論　文　概　要 (Thesis Abstract)

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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目　的
(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 involved the retinoic acid signaling to maintain spermatogenesis. However, the exact localization and function of MAFB remain unclear since mice with null mutants are not viable and conditional alleles were not available. Thus the aim of the current study was to investigate the role of MAFB in; 1) Testes morphogenesis at 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
(Material and method):

We carefully localized the expression of MAFB in embryonic and adult mice testes and then analyzed its function using *Mafb*-deficient mice. For embryonic stage, E18.5 *Mafb* KO testes were examines 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. RNA sequencing and bioinformatic analysis were done to identify the downstream targets of MAFB.

結　果
(Result):

We 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. Examination of E18.5 *Mafb* KO testes 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 indicated that testes

morphogenesis was normal in the KO mice. In postnatal testes, MAFB were found to be 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. By induce retinoic acid in the *in vitro* cultured Sertoli cells, MAFB found to be the downstream target of retinoic acid to modulate such function. Moreover, we analyzed the transcriptome profile of Sertoli cells from *Mafb*-cKO by RNA-Seq. There were 13 down-regulated genes containing MARE sites in mouse and human, 5 of them were secretory proteins that are Cxcl5, Spink8, Tex101, Prnd, and Il10. Interestingly, we 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.

考 察
(Discussion):

The present study was undertaken in an effort to define the physiological role of the *Mafb* gene in mammalian testes. Previous study on embryonic mouse gonads showed that MAFB is expressed in the XY gonads along the gonad-mesonephros border as early as E11.5 and is then restricted to Leydig cells by E14.5 (DeFalco et al., 2011). Just before birth at E18.5, we localized MAFB expression and found that MAFB is expressed in Sertoli cells in addition to being expressed in Leydig cells. However, E18.5 *Mafb* KO testes developed normal. Since the large MAF transcription factor in Drosophila melanogaster TJ, which encodes an ortholog of a typical basic Leu zipper of transcription factors MAFB and c-MAF in vertebrates, was shown to be mandatory for germ cell differentiation in Drosophila (Li et al., 2003). In addition, RA, the active metabolite of vitamin A, is known to be essential for the initial differentiation and meiotic entry of mice spermatogonia (Raverdeau et al.). Therefore, it is hypothesized that *Mafb* is the downstream of RA to induce spermatogonia differentiation in mouse. We found that MAFB is localized in Sertoli cells and pachytene spermatocytes in postnatal mouse testes and by analyzing the cKO mice, data revealed that deletion of MAFB in adult mice did not exhibit any abnormality indicating that MAFB is dispensable for the maintenance of subsequent waves of spermatogenesis. Next, to examine its role in the first spermatogenic wave, we induced MAFB deletion by tamoxifen injection before spermatogonia

differentiation started by P5. Unexpectedly, the cKO mice died soon after MAFB deletion, and therefore, could not be analyzed. To overcome this issue we used busulfan drug that deplete differentiated germ cells in adults at the dose of 20mg/kg and testes remains similar to the neonatal mice. cKO mice treated with busulfan couldn't reinitiate the spermatogenic cycle. Moreover, as our in vitro culture of Sertoli cell showed that RA induces *Mafb* expression, altogether indicating that *Mafb* is the down stream target of the RA to initiate the first spermatogenic wave in mice. Furthermore, MAFB is a transcription factor indicting that its' function is not a paracrine manner in Sertoli cells but autocrine. Thus, MAFB should regulate another factor(s) that induce the spermatogonial differentiation. We analyzed the transcriptome profile of Sertoli cells from *Mafb*-cKO by RNA-Seq. There are 13 down-regulated genes, containing MARE site in mouse and also human, were identified. Among of them 5 were considered as secretory proteins that are Cxcl5, Spink8, Tex101, Prnd, and Il10. One of them is possibly the downstream target of *Mafb* to maintain such function.

結　論
(Conclusion):

The transcription factors MAFB and c-MAF are expressed in mouse testes. MAFB localized in Leydig and Sertoli cells in testes at E18.5 while it localized in Leydig cells, Sertoli cells, and pachytene spermatocytes in adult testes. Our current examination technique revealed 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 transcriptome profile of adult *Mafb*-cKO Sertoli cells showed 13 down-regulated genes has MARE sites in both mouse and human and 5 of them are secretory proteins. One of these genes is possibly the direct target of MAFB to initiate the first A to A1 spermatogonia transition. Interestingly, we 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.