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学位論文の題名	Influence of siRNA-expressing Vector Transfection on Adult Rat Testis In Vivo （In vivo における siRNA 発現ベクター導入がラット精巣におよぼす影響） Nagoya Medical Journal (Accept for publication)
論文審査担当者	主査： 杉浦 真弓 副査： 高橋 智，郡 健二郎

Summary

Improving the rate sperm retrieval rate of patients afflicted with nonobstructive azoospermia is a critical issue. In this study, I designed an *in vivo* RNA interference experiment by using a vector plasmid that expressed siRNAs against *Numb* (numb homolog (Drosophila)) and *Numbl* (numb homolog (Drosophila)-like), which are reported to be necessary for spermiogenesis in mouse; the vector was transfected into rat testis *in vivo* and the influence of vector transfection was evaluated. I analyzed the expression levels and localization of *Numb* and *Numbl* in normal rat testes. Furthermore, I performed a loss-of-function analysis using a gene-knockdown (KD) approach *in vivo*. Normal 8-week-old rats were divided into 4 groups based on the gene KD: Group A: *Numb* KD; Group B: *Numbl* KD; Group C: *Numb*/*Numbl* co-KD; and Group D: negative control. Histological evaluations were performed at 7 and 14 days after gene transfer. The expression patterns of *Numb* and *Numbl* were distinct. No differences in the proportion of germ cells were detected between the 4 groups at 7 days after transfection. However, at 14 days after knocking down *Numb* and *Numbl* expression, the number of spermatids decreased substantially in the Group-A rats, and the number of elongated spermatids decreased markedly in the rats in Groups B and C. No remarkable damage was observed in vector-transfected testes. Thus, siRNA-expressing vectors were

safely transfected into testes and they expressed siRNAs *in vivo* and caused minimal damage. Furthermore, I found that *Numb* and *Numbl* regulate the differentiation of spermatids during rat spermatogenesis. It is expected that these observations to form the basis for the future development of gene therapy or molecule-targeted therapies for male infertility in which the Notch signaling system is modified using *Numb* and *Numbl*.