Molecular Study on the Transcription Factors, Nkx2 and Pax8, and Clusterin Expressed in Endocrine Cells

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学 位 論 文 要 旨

Abstract of Doctoral Thesis

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Title of Thesis：Molecular Biological Study on the Transcription Factors, Nkx2 and Pax8, and Clusterin Expressed in Endocrine Cells

Abstract：

In the present study, I have investigated characteristic molecules expressed in the thyroid follicular cells or C cells: Pax8, Nkx2-1, Nkx2-4, and clusterin (Clu).

Firstly, we have identified two distinct Pax8 (a and b) mRNAs from the thyroid gland of the rainbow trout (*Oncorhynchus mykiss*), which seemed to be generated by alternative splicing. Both Pax8a and Pax8b proteins were predicted to possess the paired domain, octapeptide, and partial homeodomain, while Pax8b lacked the carboxy-terminal portion due to an insertion in the coding region of the mRNA. Pax8a and Pax8b mRNAs were abundantly expressed in the thyroid. Thyroid peroxidase (TPO) promoter was found to be increased by Pax8a, but not by Pax8b. Pax8a further showed synergistic transcriptional activity with rat Nkx2-1. On the other hand, Pax8b decreased the synergistic activity of Pax8a and Nkx2-1. Furthermore, not only Pax8a but also Pax8b could bind to the TPO promoter and enhancer. The results suggest that for the trout thyroid gland, Pax8a may directly increase TPO gene expression in cooperation with Nkx2-1 while Pax8b may work as a non-activating competitor for the TPO transcription.

Secondly, we identified three distinct cDNAs encoding Nkx2-4 isoforms (Nkx2-4a, -b, and -c) from the rainbow trout thyroid tissue, and characterized their transcriptional properties. Nkx2-4a and Nkx2-4b, but not Nkx2-4c, significantly activated transcription from a cotransfected rat thyroglobulin (TG) promoter. An electrophoretic mobility shift assay indicated that all the Nkx2-4 isoforms could bind to the TG promoter, implying that the faint transcriptional activity of Nkx2-4c might result from some critical amino acid substitution(s) outside the homeodomain. The distribution patterns of Nkx2-4a mRNA was similar to that of Nkx2-4b mRNA: both mRNAs were expressed abundantly in the thyroid. On the other hand, Nkx2-4c mRNA was detected in the ovary as well as in the thyroid, and was localized to the ovarian granulosa cells and thyroid follicular cells. The results suggest that in the rainbow trout, Nkx2-4a and Nkx2-4b might play a major role in TG gene transcription whereas Nkx2-4c might have some functions in the ovary as well as the thyroid.

Thirdly, Nkx2-1 mRNA was abundantly expressed in the ultimobranchial gland and brain, but not in the thyroid gland. The transcription from trout calcitonin promoter was significantly increased by trout Nkx2-1. Electrophoretic mobility shift assay demonstrated that rainbow trout Nkx2-1 bound to the cis-element of rat Nkx2-1 in the rat calcitonin gene promoter. *In silico* prediction of transcription factor

binding site suggested the presence of eleven Nkx2-1 binding sites in the rainbow trout CT gene upstream region. Based on the *in silico* analysis, I designed DNA probes and carried out electrophoretic mobility shift assay. As a result, Nkx2-1 could bind to the CT promoter. These results suggest that in the rainbow trout, Nkx2-1 is specifically expressed in the ultimobranchial body and might play a major role in CT gene transcription.

Fourthly, we studied Clu that is known as a multifunctional protein in the mammals. In our laboratory, it was found that clu mRNA was expressed in the trout ultimobranchial gland. clu mRNA was distributed ubiquitously while Clu proteins were identified only in the ultimobranchial gland and Brockmann body of the rainbow trout. Therefore, I performed immunohistochemical analysis using medaka fish. Clu immunoreactivity was detected in the ultimobranchial gland and the Brockmann bodies of medaka. These results suggests that Clu has some roles (e.g. hormone synthesis and tissue maintenance) in the teleost ultimobranchial gland and Brockmann bodies.

Fifthly, we developed an advanced genotyping method using high-resolution melting curve (HRM) analysis. HRM analysis is useful for identifying heterozygous point mutants, but it is not routinely used to distinguish homozygous mutants from wild-type organisms. We developed an easy and fast genotyping method to distinguish homozygous mutants from their wild-type counterparts through a three-round HRM analysis: first HRM analysis for distinction of heterozygous mutant from homozygous DNAs; second HRM analysis for denaturation and reannealing; third HRM analysis for the determination of homozygous mutants. After the second HRM analysis, wild-type DNAs were added as a tester DNA to the homozygous DNA to form heteroduplex between homozygous mutant amplicon and wild-type tester DNA. By addition of tester DNA, homozygous mutants become distinguishable. The advantages of this method include its easy procedure, time-saving ability, high stability, and potential usefulness for screening mutants that are generated with not only the TILLING system, but also genome editing method such as TALENs and CRISPR/Cas9 system.

Sixthly, we generated Clu mutant medaka line by TILLING method for further investigation of teleost's Clu function. We have identified three types of Clu mutant (Clu^{K54N}, Clu^{L109P}, Clu^{Y125H}) in the Chapter 6. I have successfully established Clu^{Y125H} homozygous mutant by crossing heterozygous mutants. In the future, we will analyze the function of teleost's clusterin by using Clu^{Y125H} homozygous mutant.

In conclusion, I demonstrated that transcriptional regulatory mechanisms of thyroid follicular cells and C cells specific genes. Furthermore, Immunohistochemistry of wild type medaka and preliminary experiment using Clu^{Y125H} mutant established by TILLING method suggested that clusterin plays some role of the hormone secretion, and the tissue maintenance of endocrine organs such as ultimobranchial gland and Brockmann body. However, detailed Clu function is not clear. Therefore, further investigation using Clu mutant is required in the future.