様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Retinoid signals to control spermatogonial stem cells differentiation

研究課題名（英文）Retinoid signals to control spermatogonial stem cells differentiation

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：ビタミンAは、精原細胞や有足細胞を含むさまざまな細胞分化の重要な調節因子であるレチノイン酸（RA）に合成されます。本研究では、RAの新しいターゲットとしてMafBとcMafを同定しました。 RAは、MafBとc-Mafを直接制御し、特に、培養セルトリ細胞と有足細胞でそれらの発現を誘導することを確認しました。 一方で、MafBとc-Mafの二重欠損マウスは尿中タンパク量が増加し、糸球体RNA-seq解析により、スリット膜関連タンパク質（Magi2）と有足細胞特異的転写因子（Tcf21）のダウンレギュレーションを明らかにしました。

研究成果の学術的意義や社会的意義
レチノイン酸シグナルは、全身で多彩な役割を果たしており、その標的遺伝子を含むカスケードの全貌を明らかにすることは、脳神経疾患や各種悪性腫瘍の予防や治療に大きな貢献をすると期待される。本研究では、精子形成および腎臓足細胞におけるシグナルの下流遺伝子と生理的役割を同定しており、この分野における更なる発展が期待できる。

研究成果の概要（英文）：Vitamin A is synthesized into retinoic acid (RA), an important regulator of various cell differentiations, including spermatogonia and podocytes. In this study, we identified MafB and cMaf as new targets for RA. RA has confirmed that it directly regulates MafB and c-Maf and induces their expression, especially in cultured Sertoli cells and podocytes. On the other hand, urinary protein levels increased in MafB and c-Maf double-deficient mice, and glomerular RNA-seq analysis showed that slit diaphragm-related proteins (Magi2) and podocyte-specific transcription factors (Tcf21) were down. Clarified the regulation.

研究分野：発生生物学

キーワード：精子形成　腎足細胞　大Maf群転写因子　タンパク尿

様　式　C－１９、F－１９－１、Z－１９（共通）

１．研究開始当初の背景

Sertoli cells create the microenvironment required for spermatogonial differentiation. Vitamin A (Retinol, ROL) is synthesized into retinoic acid (RA) and then bind to retinoic acid receptor alpha (RARA) inside Sertoli cell and somehow induce spermatogonial differentiation. However, the target genes that are regulated by retinoic acid and control spermatogonia differentiation are unknown. MAF family of transcription factors is a subgroup of the basic leucine zipper (bZip) transcription factors and is an important regulator of the development and differentiation of various cell types. In Drosophila, large MAF called *traffic jam* (TJ), expresses in somatic cells of gonads and produces a soma-specific factor that showed to be mandatory for germ cell differentiation. The basic domains of TJ show 85% identity to mammalian large MAF factors MAFB and c-MAF. Therefore, MAFB and c-MAF are suggested to be the downstream targets of retinoic acid in testis as well as other organs.

２．研究の目的

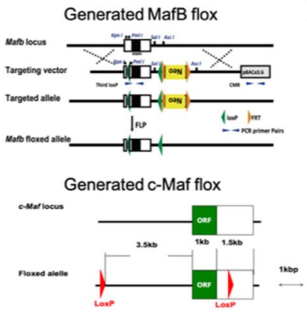
The purpose of the current research was to identify whether MAFB and c-MAF are directly regulated by retinoic acid to maintain its' *in vivo* function for 1) germ cell differentiation and/or 2) other cell types differentiation.

３．研究の方法

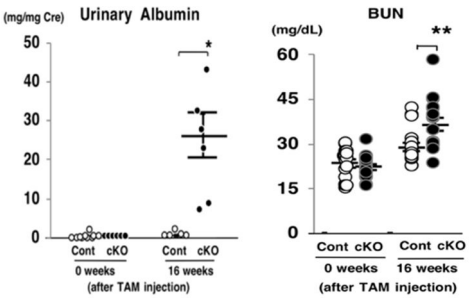
(1) Examine the interaction of RA with MAFs *in vitro* using sertoli cell culture and by luciferase assay. (2) In order to understand the retinoic acid signaling *in vivo*, it was necessary to generate MafB and c-Maf mutant mice. Since both null mutant mice are embryonic lethal, the strategy was to generate the floxed allels of MafB and c-Maf independently as well as MafB/c-Maf double floxed mouse, the three lines were crossed with CAG-Cre ERT_2 to obtain double cKO mice, in which Cre recombinase was activated upon tamoxifen treatment. (3) Phenotypic analyses examined using histology and transmission electron microscopy. (4) NGS analyses for tissues of MAFs conditional KO mice to identified proteins involving in Vitamin A signaling.

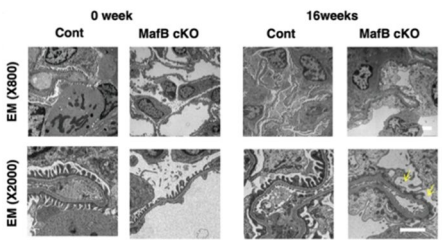
４．研究成果

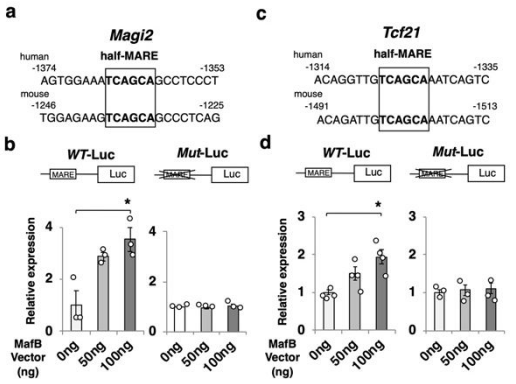
(1) RA directly regulate MafB and cMaf *in vitro*: Primary sertoli cells were isolated and cultured for 4 days followed by stimulation with RA at day 5. The expression changes in MafB and cMaf were quantified by qRT-PCR. The expression levels were increased significantly by

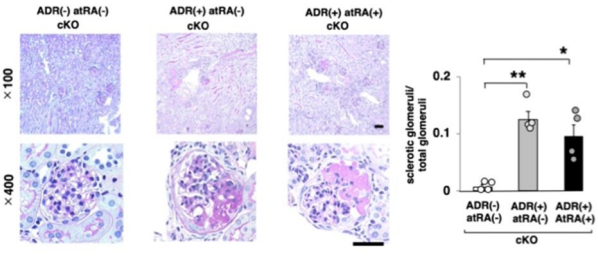


RA treatment compared to that in control cells. These data were confirmed by luciferase assay indicating that RA directly regulate MafB and cMaf. (2) Generation of MafB, c-Maf, and double cKO mice: The floxed allels of MafB and c-Maf as well as MafB/c-Maf DKO were successfully generated. We induced Cre activation at 6 weeks old mice and mouse tissues were collected and the deletion were confirmed by western blot. (3) MAFs KO lines identified podocyte as a candidate of MAFs function: The spermatogenic cycles in mouse takes around 35 days. Before the completion of one gametogenesis cycle, two weeks after Cre activation, the three generated lines developed overt proteinuria, thereby indicate that MafB and c-Maf plays an important role in podocytes. (4) Generation of MafB podocyte-specific deletion developed chronic kidney disease: We therefore generated the fourth line by crossing floxed allels of MafB with NPHS2 promoter Cre ERT^2. After MafB inactivation, the cKO mice showed also overt proteinuria, higher blood urea nitrogen BUN and serum creatinine, and most of mice died of renal failure by 40 weeks. Their kidneys had a pale appearance and renal histopathological analysis of kidneys exhibit focal glomerular segmental sclerosis (FSGS) lesions 16 weeks after tamoxifen administration. Ultrastructural analysis revealed podocyte foot process effacement in cKO glomeruli. (5) Downregulation of slit diaphragm-related genes in MafB cKO glomeruli: RNA samples were collected from the isolated glomeruli of MafB cKO and RNA-seq analysis was performed. There were 21 genes down-regulated including slit diaphragm-related protein (Magi2) and the podocyte-specific transcription factor Tcf21. Luciferase assay suggested that MafB directly regulate the transcription of Magi2 and Tcf21 by interacting with highly conserved half-MAREs. (6) All-trans retinoic acid (atRA) protect of FSGS through MafB. WT mice were induced for FSGS by Adriamycin (ADR) in the presence of absence of atRA for 7 days. Glomerular sclerosis was observed in









atRA(-) ADR mice, but was less obvious in atRA(+) ADR mice. Howerver, atRA fails to inhibit FSGS in MafB cKO mice indicating that atRA inhibit murine FSGS by MafB induction in glomeruli.

The above research results are reported below;

Usui T*****, Morito N*****, **Shawki H* (*; Equally first author)**, *et al.* Transcription factor MafB in podocytes protects against the development of focal segmental glomerulosclerosis. ***Kidney International.*** 403, 2020.

Published papers:

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5) **Shawki HH**, Ishikawa-Yamauchi Y, Kawashima A, Katoh Y, Matsuda M, Al-Soudy AS, Minisy FM, Kuno A, Gulibaikelamu X, Hirokawa T, Takahashi S. EFCAB2 is a novel calcium-binding protein in mouse testis and sperm. Plos one. 2019 Apr 1;14(4):e0214687.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　2件／うちオープンアクセス　1件）

１．著者名 Sun Jiang、Lu Yonggang、Nozawa Kaori、Xu Zoulan、Morohoshi Akane、Castaneda Julio M、Noda Taichi、Miyata Haruhiko、Abbasi Ferheen、Shawki Hossam H、Takahashi Satoru、Devlin Darius J、Yu Zhifeng、Matzuk Ryan M、Garcia Thomas X、Matzuk Martin M、Ikawa Masahito	４．巻 103
２．論文標題 CRISPR/Cas9-based genome editing in mice uncovers 13 testis- or epididymis-enriched genes individually dispensable for male reproduction	５．発行年 2020年
３．雑誌名 Biology of Reproduction	６．最初と最後の頁 183～194
掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/biolre/ioaa083	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Shawki HH, Ishikawa-Yamauchi Y, Kawashima A, Katoh Y, Matsuda M, Al-Soudy AS, Minisy FM, Kuno A, Gulibaikelamu X, Hirokawa T, Takahashi S, Oishi H.	４．巻 14
２．論文標題 EFCAB2 is a novel calcium-binding protein in mouse testis and sperm.	５．発行年 2019年
３．雑誌名 PLoS One	６．最初と最後の頁 e0214687
掲載論文のDOI（デジタルオブジェクト識別子） 10.1371/journal.pone.0214687	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関