Original Article

Conditional Knockout of Src Homology 2 Domain-containing Protein Tyrosine Phosphatase-2 in Myeloid Cells Attenuates Renal Fibrosis after Unilateral Ureter Obstruction

Jing-Fei Teng1,2, Kai Wang1, Yao Li1, Fa-Jun Qu1, Qing Yuan2, Xin-Gang Cui1, Quan-Xing Wang1, Dan-Feng Xu1

1Department of Urology, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai 200003, China
2Department of Urology, General Hospital of Beijing Military Command, Beijing 100700, China
3Department of Urology, 309 Hospital of Chinese People’s Liberation Army, Beijing 100091, China
4National Key Laboratory of Medical Immunology and Institute of Immunology, Second Military Medical University, Shanghai 200433, China

Abstract

Background: Src homology 2 domain-containing protein tyrosine phosphatase-2 (SHP-2) is a kind of intracellular protein tyrosine phosphatase. Studies have revealed its roles in various disease, however, whether SHP-2 involves in renal fibrosis remains unclear. The aim of this study was to explore the roles of myeloid cells SHP-2 in renal interstitial fibrosis.

Methods: Myeloid cells SHP-2 gene was conditionally knocked-out (CKO) in mice using loxP-Cre system, and renal interstitial fibrosis was induced by unilateral ureter obstruction (UUO). The total collagen deposition in the renal interstitium was assessed using picrosirius red stain. F4/80 immunostaining was used to evaluate macrophage infiltration in renal tubular interstitium. Quantitative real-time polymerase chain reaction and enzyme linked immunosorbent assay were used to analyze the production of cytokines in the kidney. Transferase-mediated dUTP nick-end labeling stain was used to assess the apoptotic renal tubular epithelial cells.

Results: Src homology 2 domain-containing protein tyrosine phosphatase-2 gene CKO in myeloid cells significantly reduced collagen deposition in the renal interstitium after UUO. Macrophage infiltration was evidently decreased in renal tubular interstitium of SHP-2 CKO mice. Meanwhile, the production of pro-inflammatory cytokines was significantly suppressed in SHP-2 CKO mice. However, no significant difference was observed in the number of apoptotic renal tubular epithelial cells between wild-type and SHP-2 CKO mice.

Conclusions: Our observations suggested that SHP-2 in myeloid cells plays a pivotal role in the pathogenesis of renal fibrosis, and that silencing of SHP-2 gene in myeloid cells may protect renal from inflammatory damage and prevent renal fibrosis after renal injury.

Key words: Fibrosis; Inflammation; Myeloid Cells; Obstructive Nephropathy; Src Homology 2 Domain-Containing Protein Tyrosine Phosphatase-2

Introduction

It is estimated that about 14% of American adults are suffering from chronic kidney disease, and many of them eventually progress to renal failure.1 In this process, renal fibrosis is usually the final common pathway leading to progressive loss of renal function. As the predominant infiltrating cell type in response to kidney injuries, myeloid cells play a pivotal role in the development of renal fibrosis.2 Activated macrophages secrete several pro-inflammatory cytokines and growth factors, which induce renal tubular epithelial cell apoptosis, activate fibroblast, stimulate extracellular matrix (ECM) production, and finally result in renal fibrosis.3,4 Nevertheless, the mechanism how myeloid cells regulate renal fibrosis remains unclear.

The Src homology 2 domain-containing protein tyrosine phosphatase-2 (SHP-2), characterized by two N-terminal Src homology 2 domains, a central catalytic domain and a C-terminal protein tyrosine phosphatase (PTP) domain, is an evolutionarily conserved intracellular PTP.5 It has been found that SHP-2 acts as a key regulator in various signal transduction pathways6 and is involved in many diseases, such as Noonan syndrome,7 leukemia,8 prostate cancer,9 and so on. Recent studies also revealed that the SHP-2 regulates the production of pro-inflammatory cytokines and plays important roles in several inflammatory diseases. However, the role of SHP-2 in the pathogenesis of renal fibrosis has not been studied so far.
In this work, we introduced a SHP-2 conditional knockout (CKO) mouse, and studied the function of myeloid cells SHP-2 in the pathogenesis of renal fibrosis under unilateral ureter obstruction (UUO).

**Methods**

**Animal preparation**

C57/BL6 mice with floxed exon 4 of SHP-2 (SHP-2^floxed/Cre^-/-) were mated with mice expressing Cre recombinase from the endogenous lyzs locus in myeloid cells (SHP-2^+/+Cre^-/-) (Jackson laboratory). SHP-2^floxed/Cre^-/- mice were selected from the offspring and crossed with SHP-2^floxed/Cre^-/- mice to generate SHP-2 CKO mice (SHP-2^floxed/flox/Cre^-/-). For genotyping, DNA was isolated from tails using Mouse Tail DNA Mini Kit (Foregene, Chengdu, China) following the manufacturer’s instructions. DNAs were amplified by polymerase chain reaction (PCR) using Takara PCR Amplification Kit (Takara, Dalian, China). Primers for the Cre gene were: 5'-ATGCCCAAGAAGAAGGAGGT-3' (forward), 5'-GAAATCAGTGCGTTCGAACGCTAGA-3' (reverse). For lox gene, DNA agarose gel electrophoresis was performed to identify the target genes. In this study, SHP-2^floxed/Cre^-/- mice were used as controls. Our animal experiment protocols were approved by the Ethics Committee of Second Military Medical University.

**Surgical procedure**

Unilateral ureter obstruction or sham operation was performed under general anesthesia using pentobarbital (60 mg/kg, i.p.). An abdominal median incision was made, and the left ureter was visualized. In UUO group, the ureter was permanently ligated using 9–0 silk sutures at the pyeloureteric junction. While in sham-operation group, the ureter was exposed and photographed at a magnification of ×200. For each section, 10 randomly chosen fields in the cortex of the kidney were captured. The integrated optical density of the collagen area was determined using Image-Pro Plus software version 5.1 (Media Cybernetics, USA) to assess the degree of fibrosis.

**Histological analysis**

Formalin-fixed paraffin-embedded kidneys were cut into 4-μm sections and stained with picrosiris red to evaluate interstitial fibrosis. The sections were scanned under a light microscope and photographed at a magnification of ×200. For each section, 10 randomly chosen fields in the cortex of the kidney were captured. The integrated optical density of the collagen area was determined using Image-Pro Plus software version 5.1 (Media Cybernetics, USA) to assess the degree of fibrosis.

**Immunohistochemistry**

The 4-μm-thick slide-mounted sections were deparaffinized in xylene and rehydrated with graded ethanol. The slides were incubated in 1:50 dilution of rat anti-mouse F4/80 monoclonal antibody (Abcam, Cambridge, MA, USA) overnight at 4°C. Then, the slides were incubated with 1:200 diluted biotinylated goat anti-rabbit secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 30 min, and subsequently reacted with streptavidin-peroxidase conjugate and 3', 3'-diaminobenzidine. The slides were examined under light microscope at a magnification of ×200 and macrophages positive for F4/80 were counted in 10 microscopic fields of the cortex. Results were expressed as the number of macrophages per high power field (HPF).

**Quantitative real-time polymerase chain reaction**

Total RNA was extracted using TRizol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed using ReverTra Ace-α (Toyobo, Shanghai, China) following the manufacturer’s instructions. Quantitative real-time PCR (qRT-PCR) was performed with Stepone™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using SYBR Premix Ex Taq (Takara, Dalian, China) according to the manufacturer’s instructions. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The following primers were used: GAPDH: 5'-TGACCAAGCTTCCATGACCTC-3' (forward), 5'-GACGCCACACTTGGGGTATG-3' (reverse); Exon 4: 5'-CTGAACCTTCCAGACCCCTTCTC-3' (forward), 5'-TTTGGACTTGGCGTCCTTG-3' (reverse); Exon 8, 9: 5'-GCTCGTGACCAAGCAACCG-3' (forward), 5'-ACAGGATCTCCACATCTCCAC-3' (reverse); tumor necrosis factor-α (TNF-α): 5'-AACGCCCTTGGCAGCCCCTGTA-3' (forward), 5'-GGGACCACCTATGTTGGTCTTCTTGG-3' (reverse); transforming growth factor-β (TGF-β): 5'-GGGAGCGATTCCGCACTG-3' (forward), 5'-GTGGGTTTCCATTACGAC-3' (reverse); interleukin-1β (IL-1β): 5'-GGTGAGCTGAGCCCTATCTATG-3' (forward), 5'-CATGGAAGATATCCTGGTGGTCGTG-3' (reverse). The RNA expression levels of TNF-α, TGF-β, IL-1β and IL-6 were normalized to that of GAPDH.

**Enzyme linked immunosorbent assay**

Kidney tissues were homogenized in M-PER tissue protein extraction reagent (Thermo Scientific, Waltham, MA, USA) supplemented with protease inhibitor mixture (Calbiochem, Darmstadt, Germany) at 4°C for 30 min, and then centrifuged at 4000 g for 15 min, the supernatants were collected. The protein concentrations were measured...
using BCA assay (Thermo Scientific, Waltham, MA, USA) and equalized to 4 μg/μl using extraction reagent. The TNF-α, TGF-β, IL-1β and IL-6 levels of the kidney were determined by commercial enzyme linked immunosorbent assay (ELISA) kits (Bender MedSystems GmbH, Vienna, Austria) according to the manufacturer’s instructions.

**Statistical analysis**

Data were shown as mean ± standard deviation (SD); comparisons between groups were performed with Student’s t-test or one-way analysis of variance (ANOVA) followed by SNK-q test for multiple comparisons using SPSS version 19.0 (IBM SPSS Statistics, Armonk, NY, USA). P < 0.05 was considered as statistically significant.

**RESULTS**

**Identification of Src homology 2 domain-containing protein tyrosine phosphatase-2 gene conditionally knocked-out in myeloid cells**

To generate SPH-2 CKO mice, mice with loxP flanked SPH-2 allele were crossed with transgenic mice expressing Cre recombinase under the control of lyzs promoter. PCR and DNA agarose gel electrophoresis were performed to identify SHP-2 flox/flox Cre−/− (CKO) and SHP-2 flox/flox Cre−/− (control) mice [Figure 1a]. Abdominal macrophages from SPH-2 CKO and control mice were collected and total RNA was extracted. qRT-PCR showed that SHP-2 mRNA expression was reduced by 68% in SPH-2 CKO mice compared to control mice [Figure 1b].

**Src homology 2 domain-containing protein tyrosine phosphatase-2 gene conditionally knocked-out attenuates renal fibrosis**

To determine the role of SHP-2 in renal fibrosis, SHP-2 flox/flox Cre−/− and SHP-2 flox/flox Cre−/− mice were subjected to UUO surgery and sham-operation, respectively. The degrees of collagen deposition in the tubulointerstitium were studied between both groups. Seven days after operation, kidneys were harvested and picrosirius red stain was performed to evaluate collagen deposition. Both genotypes exhibited enhanced collagen deposition in UUO group compared sham-operation group. In UUO group, control mice displayed significantly more collagen deposition than SHP-2 CKO mice, suggesting that SHP-2 gene knockout in myeloid cells may attenuate renal fibrosis under mechanical damage [Figure 2].

**Src homology 2 domain-containing protein tyrosine phosphatase-2 gene conditionally knocked-out results in reduced macrophage infiltration after unilateral ureter obstruction**

Myeloid cell infiltration, including macrophage, monocyte and dendritic cells, is the key event in the pathogenesis of renal fibrosis. F4/80 immunohistochemistry stain was performed to identify interstitial macrophages. UUO increased the number of infiltrated macrophages in both genotypes compared to sham-operation. When compared to control mice, the number of macrophages on day 7 after UUO was significantly smaller in SPH-2 CKO mice [Figure 3].

**Src homology 2 domain-containing protein tyrosine phosphatase-2 gene conditionally knocked-out prevents pro-inflammatory cytokines production after unilateral ureter obstruction**

Several pro-inflammatory cytokines were up-regulated and played important roles in the process of renal fibrosis. To test whether SPH-2 CKO may affect pro-inflammatory cytokine production, qRT-PCR and ELISA were performed. qRT-PCR revealed significantly decreased production of TNF-α, TGF-β, IL-1β and IL-6 mRNA in SPH-2 CKO mice at day 7 after UUO compared to sham-operation mice [Figure 4a]. At protein level, ELISA revealed the similar results [Figure 4b].

**Src homology 2 domain-containing protein tyrosine phosphatase-2 gene conditionally knocked-out does not affect renal tubular epithelial cells apoptosis after unilateral ureter obstruction**

Transferase-mediated dUTP nick-end labeling analysis was performed to test renal tubular epithelial cells apoptosis. Despite increased apoptotic cells after UUO in both genotypes

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**Figure 1:** Myeloid cells specific deletion of the Src homology 2 domain-containing protein tyrosine phosphatase-2 (SHP-2) gene. (a) DNA agarose gel electrophoresis. SHP-2 flox/flox Cre−/− represents a bright band at about 400 bp, SHP-2 flox/flox Cre+/+ represents a band at about 300 bp, Cre−/+ represents a bright band at about 400 bp; (b) SHP-2 is down-regulated in conditionally knocked-out (CKO) macrophages. Exon 4 of SHP-2 gene was floxed, thus in SPH-2 CKO mice, exon 4 was deleted. The expression of SHP-2 in abdominal macrophages was measured by quantitative real-time polymerase chain reaction. (n = 5, *P < 0.01, vs. control mice).
compared to sham-operation group, no significant difference was observed between SHP-2 CKO mice and control mice after UUO, suggesting SHP-2 CKO does not affect renal tubular epithelial cells apoptosis after UUO [Figure 5].

**Discussion**

Renal fibrosis could be regarded as a result of imbalanced wound healing process in response to various renal injuries.\(^{10}\) Studies have suggested that macrophages are critical players.
involving in many forms of renal injuries, repairs, and contributing to renal fibrosis.\textsuperscript{[11]-[14]} In obstructive nephropathy, hydronephrosis causes elevated intrapelvis pressure and renal hemodynamic changes, which result in ischemic oxidative stress and activation of renin-angiotensin system.\textsuperscript{[15]} With the production of reactive oxygen species and nuclear factor-kB (NF-kB), macrophages were enriched in the renal interstitium.\textsuperscript{[16,17]} Activated macrophages in the injured kidney produce TNF-\(\alpha\) and TGF-\(\beta\), which are central mediators for renal fibrosis. TNF-\(\alpha\) induces apoptosis of renal epithelial cells.\textsuperscript{[23]} TGF-\(\beta\) is a pleiotropic cytokine, it can promote renal epithelial and nonepithelial cells undergo epithelial-mesenchymal transition, becoming myofibroblasts and contribute to ECM deposition.\textsuperscript{[18]} Besides, TGF-\(\beta\) can also induce loss of PTEN,\textsuperscript{[19]} up-regulation of Notch\textsuperscript{[20]} and down-regulation of Klotho\textsuperscript{[21]} and thus promote renal fibrosis. This study also proved that reduced macrophages infiltration as well as the production of pro-inflammatory cytokines prevented renal fibrosis.

Recent studies have revealed the indispensable roles of SHP-2 in various signal transduction pathways and cellular activities. An et al\textsuperscript{[22]} used small interfering RNA technique targeting macrophage SHP-2 gene, and found that SHP-2 negatively regulate MyD88-independent pro-inflammatory cytokine and type I interferon production through directly binding to the kinase domain of TANK binding kinase. You et al\textsuperscript{[23]} found that SHP-2 positively regulates IL-1\(\alpha\) or TNF-\(\alpha\) induced IL-6 production by modulating NF-\(\kappa\)B pathway in a mitogen-activated protein kinase (MAPK)-independent manner. SHP-2 can also mediate ERK activation in several growth factor signaling, which might be necessary for IL-1 to associate with focal adhesion.\textsuperscript{[4]} Besides, SHP-2 has been reported to positively regulate the signaling pathways of insulin, epidermal growth factor, platelet-derived growth factor, fibroblast growth factor and in the context, negatively regulate the Jak-Stat signaling pathway initiated by interferon-\(\alpha\)/\(\gamma\).\textsuperscript{[24,25]} This study demonstrated that UUO strongly induced pro-inflammatory cytokine productions, including TNF-\(\alpha\), TGF-\(\beta\), IL-1\(\beta\) and IL-6, which were suppressed by SHP-2 knockout, indicating that SHP-2 took an important part in the inflammatory process of UUO-mediated renal fibrosis.

Gene knockout has been the most efficient method for function study of target genes. Cre-loxP system was a site-specific recombinase system and was widely used in conditional gene knockout. Cre recombinase recognizes loxP site and mediate loxP flanked gene mutations. To achieve tissue-specific gene knockout, a Cre transgenic mouse with Cre recombinase gene inserted into downstream of a tissue-specific promoter was needed. In the present study, transgenic mice expressing Cre recombinase under the control of the endogenous Lyz2 promoter/enhancer elements were crossed with mice in which exon 4 of SHP-2 gene were flanked by loxP sequence. qRT-PCR showed that the expression of SHP-2 in macrophages decreased about 68%.

In our study, picrosirius red stain was performed 7 days after UUO, and the result showed that the renal interstitial collagen fibrils in SHP-2 CKO group were significantly less than those in control group, indicating that SHP-2 deficient attenuates renal fibrosis. It is reported that SHP-2 promotes cell migration and invasiveness.\textsuperscript{[26-27]} We thus hypothesize that SHP-2 might affect macrophage infiltration after UUO. We then performed F4/80 stain to evaluate the macrophage infiltration in both groups and found that the number of macrophages was significantly less in SHP-2 CKO group compared to the control group. Meanwhile, another study found that the expression of SHP-2 was up-regulated in rheumatoid arthritis fibroblast-like synoviocyte (RA FLS), knockdown of SHP-2 resulted in inhibited invasiveness, migration, adhesion and spreading of RA FLS.\textsuperscript{[28]}

To test whether SHP-2 influence inflammatory factors secretion after UUO, qRT-PCR and ELISA were performed. We found that TNF-\(\alpha\), TGF-\(\beta\), IL-1\(\beta\) and IL-6 were inhibited in SHP-2 CKO group, indicating that SHP-2 might positively regulate cytokine production under UUO. These results were contrary to those of An et al\textsuperscript{[22]} However, several studies have reported that SHP-2 was positively involved in inflammatory responses. Li et al\textsuperscript{[29]} found that SHP-2 positively regulates

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**Figure 5:** Src homology 2 domain-containing protein tyrosine phosphatase-2 conditionally knocked-out does not affect renal tubular epithelial cells apoptosis after unilateral ureter obstruction. (a) Transferase-mediated dUTP nick-end labeling (TUNEL) staining detecting apoptotic cells (original magnification, \(\times 200\)); (b) Quantification of TUNEL-positive cells (counts/HPF) in the sections. (\(n = 7\)). Ctrl: control mice; CKO: SHP-2 conditional Knockout mice, Sham: sham-operation; UUO: unilateral ureter obstruction; Ctrl-Sham: control mice with sham-operation; CKO-Sham: SHP-2 conditional Knockout mice with sham-operation; Ctrl-UUO: control mice with unilateral ureter obstruction; CKO-UUO: SHP-2 conditional Knockout mice with unilateral ureter obstruction.
IL-8 production in acute cigarette smoke-mediated lung inflammation through MAPK pathway, SHP-2 CKO in lung epithelia reduced IL-8 release and pulmonary inflammation in cigarette smoke-exposed mice. Stanford et al. found that SHP-2 was positively involved in RA. In the contrast, Coulombe et al. found that epithelial SHP-2 protects against intestinal inflammation. The detailed mechanism for the up-regulated expression of inflammatory factors in the kidney was unclear, possibly SHP-2 regulates inflammatory factors expression after UUO through another signaling pathway, which was different from that reported by An et al.

In conclusion, the present study found that myeloid cells SHP-2 knockout results in decreased macrophage infiltration and inflammatory factors production after UUO, thus attenuates renal fibrosis; however, it does not affect renal tubular epithelial cells apoptosis. These results suggested myeloid cells SHP-2 is positively involved in renal fibrosis. To the best of our knowledge and belief, this is the first study discussing the function of myeloid cells SHP-2 in renal fibrosis. Our results shed light on the management of renal fibrosis caused by obstructive nephropathy.

**References**

1. U.S. Renal Data System, USRDS 2013 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD; 2013. Available from: http://www.usrds.org/atlas.aspx. [Last accessed on 2014 Jul 8].

2. Nishida M, Hamaoka K. Macrophage phenotype and renal fibrosis in obstructive nephropathy. Nephron Exp Nephrol 2008;108:E31-6.

3. Misseri R, Meldrum DR, Dinarello CA, Dagher P, Hile KL, Rink RC, et al. TNF-alpha mediates obstruction-induced renal tubular cell apoptosis and proapoptotic signaling. Am J Physiol Renal Physiol 2005;288:F406-11.

4. Boor P, Ostendorf T, Floege J. Renal fibrosis: Novel insights into mechanisms and therapeutic targets. Nat Rev Nephrol 2010;6:643-56.

5. Matthews RJ, Bowne DB, Flores E, Thomas ML. Characterization of hematopoietic intracellular protein tyrosine phosphatases: Description of a phosphatase containing an SH2 domain and another enriched in proline-, glutamic acid-, serine-, and threonine-rich sequences. Mol Cell Biol 1992;12:2396-405.

6. Chong ZZ, Maiese K. The Src homology 2 domain tyrosine phosphatases SHP-1 and SHP-2: Diversified control of cell growth, inflammation, and infection. Histol Histopathol 2007;22:1251-67.

7. Tartaglia M, Meher EL, Goldberg R, Zapponi G, Brunner HG, Kremer H, et al. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet 2001;29:465-8.

8. Xu D, Wang S, Yu WM, Chen G, Araki T, Bunting KD, et al. A germine gain-of-function mutation in PTPN11 (Shp-2) phosphatase induces myeloproliferative disease by aberrant activation of hematopoietic stem cells. Blood 2010;116:3611-21.

9. Tassidis H, Brokken LJ, Jirström K, Bjartell A, Ulmert D, Härkönen P, et al. Low expression of SHP-2 is associated with less favorable prostate cancer outcomes. Tumour Biol 2013;34:637-42.

10. Liu Y. Cellular and molecular mechanisms of renal fibrosis. Nat Rev Nephrol 2011;7:684-96.

11. Lee S, Huen S, Nishio H, Nishio S, Lee HK, Choi BS, et al. Distinct macrophage phenotypes contribute to kidney injury and repair. J Am Soc Nephrol 2011;22:317-26.

12. Vernon MA, Mylonas KJ, Hughes J. Macrophages and renal fibrosis. Semin Nephrol 2010;30:302-17.

13. Ko GJ, Boo CS, Jo SK, Cho WY, Kim HK. Macrophages contribute to the development of renal fibrosis following ischemia/reperfusion-induced acute kidney injury. Nephrol Dial Transplant 2008;23:842-52.

14. Lin SL, Li B, Rao S, Yeo EI, Hudson TE, Nowlin BT, et al. Macrophage Wnt7b is critical for kidney repair and regeneration. Proc Natl Acad Sci U S A 2010;107:4194-9.

15. Chevalier RL, Cachat F. Role of angiotensin II in chronic ureteral obstruction. Contrib Nephrol 2001;135:250-60.

16. Miyajima A, Kosaka T, Seta K, Asano T, Umezawa K, Hayakawa M. Novel nuclear factor kappa B activation inhibitor prevents inflammatory injury in unilateral ureteral obstruction. J Urol 2003;169:1559-63.

17. Tashiro K, Tamada S, Kuwabara N, Komiyavi T, Takahida K, Arai T, et al. Attenuation of renal fibrosis by proteasome inhibition in rat obstructive nephropathy: Possible role of nuclear factor kappab. J Int Med Res 2003;12:587-92.

18. García-Sánchez O, López-Hernández FJ, López-Novoa JM. An integrative view on the role of TGF-beta in the progressive tubular deletion associated with chronic kidney disease. Kidney Int 2010;77:950-5.

19. Lan R, Cheng H, Polichnowski AJ, Singh PA, Saikumar P, McEwen DG, et al. PTEN loss defines a TGF-β-β-induced tubular phenotype of failed differentiation and JNK signaling during renal fibrosis. Am J Physiol Renal Physiol 2012;302:F1210-23.

20. Bielez B, Sirin Y, Si H, Niranjani T, Gruenwald A, Ahn S, et al. Epithelial Notch signaling regulates interstitial fibrosis development in the kidneys of mice and humans. J Clin Invest 2010;120:4040-54.

21. Sugira H, Yoshida T, Shiohira S, Koeji H, Mitobe M, Kuros H, et al. Reduced Klotho expression level in kidney aggravates renal interstitial fibrosis. Am J Physiol Renal Physiol 2012;302:F1252-64.

22. An H, Zhao W, Hou J, Zhang Y, Xie Y, Zheng Y, et al. SHP-2 phosphatase negatively regulates the TRIF adapter protein-dependent type I interferon and proinflammatory cytokine production. Immunity 2006;25:919-28.

23. You M, Flickr LM, Yu D, Feng GS. Modulation of the nuclear factor kappa B pathway by Shp-2 tyrosine phosphatase in mediating the induction of interleukin (IL)-6 by IL-1 or tumor necrosis factor. J Exp Med 2001;193:101-10.

24. Qu CK. The SHP-2 tyrosine phosphatase: Signaling mechanisms and biological functions. Cell Res 2000;10:279-88.

25. You M, Yu DH, Feng GS. SHP-2 tyrosine phosphatase functions as a negative regulator of the interferon-stimulated Jak/STAT pathway. Mol Cell Biol 1999;19:2416-24.

26. Yu DH, Qu CK, Hengarau O, Lu X, Feng GS. Protein-tyrosine phosphatase Shp-2 regulates cell spreading, migration, and focal adhesion. J Biol Chem 1998;273:21125-31.

27. Wang S, Yu WM, Zhang W, McCrae KR, Neel BG, Qu CK. Noonan syndrome/leukemia-associated gain-of-function mutations in SHP-2 phosphatase (PTPN11) enhance cell migration and angiogenesis. J Biol Chem 2009;284:913-20.

28. Stanford SM, Maestre MF, Campbell AM, Bartok B, Kiosses WB, Boyle DL, et al. Protein tyrosine phosphatase expression profile of rheumatoid arthritis fibroblast-like synovocytes: A novel role of SH2 domain-containing phosphatase 2 as a modulator of invasion and survival. Arthritis Rheum 2013;65:1171-80.

29. Li FF, Shen J, Shen HJ, Zhang X, Cao R, Zhang Y, et al. Phosphatase Shp-2 plays an important role in acute cigarette smoke-mediated lung inflammation. Clin Immunol 2012;139:1587-97.

30. Coulombe G, Leblanc C, Cagnol S, Maloum F, Lemieux E, Perreault N, et al. Epithelial tyrosine phosphatase Shp-2 protects against intestinal inflammation in mice. Mol Cell Biol 2013;33:2275-84.