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

謝榮振、陳志毅、黃尉東

E-mail: 9418529@mail.dyu.edu.tw

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 (GtHα, common α) and a hormone-specific β subunit, such as FSHβ and LHβ, 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 GtHα and FSHβ 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 GtHα subunit expression as much as 89.53%. This study also suppressed the GtHα gene expression by morpholino, the data indicated that GtHα morpholino led to the suppression of embryonic development and also embryonic mutant as the result of the injection of GtHα-shRNA. In the result of co-injection morpholino can knockdown GtHα 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

Table of Contents

封面內頁 簽名頁 授權書 中文摘要 英文摘要 目錄 資料謝 感 前言 第一章 文獻討論 第二章 材料與方法 第三章 結果與討論 第四章 論文之結論 參考文獻

第一章 文獻討論

第二章 材料與方法

第三章 結果與討論

第四章 論文之結論

參考文獻


Structural requirements of double-stranded RNA for the activation of 2',5'-oligo(A) polymerase and protein kinase of interferon-treated HeLa cells.


Molecular characterization of sea bass gonadotropin subunits (α, FSHβ, and LHβ) and their expression during the reproductive cycle. Neuropeptides 33:125-33

Kah O 1999 Involvement of γ-aminobutyric acid in the control of GTH-1 and GTH-2 secretion in male and female rainbow trout. Neuroendocrinology 69:269-80


Zhang FP, Poutanen M, Wilbertz J, Huhtaniemi I 2001 Normal prenatal but arrested postnatal sexual development of luteinizing hormone.


Yu JY, DeRuiter SL, Turner DL 2002 RNA interference by rainbow trout, Oncorhynchus mykiss. Gen Comp Endocrinol 100:327-33


Valotaire Y 1995 Preliminary evidence suggesting variations of GtH 1 and GtH2 mRNA levels at different stages of gonadal development in salmonid fish. Gen Comp Endocrinol 71:292-301

Swanson P 1991 Effects of salmon pituitary gonadotropin, ovine luteinizing hormone and testosterone on the testes and seminal vesicles of hypophysectomized catfish Heteropneustes fossilis (Bloch). Gen Comp Endocrinol 71:73-83


Nozaki M, Naito N, Swanson P, Nakai Y, 1991 Differential production and regulation of gonadotropins (GTH I and GTH II) and their plasma concentrations throughout the reproductive cycle in male and female rainbow trout (Oncorhynchus mykiss). Biol Reprod 54:1375-82


Querat B 1995 Structural relationships between Fish and Tetrapod gonadotropins. In: Goetz FW, Thomas P (Eds), Reproductive Physiology of Fish. FishSymp95, University of East Anglia, UK, 2–7

Querat B, Sellouk A, Salmon C 2000 Phylogenetic analysis of the vertebrate glycoprotein hormone family including new sequences of gonadotropins (GTH I and GTH II) and their plasma concentrations throughout the reproductive cycle in male and female rainbow trout (Salmo gairdneri irideus). Gen Comp Endocrinol 77:358-67


Peter RE and Tobe SS Editors, Perspectives in Comparative Endocrinology, National Research Council of Canada, 27–35


Querat B, Sellouk A, Salmon C 2000 Phylogenetic analysis of the vertebrate glycoprotein hormone family including new sequences of gonadotropins (GTH I and GTH II) and their plasma concentrations throughout the reproductive cycle in male and female rainbow trout (Salmo gairdneri irideus). Gen Comp Endocrinol 77:358-67


Mulchahey JJ, DiBlasio