Effect of Youthful Blood Environment and Its Key Factor SCF on Renal Interstitial Fibrosis in Elderly Mice

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Abstract

Background: youthful blood environment was shown to decelerate the aging process of kidney and to attenuate senile renal fibrosis in a young-old parabiotic animal model; in addition, we identified a stem cell factor (SCF) that is closely linked with the process. To further investigate the effect of youthful blood environment on renal interstitial fibrosis and the underlying mechanisms, we bred SCF receptor c-Kit gene loss-of-function Wps/Wps mice and established a combination mice model that was subjected to unilateral ureteral obstructive (UUO) and parabiotic surgeries.

Methods: Parabiotic mice were divided into isochronic parabiotic (young-young, Y-IP and old-old, O-IP) and heterochronic parabiotic (young-old, HP) groups. UUO surgery was performed in one of the parabiotic pairs in the IP group (Y-IPuuo and O-IPuuo) and in the elderly mice in the HP group (O-HPuuo). In order to study the role of SCF/c-kit on renal interstitial fibrosis, UUO surgery was performed in wildtype (WT) and Wps/Wps mice.

Results: Fourteen days after UUO surgery, the kidney interstitial fibrosis area, kidney function, and the expressions of SCF/c-Kit, pNF-κB, and fibrosis-related proteins in the O-HPuuo group were significantly lower than those in the Ouuo and O-IPuuo groups. Compared with wildtype UUO mice, the expressions of pNF-κB and fibrosis-related proteins, kidney interstitial fibrosis area, and the kidney function were all significantly decreased in Wps/Wps UUO mice.

Conclusions: Youthful blood environment downregulated the expressions of SCF/c-Kit in elderly UUO mice, and ameliorated UUO-induced kidney fibrosis and function loss, which may be mediated via the NF-κB pathway.

Background

Renal interstitial fibrosis is the basis of renal senescence, and is one of the most common mechanisms of deterioration of kidney disease into end-stage renal disease. Therefore, effective control or reversal of renal fibrosis is the key to prevent renal senescence and deterioration of a variety of kidney diseases [1–3].

Parabiotic animal model is characterized by shared blood circulation established via surgical linkage between the muscle flap and skin of two animals. Parabiotic animal models are categorized into isochronic parabiotic (IP) and heterochronic parabiotic (HP) models, depending on the age of the two parabiotic animals. Parabiotic animal models have been gradually applied in recent years for the study of senescence of heart [4], liver [5], nerves [6, 7], and muscle [8] tissues. The results have consistently indicated that youthful blood environment can promote proliferation, reduce tissue injury, and help recover organ function to varying degrees.

In our previous study, we observed lower expressions of renal senescence markers and decreased kidney fibrosis area in elderly HP mice after 5 weeks of shared blood circulation, but with no obvious change in

renal function [9]. Furthermore, the expressions of renal senescence markers were decreased 16 weeks after transplantation of elderly rat kidney to young rat; however, no obvious changes were observed with respect to kidney fibrosis and renal function [10]. Kidney tissue damage and function loss were significantly alleviated in elderly HP mice after ischemia-reperfusion injury (IRI), compared to the control old IRI mice [11]. However, the effect of youthful blood environment on renal interstitial fibrosis and the associated underlying mechanisms are not clear. In addition, in earlier studies, we compared serum cytokine levels in young and elderly mice with 5 weeks of IP or HP, and found that 6 types of blood factors including stem cell factor (SCF) may be associated with renal senescence; in addition, we also confirmed higher expressions of SCF in elderly mice blood.

Stem cell factor is also referred to as the mast cell growth factor (MGF) or c-Kit ligand (KL). It has been shown to promote cell proliferation, mobilization, and adhesion [12]. Early studies about SCF largely pertained to the treatment of blood system diseases [13]. Several recent studies have indicated that SCF/c-kit plays a critical role in the process of reconstruction and fibrosis in a variety of diseased organs, such as lung, skin, liver and kidney (14–17). SCF is associated with development of renal fibrosis in multiple kidney diseases. Moreover, SCF is highly expressed in a variety of kidney diseases, such as IgA nephropathy, membranous nephropathy, crescent nephritis, hypertensive nephropathy, diabetic nephropathy, late AKI, and graft-versus-host reaction after kidney transplantation (18–20). However, the role of SCF in elderly renal fibrosis and the associated mechanisms are not clear.

In this study, by establishing a combination mice model with unilateral ureteral obstructive (UUO) and parabiotic surgeries, we investigated the effect of youthful parabiotic circulation on senile renal interstitial fibrosis and studied the associated mechanisms. In order to understand the role of SCF/c-Kit on renal interstitial fibrosis, the UUO model was also established using Wps/Wps mice with loss-of-function mutation in the SCF receptor c-Kit.

Methods

Establishment of animal model

Young (age: 3 months) and elderly (age: 22 months) SPF graded mice were obtained from the Experimental Animal Center at the Chinese PLA General Hospital. Three-month-old Wps/Wps mice with a C57BL/6 strain background were obtained from the MOE Key Laboratory of Model Animal for Disease Study, Model Animal Research Center, Nanjing University. The methods for parabiosis surgical procedures and the verification of shared circulation in parabiotic mice are described elsewhere [9]. For establishment of unilateral ureteral obstruction (UUO) mice model, the left ureter was ligated using 5 − 0 silk via a mid-abdominal incision. All surgical procedures were performed under isoflurane anesthesia. Mice were sacrificed 7 or 14 days after UUO surgery. The contralateral kidneys were harvested as internal controls. All animal experiments were approved and performed in accordance with the Chinese PLA General Hospital's Committee on Animal Protection and Utilization.

Experimental animal groups

In order to study the effect of blood environment on senile kidney interstitial fibrosis, experimental animals were divided into parabiotic and normal mice groups. Normal mice group comprised of young control group (Ycon) and old control group (Ocon). Parabiotic mice group comprised of isochronic parabiotic (IP) and heterochronic parabiotic (HP) groups. Young-young (Y-IP) and old-old (O-IP) pairings were performed in the IP group, while young-old pairing was performed in the HP group. Three weeks after parabiosis, UUO surgery was performed in one of the parabiotic pairs in the IP group and in the elderly mice in the HP group. According to duration of UUO obstruction, these mice were divided into 7 d and 14 d groups: young control groups (Yuuo7 and Yuuo14); old control groups (Ouuo7 and Ouuo14); Y-IP groups with UUO in the young mice (Y-IPuuo7 and Y-IPuuo14); O-IP groups with UUO in the old mice (O-IPuuo7 and O-IPuuo14); HP groups with UUO in the old mice (O-HPuuo7 and O-HPuuo14).

In order to study the role of SCF/c-kit on renal interstitial fibrosis, UUO surgery was performed in 3-month old wildtype (WT) and Wps/Wps mice. These mice were divided into WTuuo7, WTuuo14, Wps/Wpsuuo7, and Wps/Wpsuuo14 groups, according to the length of the postoperative period.

Blood biochemistry analysis

Mouse serum creatinine and blood urea nitrogen (BUN) levels were determined using a Hitachi 7150 automatic biochemistry analyzer. Mouse serum SCF was examined using Quantikine ELISA Kit (R&D Systems) according to the protocol.

Histopathological examination

Kidney tissues were fixed using 10% neutral-buffered formalin. The kidney tissue blocks were embedded with paraffin and sections stained with periodic acid-Schiff (PAS). The degree of fibrosis in young and old kidney samples were determined using Sirius-red staining and Masson's Trichrome staining, as described elsewhere [21].

Western blot analysis

About 60–100 μg of protein was loaded on each well of 10–15% SDS-polyacrylamide gel. The protein was transferred to a nitrocellulose membrane and blocked with casein buffer at room temperature for 1 hour. The following antibodies were added to the membrane and incubated at 4 °C overnight: rabbit polyclonal anti-ColI (abcam; cat no. ab34710), rabbit monoclonal anti-α-SMA (abcam; cat no. ab32575), rabbit polyclonal anti-TGFβ1 (abcam; cat no. ab92486), goat polyclonal anti-SCF (R&D; cat no.AF-455-NA), goat polyclonal anti-c-Kit (R&D; cat no.AF1356), rabbit monoclonal anti-Phospho-NF-κB p65 (CST company; cat no. 3033), and rabbit monoclonal anti-NF-κB p65 (CST company; cat no. 8242). HRP-labeled goat anti-rabbit or goat anti-mouse IgG (Santa Cruz Biotechnology) were used as secondary antibodies (dilution 1:1000). Target bands were detected by electrochemiluminescence (ECL). Bio-Rad Quantity One (29.0 version) software was used for semi-quantitative analysis.

Immunofluorescence

Frozen young and old kidney sections were first blocked with 5% bovine serum albumin (BSA) in PBS for about half an hour, and incubated overnight with primary antibodies anti-α-SMA and anti-TGF-β1 at 4 °C. After washing with PBS, the sections were incubated with secondary antibodies goat anti-rabbit IgG (H + L) antibody (Alexa Fluor-488, Invitrogen) and goat anti-Mouse IgG (H + L) antibody (Rhodamine RedTM-X, Jackson ImmunoResearch) for 2 h in dark at room temperature. Cell nuclei were stained with 1 μg/mL Hoechst for about 5 min. Confocal images of mouse kidney section were obtained using FluoView FV10i Confocal Laser Scanning Microscope (Olympus, Tokyo).

Statistical analysis

All data analyses were performed using SPSS v21.0 software. Normally distributed quantitative data were presented as mean ± standard deviation (SD). Enumeration data are expressed as percentage. For assessing statistical significance, the changes of quantitative data among groups were tested by one-way ANOVA; inter-group differences were analyzed using post hoc Dunnett-*t* test. The Chi-squared test was used to analyze the changes in enumeration data. Values of $P < 0.05$ were considered statistically significant.

Results

Survival condition of parabiotic mice after UUO surgery

No perioperative deaths occurred after UUO surgery of parabiotic mice with shared circulation for 3 weeks. One young-old paired and 1 old-old paired mice without UUO surgery died 1 week later, which suggested that the animal deaths were likely related with the parabiotic surgery but not with the UUO treatment. Two weeks after UUO surgery, death of 1 pair each occurred in the young-young paired, old-old paired, and young-old paired groups. Of these, the receptor mouse died in the young-young paired group, both donor and receptor mice died in old-old paired and young-old paired groups. The survival rate was high in UUO surgery groups, and the death rate in the young-young paired groups was slightly lower than that in the other groups (Table S1). Two weeks after UUO surgery, the survival rate was 100% for the control young or old mice groups. Finally, there were 4 pairings of Y-lPuuo7, O-lPuuo7 and O-HPuuo7 mice, respectively; 5 pairings of Y-lPuuo14 mice; 4 pairings of O-lPuuo14 and O-HPuuo14 mice, respectively.

Youthful blood environment promotes renal function recovery in old mice after UUO surgery

The serum creatinine levels of young UUO mice showed a significant increase to 1.92 times of the baseline at 7 days after surgery, remained unchanged from 7–10 days after surgery, and subsequently showed a significant decrease to 1.49 times of the baseline at 14 days after surgery. A similar trend was observed in the BUN levels; the BUN level reached a peak at 7 days after surgery (2.18 times of baseline level) and then decreased to 1.72 times of the baseline level at 14 days after surgery. The serum creatinine levels of old UUO mice showed a significant increase to 1.43 times of the baseline at 7 days after surgery, and continued to rise to 1.87 times of baseline at 14 days after surgery. The BUN level also

maintained the increasing trend, and increased to 2.37 times of the baseline at 14 days after surgery (Figure S1C). There was no significant difference with respect to serum creatinine or BUN peak levels between the old and young mice at 14 days after UUO surgery. The only difference was that no sign of slowing down was observed in the old UUO mice groups (Figure S1A, S1B).

The trend of serum creatinine and BUN levels in Y-IPuuo and O-IPuuo groups was identical or a slightly lower than that in the Yuuo and Ouuo groups, respectively. The serum creatinine levels in the O-HPuuo7 and Ouuo7 groups increased to 1.44 and 1.43 times of baseline value, respectively. However, 14 days after UUO surgery, the serum creatinine level in the O-HP mice remained at a level (1.49 times baseline value) similar to that at 7 days after surgery, which was significantly lower than that in the Ouuo14 (1.87 times baseline value) and O-IPuuo14 (1.79 times baseline value) groups. The trend of BUN level in O-HP mice was similar to that of serum creatinine level. The BUN level in the O-HPuuo14 group (1.83 times baseline value) was significantly lower than that in the Ouuo14 and O-IPuuo14 groups (2.23 times and 2.45 times baseline values, respectively) (Figure S1D–F).

Youthful blood environment alleviates renal tissue injury of elderly UUO mice

Histological examination of kidney tissues showed renal tubular epithelial cell necrosis and shedding, inflammatory cell infiltration and fibrosis in the Yuuo7 group. The degree of pathological damage in the Ouuo7 and Yuuo7 groups was similar. However, 14 days after surgery, Ouuo14 group showed more obvious renal tubular epithelial cell necrosis, formation of casts, and interstitial fibrosis than that in the Yuuo14 group (Figure S2).

The pathological manifestations in parabiotic UUO mice were similar to those in the control UUO mice of the same age, i.e., there was no significant difference between Y-IPuuo and Yuuo groups or between O-IPuuo and Ouuo groups (images not shown). The extent of pathological damage in the O-HPuuo7 group was similar to that in the O-IPuuo7 and Ouuo7 groups (Figure S2). The trend of kidney damage in O-HPuuo14 was comparable to that in Y-IPuuo14 or Yuuo14 group, i.e., the O-HPuuo14 group did not exhibit aggravation of renal tubular epithelial cell necrosis and shedding. In addition, the extent of interstitial fibrosis was significantly lower than that in the O-IPuuo14 and Ouuo14 groups (Figure S2). These findings indicate that youthful blood environment during parabiosis alleviated kidney tissue damage in elderly UUO mice.

Youthful blood environment alleviates renal interstitial fibrosis in elderly UUO mice

The expressions of fibrosis-associated proteins such as ColI, α-SMA, and TGF-β1 in the Ouuo14 group were significantly higher than those in the Yuuo14 group. These protein expressions in the Y-IPuuo14 and O-IPuuo14 groups were similar to those in the Yuuo14 and Ouuo14 groups, respectively. The expressions of these three proteins in the O-HPuuo14 group were significantly lower than those in the O-IPuuo14 and Ouuo14 groups, and similar to those in the Y-IPuuo14 and Yuuo14 groups (Figure S2). These results indicate that youthful blood environment may alleviate renal interstitial fibrosis in elderly UUO mice.

Blood factor SCF expression is closely related to increasing age and renal interstitial fibrosis

The serum SCF expression in elderly mice was significantly higher than that in young mice, as indicated by ELISA assay (Fig. 1A). The serum expression of SCF in healthy people exhibited a positive correlation with age (Fig. 1B). The expressions of SCF and its receptor c-Kit in elderly mice kidney tissues were significantly higher than those in young mice (Fig. 1C, 1D).

In order to verify the role of SCF/c-Kit in renal interstitial fibrosis, the expressions of SCF and its receptor c-Kit were determined at 7 and 14 days after UUO surgery. Compared with the sham group, the expressions of these two proteins were significantly upregulated 7 days after UUO surgery. It is noteworthy that their expressions at 14 days were significantly higher than those at 7 days and in the sham groups (Fig. 1E, 1F). These findings indicate that SCF/c-Kit may be involved in renal interstitial fibrosis caused by UUO surgery.

c-Kit gene mutation facilitates renal function recovery after UUO surgery

In order to further understand the role of SCF/c-Kit in renal fibrosis, homozygous *c-Kit* mutant Wps/Wps mouse strain was used in the subsequent experiments. Wps/Wps mouse strain is characterized by loss-of-function mutation in the *c-Kit* gene. No obvious difference of pathological staining and renal function were observed between Wps/Wps mice and wildtype mice of the same age (Fig. 2A, 2B). The degree of increase in serum creatinine level in Wps/Wps 7 days after UUO surgery was significantly lower than that in wildtype mice (1.18 times and 1.82 times the baseline level, respectively). This was also the case with respect to the BUN level, which was 1.28 times and 2.14 times the baseline value, respectively. The serum creatinine and BUN levels of Wps/Wps mice almost returned to baseline levels 14 days after UUO surgery (Fig. 2B-2D). These results indicate that deficiency of c-Kit facilitates renal function recovery after UUO surgery.

c-Kit gene mutation alleviates kidney tissue damage, interstitial fibrosis, and NF-κB phosphorylation in UUO model

Renal tubular epithelial cell swelling, necrosis and shedding, formation of casts, interstitial edema, and fibrosis were observed in the WTuuo14 group. Compared to the WTuuo14 group, the kidney damage and the fibrosis areas were significantly reduced in the Wps/Wpsuuo14 group (Fig. 2E, 2F), which suggests that *c-Kit* gene mutation may alleviate UUO-induced kidney tissue damage and renal interstitial fibrosis.

The expressions of fibrosis-related proteins such as ColI, α-SMA, and TGF-β1 in the WTuuo14 group were significantly greater than that in the control and the Wps/Wpsuuo14 groups (Fig. 3A–3D). The immunofluorescence results indicated that the α-SMA- and TGF-β1-positive cell ratios in the Wps/Wpsuuo14 group were significantly lower than that in wildtype mice (Fig. 3F-3I), which further indicates that c-Kit deficiency contributed to the alleviation of UUO-induced renal interstitial fibrosis. The ratio of phosphor-NF-κB/total NF-κB was upregulated after UUO surgery. Moreover, the elevated level of

the ratio in the Wps/Wpsuuo14 group was significantly lower than that in the WTuuo14 group (Fig. 3A, 3E), which suggests that SCF/c-Kit may activate NF-κB.

Youthful blood environment may alleviate elderly mice kidney fibrosis by reducing NF-κB activation

The expressions of SCF, c-Kit, TGF-β1, and the phosphor-NF-κB/total NF-κB ratio in the Ouuo14 group were significantly higher than those in the Yuuo14 group ($p < 0.01$). Compared to the Ouuo14 and O-lPuuo14 groups, their expressions in the O-HPuuo14 group decreased significantly ($p < 0.01$). The expressions of SCF and TGF-β1 in the O-HPuuo14 group were similar to those in the Y-lPuuo14 group ($p > 0.05$) (Figure S3A-S3E). The phosphor-NF-κB/total NF-κB ratio in the O-HPuuo14 group was remarkably lower than that in the Ouuo14 and O-lPuuo14 groups. These results suggest that youthful blood environment downregulates the expression of SCF/c-Kit in elderly UUO mice and inhibits renal fibrosis, and that these effects may be mediated via the NF-κB pathway.

Discussion

In our previous study, we have identified that youthful blood environment can reduce the extent of age-related increase in kidney fibrosis area using parabiotic mice model [9]. However, when the elderly rat kidney was transplanted into a young rat, no obvious change in kidney fibrosis of the elderly rat was observed [10]. In this study, we further sought to clarify the effect of blood environmental change on senile renal interstitial fibrosis. We established the UUO model using parabiotic mice. UUO surgery was performed 3 weeks after parabiosis. One reason is that the stable common circulation is formed usually 2 weeks after parabiotic surgery. In addition, the death rate is high in the first 2–3 weeks after parabiotic surgery. Therefore, by selecting parabiotic mice 3 weeks after parabiosis, the failure of modeling caused by ‘parabiosis disease’ can be avoided to save time and costs. The success rate of the parabiotic mice model combined with UUO surgery was 80–90%, which laid the foundation for further research.

Compared with the young individuals, older individuals have poor tolerance for various types of trauma, and it is not easy to recover after more serious injury [22]. It has been shown that a significantly higher proportion of elderly patients progress to end-stage renal disease after acute kidney injury or unilateral ureteral obstruction as compared to young patients [23]. In this study, the levels of serum creatinine and BUN in young mice reached peak levels at 7–10 days after UUO surgery, and subsequently recovered to varying degrees at 14 days after surgery; these changes may be associated with the compensatory effect of the contralateral kidney [24]. However, in case of elderly mice, the renal function continued to deteriorate until 14 days after surgery, which suggests decreased compensatory function and lower ability to repair damaged kidney of elderly mice. The renal function of O-HPuuo14 mice did not get worse or even improved, which suggests that youthful blood environment can reduce the renal injury caused by UUO, and promote renal function recovery after damage.

The pathological process of renal fibrosis can be divided into the following phases [15]: (1) start-up phase: cell activation and damage; (2) activation phase: activation of fibrosis signaling pathway; (3) fibrosis stage: extracellular matrix accumulation period; (4) fibrosis progression phase. Renal blood flow

and glomerular filtration rate were shown to decrease within 24 hours of complete unilateral ureteral obstruction, followed by development of hydronephrosis, tubulo-interstitial inflammatory infiltration, and kidney tubular cell apoptosis or necrosis [25]. Severe hydronephrosis and atrophy of renal parenchyma typically occurs 1–2 weeks after UUO surgery [26]. In this study, the UUO-induced kidney tissue injury was more severe in elderly mice compared to young mice, and was accompanied with severe renal interstitial fibrosis at 2 weeks after surgery. The extent of kidney damage or fibrosis in the O-HPuuo mice was lower than that in the Ouuo or O-IPuuo mice. The difference was especially obvious at 2 weeks after surgery, i.e., around the time of the formation and accumulation of extracellular matrix [27]. This indicates that some 'anti-fibrosis' factors may exist in youthful blood environment which play a role in inhibiting kidney fibrosis after UUO. Studies have shown that multiple signaling pathways are involved in renal fibrosis process, including the TGF-β/Smad [20, 28], Wnt/beta-catenin [29], and p38MAPK [30] pathways. Moreover, the sharp decline in renal blood flow and glomerular filtration rate after UUO surgery and the elevated angiotensin II level promote secretion of TGF-β1 by macrophages, which triggers the proliferation of fibroblasts and their differentiation to myofibroblasts; the myofibroblasts secrete collagen that is helpful for renal tubular interstitial fibrosis [31]. High expression of TGF-β1 has been shown to be closely related to renal fibrosis [32–34]. Our research shows that youthful blood environment can significantly reduce the expression of TGF-β1 in elderly renal tissue after UUO, and can also reduce the protein expressions of myofibroblast surface markers α-SMA and collagen ColI.

In previous study, we compared the serum cytokine levels in young and elderly mice with 5 weeks of IP or HP, and found that stem cell factor (SCF) may be associated with renal senescence; in addition, we confirmed higher expression of SCF in blood of elderly mice. Several studies have shown high expression levels of SCF and its receptor c-Kit in renal fibrosis tissue [18–20]. In this study, the expressions of SCF and c-Kit in kidney tissue of elderly mice were significantly higher than those in young mice. The expressions of SCF and c-Kit in renal tissue after UUO were significantly higher than those in the sham group, and continued to increase with increase in the duration of renal obstruction; these findings suggest that SCF/c-Kit are important for UUO-induced renal tissue damage. We further used loss-of-function *c-Kit* gene mutant mice Wps/Wps strain to construct UUO model, and the mutant mice showed lower degree of kidney fibrosis, lower levels of serum creatinine and BUN, and decreased expressions of key fibrosis-related cytokines TGF-β, and myofibroblast surface markers α-SMA and collagen ColI, compared to wildtype mice. In addition, the serum creatinine and BUN levels almost recovered to baseline level 2 weeks after UUO. These results further confirmed the pro-fibrogenic effects of SCF/c-Kit during renal tubular interstitial fibrosis. Blockade of the SCF/c-Kit signaling pathway can alleviate UUO-induced renal interstitial fibrosis and promote renal function recovery after UUO injury.

Binding of SCF to its receptor c-Kit has been shown to promote the expressions of a variety of inflammatory cytokines, including histamine, tryptase, transforming growth factor (TGF-β), fibroblast growth factor (FGF-β), and NF-κB [35, 36]. Studies have shown that SCF/c-Kit can mediate airway inflammation through activation of the NF-κB signaling pathway [37], regulate tumor cell inflammatory microenvironment [38], promote pulmonary interstitial fibrosis [39], and remodel the vascular neural network in chronic stroke patients [40]. In a fundamental research about spontaneous hypertension, high

expressions of SCF/c-Kit were shown to activate NF-κB expression, and promote myocardial hypertrophy and myocardial fibrosis [41]. In this study, more severe UUO-induced injury was observed in elderly mice with high expressions of SCF/c-Kit and NF-κB, compared to young mice. The youthful blood environment in O-HPuuo mice downregulated the expressions of SCF/c-Kit and NF-κB, and the extent of kidney damage in O-HPuuo14 was similar to that in the young mice group. Moreover, the c-Kit loss-of-function mutant Wps/Wps mice exhibited decreased expression of NF-κB and reduced extent of kidney interstitial fibrosis. These results indicate that youthful blood environment downregulates the high expression of SCF/c-Kit in elderly kidney tissue, and subsequently inhibits NF-κB expression and decreases elderly renal interstitial fibrosis.

Conclusions

We established a combination mice model of unilateral ureteral obstructive (UUO) and parabiotic surgeries, and found that youthful blood environment can significantly reduce UUO-induced renal interstitial fibrosis in elderly mice, and promote kidney function recovery by downregulating the expression of SCF/c-Kit in elderly kidney tissue. Using Wps/Wps mice with loss-of-function mutation in SCF receptor c-Kit, we demonstrated for the first time the role of SCF/c-Kit in the process of senile renal fibrosis, which may be mediated via the NF-κB pathway. These results provide the basic experimental evidence for prevention and treatment of renal interstitial fibrosis, which may help formulate strategies to attenuate kidney disease progression and age-related renal damage.

Abbreviations

SCF: stem cell factor;

UUO: unilateral ureteral obstructive;

IP: isochronic parabiotic;

HP: heterochronic parabiotic;

WT: wildtype;

IRI: ischemia-reperfusion injury;

MGF: mast cell growth factor;

KL: c-Kit ligand;

BSA: bovine serum albumin;

TGF-β: transforming growth factor;

FGF-β: fibroblast growth factor.

Declarations

Ethics approval

All animal experiments were approved and performed in accordance with the Chinese PLA General Hospital's Committee on Animal Protection and Utilization.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files]

Competing interests

The authors declare that they have no competing interests

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Author contributions

Xuefeng Sun, Xiangmei Chen and Guangyan Cai designed the experiment; Qi Huang, Dong Liu, Shaoyuan Cui, Zhong Yin and Yinping Zhang performed the experiment; Zan Huang provided the Wps/Wps mice; Qi Huang completed the paper; and Xuefeng Sun revised the paper.

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Figures

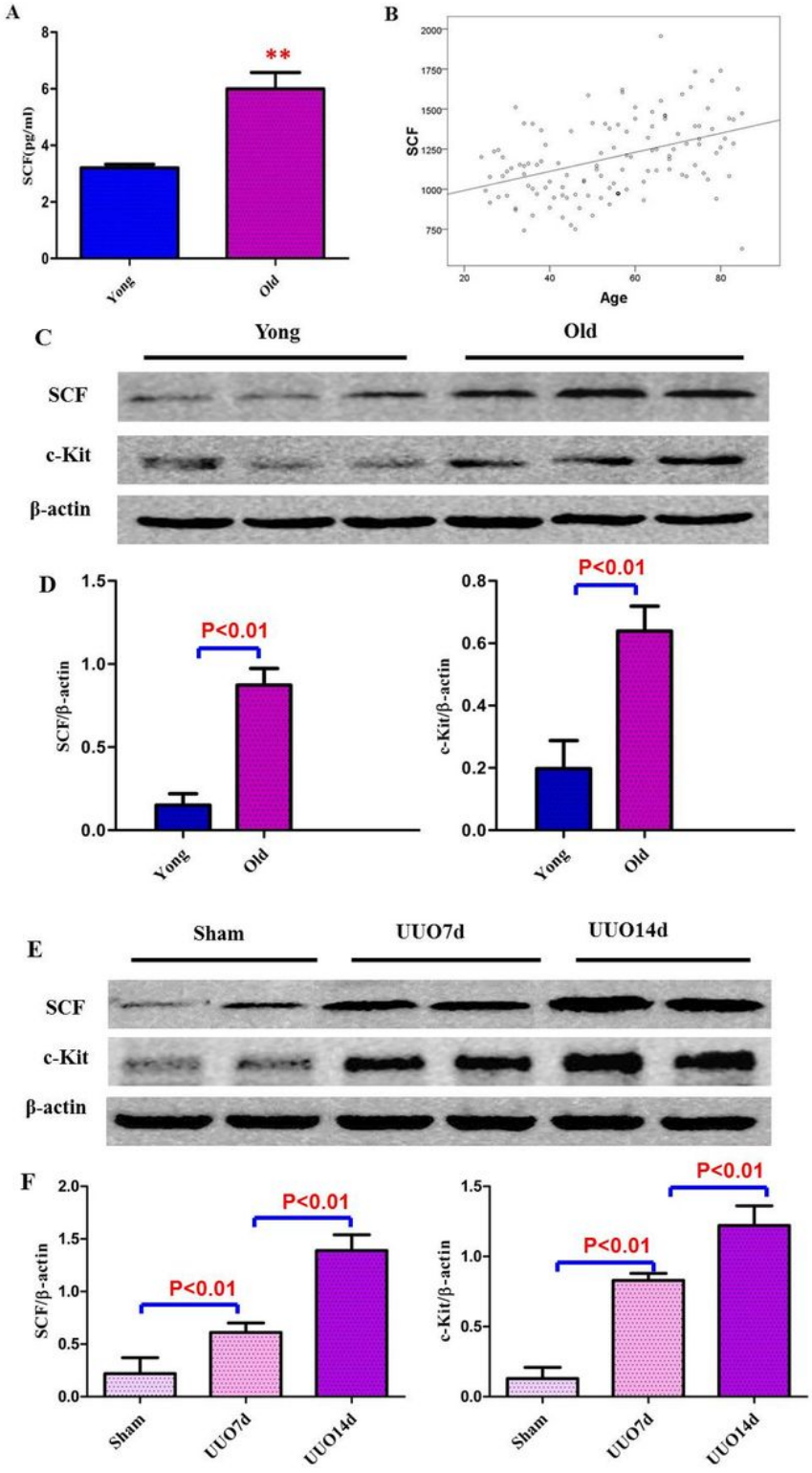
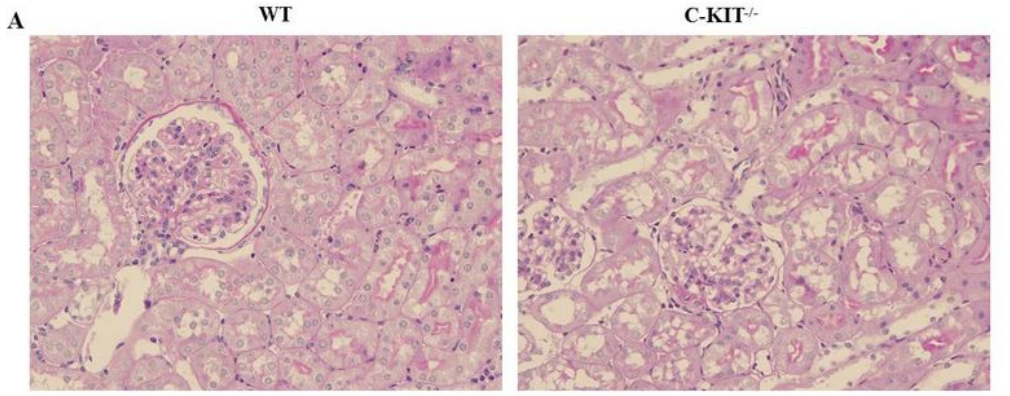


Figure 1

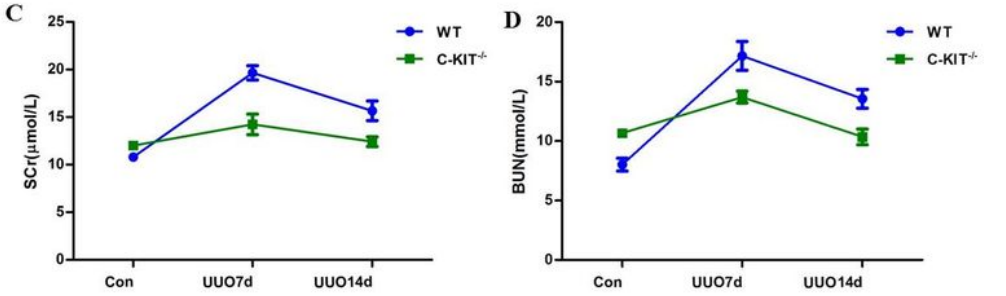
Blood factor SCF expression is closely related to increasing age and renal interstitial fibrosis. (A) The serum SCF expression in young and old groups. ** P < 0.01 vs. Young. (B) The serum SCF in healthy people with different age. (C) The expressions of SCF and its receptor c-Kit in young and old groups. Western Blot analysis showed both of the proteins in elderly mice kidney tissues were significantly higher than those in young mice. (D) Graph representing quantitative analysis results of SCF and c-Kit. (E) The

expressions of SCF and c-Kit at different time after surgery. Western Blot analysis showed 14 days after UUO surgery, both of proteins were significantly higher than those at 7 days and in the sham groups. (F) Graph representing quantitative analysis results of SCF and c-Kit. UUO: unilateral ureteral obstructive; UUO7d: 7 days after UUO surgery; UUO14d: 14 days after UUO surgery.



B

	Group	Con	UUO7d	UUO14d
SCr (μmol/L)	WT	10.8±0.31	19.66±0.75[a]	15.66±1.03[a]
	C-KIT-/-	12.02±0.29	14.24±1.08	12.42±0.51
BUN (mmol/L)	WT	8.02±0.54	17.16±1.22[a]	13.55±0.79[a]
	C-KIT-/-	10.68±0.16	13.70±0.49	10.35±0.66



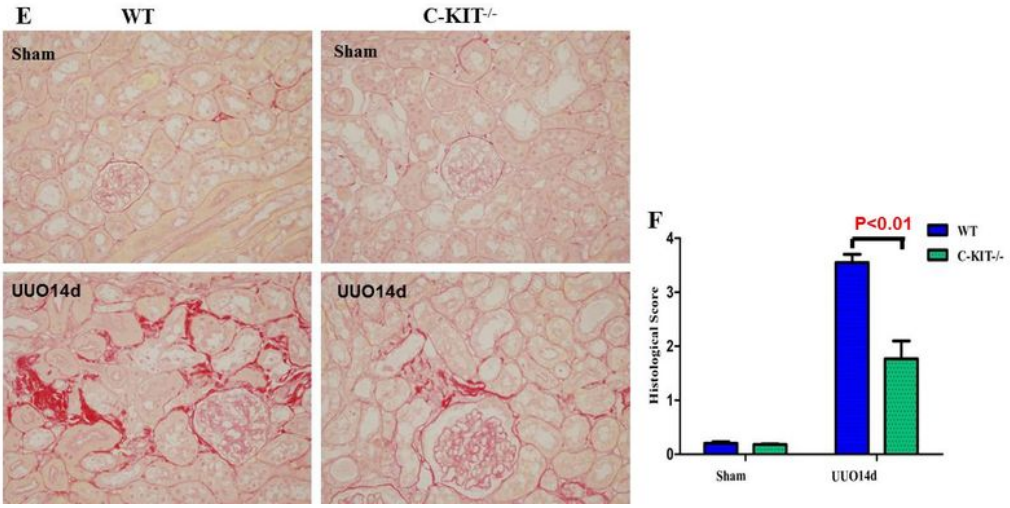


Figure 2

c-Kit gene mutation facilitates renal function recovery and alleviates kidney tissue damage after UUO surgery. (A) Periodic Acid-Schiff (PAS) staining results of WT and C-KIT-/- groups, no obvious difference of pathological staining between these two groups. (C) SCr levels at different time after surgery in the two groups. (D) BUN levels in at different time after surgery in the two groups. (B) Detailed data of SCr and BUN levels in each group; a, $P < 0.01$ vs. con. (E) Sirius-red staining results after 14 days surgery in the two groups (original magnification, 400×). Compared to the WTuuo14 group, the kidney damage and the fibrosis areas were significantly reduced in the C-KIT-/-uuo14 groups. (F) Graph representing interstitial fibrosis lesions measured by NIH semi quantitative scoring method after Sirius-red staining. Ten fields were randomly selected from each slide to calculate the pathological scores, data shown as mean±sd. SCr: serum creatinine; BUN: blood urine nitrogen; WT: wild type; C-KIT-/-: loss-of-function mutation in the c-Kit gene; Con: control; UUO: unilateral ureteral obstructive; UUO7d: 7 days after UUO surgery; UUO14d: 14 days after UUO surgery.

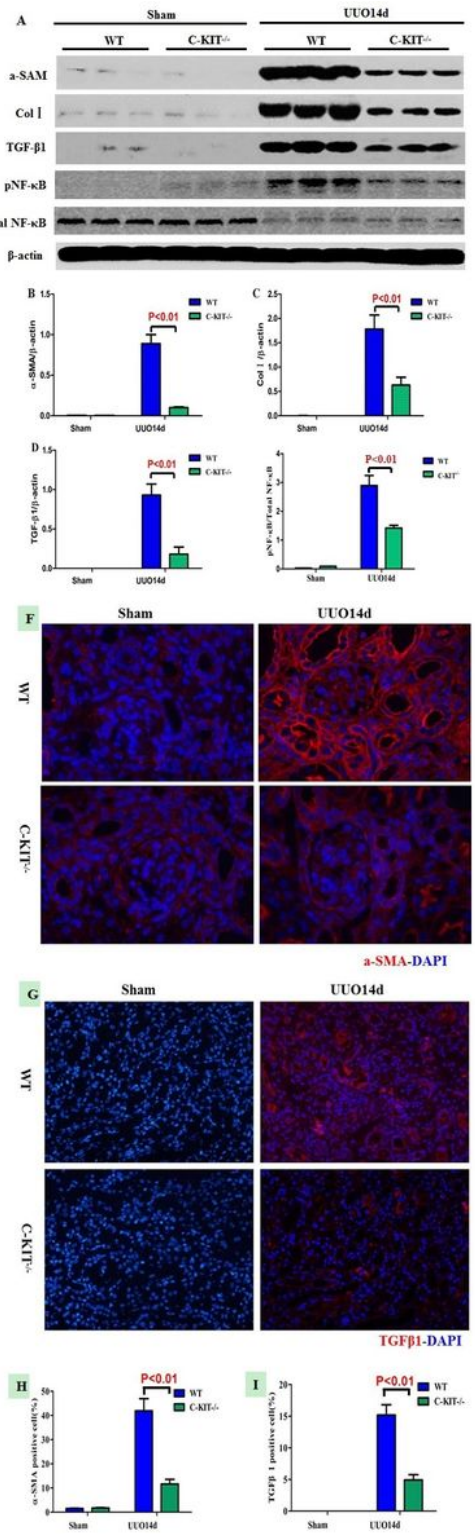


Figure 3

c-Kit gene mutation alleviates kidney interstitial fibrosis and NF-κB phosphorylation in UUO model. (A) The expressions of phosphor-NF-κB and fibrosis-related proteins ColI, α-SMA, and TGF-β1 in the four groups. Western Blot analysis showed 14 days after UUO surgery, all these fibrosis-related proteins and the ratio of phosphor-NF-κB/total NF-κB in WT groups were significantly higher than those in C-KIT-/- groups. (B-E) Graph representing quantitative analysis results of these four proteins. (F)

Immunofluorescence staining of fibrosis-related proteins α-SMA. Representative photographs from independent groups (original magnification, 400×). (H) Graph representing the percentage of α-SMA positive cells. (G) Immunofluorescence staining of fibrosis-related proteins TGF-β1. Representative photographs from independent groups (original magnification, 200×). (I) Graph representing the percentage of TGF-β1 positive cells. WT: wild type; C-KIT-/-: loss-of-function mutation in the c-Kit gene; UUO: unilateral ureteral obstructive; UUO14d: 14 days after UUO surgery.

Supplementary Files

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