The role of RSBN1 in mouse spermatogenesis

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（マウス精子形成過程における RSBN1 の役割）

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Thesis Summary

During spermatogenesis, chromatin conformation of germ cells is dramatically changed and is regulated by epigenetic modifications especially methylation of histones, which contribute to differentiation of germ cells. Although the roles of Histone H3 Lysine methylation as well as methyltransferases and demethylases during spermatogenesis have been shown, the roles of Histone H4 methylation and its modifiers were rarely reported. Recently, mouse RSBN1 that has been detected to solely express in nucleus of round spermatids was identified as an ortholog of DPY-21 in *C.elegans* and a demethylase for di-methylated H4K20 (H4K20me2). To explore the dynamic change of H4K20 and the role of RSBN1 during spermatogenesis, the localization of three different levels of H4K20 and RSBN1 was confirmed. Immunostaining results indicated in our report that RSBN1 was not only expressed in nucleus of round spermatids but also in whole of elongated spermatids. Furthermore, results in this study showed RSBN1 was likewise expressed in other tissues, including ovary and brain. Unlike the previous report, RSBN1 could demethylate both H4K20me3 and H4K20me2 but not H4K20me1, when RSBN1 was expressed in HeLa cells. When dynamic change of methylated H4K20 was examined, I found each methylated H4K20 was distributed in almost every stage of germ cell before spermiogenesis, although the intensity of signal is slightly different. Nevertheless, the intensity of each methylated H4K20 was largely different in the occurrence of spermiogenesis. Results of immunostaining indicated that tri-methylated H4K20 and di-methylated H4K20 were significantly decreased in nucleus of round spermatids, but mono-methylated H4K20 was increased during spermiogenesis, strongly suggesting that different methylated H4K20 may play different regulatory roles in round spermatids. When the distribution pattern of RSBN1 in the seminiferous tubule was then compared to that of methylated H4K20, di-methylated H4K20 and tri-methylated H4K20 but not mono-methylated H4K20 were disappeared from RSBN1 positive germ cells. Thus, these results suggested that RSBN1 could be a very important modifier to construct testis-specific landscape of methylated H4K20.