Pharmacological,toxicological and genetical studies on zebrafish gonad

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学 位 論 文 要 旨

Abstract of Doctoral Thesis

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Abstract：

Several pharmaceuticals are now using Aromatase inhibitors (AIs) to reduce the synthesis of estrogen as cancers treatment agent. Treatment with the aromatase inhibitor (AI) during sex differentiation have huge impact on sex changes and it has been experimentally proved in Japanese flounder and tilapia during sex differentiation the genetic behavior of females develop into phenotypically normal males. Furthermore, successful sex change in sex-differentiated fish has been achieved in Medaka, tilapia and zebrafish by long-term treatment with AIs. The sex differentiation of zebrafish is known to involve juvenile hermaphroditism. Initially, undifferentiated ovary-like gonads are formed during gonadal development in all juvenile zebrafish, regardless of genotypic sex. In genotypically male zebrafish, oocytes disappear from the gonad by apoptosis, and spermatocytes develop concomitant with testicular differentiation. In contrast, oocyte in the female ovaries continue to grow to maturation. In zebrafish, the gonadal masculinization of juvenile genetic females can be induced by the dietary administration of an AI (fadrozole).

Our previous work in zebrafish showed that newly synthesized testes formed separately on the ventral side of the ovaries after ovarian degeneration. The results suggested that undifferentiated germ stem cells that remained alongside the ovaries developed into testes under these conditions. To make it easy to search this germ stem cells, I tried to shorten the time for inducing sex change by injection of AI. By the improvement of way to inject, I succeeded to induce sex change within 3 months. This technique will support the study about hypothesized germ stem cell. The results described in Chapter I.

However, many chemicals in the environment have some specific role in the reproductive system of living organisms. These chemical sometimes act as an inducer or a repressor in reproduction. Bisphenol A (BPA) is one of the important and frequently used chemicals which has abundant commercial applications and many countries are now producing huge amount per year worldwide. Thus, the excessive use of this chemical in daily life may increases the environmental waste which further leads a serious potential risk to the public and wildlife health. It has been

considered a highly suspect human endocrine disruptor likely affecting both male and female reproduction system.

In fish a line of evidence of transgenerational effect were reported in many species, including parental exposure of EE2 during reproductive season reduced eggs, embryos and F1 juvenile production in fathead minnows, embryonic exposure to BPA or EE2 to medaka caused transgenerational abnormalities and health outcomes at later life stage and more recently deregulation of epigenetic patterns by BPA treatment was observed, although the mechanisms mediating these effects remains an active area of research. By previous study in our laboratory, we have demonstrated the adverse effects of BPA on reproductive function of zebrafish. Surprisingly the effects were inherited over generations even after the treatment of fishes with BPA. I tried to determine the lowest amount of BPA to cause this tarnsgenerational effects. I got results that showed 1μg/g of food could induce tarnsgenerational effects. This amount corresponds to the amount lower than human intake of BPA from daily intake. These results are described in Chapter II.

As a result of genome analysis, mPR was classified into the PAQR (progestin and adipo Q receptors) family, which is a novel family of GPCR (G-protein couple receptor). In this family, three subtypes α, β, γ of mPR molecule are correspond to PAQR 7, PAQR 8 and PAQR 5 respectively. Subsequent studies identified mPRδ and mPRε are correspond to PAQR 6 and PAQR 9 and were presumed to have progestin binding activity.

For fish to begin oocyte maturation, progestin binds to mPR on the egg cell membrane and meiosis is resumed. In this pathway, luteinizing hormone (LH), a gonadotropic hormone secreted from the pituitary gland, acts on the follicle cells and induces production of steroidal oocyte maturation-inducing hormone (MIH), 17, 20β-DHP. It is thought that MIH acts on the progestin membrane receptor (mPR) localized on the oocyte membrane and signal transduction of egg maturation. mPR activates the α subunit (Giα) of G protein that is intracellularly coupled by binding to extracellular progestin (17, 20β - DHP). G protein suppresses intracellular adenylyl cyclase activity and the concentration of cyclic AMP (cAMP) decreases. Suppression of cAMP synthesis by progestin stimulation promotes new synthesis of cyclin B and activates maturation promoting factor (MPF) that MPF eventually causes a germinal vesicle breakdown (GVBD). Involvement of mPR as receptors for MIH in induction of oocyte maturation were investing by the experiments using antisense morpholino oligo in zebrafish and goldfish. However more confident results of physiological role of mPR by gene-knock out was not yet reported. Thus, we are trying to establish gene-knock out strains of 7 genes of paqrs in zebrafish. Among these I tried to be established paqr5b gene-knock out strain. Fortunately, I found serious abnormality in embryos from established homozygous mutants of paqr5b. This is a first finding of serious phenotypes of mPR mutant. I would like to continue detailed analysis of this paqr5b phenotype and show the role of paqr5b in early development. These results are described in Chapter III.