Functional analysis of sex chromosomes in adult Leydig cells

柳井, 翔吾

（様式３）

氏　　名　：柳井 翔吾

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（成獣ライディッヒ細胞における性染色体の機能解析）

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論 文 内 容 の 要 旨

The sex-determining gene Sry induces testis development even in chromosomally female (XX) individuals. The XX/Sry testes are apparently normal and the interstitial space of the testes is filled with a large number of Leydig cells, which are essential for masculinization through testosterone production. As for the function, XX/Sry Leydig cells were reported to produce less testosterone than the XY cells, and the reason for the decreased activity of testosterone production in XX/Sry Leydig cells remains unclear. To explore it, I performed transcriptomic analysis of XY and XX/Sry adult Leydig cells (ALCs) and found that cholesterogenic genes were upregulated while immediate early genes and ALC-enriched genes were downregulated. In addition, of the six genes required for testosterone production, four were downregulated. To investigate whether these transcriptional changes affected steroid synthesis, I carried out a collaboration to quantify all intermediates of the testosterone synthetic pathway. The result showed that the amount of 17α-hydroxypregnenolone in the XX/Sry testes was significantly larger than that in the XY testes, while that of androstenedione in the XX/Sry testes was smaller than that in the XY testes, strongly suggesting that 17,20-lyase activity of CYP17A1 was substantially reduced in the XX/Sry testes. Another activity of CYP17A1, 17α-hydroxylation, was not likely to be affected. Activities of other steroidogenic enzymes including HSD3B and HSD17B were also not. Taken together, these results showed that the poor testosterone production in XX/Sry testes is possibly attributable to dysregulation of CYP17A1 enzymatic activity rather than the expression levels of steroidogenic enzymes.

Next, I explored how the sex chromosomes impact differential transcription between XY and XX/Sry ALCs. I focused on epigenetic modification because the loss of Y chromosome and gain of extra X chromosome lead to loss of Uty and Smcy, and gain of extra Utx and Smcx, respectively. These genes on the sex chromosomes encode histone demethylases. To examine the possibility that the sex chromosome composition affects histone methylation status, I compared H3K4me3 and H3K27me3 profiles between XY and XX/Sry ALCs. This result showed that H3K4me3 (but not H3K27me3) levels at the loci of cholesterogenic genes were consistently and significantly high in XX/Sry ALCs. Both H3K4me3 and H3K27me3 levels at the loci of immediate early genes and ALC-enriched genes were not largely changed. These results showed that some of the differentially expressed genes including cholesterogenic genes were accompanied by changes of

the histone methylation while others were independent of the histone methylation.

Finally, I examined the role of UTY in transcriptional regulation. Transcriptomic analyses of wild type and Uty-knockout (KO) ALCs demonstrated that expression of the immediate early genes and ALC-enriched genes was generally decreased in Uty-KO ALCs whereas that of cholesterogenic genes was not affected, suggesting that the transcriptional changes in XX/Sry ALCs are partially attributable to the lack of Uty. Considering that the histone methylation was unlikely to be correlated with the decreased expression of the immediate early genes and ALC-enriched genes in XX/Sry ALCs, UTY may regulate expression of these genes in the histone methylation-independent manners.