Expression of PTEN-long nephritis and its effect on renal inflammation

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Abstract. Based on the important functions of phosphatase and tensin homolog (PTEN)-Long for renal diseases, the present study aimed to investigate the expression of PTEN-Long in patients and mice with nephritis and its effect on nephritis. Expression levels of PTEN-Long in serum of patients with nephritis, renal cell carcinoma (RCC) as well as normal controls, and in serum and renal tissues of mice were measured by western blotting. PTEN-Long knock-in and knock-out mice were constructed via the CRISPR-Cas9 technique. Intraperitoneal injection of lipopolysaccharide+renal homogenate was performed to construct a mouse nephritis model. Mice were divided into control group, model group, knock-in group and knock-out group. A Bio-Plex system was used to detect secretion of serum inflammatory factors. Expression of inflammatory factors in renal tissues of different groups was detected by reverse transcription semi-quantitative polymerase chain reaction. Hematoxylin and eosin staining was used to observe the pathological changes of renal tissue. PTEN-Long was downregulated in patients with nephritis and RCC compared with controls, whereas the expression levels of inflammatory factors were increased. PTEN-long knock-in significantly reduced the serum content and expression levels of tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β and IL-18. PTEN-long knock-out also decreased the expression levels of TNF-α and IL-6 but exhibited no effects on expression of IL-1β and IL-18. Compared with knock-out and model groups, renal tissue inflammation was significantly reduced in knock-in group. The protein level of PTEN-Long was significantly lower in serum than in renal tissue. These findings suggest that PTEN-long can inhibit the progression of nephritis by interacting with inflammatory factors to protect kidney.

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Introduction

Renal cell carcinoma (RCC), which has the highest mortality rate among all types of genitourinary cancers, represents a serious problem to public health (1). Over 300,000 patients are diagnosed with RCC each year worldwide and the incidence rate of RCC still exhibits an increasing trend (2). Although various drugs, including axitinib, sorafenib, nivolumab and everolimus have been developed to treat patients with RCC (3,4), treatment of advanced RCC has typically failed to provide satisfactory outcomes due to a series of problems, such as drug tolerance and individual difference in response to drugs (5). It has previously been demonstrated that, although recurrence will occur in a minority group of patients, RCC is generally curable in early stages (1). Therefore, early diagnosis and treatment is the key to the treatment of RCC. Nephritis occurs in tubules, glomeruli and interstitial tissue surrounding the tubules and glomeruli. It has been demonstrated that the onset and development of RCC may be associated with certain risk factors of kidney inflammation, including oxidative stress (6). Therefore, proper treatment of nephritis will inhibit the progression of RCC, and therefore potentially reduce the mortality rate.

Phosphatase and Tensin Homolog (PTEN)/phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway serves essential roles in the regulation of the balance between survival and apoptosis as well as cell migration (7,8). The pathway also has inhibitory effects on a number of human diseases including different types of cancer, such as prostate cancer (9), breast cancer (10), hepatocellular carcinoma (11) and RCC (12). PTEN-Long, which is a translational variant of PTEN produced by in-frame alternative translation, has been demonstrated to have similar roles to PTEN in inhibiting the growth of tumors by inhibiting PI3K signaling through a phosphatase-dependent manner (13). Compared with PTEN, PTEN-Long protein contains an additional N-terminal region, which gives it the ability to cross the cell membrane (14), and due to its functions, PTEN-Long has been selected as a therapeutic target for the treatment of pancreatic cancer (15,16). A previous study has demonstrated that expression levels of

PTEN-Long were increased in patients with clear cell RCC (ccRCC), and that PTEN-Long overexpression can inhibit cell proliferation, migration and invasion, which in turn leads to the inhibition of tumor growth (14), indicating the inhibitory effects of PTEN-Long on ccRCC. Although functions of PTEN-Long have been studied previously in various cancers including RCC, its specific roles in the progression of nephritis have not yet been investigated, to the best of our knowledge. Due to the pathogenesis shared by RCC and nephritis, it may be hypothesized that PTEN-Long also has important functions in the progression of nephritis.

CRISPR/Cas9 is a novel gene-editing tool derived from prokaryotic adaptive immune system against phage/virus and foreign DNA invasion. It is widely used in mammalian genomic editing, including repressing gene expression (17), gene knock-in (18), repairing disease-associated genes (19) and development of animal models of cancer (20). In the present study, effects of PTEN-Long on nephritis have been investigated in patients and animal models. In addition, effects of PTEN-Long on inflammatory factors were also evaluated using PTEN-Long knock-in and knock-out mice established by the CRISPR/Cas9 technique.

Materials and methods

Specimen collection. Blood samples from patients with nephritis, RCC and normal healthy controls (n=60 each) treated at the Institute of Urology of The Affiliated Yantai Yuhuangding Hospital of Qingdao University (Yantai, China) between May 2014 to December 2016 were collected. No patient received chemoradiotherapy or other antitumor therapy prior to surgery. The median age is 58 years (range, 45-77 years) and the ratio of male to female was 0.68 (73/107). The present study was approved by the Ethics Committee of The Affiliated Yantai Yuhuangding Hospital of Qingdao University, and all participants provided written informed consent. All surgery was performed according to the Declaration of Helsinki.

Preparation of mouse nephritis model. All mice used in the present study were 8 weeks old with a body weight of 20-25 g and were housed in a pathogen-free environment with 12-h light/dark cycles at room temperature (20±2˚C) with a relative humidity of 50-70%. Animals had free access to food and water. PTEN-Long knock-in and knock-out mice were constructed using CRISPR-Cas9 technique by Cyagen Biosciences, Inc. (Santa Clara, CA, USA). The efficiency of knock-in and knock-out was detected by western blotting. Female transgenic BALB/c mice (Cyagen Biosciences), including 10 knock-in mice and 10 knock-out mice, were used in the present study. A total of 20 transgenic mice (10 knock-in mice and 10 knock-out mice) were treated with intraperitoneal injection of lipopolysaccharide (30 mg/kg, 50 μl; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and renal homogenate (50 μl; renal tissue:saline, 1:6 g/ml; Cyagen Biosciences) and wild type BALB/c mice (n=10) in the control group were administered 100 μl saline. The present study was approved by the Ethics Committee of The Affiliated Yantai Yuhuangding Hospital of Qingdao University.

Determination of serum levels of inflammatory factors. Following intraperitoneal injection, blood samples (0.2 ml) were extracted from the fundus vein at 12, 24 and 48 h later. Blood samples were injected into EDTA anticoagulant tubes and maintained at 4˚C for 30 min, followed by centrifugation at 800 x g for 10 min at 4˚C to collect supernatant. A Bio-Plex system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to compare the secretion of tumor necrosis factor (TNF)-α (cat. no. ab6671), interleukin (IL)-6 (cat. no. 100712), IL-1β (cat. no. ab100704) and IL-18 (cat. no. ab216165; all Abcam, Cambridge, UK) in serum of different groups. All kits used to detect inflammatory markers were purchased from Abcam and the manufacturer's protocols were followed.

Detection of inflammatory factors' gene expression in renal tissue by reverse transcription semi-quantitative polymerase chain reaction (PCR). Mice were sacrificed at 48 h following intraperitoneal injection. Left kidneys were harvested and cut, added into normal saline prior to preparing a renal tissue homogenate using an electric homogenizer. Total RNA was extracted from tissue homogenate using TRIzol (Life Technologies; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. Purity and integrity of total RNA were detected and cDNA was synthesized via reverse transcription using PrimeScript™ RT reagent kit (cat. no. RR037Q; Takara Biotechnology Co., Ltd., Dalian, China). SYBR Green Master Mix kit (Thermo Fisher Scientific, Inc.) and 1 μl cDNA were used to prepare the PCR reaction system. All primers used for PCR reaction are listed in Table I. PCR reaction conditions were as follows: 95˚C for 5 min, and 40 cycles of 95˚C for 20 sec, 60˚C for 30 sec and 72˚C for 30 sec. Subsequently, PCR product was subjected to 1.2% agarose gel horizontal electrophoresis. Ethidium bromide was visualized using the Gel Doc XR gel imaging system (Bio-Rad Laboratories, Inc.) that allowed images to be captures, and ImageJ software 1.48u (National Institutes of Health, Bethesda, MD, USA) was used to analyze the results. The relative expression level of TNF-α, IL-6, IL-1β and IL-18 mRNA were quantified according to the endogenous control (β-actin) using the aforementioned software.

Observation of pathological changes by hematoxylin and eosin staining. At room temperature, mouse renal tissue was fixed in 4% paraformaldehyde for 12-24 h, followed by paraffin-embedding for 1 h. The embedded tissue was then cut into 4-μm sections. Dewaxing was performed twice with xylene for 15 min at 24˚C. Following hydration by passing a series of graded ethanol concentrations (95% ethanol for 5 min, 90% ethanol for 5 min, 70% ethanol for 2 min and distilled water for 5 min), hematoxylin staining was performed for 10 min, followed by washing with water for 10 min at room temperature. Following treatment with 95% ethanol for 5 sec, eosin (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) staining was performed for 2 min at 24˚C. Following staining, dehydration was performed by passing a series of graded ethanol concentrations (70% ethanol for 2 min, 80% ethanol for 2 min, 90% ethanol for 5 min and twice with 100% ethanol for 5 min). Then, tissue sections were then treated twice with xylene, 10 min each. Neutral gum was used to seal the sections and optical microscopy

Table I. Primer sequences.

Gene	Forward	Reverse
TNF-α	5'-TACTCCCAGGTTCTCTTCAAGG-3'	5'-GGAGGCTGACTTTCTCCTGGTA-3'
IL-6	5'-GAGTTGTGCAATGGCAATTC-3'	5'-ACTCCAGAAGACCAGAGCAG-3'
IL-1β	5'-CACCTCTCAAGCAGAGCACAG-3'	5'-GGGTTCCATGGTGAAGTCAAC-3'
IL-18	5'-AAACCCGCCTGTGTTCGA-3'	5'-TCAGTCTGGTCTGGGATTCGT-3'
β-actin	5'-TTGTTACCAACTGGGACG-3'	5'-GGCATAGAGGTCTTTACGG-3'

TNF, tumor necrosis factor; IL, interleukin.

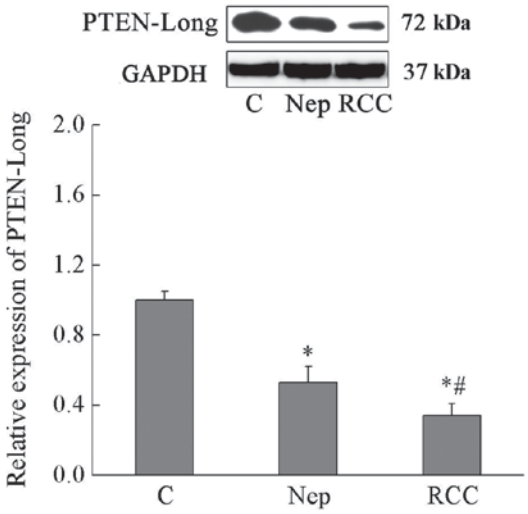

Figure 1. Serum levels of PTEN-Long protein in serum of patients with NEP, RCC and controls. *P<0.01 vs. C; #P<0.01 vs. Nep. C, control group; NEP, nephritis; RCC, renal cell carcinoma; PTEN, phosphatase and tensin homolog.

(magnification, x200; Olympus Corporation, Tokyo, Japan) was used to observe the signals.

Western blotting. Total protein was extracted from human blood samples and mice renal tissue by TPE™ kit (Beijing Solarbio Science & Technology Co., Ltd.) and quantified using the bicinchoninic acid protein assay method. Protein samples were boiled for 3 min and then loaded into 10-20% Ready Gels (40 μg/lane; Bio-Rad Laboratories, Inc.) for electrophoresis, followed by transferring to a polyvinylidene difluoride membrane. The membrane was blocked with 5% skimmed milk for 2 h at room temperature. Following washing, membrane was incubated with PTEN-Long primary antibody (1:500; cat. no. ABM-2052; Cascade Bioscience, Inc., Winchester, MA, USA), β-actin polyclonal antibody (1:500; cat. no. orb129534; Biorbyt Ltd., Cambridge, UK) overnight at 4˚C. Following washing with TBS-Tween-20 (3X), the membrane was incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G secondary antibody (1:1,000; cat. no. ab150084; Abcam) at room temperature for 2 h. Enhanced chemiluminescence (cat. no. 34580; Thermo Fisher Scientific, Inc.) was used to detect the signals. ImageJ software 1.48u was used to analyze the data and the relative expression level of PTEN-Long was quantified relative to the endogenous control, β-actin.

Statistical analysis. Statistical analysis was performed using SPSS 19.0 statistical software (IBM, Corp., Armonk, NY, USA). Data are presented as mean + standard deviation. All data were analyzed using one-way analysis of variance with Bonferroni's correction for multiple group comparisons and Student's t-test for the comparisons between two groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Serum levels of PTEN-Long protein in serum of patients with nephritis, RCC and normal controls. As presented in Fig. 1, Serum levels of PTEN-Long in patients with nephritis and RCC carcinoma were significantly reduced by 47 and 66% compared with normal controls (P<0.01). In addition, serum levels of PTEN-Long in patients with nephritis was significantly higher than that of patients with RCC (P<0.01). These data suggest that serum level of PTEN-Long was negatively associated with the severity of renal disease (Fig. 1).

Expression of PTEN-Long protein in serum and rental tissue of different groups. As presented in Fig. 2, expression levels of PTEN-Long protein were significantly lower in serum than in rental tissue. Expression levels of PTEN-Long protein in serum and renal tissue of the model group were significantly reduced by 64 and 36%, respectively, compared with the control group (P<0.01). Compared with the model group, expression levels of PTEN-Long protein were significantly increased in serum and rental tissue of knock-in group, but remained lower than those of control group (68 and 92% of the control group, respectively; P<0.01). Compared with the model group, expression levels of PTEN-Long protein were significantly reduced in knock-out group (P<0.01). The data suggested that low expression of PTEN-Long protein may promote the progression of nephritis, whereas PTEN-Long protein overexpression may inhibit it (Fig. 2).

Effects of PTEN-Long knock-in and knock-out on serum levels of inflammatory factors. In order to investigate the effects of PTEN-Long on serum levels of inflammatory factors, PTEN-Long knock-in and knock-out mice were constructed, and serum levels of several selected inflammatory factors were determined. As presented in Fig. 3, serum levels of TNF-α, IL-6, IL-1β and IL-18 in the model group were 51.07±4.18, 33.64±3.12, 21.63±2.0 and 32.75±3.19 pg/ml,

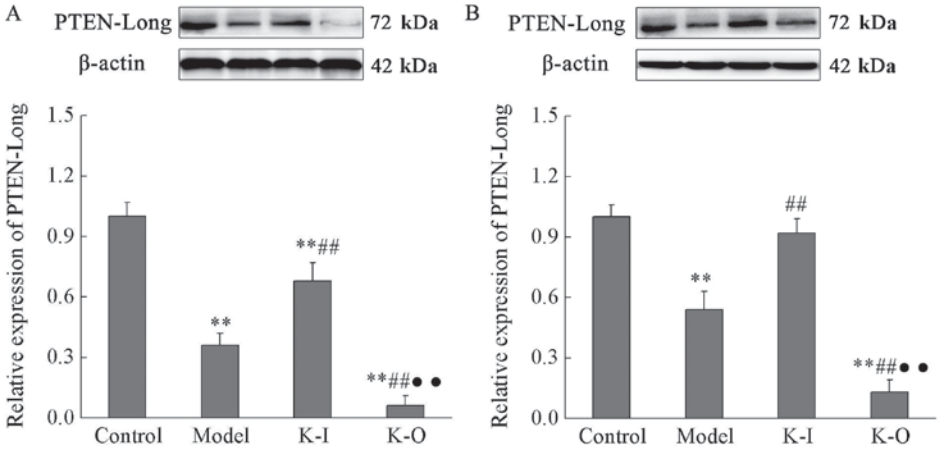

Figure 2. Expression of PTEN-Long protein in serum and rental tissue of different groups. (A) Expression of PTEN-Long protein in serum; (B) Expression of PTEN-Long protein in renal tissue. K-I, knock-in; K-O, knock-out; **P<0.01 vs. control; ##P<0.01 vs. model; ••P<0.01 vs. K-I. PTEN, phosphatase and tensin homolog; K-I, knock-in; K-O, knock-out.

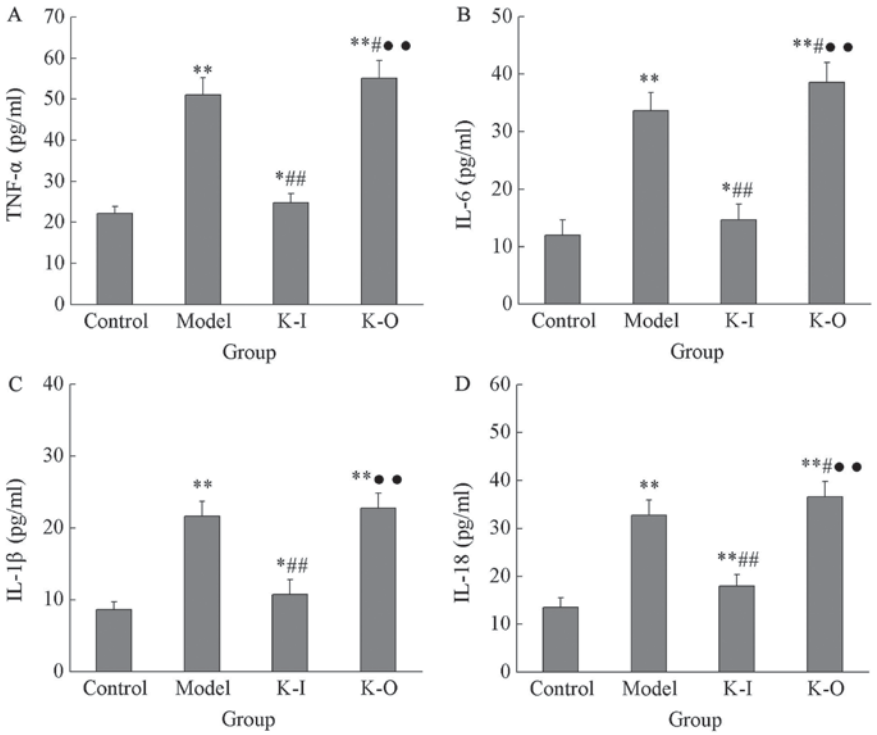

Figure 3. Comparison of serum levels of inflammatory factors between mice in different groups. (A) TNF-α; (B) IL-6; (C) IL-1β; (D) IL-18. *P<0.05, **P<0.01 vs. control; #P<0.05, ##P 0.01 vs. model; ••P<0.01 vs. K-I. TNF, tumor necrosis factor; IL, interleukin; K-I, knock-in; K-O, knock-out.

respectively, which were significantly higher than those in the control group (22.15±1.69, 11.98±2.64, 8.65±1.09 and 13.46±2.03 pg/ml, respectively; P<0.05). Compared with the model group, PTEN-Long knock-out further significantly increased serum levels of TNF-α, IL-6, IL-1β and IL-18 to 55.17±4.23, 38.59±3.46, 22.76±2.09 and 36.62±3.21 pg/ml, respectively (P<0.05). Compared with PTEN-Long knock-out, PTEN-Long knock-in significantly decreased serum levels of TNF-α, IL-6, IL-1β and IL-18 to 24.72±2.36, 14.61±2.79, 10.76±2.05 and 17.93±2.46, respectively (P<0.01). These results suggested that, PTEN-Long can inhibit inflammation caused by nephritis through its interactions with inflammatory factors (Fig. 3).

Expression of inflammatory factors in renal tissue of mice with different backgrounds. As presented in Fig. 4, expression levels of TNF-α, IL-6, IL-1β and IL-18 in the model group were 1.92±0.13, 2.02±0.14, 1.63±0.12 and 1.47±0.11, respectively, which were significantly higher than those of the control group (P<0.05). Compared with the model group, expression levels of TNF-α and IL-6 mRNA in the knock-out group were significantly increased to 2.17±0.16 and 2.29±0.16, respectively, and no significant differences were found in expression levels of IL-1β and IL-18 between those two groups. In contrast, compared with the model group, expression levels of TNF-α, IL-6, IL-1β and IL-18 in PTEN-Long knock-in group were significantly reduced to 1.12±0.07, 1.21±0.13,

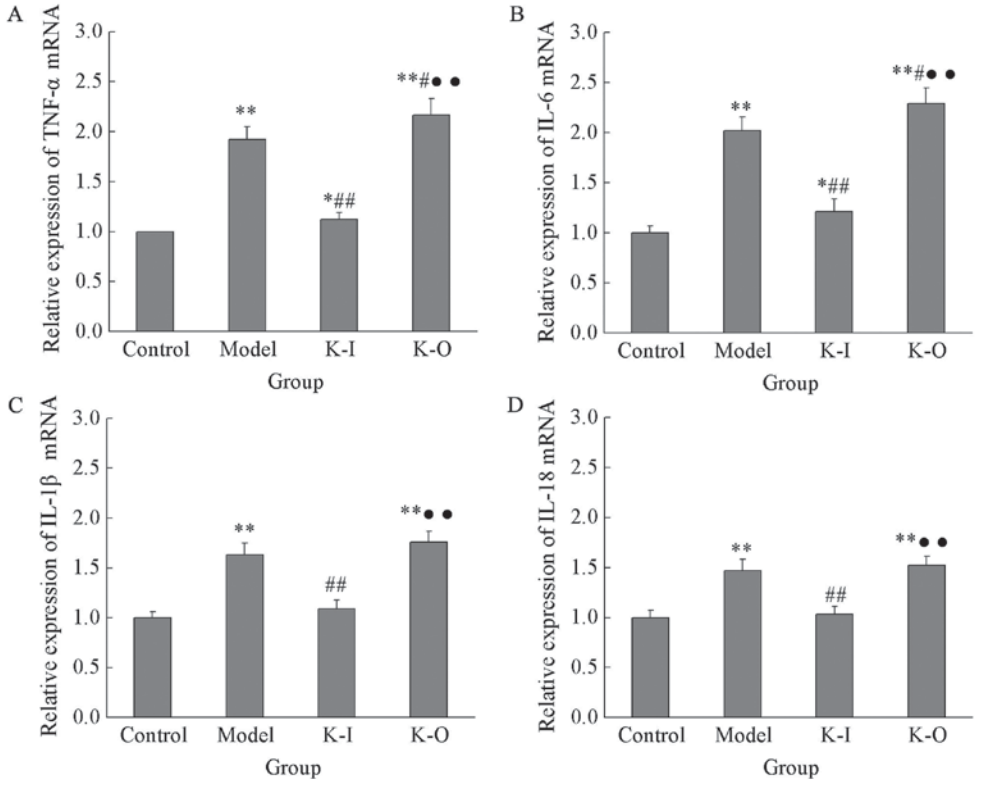

Figure 4. Expression levels of inflammatory factors in mice in different groups. (A) TNF-α; (B) IL-6; (C) IL-1β; (D) IL-18. *P<0.05, **P<0.01 vs. control; $^{\#}$P<0.05, $^{\#\#}$P 0.01 vs. model; $^{\bullet\bullet}$P<0.01 vs. K-I. TNF, tumor necrosis factor; IL, interleukin; K-I, knock-in; K-O, knock-out.

1.09±0.09 and 1.03±0.08, respectively (P<0.05). Expression levels of TNF-α and IL-6 mRNA were significantly higher in the PTEN-Long knock-in group than in the control group, but no significant differences in expression levels of IL-1β and IL-18 mRNA were observed between these groups. These data demonstrated that PTEN-Long can inhibit the expression of inflammatory factors to protect kidney from the injuries caused by nephritis (Fig. 4).

Histological changes of kidney of mice in different groups. As presented in Fig. 5, the structure of glomerular cells in the control group is clear, no interstitial inflammatory cell infiltration and edema were exhibited and no proliferation of mesangial cells and matrix was observed. In the model group, glomerular adhesions were observed, glomerular blood vessels and basement membrane were markedly thickened, and inflammatory cell infiltration was also observed; compared with the model group, structure of the glomerular was improved, the thickening of glomerular blood vessels and basement membrane and inflammatory cell infiltration were reduced in PTEN-Long knock-in group; in PTEN-Long knock-out group, renal tissue structure was destroyed, no clear glomerular was observed and severe inflammatory cell infiltration was observed. The results indicate that PTEN-Long can protect renal tissue structure in patients with nephritis.

Discussion

It is well known that the PI3K/Akt pathway is closely associated with the onset and progression of a variety of cancers (21). PI3Ks can phosphorylate phosphatidylinositol (4,5)-bisphosphate (PIP2) to produce phosphatidylinositol 3, 4, 5 triphosphate (PIP3), and the produced PIP3 can active Akt to phosphorylate a variety of target proteins associated with the regulation of cell growth, survival, proliferation and other cellular processes (22). PTEN protein, which is a tumor suppressor, serves essential roles in the regulation of the PI3K/Akt pathway. PTEN can convert PIP3 to the inactive form of PIP2 through hydrolyzation, so as to inhibit signaling transduction of PI3k/Akt pathway (23). PTEN-Long, which is derived from PTEN, is the permeable form of PTEN that can be secreted by one cell to enter neighboring cells. Similar to the functions of PTEN, PTEN-Long can also dephosphorylate PIP3 to reduce the signaling transduction of the PI3K-Akt pathway, which in turn induces cell death and inhibit tumor growth (24). Previous studies have demonstrated that PTEN-Long serves pivotal roles in the development and progression of various cancers (13,25,26). A previous study on the treatment of pancreatic cancer have demonstrated that PTEN-Long targeted therapy combined with chemotherapy may significantly extend overall survival in mice model (15). In the clinical study of ccRCC, expression levels of PTEN-Long protein were observed to be significantly reduced in patients with ccRCC, and the reduced level of PTEN-Long protein caused the increased phosphorylation levels of Akt (16). In contrast, PTEN-Long overexpression in the ccRCC cell line 786-0 decreased the phosphorylation level of Akt, which in turn inhibit the proliferation, migration and invasion of cancer cells or may induce cells death (16). Therefore, expression level of PTEN-Long protein was negatively associated with the development of ccRCC. In the present study, reduced serum level of PTEN-Long protein was observed in patients

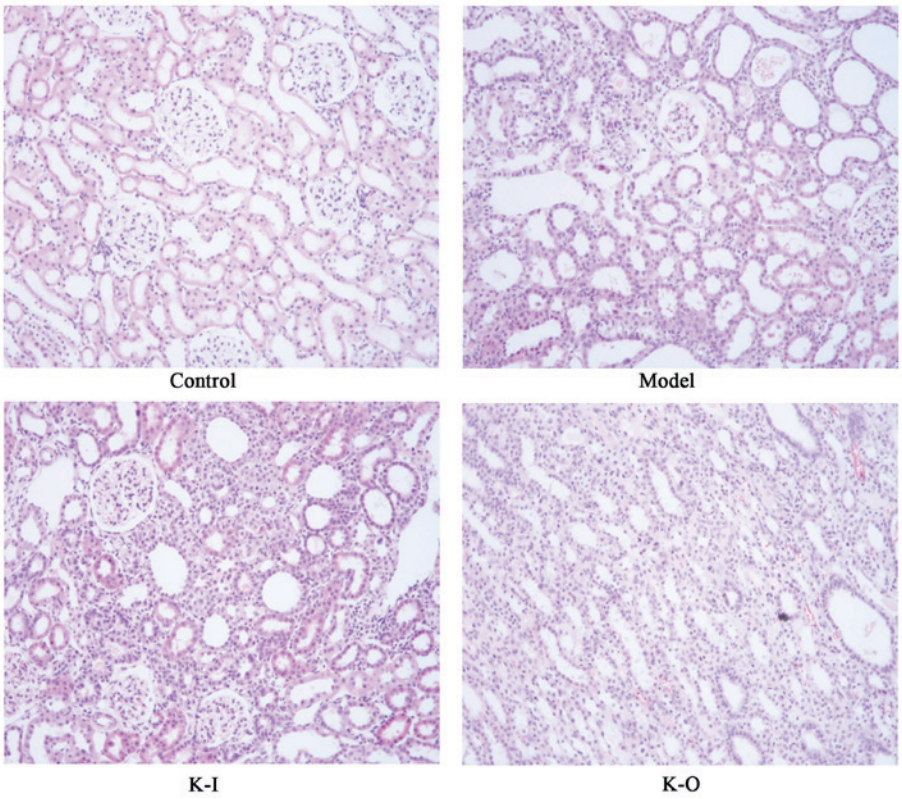

Figure 5. Histological changes of kidney of mice in different groups (original magnification, x200).

with RCC compared with healthy controls. In addition, serum levels of PTEN-Long protein in patients with nephritis were lower than that of healthy controls but higher than that of patients with RCC. These data suggest that PTEN-Long is also associated with the development of nephritis and its expression level is negatively associated with the severity of kidney diseases.

The development of nephritis is a complex process with a variety of associated factors, particularly inflammatory factors. Expression levels of TNF-α, IL-6, IL-12 and IL-18, which are proinflammatory cytokines, may be used to sensitively reflect the status of inflammation (27,28). It has previously been demonstrated that expression levels of IL-1β, IL-18 and IL-6 were significantly increased in patients with acute renal failure (29). TNF-α can mediate the expression of chemokines and cytokines to interact with inflammatory responses caused by renal injury (30). In the present study, serum levels of TNF-α, IL-6, IL-12 and IL-18 were significantly increased in mice with nephritis than in healthy control mice. In addition, expression levels of TNF-α, IL-6, IL-12 and IL-18 mRNA in renal tissue were also increased in nephritis mice compared with controls, indicating the inflammation response caused by nephritis. Furthermore, PTEN knock-in significantly decreased the serum levels of TNF-α, IL-6, IL-12 and IL-18 and their expression in retinal tissue. In contrast, PTEN knock-out served an opposite role. The development of nephritis is accompanied by histological changes in renal tissue that can significantly reduce renal function (31). In the present study, PTEN knock-in was demonstrated to improve the histological changes caused by nephritis, but PTEN knock-out promoted those changes. These data suggest that PTEN-Long can protect kidney from nephritis injuries by inhibiting the expression of inflammatory factors.

Based on these findings, PTEN-Long expression was significantly reduced due to kidney diseases, and the reduced level of PTEN-Long expression was positively correlated with the severity of disease. Therefore, PTEN-Long protein can potentially serve as a biomarker for the diagnosis of nephritis. The protein levels of PTEN-Long in serum and renal tissue of mice in different groups were evaluated, and protein levels of PTEN-Long were observed to be significantly lower in serum than in retinal tissue. Therefore, serum level of PTEN-Long may more sensitively reflect the status of kidney diseases.

In conclusion, serum level of PTEN-long was negatively associated with the severity of kidney disease, and PTEN-long may protect retinal tissue from the damage caused by nephritis. Therefore, PTEN-long may potentially be a target for the diagnosis and treatment of nephritis.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

HW and QY conceived and designed the experiments. HW, QY, LW, YLi, MX, YLu and YC performed the experiments and prepared the manuscript. HW, QY, LW, YLi, MX, YLu and YC analyzed the data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The Affiliated Yantai Yuhuangding Hospital of Qingdao University, and all participants provided written informed consent.

Patient consent for publication

All participants provided written informed consent.

Competing interests

The authors declare that they have no competing interests.

References

1. Cairns P: Renal cell carcinoma. Cancer Biomarkers 9: 461-473, 2010.
2. Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, Tykodi SS, Sosman JA, Procopio G, Plimack ER, *et al*: Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med 373: 1803-1813, 2015.
3. Motzer RJ, Escudier B, Tomczak P, Hutson TE, Michaelson MD, Negrier S, Oudard S, Gore ME, Tarazi J, Hariharan S, *et al*: Axitinib versus sorafenib as second-line treatment for advanced renal cell carcinoma: Overall survival analysis and updated results from a randomised phase 3 trial. Lancet Oncol 14: 552-562, 2013.
4. Tomita Y, Fukasawa S, Shinohara N, Kitamura H, Oya M, Eto M, Tanabe K, Kimura G, Yonese J, Yao M, *et al*: Nivolumab versus everolimus in advanced renal cell carcinoma: Japanese subgroup analysis from the CheckMate 025 study. Jpn J Clin Oncol 47: 639-646, 2017.
5. Tanriverdi O: Review on targeted treatment of patients with advanced-stage renal cell carcinoma: A medical oncologist's perspective. Asian Pac J Cancer Prev 14: 609-617, 2013.
6. Chow WH, Dong LM and Devesa SS: Epidemiology and risk factors for kidney cancer. Nat Rev Urol 7: 245-257, 2010.
7. Perry JM, He XC, Sugimura R, Grindley JC, Haug JS, Ding S and Li L: Cooperation between both Wnt/{beta}-catenin and PTEN/PI3K/Akt signaling promotes primitive hematopoietic stem cell self-renewal and expansion. Genes Dev 25: 1928-1942, 2011.
8. Dasari VR, Kaur K, Velpula KK, Gujrati M, Fassett D, Klopfenstein JD, Dinh DH and Rao JS: Upregulation of PTEN in glioma cells by cord blood mesenchymal stem cells inhibits migration via downregulation of the PI3K/Akt pathway. PLoS One 5: e10350, 2010.
9. Carver BS, Chapinski C, Wongvipat J, Hieronymus H, Chen Y, Chandarlapaty S, Arora VK, Le C, Koutcher J, Scher H, *et al*: Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. Cancer Cell 19: 575-586, 2011.
10. Esteva FJ, Guo H, Zhang S, Santa-Maria C, Stone S, Lanchbury JS, Sahin AA, Hortobagyi GN and Yu D: PTEN, PIK3CA, p-AKT, and p-p70S6K status: Association with trastuzumab response and survival in patients with HER2-positive metastatic breast cancer. Am J Pathol 177: 1647-1656, 2010.
11. Li X, Yang Z, Song W, Zhou L, Li Q, Tao K, Zhou J, Wang X, Zheng Z, You N, *et al*: Overexpression of Bmi-1 contributes to the invasion and metastasis of hepatocellular carcinoma by increasing the expression of matrix metalloproteinase (MMP)-2, MMP-9 and vascular endothelial growth factor via the PTEN/PI3K/Akt pathway. Int J Oncol 43: 793-802, 2013.
12. Brenner W, Färber G, Herget T, Lehr HA, Hengstler JG and Thüroff JW: Loss of tumor suppressor protein PTEN during renal carcinogenesis. Int J Cancer 99: 53-57, 2002.
13. Hopkins BD, Fine B, Steinbach N, Dendy M, Rapp Z, Shaw J, Pappas K, Yu JS, Hodakoski C, Mense S, *et al*: A secreted PTEN phosphatase that enters cells to alter signaling and survival. Science 341: 399-402, 2013.
14. Hopkins BD, Hodakoski C, Barrows D, Mense SM and Parsons RE: PTEN function: The long and the short of it. Trends Biochem Sci 39: 183-190, 2014.
15. Ahmed RA, Hopkins B, Palermo C, Sastra SA, Rapp Z, Parsons R and Olive KP: Abstract A86: PTEN-Long and gemcitabine combination treatment as a therapeutic for pancreatic cancer. Cancer Res 75 (13 Suppl): A86, 2015.
16. Wang H, Zhang P, Lin C, Yu Q, Wu J, Wang L, Cui Y, Wang K, Gao Z and Li H: Relevance and therapeutic possibility of PTEN-long in renal cell carcinoma. PLoS One 10: e114250, 2015.
17. Zhang R, Miner JJ, Gorman MJ, Rausch K, Ramage H, White JP, Zuiani A, Zhang P, Fermandez E, Zhang Q, *et al:* A CRISPR screen defines a signal peptide processing pathway required by flaviviruses. Nature 535: 164-168, 2016.
18. Zhou J, Wang C, Zhang K, Wang Y, Gong X, Wang Y, Li S and Luo Y: Generation of human embryonic stem cell line expressing zsGreen in cholinergic neurons using CRISPR/Cas9 system. Neurochem Res 41: 2065-2074, 2016.
19. Yin H, Song CQ, Dorkin JR, Zhu LJ, Li Y, Wu Q, Park A, Yang J, Suresh S, Bizhanova A, *et al*: Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo. Nat Biotechnol 34: 328-333, 2016.
20. Zuckermann M, Kawauchi D and Gronych J: Applications of the CRISPR/Cas9 system in murine cancer modeling. Brief Funct Genomics 16: 25-33, 2017.
21. Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C and González-Barón M: PI3K/Akt signalling pathway and cancer. Cancer Treat Rev 30: 193-204, 2004.
22. Luo J, Manning BD and Cantley LC: Targeting the PI3K-Akt pathway in human cancer: Rationale and promise. Cancer Cell 4: 257-262, 2003.
23. Carnero A and Paramio JM: The PTEN/PI3K/AKT pathway in vivo, cancer mouse models. Front Oncol 4: 252, 2014.
24. Bassi C, Ho J, Srikumar T, Dowling RJ, Gorrini C, Miller SJ, Mak TW, Neel BG, Raught B and Stambolic V: Nuclear PTEN controls DNA repair and sensitivity to genotoxic stress. Science 341: 395-399, 2013.
25. Wu Q, Li Z and Liu Q: Treatment with PTEN-Long protein inhibits hepatitis C virus replication. Virology 511: 1-8, 2017.
26. Pan MW and Hou LL: Relationship and tumor inhibition between PTEN and PTEN-long. Sheng Li Ke Xue Jin Zhan 47: 355-360, 2016. (In Chinese)
27. Krishnan SM, Sobey CG, Latz E, Mansell A and Drummond GR: IL-1β and IL-18: Inflammatory markers or mediators of hypertension? Br J Pharmacol 171: 5589-5602, 2014.
28. Daniele G, Guardado Mendoza R, Winnier D, Fiorentino TV, Pengou Z, Cornell J, Andreozzi F, Jenkinson C, Cersosimo E, Federici M, *et al*: The inflammatory status score including IL-6, TNF-α, osteopontin, fractalkine, MCP-1 and adiponectin underlies whole-body insulin resistance and hyperglycemia in type 2 diabetes mellitus. Acta Diabetol 51: 123-131, 2014.
29. Faubel S, Lewis EC, Reznikov L, Ljubanovic D, Hoke TS, Somerset H, Oh DJ, Lu L, Klein CL, Dinarello CA and Edelstein CL: Cisplatin-induced acute renal failure is associated with an increase in the cytokines interleukin (IL)-1beta, IL-18, IL-6, and neutrophil infiltration in the kidney. J Pharmacol Exp Ther 322: 8-15, 2007.
30. Ramesh G and Reeves WB: TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. J Clin Invest 110: 835-842, 2002.
31. Risdon RA, Sloper JC and De Wardener HE: Relationship between renal function and histological changes found in renal-biopsy specimens from patients with persistent glomerular nephritis. Lancet 2: 363-366, 1968.