論文内容の要旨

FUNCTION AND EXPRESSION OF NANOS3, A GERM CELL SPECIFIC PROTEIN IN MOUSE

(マウス生殖細胞特異的タンパク質 Nanos3 の機能と発現)

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The germ cell is the only cell population that transmits genome information to the next generation. In spite of its importance, only a few genes are identified to be functional for the generation and development of germ cells. *nanos* homologues encode RNA binding proteins conserved among many organisms and one of gene families implicated in the germ cell development. The mouse genome encodes three *nanos* homologues, *Nanos1-3* in which Nanos3 expression is restricted in germ cells immediately after its formation and the function is essential for the germ cell development. I studied the mechanism of anti-apoptotic function of Nanos3 and the expression of Nanos3 during embryogenesis and in the adult spermatogenesis. In this thesis, I report my accomplishments in three independent chapters: 1) Function of Nanos3, 2) Regulatory mechanisms of Nanos3 expression, 3) Expression of Nanos proteins in adult testis.

Chapter 1. Function of Nanos3

Nanos3 maintains the germ cell lineage in the mouse by suppressing both Bax-dependent and -independent apoptotic pathways

Cell death in the germ line is controlled by both positive and negative mechanisms that maintain the appropriate number of germ cells and that prevent the possible formation of germ cell tumors. In the mouse embryo, Steel/c-Kit signaling is required to prevent migrating primordial germ cells (PGCs) from

undergoing Bax-dependent apoptosis. In the chapter 1, I show that migrating PGCs also undergo apoptosis in Nanos3-null embryos. I assessed whether the Bax-dependent apoptotic pathway is responsible for this cell death by knocking out the Bax gene together with the Nanos3 gene. Differing from Steel-null embryos, however, the Bax elimination did not completely rescue PGC apoptosis in Nanos3-null embryos, and only a portion of the PGCs survived in the double knockout embryo. I further established a mouse line, *Nanos3-Cre-pA,* to undertake lineage analysis and my results indicate that the most of the Nanos3-null PGCs die rather than differentiate into somatic cells, irrespective of the presence or absence of Bax. In addition, a small number of surviving PGCs in Nanos3/Bax-null mice are maintained and differentiate as male and female germ cells in the adult gonads. My findings thus suggest that heterogeneity exists in the PGC populations and that Nanos3 maintains the germ cell lineage by suppressing both Bax-dependent and Bax-independent apoptotic pathways.

Chapter 2. Regulatory mechanisms of Nanos3 expression

Nanos3-3'UTR is required for germ cell specific Nanos3 expression in mouse embryo

The significance of 3' untranslated region (UTR) in the control of gene expression has been demonstrated in many organisms and tissues. In the early embryogenesis of nematodes, fly, fish and frog, several protein expressions are temporally and spatially regulated via mechanisms depending on 3'UTR of maternal mRNAs, which is a critical step to establish the elaborate body patterning. In mouse, 3'UTRs are responsible for the development of specific cell types in neurogenesis, erythropoiesis and spermatogenesis by organizing the timing and region of mRNA translation. Here, I show that *Nanos3* mRNA is detected in both germ cells and somatic cells although Nanos3 protein is expressed specifically in germ cells. To investigate the regulatory mechanism of Nanos3 expression, I generated several BAC transgenic mouse lines using BAC modification technologies and assessed whether the expression is altered by the replacement or deletion of elements in *Nanos3* gene. The results indicate that *Nanos3* is transcribed but the translation in somatic cells is suppressed via mRNA destabilizing mechanism mediated by *Nanos3*-3'UTR. Surprisingly, even though *mRFP* was driven by CAG promoter which induce strong and ubiquitous transcription, the addition of *Nanos3*-3'UTR was effective to restrict mRFP expression in germ cell. In addition, I also find that *Nanos3* exons and intron sequences may be involved in the transcriptional regulation. My current study suggests that Nanos3 expression is regulated by multiple mechanisms at transcriptional and translational levels. Moreover *Nanos3*-3'UTR has a great deal of capability of translational control: it is not only required for the expression of Nanos3 protein in germ cells, but also sufficient for the establishment of germ cell specific expression patterning in the mouse embryo.

Chapter 3. Expression of Nanos proteins in the adult testis

The spermatogonial heterogeneity revealed by their topology and marker expressions including germ cell-specific proteins Nanos2 and Nanos3

Spermatogonial stem cells (SSCs) reside in undifferentiated type-A spermatogonia and contribute to continuous spermatogenesis, by keeping the balance between self-renewal and differentiation to meet the biological demand in the testis. Despite their critical importance, spermatogonia has been characterized principally through their morphology. I herein report a detailed characterization of undifferentiated spermatogonia in mouse testes based on the gene expression profiles in combination with topological features. The detection of the germ cell-specific proteins Nanos2 and Nanos3 as markers of spermatogonia enabled the clear dissection of complex populations of these cells. Nanos2 was found to be expressed exclusively in A_s to A_{al4} cells, whereas Nanos3 was detectable in all undifferentiated spermatogonia (A_s to A_{al}) and differentiating A_1 spermatogonia. In addition, we found that A_s and A_{pr} can be basically classified into three categories: 1) GFRα1$^+$Nanos2$^+$Nanos3$^-$*Ngn3*$^-$, 2) GFRα1$^+$Nanos2$^+$Nanos3$^+$*Ngn3*$^-$ and 3) GFRα1$^-$Nanos2$^\pm$Nanos3$^+$*Ngn3*$^+$, the first of which is most likely to include the stem cell population. Taken together, we suggest from my current data that Nanos2 is involved in the maintenance of stem cells with GFRα1 and Plzf, whilst Nanos3 may function in transit amplifying cells.

In conclusion, my thesis studies clarified mechanisms how Nanos3 maintains embryonic germ cells and how this important gene expression is regulated. In addition, the detail analysis of Nanos expression pattern in adult testes implied the difference in functions between Nanos2 and Nanos3 during spermatogenesis and gave us new insights on the notion of spermatogonial stem cell.