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

令和　元 年　 ８ 月 ３０ 日現在

機関番号：１４３０１
研究種目：研究活動スタート支援
研究期間：2017～2018
課題番号：１７Ｈ０６７９９

研究課題名（和文）Regulation and the role of serine-threonine protein kinase (PBK/TOPK) in spermatogenesis

研究課題名（英文）Regulation and the role of serine-threonine protein kinase (PBK/TOPK) in spermatogenesis

研究代表者

ゴエル　サンディープ（Sandeep, Goel）

京都大学・農学研究科・特定准教授

研究者番号：８０８０１７４５

交付決定額（研究期間全体）：（直接経費）　　2,100,000円

研究成果の概要（和文）：PBKは精巣でのみ発現する遺伝子であり、そのKOマウスを作製し精巣における役割を調べた。KOマウスの精巣の重量、精細管の直径、精巣上体の精子数、生存率、精子の形態、精子の受精能、受精後の卵母細胞の発生能はWTと差異がなかった。タンパク発現解析の結果、P-P38発現レベルがWTに比べ、KOマウスで増加していた。しかしながら、アポトーシス、細胞増殖、精原細胞、精原幹細胞、減数分裂細胞に特異的なタンパクの発現レベルはKOマウスとWTの精巣で同程度だった。結論として、PBK遺伝子の消失は雄マウスの妊性に全く影響を及ぼさず、これは精巣内の何らかの他のキナーゼがPKBを補完するためであると考えられる。

研究成果の学術的意義や社会的意義
PBK is a cancer-testis antigen expressed exclusively in adult testis and in highly metastatic tumors. Since its role in testis remains unknown, we hypothesized that PBK may have a critical role in male fertility. Results show that PBK is dispensable for fertility in males.

研究成果の概要（英文）：PBK expression is exclusively detected in the testis of adult males. However, its role in testis remains elusive. To investigate, PBK knockout (KO) mice were generated. Parameters such as testis, epididymis, seminal vesicle weights, tubular diameter, litter size, epididymal sperm number, percentage of viable and morphologically normal sperm did not differ between the KO and wild type (WT) mice ($P > 0.05$). Fertilization competence of sperm of KO mice and oocyte development competence post-fertilization was comparable to WT mice. Protein expression analysis showed that P-P38 expression was elevated in KO mice as compared to WT mice ($P < 0.05$). However, expression of apoptosis, cell proliferation, spermatogonia, spermatogonial stem cell and meiotic cell -specific proteins were comparable between KO and WT mice testis ($P > 0.05$). In conclusion, deletion of PBK gene has no effect on the fertility of male mice possibly due to compensation of loss of PBK by some other kinase in the testis.

研究分野：Reproductive biology

キーワード：testis　spermatogenesis　protein kinase　gene knockout

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９、ＣＫ－１９（共通）

１．研究開始当初の背景

PDZ-binding kinase/T-LAK cell-originated protein kinase (PBK/TOPK), a serine-threonine protein kinase, functions as mitogen-activated protein kinase kinase (MAPKK) to activate p38 MAPK. PBK expression has been shown to be upregulated in lymphomas and myelomas and in primary hematological neoplasms. Overexpression/activation of PBK promote tumor cell proliferation and poor survival of patients with squamous cell carcinoma. On the contrary, suppression of *PBK* expression leads to significant suppression of tumor cell growth. The MAPK pathways are also known to be involved in germ cell development such as sex determination, spermatogenesis, cell cycle progression, apoptosis, and acquisition of sperm motility, sperm capacitation, and acrosome reaction. In normal adult organs, PBK is exclusively expressed in the testis, mainly in proliferating spermatocytes.

２．研究の目的

Since PBK is expressed exclusively in the testis in the normal adult, it is likely that PBK may have a critical role in spermatogenesis. Therefore, the objective of the present project was to decipher the precise role of PBK in the testis and how its absence can influence male fertility.

３．研究の方法

a) Generation of PBK knockout (KO) mice

Using CRISPER/Cas9 nuclease system, PBK KO mouse lines were established. The PBK KO mice were produced in collaboration with Dr. Tsuyoshi Koide, NIG, Mishima. Exon 3 of the PBK gene was targeted for a mutation in the KO mice

b) Fertility analysis of PBK KO mice

Adult PBK KO male mice were mated with wild type (WT) females and litter size were determined. Testis, epididymis and seminal vesicle weights were determined. Epididymal sperm was analyzed for number, viability and morphological defects. In vitro fertilization (IVF) with sperm from PBK KO mice were also investigated.

c) Protein expression profile of PBK KO mice

Protein isolated from PBK KO mice was analyzed for the expression of cell proliferation, spermatogenic cell, and apoptosis-specific proteins. Testis was also analyzed for expression of kinases (total and phosphorylated) such as P38, ERK, JNK, and MEK proteins.

４．研究成果

a) PBK KO mice line could be established

Two independent PBK KO mouse lines were generated with 5 bp and 35 bp deletion respectively in the exon 3 of PBK gene. Both lines showed the absence of PBK protein expression from testis (Fig. 1).

b) PBK KO mice are fertile

Adult PBK KO mice showed no significant difference in testis, epididymis and seminal vesicle weight as compared to adult WT mice. Sperm concentration, litter size, viability, and morphology were also similar to adult WT mice. IVF experiment showed that the fertilization competence of sperm of PBK KO mice and development competence of oocytes post-fertilization was similar to WT mice (Table 1).

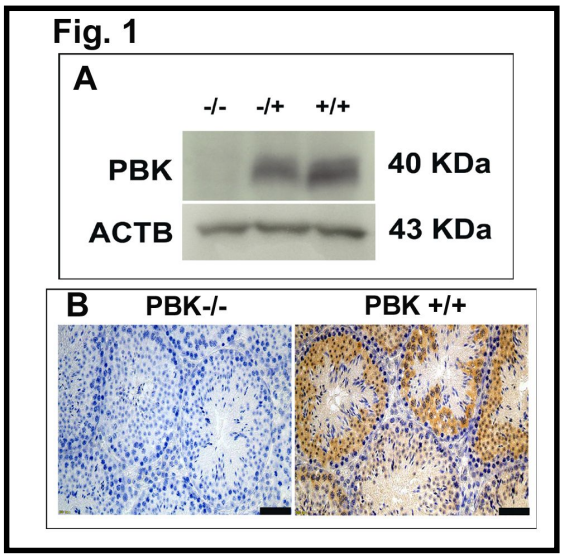


Table 1. In vitro fertilization (IVF) ability of sperm from PBK KO mice and development of oocytes in culture

Male mice	Number of oocytes subjected to IVF	Number of oocytes fertilized (%)	Number of blastocysts (%)
PBK -/- (n =3)	203	145 (71 ± 3)	108 (74 ± 3)
PBK +/+ (n = 3)	195	138 (70 ± 2)	103 (74 ± 2)
P value		N.S.	N.S.

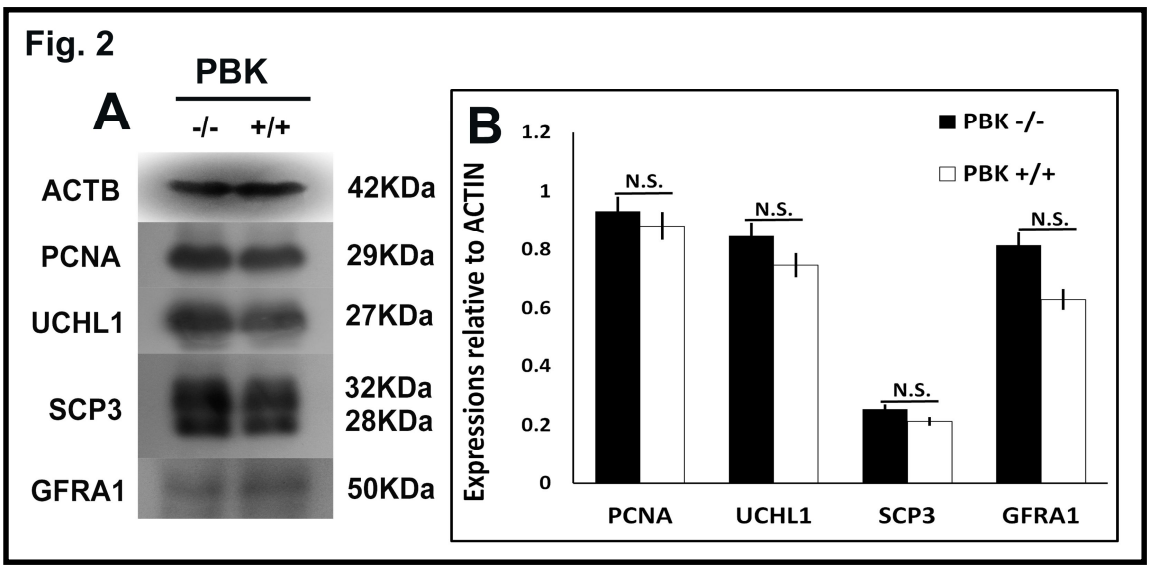
Values are Mean ± S.E.M

The level of significance was set at P < 0.05.

N.S..- Not significant

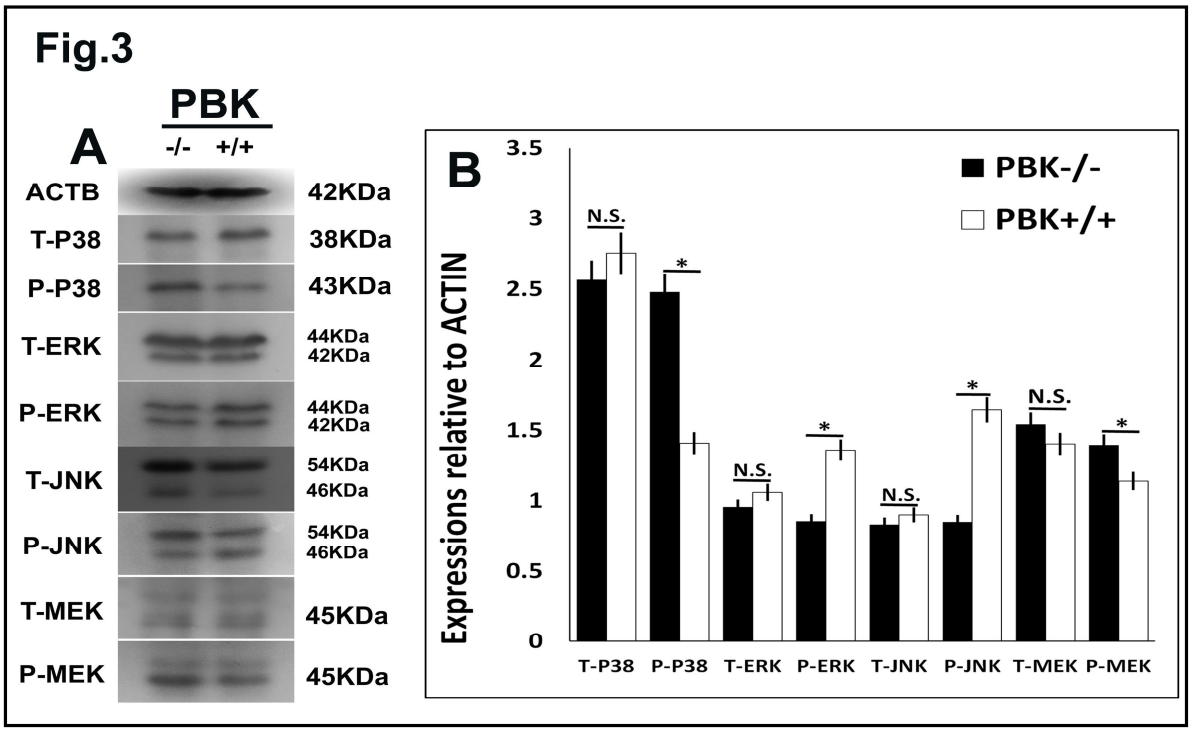
c) Expression of cell proliferation, spermatogonia, spermatogonial stem cell, and meiosis-specific proteins was unchanged

The expression of PCNA, UCHL1, GFRA1 and SCP3 proteins in the testis of PBK KO mice was comparable to WT mice (Fig. 2).



d) Expression of kinases was altered in PBK KO mice

Although the expression of total kinases (P38, ERK, JNK, and ERK) was not affected, loss of PBK leads to significantly enhanced expression of activated P38 (P-P38) protein (Fig. 3). However, expression of activated ERK (P-ERK) and activated JNK (P-JNK) was downregulated in KO mice testis. On the contrary, expression of activated MEK (P-MEK) was upregulated in KO mice testis. Since PBK functions as the upstream kinase that activates P38 signalling, an increased expression of P-P38 is intriguing in the KO mice testis. It is quite likely that upregulation of P-MEK may be compensating for the loss of PBK.



e) Conclusions

In conclusion, loss of PBK protein expression is not lethal for mice and it does not affect the fertility of male mice. It is quite likely that the loss of PBK is compensated by overexpression of other kinases (eg. MEK). However, further investigations are warranted.

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②Devi L, Pothana L and **Goel S*** (2017). Dysregulation of angiogenesis-specific signaling in adult testis results in xenograft degeneration. *Scientific Reports DOI SREP-17-01008A*

③ **Goel S** *and Minami N (2019). Altered hormonal milieu and dysregulated protein expression can cause spermatogenic arrest in ectopic xenografted immature rat testis. *Scientific Reports DOI: 10.1038/s41598-019-40662-y.*

(*Corresponding author)

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〔図書〕（計　　件）
①
〔産業財産権〕
○出願状況（計　　件）

名称：
発明者：
権利者：
種類：
番号：
出願年：
国内外の別：

○取得状況（計　　件）

名称：
発明者：
権利者：
種類：
番号：
取得年：
国内外の別：

〔その他〕
ホームページ等

６．研究組織

(1)研究分担者
研究分担者氏名：

ローマ字氏名：

所属研究機関名：

部局名：

職名：
研究者番号（8 桁）：

(2)研究協力者
研究協力者氏名：小出　剛
ローマ字氏名：Tsuyoshi KOIDE
Professor, Mouse Genome Resource Laboratory,
National Institute of Genetics (NIG), Mishima, Shizuoka 411-8540