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学位の種類	博士（医学）
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審査研究科	人間総合科学研究科
学位論文題目	The role of transcription factor MAFB in the initiation of spermatogenic cycle（精子形成サイクルの開始における転写因子 MAFB の役割）
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論文の内容の要旨
Abstract of thesis

【背景・目的　Background/Purpose】

The transcription factor MAFB is an important regulator of the development and differentiation of various organs and tissues. Previous studies showed that MAFB is expressed in early embryonic gonads and in adult mouse testes. It was expected that MAFB modulate the retinoic acid signaling to maintain spermatogenesis. However, the exact localization and function of MAFB remain unclear through a genetic approach in the mouse as null mutants are not viable due to defective respiratory rhythmogenesis and conditional alleles of MAFB were not available. In this study, the author aimed to investigate the role of MAFB in three stages; 1) Testes morphogenesis in E18.5 embryo, 2) Initiation of the first spermatogenic wave in postnatal mice, and 3) Maintenance of spermatogenesis after first spermatogenic wave.

【対象と方法　Materials and methods】

The author localized the expression of MAFB in embryonic and adult testes and then analyzed its function using Mafb-deficient mice. For embryonic stage, E18.5 Mafb KO testes were examined via testes somatic cell counts, histological analysis, and expression level of genes related to normal development. For postnatal stage, tamoxifen-induced time-dependent deletion of conditional Mafb alleles were generated and examined via histological analysis, testosterone levels, and germ cell stage-specific markers. The author also performed RNA sequencing and bioinformatic analysis to identify the downstream targets of MAFB.

【結果・考察　Results】

The author found that MAFB and c-MAF are the only large MAF transcription factors expressed in testes,

while MAFA and NRL are not. MAFB was localized in Leydig and Sertoli cells at embryonic day 18.5 but in Leydig cells, Sertoli cells, and pachytene spermatocytes in adults. The author examined E18.5 Mafb KO testes and found that they showed a fully formed seminiferous tubules with no abnormal structure or differences in testicular somatic cell numbers and the expression level of genes related to development and function of primordial germ cells, Leydig cells, Sertoli cells and sex determination did not significantly differ between genotypes, which indicated that testes morphogenesis was normal in the KO mice. The author found that, in postnatal testes, MAFB was essential for the initiation of the first spermatogenic wave, by inducing the transition of spermatogonia type A to A1, but not the subsequent waves as indicated by histological analysis, testosterone levels, and germ cell stage-specific markers. The author performed retinoic acid-mediated induction of the *in vitro* cultured Sertoli cells and found that MAFB is the downstream target of retinoic acid to modulate such function. Moreover, the author analyzed the transcriptome profile of Sertoli cells from Mafb-cKO by RNA-Seq and identified 13 down-regulated genes containing MARE sites in mouse and human, five of them are secretory proteins; namely, Cxcl5, Spink8, Tex101, Prnd, and Il10. The author also found that MAFB is critical for life maintenance during the pre-adulthood period, likely due to its role in preventing the development of chronic kidney disease observed in the Mafb-deficient mice.

審査の結果の要旨
Abstract of assessment result

【批評　General Comments】

The author demonstrated that the transcription factors MAFB and c-MAF are expressed in mouse testes and localized in Leydig and Sertoli cells in testes at E18.5 whereas it localized in Leydig cells, Sertoli cells, and pachytene spermatocytes in adult testes. In addition, the author found that MAFB-deficient testes developed normally by E18.5, and spermatogenesis maintenance was not disrupted in adult mice. However, MAFB was indispensible for the initiation of the first spermatogenic wave. The author analyzed the transcriptome profile of adult Mafb-cKO Sertoli cells to identify 13 down-regulated genes, five of which are secretory proteins, that have MARE sites in both mouse and human and thus are possibly the direct target of MAFB to initiate the first A to A1 spermatogonia transition. The author also found that MAFB is critical for life maintenance during the pre-adulthood period, likely due to its role in preventing the development of chronic kidney disease in the Mafb-deficient mice. These investigations by the author provide novel findings into the role of MAFB in spermatogenesis, in particular, with regards to the functional interactions among Leydig cells, Sertoli cells, and spermatocytes. The author's investigations were thoroughly performed and technically sound, and not only provide some novel insights but also open new avenues for further research in the process of spermatogenesis.

【最終試験の結果　Assessment】

The final examination committee conducted a meeting as a final examination on June 7, 2017. The applicant provided an overview of dissertation, addressed questions and comments raised during the Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結果　Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.