論文の内容の要旨

獣医学専攻
平成 26 年度博士課程入学
猪村（貴志） かさね
指導教員　九郎丸 正道

論文題目

Study of the Sertoli Valve Epithelia in the Terminal Segment of Mouse Seminiferous Tubules

（マウス精細管基部のセルトリバルブ上皮に関する研究）

In adult mammalian testes, spermatozoa are produced through almost all their life, and this is because males have spermatogonial stem cells (SSCs) inside testes, and SSCs self-renew or differentiate in a well-balanced manner. SSCs are settled in seminiferous tubules with nursing cells called Sertoli cells (SCs) and SCs support the SSCs. SCs supply secreting factors and regulate the balance of self-renewing and differentiation of undifferentiated spermatogonia including SSCs. A major niche factor for SSCs' self-renewing is glial cell line-derived neurotrophic factor (GDNF). Undifferentiated spermatogonia including SSCs express GDNF family receptor alpha 1 (GFRA1). On the other hand, major SSCs' differentiating factor is retinoic acid (RA).

Undifferentiated spermatogonia differentiate into differentiated spermatogonia and after that, they differentiate into spermatocytes and meiosis occurs. By meiosis, haploid cells, called spermatids, are produced. Spermatids undergo the morphological change and develop into matured spermatids. Finally, they are released into the lumen of seminiferous tubules as spermatozoa and transferred into the rete testis (RT) by the luminal fluid flow. This series of events is called spermatogenesis. Spermatogenesis is precisely coordinated in time and space and occurs with periodical cycle called seminiferous epithelial cycle. In mice, it has been divided into 12 stages. These cyclical differentiations might be explained by periodical GDNF secretion and RA secretion. The stages highly expressing GDNF and RA occur alternatively like a seesaw.

The above periodical differentiation is the story inside convoluted seminiferous tubules (ST), which account for a large portion of seminiferous tubules. In the terminal segment of seminiferous tubules, where they are connected to rete testis, a gradual depletion of germ cells occurs and finally tubules are lined only by SCs and a few spermatogonia. This region is known as straight seminiferous tubule, transitional zone or transition region, and this region is seen in many species including human. The SCs in this region form 'plug' or 'valve' -like structure and are considered to regulate the luminal fluid flow of seminiferous tubules. In our laboratory, we call this structure "Sertoli valve (SV)" and our previous data on hamsters show that SV epithelium constitutively expresses GDNF and supports the stable proliferation and selective maintenance of undifferentiated spermatogonia. The SCs in SV region support GFRA1-positive undifferentiated spermatogonia, while the region lacks KIT-positive differentiated spermatogonia and further differentiated spermatogenic cells. These data suggest that in SV region, balance between self-renewal and differentiation of SSCs is inclined more to self-renewal, so that periodical cycle is missing.

SCs proliferate from fetal stage to neonatal stage, however for a long time, it was considered that proliferation of SCs is not observed in adult stage in most of mammals. Our previous data show that SCs in SV region of hamsters are still capable of proliferation even in adult stage. Moreover, the evidences of proliferating adult SCs in SV region were also found in normal rat testes and cultured mouse SCs.

As stated above, SV region shows interesting feature, so that many researchers hope to know the cellular or molecular characteristics. In this study, I did the histological observation, developmental engineering analysis, and transciptomic analyses to clarify the development and molecular basis of SV structure. For the histological observation, I focused on the Akt signaling and performed the immunohistochemistry using anti-phospho-Akt (active form of Akt) antibody. In the developmental engineering analysis, I used Amh-Treck transgenic mice to deplete the SV structure and after that, I reconstruct the SV structure by SCs of convoluted seminiferous tubules. Finally, I performed the microarray and detected the SV specifically highly expressed genes.

In Chapter 1, I analyzed the activation of Akt signaling in SCs using immunostaining of anti-p-Akt antibody. In ST region, Akt signaling was seminiferous epithelial cycle-dependently activated, while in SV region, Akt signaling was

constitutively activated. This pattern of Akt activation in SV region was not observed in 1-week-old neonatal mouse testes, but from 2 to 4-week-old, this pattern was observed and the valve-like structure was constructed. Transplanting the immature 1-week-old mouse SCs of ST region into SCs-depleted Amh-Treck Tg mouse testes revealed that SCs of ST can act as SCs of SV such as supporting few spermatogenic cells, activation of Akt signaling, and construction of valve-like structure positive for ace-Tub, when they are settled in SV region. These data suggest that Akt signaling is region-specifically constitutively activated after birth, and the construction of valve-like structure non-cell autonomously occurs dependent on information of the place where SCs are settled. I cannot understand the activation of Akt signaling in SV is the result or cause of the construction of valve-like structure from these data, but activation of Akt signaling in SV region might play an important role in constructing SV structure.

In Chapter 2, I show that *Cyp26a1*, the RA-metabolizing enzyme, was highly expressed in SCs of SV region of W/W^v mice (lack spermatogenic cells due to a germ cell-autonomous defect) compared to RT and ST regions by microarray, quantitative PCR analysis and *in situ* hybridization. In contrast, *Aldh1a2*, the RA-synthesizing enzyme, was highly expressed in ST region of wild-type mice, which include spermatogenic cells, compared to RT, SV, and ST regions of W/W^v mice. RA is the differentiation factor of spermatogenic cells. The data that *Cyp26a1* is highly expressed in SV region suggest that RA degradation is constitutively occurring in SV region, so that SV region is missing seminiferous epithelial cycle, and balance between self-renewal and differentiation of SSCs is inclined more to self-renewal. This is consistent with the previous data that SV epithelia constitutively express GDNF and support the stable proliferation and selective maintenance of undifferentiated spermatogonia. RA degradation in SV region may play an important role in regulating the differentiation of spermatogenic cells in SV region. I also show that *Fgf9* is highly expressed in RT region, and exogenous FGF9 can activate the Akt signaling in SCs even in ST region.

In the present study, I show the constitutive activation of Akt signaling in SV region, and the activation of Akt signaling and formation of valve-like structure non-cell autonomously occurred. Based on these findings, I proposed the hypothesis that RT

secretes FGF9 and SCs that received FGF9 will be activated of Akt signaling, and then the SCs show the feature of SV structure. In addition, I cannot forget about the seminiferous epithelial cycle-dependent activation of Akt signaling in wild-type mouse ST region. Clarifying the factors of activating Akt signal in STs and the function of cyclical activation of Akt signaling is required. This cyclical activation of Akt signaling in ST region might have a relation with constitutive activation of Akt signaling in SV region. In the present study, I show that *Cyp26a1* is highly expressed in SV region and that data suggest that RA degradation is constitutively occurring in SV region, and balance between self-renewal and differentiation of SSCs is inclined more to self-renewal in SV region. I can hypothesize that this situation is produced by constitutive activation of Akt signaling. If I apply this hypothesis to ST region, cyclical activation of Akt signaling in ST region may produce the cyclical balance of self-renewing and differentiation of SSCs.

RA signaling plays crucial roles during vertebrate development. There is a concept of RA-FGF antagonism and it is applied to several mechanisms of development. In the chick embryo, FGF signaling has been shown to antagonize the RA gradient and to maintain the undifferentiated state of cells in the posterior part of the embryo throughout somitogenesis. On the research of mouse somitogenesis, conditional deletion in the mesoderm of *Fgfr1*, the only FGF receptor expressed in the mouse paraxial mesoderm, resulted in the absence of the RA-degrading enzyme CYP26 in the posterior part of the embryo. In addition, on the research of mouse limb bud development, culturing of wild-type mouse forelimb bud in the presence of an inhibitor of the FGFR tyrosin kinase (SU5402) resulted in almost complete loss of *Cyp26b1* expression, and conversely, the implantation of FGF4-loaded beads into *Shh*-deficient mouse limb buds, of which the expression of *Cyp26b1* was reduced in the distal mesenchyme and RA activity was increased, resulted in striking restoration of *Cyp26b1* expression. Applying these findings to the present study, I can consider that FGF9 signaling in SV epithelia might activate the expression of *Cyp26a1* in SV epithelia.