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Dispensable role of PTEN in mouse spermatogenesis

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Abstract

PTEN (phosphatase and tensin homologue deleted on chromosome ten) plays critical roles in multiple cellular processes, including cell proliferation, survival, migration and transformation. A role of PTEN in mammalian spermatogenesis, however, has not been explored. To address this question, we generated a mouse model with PTEN conditional knockout in postnatal male germ cells. We found that spermatogenesis was normal in PTEN-deleted male germ cells. PTEN conditional mutant males produced sperm and sired offspring as competently as wild-type littermates. Moreover, our biochemical analysis also indicated that the Akt (acutely transforming retrovirus AKT8 in rodent T cell lymphoma) signalling pathway was not affected in mutant testis. Taken together, these findings demonstrate that PTEN is dispensable in mouse spermatogenesis.

Keywords

Akt; conditional knockout; male germ cell; PTEN; spermatogenesis

1. Introduction

The PTEN (phosphatase and tensin homologue deleted on chromosome ten) gene encodes a lipid phosphatase that catalyses the D3 dephosphorylation of second-messenger PIP₃ [phosphatidylinositol-(3,4,5)-triphosphate] (Maehama et al., 2001; Sansal and Sellers, 2004), thereby antagonizing the role of PI3K (phosphoinositide 3-kinase) and Akt (acutely transforming retrovirus AKT8 in rodent T cell lymphoma)/PKB (protein kinase B) (Sulis and Parsons, 2003). The PI3K/Akt signalling pathway is fundamental for the regulation of multiple cellular processes, including cell proliferation, survival, migration and metabolism (Blume-Jensen and Hunter, 2001; Cantley, 2002; Stokoe, 2005). Accordingly, it is no surprise to find that PTEN, as a negative regulator of PI3K/Akt, is mutated or deleted with high frequency in various human cancer tissues to promote tumorigenesis (Sansal and Sellers, 2004).

Recently, several studies have demonstrated the importance of PI3K/Akt signalling in mouse spermatogenesis. It has been shown that activation of the PI3K/Akt pathway,

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Author contribution

Guo-Zhang Zhu designed the research. Yue Huang, Xia Mao and Terry Boyce performed the research. Yue Huang, Xia Mao, Terry Boyce and Guo-Zhang Zhu analysed the data. Yue Huang and Guo-Zhang Zhu wrote the paper.

downstream of GDNF (glial cell line-derived neurotrophic factor), plays a critical role in the self-renewal division of SSC (spermatogonial stem cells) (Lee et al., 2007). Ciraolo et al. (2010) reported that activation of the p110 β PI3K catalytic subunit was essential in c-Kit-mediated spermatogonial expansion, proliferation and survival of pre- and post-meiotic spermatogenic cells. PI3K activation has also been shown to mediate meiotic entry induced either by ATRA (all-*trans*-retinoic acid derivative) and KL (Kit Ligand) (Pellegrini et al., 2008) or by simulated microgravity (Pellegrini et al., 2010). On the other hand, male mice with conditional loss of PTEN in PGCs (primordial germ cells) exhibited bilateral testicular teratoma, which apparently resulted from enhanced embryonic germ cell proliferation (Kimura et al., 2003). Despite these findings, a role of PTEN in the biology of postnatal male germ cells has not been explored.

Herein, we report a mouse model with PTEN conditional deletion in postnatal male germ cells. Through this mouse model, we demonstrate that PTEN is dispensable during spermatogenesis.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma–Aldrich unless stated otherwise.

2.2. Mice

Transgenic mice *Pten*^{tm1Hwu/J} (indicated as *Pten*^{f/f} in this report) and Tg[Stra8 (stimulated by retinoic acid gene 8)-cre (Cre recombinase)]1Reb/J (indicated as Stra8-cre in this report) were previously described (Lesche et al., 2002; Sadate-Ngatchou et al., 2008) and purchased from the Jackson Laboratory. *Pten*^{f/f} males were crossed with Stra8-cre females to generate (*Pten*^{f/f}; Stra8-cre) females, which were crossed with *Pten*^{f/f} males to generate wild-type (*Pten*^{f/f}) and mutant (*Pten*^{f/f}; Stra8-cre) littermates. Mouse genotype was determined by PCR on tail genomic DNA, using the primer pairs described previously (Lesche et al., 2002; Sadate-Ngatchou et al., 2008). All animal care and use procedures described within were reviewed and approved by the Institutional Animal Care and Use Committee of Marshall University and were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals.

2.3. Testis histology and sperm count

These experiments were carried out essentially as described previously (Fan et al., 2006a).

2.4. *In vivo* fertility assay

Each male (10 weeks old) was mated with two females for 2 weeks. The number of offspring from each pregnant female was counted after birth.

2.5. RT (reverse transcription)–PCR

Total RNAs from purified spermatogenic cells were isolated as reported previously (Fan et al., 2006b). RT–PCR was conducted by the random primer method as described in the RT-for-PCR kit (Clontech). The primers used in the PCR were PTEN-5' (forward primer, 5'-

AGGACCAGAGGAAACCTCAG-3', located on exon 8) and PTEN-3' (reverse primer, 5'-CTCTGGATCAGAGTCAGTGG-3', located on exon 9). PCR parameters: 94°C for 2 min, 1 cycle; 94°C for 20 s, 60°C for 20 s, 72°C for 45 s, 30 cycles; followed by a 6-min extension at 72°C. The expected size of PCR products is 322 bp.

2.6. Western blot

Protein samples from mouse testes were prepared in lysis buffer (50 mM Tris/HCl pH 7.4, 300 mM NaCl, 5 mM EDTA, 1% Triton X-100) supplemented with protease inhibitors (Roche). Western blot was carried out as described previously (Huang et al., 2009). The primary antibodies against PTEN, phospho-Akt(Thr³⁰⁸), phospho-GSK-3 β (Ser⁹) and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were purchased from Cell Signaling Technology.

3. Results

3.1. Expression of PTEN in male germ cells

As a first step to investigate whether PTEN plays a role in mouse spermatogenesis, we wanted to determine the expression pattern of PTEN in male germ cells. To address this, we performed RT-PCR on the total RNAs of purified male germ cells. As shown, PTEN is ubiquitously expressed in different developmental stages of postnatal germ cells, including spermatogonia, primary spermatocytes of leptotene, zygotene and pachytene and round spermatids (Figure 1). This result is consistent with a previous report (Di Vizio et al., 2005), which used immunohistochemical analysis and Western blot to examine PTEN expression in mouse testicular cells.

3.2. PTEN conditional knockout in postnatal male germ cells

After we confirmed that PTEN is expressed in spermatogenic cells (Figure 1), we wanted to conditionally delete PTEN in postnatal male germ cells, since conventional PTEN knockouts are embryonic lethal. Recently, a transgenic Stra8-cre mouse line has been created to direct the expression of an optimized variant of Cre recombinase (iCre) in postnatal, premeiotic, male germ cells (Sadate-Ngatchou et al., 2008). Thus, we crossed Stra8-cre mice with Pten^{f/f} mice (possess loxP sites on either side of exon 5 of the PTEN gene) (Lesche et al., 2002) to generate wild-type (Pten^{f/f}) and conditional knockout (Pten^{f/f}; Stra8-cre) littermates. As shown, the protein levels of PTEN were substantially decreased in mutant testes, indicating that conditional knockout of PTEN in postnatal germ cells was successful (Figure 2). The residual amount of PTEN protein detected in mutant testes is likely derived from testicular somatic cells.

Since PTEN is a well-known negative regulator of PI3K/Akt signalling, we predicted that Akt signalling would be up-regulated upon PTEN deletion in germ cells. Surprisingly, we could not detect an overt change in the levels of Akt and GSK-3 β phosphorylation (Figure 2). On the other hand, our result suggests that Akt signalling is constitutively active in testis and varies among different mice. The variability in the level of phosphorylated Akt and GSK-3 β in various mice might be due to different genetic background carried from the

crossing of Stra8-cre mice and Pten^{f/f} mice, since this variance was consistently observed in repeated Western blots where the protein samples were prepared from mice of similar age.

3.3. Normal spermatogenesis in PTEN-deleted male germ cells

After successfully establishing a mouse model in which PTEN was conditionally deleted in postnatal male germ cells, we next investigated whether spermatogenesis was affected in mutant mice. To test this, we carried out different sets of experiments. First, we found that there was no weight difference between wild-type and mutant testes (data not shown). Then, we examined testis histology. As shown, mutant testes exhibit typical seminiferous tubules with different stages of spermatogenic cells (from spermatogonia to spermatozoa), suggesting that spermatogenesis is normal in PTEN-deleted male germ cells (Figure 3).

We then looked at cauda sperm and noticed that, compared with wild-type (Pten^{f/f}) littermates, mutant mice (Pten^{f/f}; Stra8-cre) produced sperm in the same number (Table 1, $P=0.35$) and with the same morphology (data not shown). Lastly, we executed an *in vivo* fertility assay. We found that mutant mice (Pten^{f/f}; Stra8-cre) sired offspring as competently as wild-type (Pten^{f/f}) littermates (Table 1, $P=0.38$).

Taken together, these results (Figure 3 and Table 1) indicate that PTEN conditional knockout in postnatal male germ cells does not functionally affect spermatogenesis, although we cannot rule out minor defects beyond our observation.

4. Discussion

In this report, we provide the evidence to demonstrate a dispensable role of PTEN in mouse spermatogenesis. This result is somewhat out of line with our initial expectation, since PTEN is a major negative regulator of the PI3K/Akt signalling, which has been shown to play crucial roles in different aspects of spermatogenesis (Lee et al., 2007; Pellegrini et al., 2008; Cirao et al., 2010; Pellegrini et al., 2010). We speculate that male germ cells might express other PTEN-like phosphatases that are functionally redundant to PTEN. Indeed, four PTEN-related genes, *PTEN2* (Wu et al., 2001), *TPTE* (transmembrane phosphatase with tensin homology) (Chen et al., 1999), *TPIP* (TPTE and PTEN homologous inositol lipid phosphatase) (Walker et al., 2001) and *PLIP* (PTEN-like phosphatase) (Pagliarini et al., 2004), have been found to be expressed in testis. This functional redundancy might also explain why the Akt/GSK-3 β signalling is not up-regulated upon PTEN deletion in male germ cells. In line with this, we did not observe germ cell hyperplasia or germ cell tumours in aged (12-month-old) mutant (Pten^{f/f}; Stra8-cre) mice (data not shown).

Recently, the PTEN protein has been detected in human, mouse and boar spermatozoa (Aparicio et al., 2007; Aquila et al., 2007; Jungnickel et al., 2007), raising a speculation that PTEN might play a role in the regulation of sperm function, e.g. sperm motility, sperm capacitation and acrosome reaction. By using membrane-permeable, non-specific lipid phosphatase inhibitors, Jungnickel et al. (2007) suggested that a phosphoinositide D3 phosphatase activity was involved in regulating spontaneous or ZP3 (zona pellucida glycoprotein 3)-triggered acrosome reaction. In this report, we did not extend into *in vitro*

assays on sperm function. However, our *in vivo* fertility data strongly suggest that PTEN is not essential in sperm function.

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Abbreviations

Akt	acutely transforming retrovirus AKT8 in rodent T cell lymphoma
cre	Cre recombinase
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GSK-3β	glycogen synthase kinase 3 β
PI3K	phosphoinositide 3-kinase
PKB	protein kinase B
PTEN	phosphatase and tensin homologue deleted on chromosome ten
RT	reverse transcription
Stra8	stimulated by retinoic acid gene 8

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Figure 1. Expression of Pten in mouse male germ cells

RT-PCR was performed on the RNA isolated from spermatogonia (lane 1), leptotene/zygotene spermatocytes (lane 2), pachytene spermatocytes (lane 3), round spermatids (lane 4) and adult testes (lane 5). β -Actin was used as the RT-PCR control.

Figure 2. Pten conditional knockout in postnatal male germ cells

Testis protein samples were prepared from four individual mice of either conditional knockout ($Pten^{f/f}; Stra8\text{-}cre$) or wild-type ($Pten^{f/f}$) littermate. Subsequently, Western blot was performed to examine PTEN expression, Akt phosphorylation and GSK-3 β phosphorylation. GAPDH served as loading control.

Figure 3. Histological analysis of the testes

The testis sections of control ($Pten^{f/f}$) and mutant ($Pten^{f/f}; Stra8\text{-cre}$) mice were stained with haematoxylin and eosin (original magnification, $\times 35$). Normal spermatogenesis was seen in mutant testes.

Table 1
Sperm count and *in vivo* fertility assay

Sperm count and *in vivo* fertility assay was performed as described in the Materials and methods section. All mated females were pregnant. The number of tested males is indicated in parentheses.

Parameter	Pten ^{fl/fl}	Pten ^{fl/fl} ; Stra8-cre
Cauda sperm count ($\times 10^5$)	219 $\pm$ 44 (5) ^a	228 $\pm$ 30 (5) ^a
Sired litter size	6.8 $\pm$ 1.4 (6) ^b	6.6 $\pm$ 1.3 (6) ^b

^a $P=0.35$.

^b $P=0.38$.

Results are means $\pm$ S.E.M. (*n*).