Pax2a is expressed in oocytes and is responsible for early development and oogenesis in zebrafish

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学 位 論 文 要 旨

Abstract of Doctoral Thesis

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論文題目：

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Abstract：

Oocyte maturation and ovulation are two important processes that can produce the next generation and maintain the species. For a long time, many molecular biologists have attempted to study two mechanisms of oocyte maturation and ovulation in many species, including the fish model zebrafish. A large part of the molecular system of genes that control these two processes remains unclear. The process of oocyte maturation and ovulation is actually related and overlapping. It is almost indistinguishable. The in vivo assay is a new system for discovering only ovulation-inducing genes that do not contain oocyte maturation-inducing genes. Ethanol, diethylstilbestrol (DES), testosterone (Tes), and 17α, 20ß-dihydroxy-4-pregnane-3-one (17α, 20ß-DHP) were treated in vivo into zebrafish. Ethanol did not alter immature oocyte maturation, but DES and Tes could only induce oocyte maturation, and 17α, 20ß-DHP could induce both of oocyte maturation and ovulation. Due to these characteristics, the ovulation-inducing gene was isolated from the oocyte maturation-inducing gene by gene profile comparison using microarray and RNA sequencing. From these experiments, 11 probable ovulation-inducing genes were selected.

Pax2a is an interesting gene found in 11 genes that are likely related to induce ovulation. *Pax2a* has been reported to be involved in the regulation of development of many organs of zebrafish embryos, such as transcription factors in the brain, eye, thyroid and kidney. Nevertheless, the function of *pax2a* in the ovulation process is not understood. In this study, genome editing was used to investigate the function of zebrafish *pax2a*. CRISPR / Cas9 has been applied to edit *pax2a*. Microinjection of CRISPR/Cas9 solution into 1-cell stage embryos. F0 mosaic zebrafish were bred under appropriate conditions, and after adulthood, F0 mosaic was paied with wild type to produce F1 heterozygous zebrafish. HMA (heteroduplex mobility assay) and DNA sequencing were performed to check for mutations. The same F1 heterozygous zebrafish were paired to obtain F2 homozygous mutant

zebrafish. Then, ovulation, fertilization, and early developmental phenotypes of the embryo were observed. Histological analysis was used to check oocytes in fish for wild-type and *pax2a* homozygous mutants. In addition, *pax2a* expression was checked using Western blotting, quantitative polymerase chain reaction (qPCR), and immunohistochemistry.

This study established a line lacking 6 nucleotides, including the start codon of *pax2a*. This mutation could result in complete knockout of *pax2a* or a shift of the start codon to the next ATG. The results of expression analysis of *pax2a* using Western blot, qPCR, and immunohistochemistry showed that the *pax2a* homozygous mutant expresses the Pax2a protein. This meant that the start codon of the *pax2a* mutant was shifted to the next ATG and only 12 amino acids were deleted. *Pax2a* homozygous mutant fish were fertile and were able to lay eggs. It means that the *pax2a* is not responsible for ovulation. However, embryos born from homozygotes had a high proportion of unfertilized eggs, and F3 homozygous embryos showed some unusual characteristics. For example, the epidermal embryo becomes oval. The oval embryo then showed other significant abnormalities such as cardiac edema, abnormal tail formation, and abnormal yolk morphology. These abnormalities were found when parents of female *pax2a* homozygous mutants were paired with wild-type or male *pax2a* homozygous mutants. However, when the male homozygous mutant was paired with the wild type, the embryos showed different types of abnormal characteristics. No oval abnormalities were found in embryos from male *pax2a* homozygous mutant zebrafish and female wild-type parents. These results suggest that *pax2a* is a gene whose effects are passed on to the next generation by the maternal effect. Very few homozygous embryos survived to adulthood. The surviving adult F3 homozygotes had very small bodies and the ovaries were barely mature in females. Histological analysis showed that the number of oocytes was extremely low. These results indicate that *pax2a* is involved in oogenesis, fertilization and early development.

The results of this study revealed that *pax2a* is not an ovulation-inducing gene but showed a new role for *pax2a* in its involvement in oogenesis, fertilization, and early embryonic development. These new findings may help us understand the molecular pathways of oogenesis, fertilization, and early embryonic development. It is also expected to help, for example, solve the problem of human infertility.