Candidate gene identification of ovulation-inducing genes with in vivo assay in zebrafish

（課程博士・様式７）（Doctoral qualification by coursework,Form 7）

学 位 論 文 要 旨

Abstract of Doctoral Thesis

専　　攻：　　氏　名：

Course：Bioscience　　Name：Wanlada Klangnurak

論文題目：

Title of　Thesis : Candidate gene identification of ovulation-inducing genes with *in vivo* assay in zebrafish

論文要旨：

Abstract :

Two essential processes, oocyte maturation and ovulation, are independently induced but co-operatively activated at the final step in oogenesis. Even though these two processes are induced by same maturation-inducing steroid, 17α, 20β- dihydroxy- 4- pregnen- 3- one (17, 20β-DHP) in teleost, the receptor for each pathway is suggested to be different and thus signal transduction pathways are different. There was no way to separate ovulation-inducing pathway from maturation-inducing pathway. It was difficult to identify ovulation-inducing genes.

In vivo induction technique which enables to induce oocyte maturation and ovulation in living zebrafish (referred to *in vivo* assay) have been applied to select highly up-regulated genes (ovulation-inducing genes) in this study. Living fishes, which treated by 17, 20β- DHP *in vivo*, can be induced both maturation and ovulation. It have been demonstrated that 17, 20β- DHP bind to membrane progestin receptor (mPR) and act via non-genomic pathways to induce oocyte maturation, in addition, ovulation can be activated by 17, 20β- DHP with binding to nuclear progesterone receptor (nPR) via genomic pathways. On the other hand, a synthetic estrogen, diethylstilbestrol (DES) can induce only maturation. DES binds on mPR and activates non-genomic pathways to induce oocyte maturation. Thus, it became possible to prepare ovarian samples that were induced only maturation and both induced maturation and ovulation. By comparing the gene expression profile in these samples, it became possible to distinguish maturation-inducing genes from ovulation-inducing genes for the first time.

Gravid female fishes were exposed to ethanol (EtOH) (none-activated group), diethylstilbestrol (DES) and testosterone (Tes) (maturation induced group), and 17, 20β- DHP (maturation and ovulation induced group) by *in vivo* assay. Using genome wide microarray of zebrafish, gene expression levels in ovaries of these 4 groups were compared. Specifically up-regulated genes during induction of ovulation was selected by Subio Platform. Fold change of expression in 17-20β- DHP treated sample against EtOH, DES and Tes were calculated. The venn diagram analysis was performed to select genes that specifically up-regulated for 1.8 times

in 17-20β- DHP treated samples. ANOVA was performed across all groups to detect significantly changed among all treated groups. Candidate genes (33 genes) for ovulation-inducing genes were selected as overlapping genes in fold change analysis and ANOVA. The mRNA expression levels of the first 20 of 33 have been checked by quantitative polymerase chain reaction (qPCR). However only 2 genes, slc37a4a and zgc:65811, showed ovulation specific up-regulation. Thus, we tried to select genes by just fold change analysis. Again only one gene, zgc:92184, was selected as a candidate for ovulation-inducing gene. Although microarray analysis resulted in a large number of false-positive, it is indicated that *in vivo* assay will be a new way to select ovulation-inducing genes. Therefore, we were going to apply newly developed technique, RNA-sequencing, for selection of ovulation-inducing genes. Then we applied novel gene expression analysis technique, RNA-seq, by using genome sequencer. By RNA-seq analysis, 8 another candidates, ctrb1, prss59.1, ctsbb, stm, adamts15a, sik1, pax2a and rbm47 were selected. qPCR confirmed its specific up-regulation during ovulation. New molecular pathway for fish ovulation has been speculated here.