of related genes in the axis play an important role in controlling the reproduction.Follicle-stimulating hormone receptor (FSHR) and luteinzing hormone receptor (LHR) are the members of G protein-coupled receptors, and the intracellular loop 2 and 3 (IL2 and IL3) of these receptors can regulate the G protein activity. In this study, wild type (WT) and point mutated amino acid sequences in conserved regions and intracellular loop 2 regions of zebrafish (Danio rerio) FSHR and LHR genes were constructed into reporter vectors and then transfected into human embryonic kidney 293 cells (293T cells). Pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) were applied to evaluate the cAMP accumulation in the transfected 293T cells, respectively. Dominant negative mutant (DNM) is a competitive effect between wild type and mutant type genes. It may competition with another one. Results of enzyme immunoassay showed that PMSG induced cAMP production only in zfFSHR-WT transfected cells, however, cAMP production of zfLHR-WT cells could be induced by either PMSG or hCG. The cAMP levels of the 293T cells transfected with zfFSHR- or zfLHR-DNM were not significantly higher than those of zfFSHR- or zfLHR-WT cells. After hCG induction,the cAMP levels of zfFSHR-DNM cells were significantly higher than those zfFSHR-WT, however, contrast results were observed in zfLHR-DNM and zfLHR-WT cells. Similarly, significantly higher cAMP levels of zfFSHR-R448H and zfLHR-R474A cells after PMSG induction were observed, indicating negative and positive feedback-like mechanisms, respectively. These DMMs may result in sterile of animals, however, further in vivo experiments should be conducted to verity this hypothesis. The current results may be applied in the fertility control of transgenic or cloned animals and in further biomedical researches.

Keywords : zebrafish, dominant negative mutant, point mutation, G protein-coupled receptors, follicle stimulating hormone receptor, luteinzing hormone receptor, cAMP

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