Center for Experimental Medicine and Systems Biology

Laboratory of Reproductive Systems Biology
生殖システム研究分野

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In the "post-genome project era," genetically modified animals play a key role in basic molecular biological investigations and act as models of human disease. Our laboratory studies the mechanisms underlying the mammalian reproductive system in gene-manipulated mice. We are the first group in the world to generate transgenic mice expressing GFP throughout the body (Green mice). We also established the ES cells that give green fluorescent spermatozoa to trace their movement and acrosome reaction during fertilization. Another tool invented in our laboratory is the placenta-specific gene manipulation system using lentiviral (LV) vectors. Using these techniques, we are trying to elucidate the mechanism underlying gametogenesis, fertilization, implantation, and placentation. Our recent interest is using the CRISPR/Cas9 system as a genome-editing tool. The combination of GWAS studies with genome editing will pave the way to understand and control human fertility problems.

1. Age-related decline in spermatogenic activity accompanied with endothelial cell senescence in male mice.

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Male fertility decreases with aging, with spermatogenic decline being one of its causes. Altered testis environment is suggested as a cause of the phenotype; however, the associated mechanisms remain unclear. Herein, we investigated the age-related changes in testicular somatic cells on spermatogenic activity. The number and proliferation of spermatogonia significantly reduced with aging in mice. Interestingly, senescence-associated β-galactosidase-positive cells appeared in testicular endothelial cell (EC) populations, but not in germ cell populations, with aging. Transcriptome analysis of ECs indicated that senescence occurred in the ECs of aged mice. Furthermore, the support capacity of ECs for spermatogonial proliferation significantly decreased with aging; however, the senolytic-induced removal of senescent cells from aged ECs restored their supporting capacity to a comparable level as that of young ECs. Our results suggest that the accumulation of senescent ECs in the testis is a potential factor contributing to the age-related decline in spermatogenic activity.

2. Ptbp1 deletion does not induce astrocyte-to-neuron conversion.

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Although in vivo reprogramming of astrocytes into neurons holds promise for treating neurodegen-

erative disorders, protocols to achieve it are complex and inefficient. A recent study reported that knockdown of *Ptbp1* in the cortex, striatum and substantia nigra of the midbrain efficiently reprogrammed astrocytes into functional neurons that rescued motor defects in a mouse model of Parkinson's disease1, but it did not convincingly demonstrate that astrocyte-to-neuron conversion had actually occurred. Using genetic disruption of *Ptbp1* in combination with cell-lineage analysis, we observe no astrocyte-to-neuron conversion or substantial changes in gene expression in *Ptbp1*-deficient astrocytes. We conclude that the results of astrocyte-to-neuron conversion in the previous study1 most likely reflect leaky neuronal expression of the $Gfap^{cre}$ mouse lines used to label astrocytes, and more-rigorous experimental methods must be used to support their observations of glial reprogramming in vivo.

3. The histone H3K36 demethylase Fbxl11 plays pivotal roles in the development of retinal late-born cell types.

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Histone methylation plays a vital role in retinal development. However, the role of histone H3K36 methylation in retinal development is not clear. We examined the role of H3K36 methylation by loss-of-function analysis of H3K36me1/2 demethylases, Fbxl10, and Fbxl11. We analyzed the effect of knockout of these genes in the developing and mature retina on retinal development. Knockout of Fbxl10 specifically in the developing retina did not result in gross developmental abnormalities. Although adult rod photoreceptor-specific knockout of Fbxl11 in mature retinas did not result in morphological abnormalities, Fbxl11 knockout in developing retinas increased apoptosis, suppressed the proliferation of retinal progenitor cells, and resulted in microphthalmia. Morphological analysis revealed perturbed differentiation of rod photoreceptor and bipolar cells. RNA-seq of retinas at P7 showed markedly decreased expression of genes characterizing rod photoreceptor and bipolar cells in Fbxl11-knockout retinas. In addition, perturbation of alternative splicing increased intron retention in Fbxl11-knockout retinas. Genome-wide evaluation of the H3K36 methylation status revealed that Fbxl11 knockout altered the distribution of H3K36me2/3 in genes important for rod photoreceptor development. Taken together, we show that Fbxl11 plays pivotal roles in the development of retinal late-born cell types and may contribute to tight control of H3K36 methylation during retinal development.

4. CCDC183 is essential for cytoplasmic invagination around the flagellum during spermiogenesis and male fertility.

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Sperm flagellum plays a crucial role in male fertility. Here, we generated Ccdc183 knockout mice using the CRISPR/Cas9 system to reveal the protein function of the testis-specific protein CCDC183 in spermiogenesis. We demonstrated that the absence of CCDC183 causes male infertility with morphological and motility defects in spermatozoa. Owing to the lack of CCDC183, centrioles after elongation of axonemal microtubules do not connect the cell surface and nucleus during spermiogenesis, which causes subsequent loss of cytoplasmic invagination around the flagellum. As a result, the flagellar compartment does not form properly and cytosol-exposed axonemal microtubules collapse during spermiogenesis. In addition, ectopic localization of accessory structures, such as the fibrous sheath and outer dense fibers, and abnormal head shape as a result of abnormal sculpting by the manchette are observed in Ccdc183 knockout spermatids. Our results indicate that CCDC183 plays an essential role in cytoplasmic invagination around the flagellum to form functional spermatozoa during spermiogenesis.

5. A small secreted protein NICOL regulates lumicrine-mediated sperm maturation and male fertility.

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The mammalian spermatozoa produced in the testis require functional maturation in the epididymis for their full competence. Epididymal sperm maturation is regulated by lumicrine signalling pathways in which testis-derived secreted signals relocate to the

epididymis lumen and promote functional differentiation. However, the detailed mechanisms of lumicrine regulation are unclear. Herein, we demonstrate that a small secreted protein, NELL2-interacting cofactor for lumicrine signalling (NICOL), plays a crucial role in lumicrine signalling in mice. NICOL is expressed in male reproductive organs, including the testis, and forms a complex with the testis-secreted protein NELL2, which is transported transluminally from the testis to the epididymis. Males lacking Nicol are sterile due to impaired NELL2-mediated lumicrine signalling, leading to defective epididymal differentiation and deficient sperm maturation but can be restored by NICOL expression in testicular germ cells. Our results demonstrate how lumicrine signalling regulates epididymal function for successful sperm maturation and male fertility.

Publication

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