論文の内容の要旨

Study on regulatory mechanisms governing the expression of transcription factor genes during mouse heart development

（マウス心臓発生過程における転写因子遺伝子の制御メカニズムに関する研究）

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Abstract

Congenital heart diseases occur frequently among newborns, indicating that heart development is a complex process that requires precise control of differentiation and growth of cardiac cells and also precise patterning of these cells. Although the mechanism underlying heart development is still not fully understood, previous studies have suggested that dose-sensitive characteristics of cardiac transcription factors play critical roles for heart development. Many of the cardiac transcription factor genes require both alleles for proper heart development and their mutations result in highly variable phenotypes even among inbred strain mice.

Recent studies about regulatory mechanism of gene expression in mammals have revealed

that long non-coding RNAs (lncRNAs) are an important modulator at the transcriptional and translational levels. Based on the hypothesis that lncRNAs also play important roles in mouse heart development, I attempted to comprehensively identify lncRNAs by comparing embryonic and adult mouse hearts.

RNA-seq analysis of the ventricles of E10.5, E13.5 and 8 week mice identified 787 lncRNA candidates. By comparing the expression of these candidates in mouse brain RNA-seq data, I identified 316 lncRNA candidates among them as non-ubiquitous and heart-selective ones. Next, I examined the distribution of lncRNAs in the mouse genome. Gene ontology analysis revealed that many heart-selective lncRNAs are present near genes important for the heart development. Importantly, many of them are transcribed from the promoters of neighboring genes in a head-to-head divergent manner. I next tried to address significance of the enrichment of genes with bidirectional lncRNAs among haploinsufficient genes. Based on the report that identified haploinsufficient genes and RefSeq transcript annotation database, I showed that the enrichment is observed not only in the heart but also in many other tissues.

In this study, I focused on the lncRNA divergently transcribed from *Tbx5*, since it is evolutionarily well conserved and *Tbx5* is also a haploinsufficient gene. Quantitative RT-PCR (qRT-PCR) analysis demonstrated that the expression pattern of the lncRNA is slightly different from that of *Tbx5*, indicating that they are regulated separately, though they share the same promoter. Using ES cells, I knocked down the lncRNA by inserting strong transcription stop signals into the second exon using the Crispr/Cas9 system. Knockdown (KD) mice derived from the ES cells were embryonic lethal and exhibited severe hypoplasia of ventricle. The expression pattern of *Tbx5* mRNA was unchanged and the RNA-seq of KD mice suggested normal differentiation of cardiomyocytes. Thus, although the precise mechanism is still unsolved, this transcript is likely to function in the morphogenesis of the mouse heart.

During the study of Chapter I, I found that *Tbx5* has multiple promoters and the expression pattern of Tbx5 protein could be altered in the lncRNA KD mice. Based on these findings, I tried to understand the translational regulation of *Tbx5* in Chapter II. *Tbx5* has been shown to be expressed at the left-side of the ventricle and crucial for the proper formation of the ventricular septum. Although disturbance of this left-right gradient is well-known to cause univentricular heart completely lacking the left-right identity, the molecular mechanism underlying the formation of this gradient is yet to be understood.

First, by conducting qRT-PCR and immunohistochemistry on*Tbx5*, I found a distinct expression pattern at the mRNA and protein levels in the ventricle. Even in postnatal mice that lack Tbx5 protein signal, the mRNA level was comparable to that in embryos. I reanalyzed the RNA-seq data I used in Chapter I and found that there are three *Tbx5* promoters and one of them, which I call promoter A, showed the embryo-specific expression pattern. I compared the expression levels of these alternative promoter isoforms by qRT-PCR and revealed that the expression pattern of promoter A isoform was consistent with that of Tbx5 protein. The spatial expression pattern of promoter A isoform in the ventricle of embryos was also remarkably consistent with the protein expression pattern. Furthermore, by luciferase assay, the 5' UTR of the highly-expressed isoform with an inconsistent expression pattern exhibited strong translational repression activity. However, knockout (KO) of isoform A turned out to have little impact on Tbx5 protein production in ESC differentiation system, indicating that *Tbx5* is subject to a complex post-transcriptional control.

In conclusion, these findings suggested that lncRNAs, especially bidirectionally transcribed ones, might play a role in the precise expression regulation of dose-sensitive transcription factor genes that are close to them. In addition, the study in Chapter II suggested various potential strategies to achieve complex expression patterns of these genes.