A Mouse Testis-specific Long Noncoding RNA, *LncRNA-HSVIII*, Regulates the Testosterone Production and the Morphology of Testis, Cauda Epididymis, and Sperm

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Long noncoding RNAs (lncRNAs) are functional without being translated, and thousands of lncRNAs are expressed in various tissues. Among them, the testis expresses more lncRNAs than other tissues, but their functions are unclear. The mouse *Prss/Tessp* locus is composed of six testis-specific serine protease genes and three testis-specific lncRNAs and play important roles in the testis function. *LncRNA-HSVIII*, one of the lncRNAs at the *Prss/Tessp* locus, is specifically expressed in the testis, especially in Leydig cells and spermatocytes, but its function was unrevealed. An objective of this study is to elucidate the physiological function of *lncRNA-HSVIII*. To this end, we generated *lncRNA-HSVIII* knockout (KO) mice by the CRISPR/Cas9 system. Although *lncRNA-HSVIII* KO mice were not infertile, here we report the phenotype at 2 months and 6 months of age. We first performed the RNA-seq analysis using testes of wild-type and *lncRNA-HSVIII* KO mice and found more than 200 genes were differentially expressed in both 2-month-old and 6-month-old testes. Because five sex steroidogenic genes were downregulated in *lncRNA-HSVIII* KO testes, we analyzed the testosterone level by ELISA and found that *lncRNA-HSVIII* KO mice had lower testosterone levels. To see whether the low testosterone level and/or the differential gene expression affected the morphology, we performed the microscopic observation of testis and cauda epididymis as well as the sperm evaluation by the computer-assisted sperm analysis (CASA) system. In both 2-month-old and 6-month-old testes, the number of seminiferous tubule stages were altered, and the number of blood vessels was increased in the interstitial tissue by *lncRNA-HSVIII* KO. Interestingly, some phenotypes were observed

specifically at 6 months of age. In 6-month-old *lncRNA-HSVIII* KO testes, we found clusters of germ cell degeneration, possibly leading to the vacuolation, and enlargement of round spermatids. The number of Sertoli cells was increased in the seminiferous tubule. In the mediastinum testis, the transition zone was elongated, and the interstitial tissue contained more fibroblasts. In 6-month-old cauda epididymis, the extravascular leukocytes were increased in the lumen and interstitium. Furthermore, the sperm evaluation results showed sperm morphological defects mainly at the head and tail in 6-month-old mice, although the total sperm number and motility were not significantly different. Collectively, our results suggest that *lncRNA-HSVIII* regulates the testosterone production and the morphology of sperm, testis, and epididymis especially at an older age.