INTRODUCTION

Kidney ischemia-reperfusion (I/R) injury occurs in a variety of clinical scenarios, including kidney transplantation, and accounts for the major cause of acute kidney injury (AKI). As a common and serious clinical problem, AKI in postsurgical patients often results in renal dysfunction and is negatively associated with the outcome of kidney transplantation. Even though great advances have been made, AKI is still associated with the high morbidity and mortality (1, 2). Accordingly, there is an urgent need to develop new effective therapeutic strategies to promote kidney repair and regeneration.

Side population (SP) cells were initially identified from mouse bone marrow hematopoietic stem cells using the Hoechst 33342 dye stain (3). Over the past decade, increasing evidences rank SP cells as highly enriched stem cells that can exert the crucial roles in injury repair and regeneration by possessing their stem cell-like features, such as self-renewal and multiple differentiation ability (4, 7). The ATP-binding cassette transporter ABCG2, an essential element for SP-mediated kidney regeneration after renal I/R injury. Moreover, AMD3100 pretreatment strikingly attenuated ABCG2 elevation in SP cells. Additionally, sonic hedgehog (SHH)-Gli 1 signaling was involved in SDF-1/CXCR4-mediated ABCG2 expression. When SP cells pretreated with AMD3100 were intravenously injected into I/R mice, SP cell-mediated decreases in blood urea nitrogen, serum creatinine, and histological score of kidney were noticeably attenuated, indicating that blocking CXCR4 pathway mitigated the therapeutic function of SP cells in renal I/R injury. Together, this research suggests that SDF-1/CXCR4 axis might act, via Shh-Gli1-ABCG2 signaling, as a positive regulator of SP cell-based therapies for renal I/R by Shh-Gli 1-ABCG2 signaling.

KEYWORDS—ABCG2, renal I/R injury, SDF-1/CXCR4, Shh signaling, SP cells

THE FUNCTION OF SDF-1-CXCR4 AXIS IN SP CELLS-MEDIATED PROTECTIVE ROLE FOR RENAL ISCHEMIA/REPERFUSION INJURY BY SHH/GLI1-ABCG2 PATHWAY

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ABSTRACT—Renal ischemia-reperfusion (I/R) injury ranks as the primary cause of acute renal injury with severe morbidity and mortality. Side population (SP) cells have recently drawn increasing attention due to their critical role in injury repair and regeneration. Unfortunately, the underlying mechanism involved in renal I/R remains poorly elucidated. Here, pronounced increases of stromal cell-derived factor-1 (SDF-1) and its receptor CXC chemokine receptor 4 (CXCR4) were substantiated in I/R kidneys from C57BL/6 mice subjected to clamp the bilateral renal pedicles to mimic renal ischemia. Similar up-regulation of them was also determined in SP cells upon simulated ischemia/reperfusion (SI/R). In contrast to non-SP cells, SP cells exhibited higher viability, apoptosis resistance, chemotaxis, and paracrine actions following SI/R treatment, and these were further enhanced after SDF-1 stimulation. Interestingly, blocking CXCR4 signaling with AMD3100 notably ameliorated the above effects. Mechanism analysis corroborated that SDF-1/CXCR4 further induced the expression of ATP-binding cassette transporter ABCG2, an essential element for SP-mediated kidney regeneration after renal I/R injury. Moreover, AMD3100 pretreatment strikingly attenuated ABCG2 elevation in SP cells. Additionally, sonic hedgehog (SHH)-Gli 1 signaling was involved in SDF-1/CXCR4-mediated ABCG2 expression. When SP cells pretreated with AMD3100 were intravenously injected into I/R mice, SP cell-mediated decreases in blood urea nitrogen, serum creatinine, and histological score of kidney were noticeably attenuated, indicating that blocking CXCR4 pathway mitigated the therapeutic function of SP cells in renal I/R injury. Together, this research suggests that SDF-1/CXCR4 axis might act, via Shh-Gli1-ABCG2 signaling, as a positive regulator of SP cell-based therapies for renal I/R by Shh-Gli 1-ABCG2 signaling.

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performed to address the role of the SDF-1/CXCR4 axis in SP cell-mediated injury repair.

In this study, we first conferred the up-regulation of SDF-1/CXCR4 in kidney SP cells upon simulated ischemia/reperfusion (SI/R) injury. Furthermore, the possible involvement of SDF-1/CXCR4 signaling in SP cell injury, chemotaxis, and paracrine action was investigated in vitro. Moreover, its effect on SP-regulated repair was also explored in vivo.

MATERIALS AND METHODS

Antibodies and reagents

The primary antibodies against mouse SDF-1, CXCR4, caspase-3and proliferating cell nuclear antigen (PCNA) were from Santa Cruz Biotechnology (Santa Cruz, Calif). Rabbit polyclonal antibodies to sonic hedgehog (Shh), Glioma-associated oncogene homolog 1 (Gli 1), and ABCG2 were obtained from Abcam (Cambridge, UK). The anti-mouse Bax and Bcl-2 antibodies were purchased from Cell Signaling Technology (Beverly, Mass). The specific CXCR4 antagonist, AMD3100, was bought from Sigma (St. Louis, Mo). A specific Shh signal inhibitor KAAD-cyclopamine was obtained from Toronto Research Chemicals (North York, Ontario, Canada).

Animals

For use in this experiment, male 7 to 8-week-old C57BL/6 mice (20–23 g each) were purchased from the Center of the Fourth Military Medical University (Xi’an, China). All animals were maintained in accordance with the ARRIVE Guidelines and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. All animals were housed in a temperature-controlled facility with a 12h light/dark cycle and maintained on a diet of standard laboratory chow and water ad libitum. The mice were acclimatized for at least 2 weeks prior to the subsequent treatments. Animal procedures were approved by the Institutional Animal Care and Use Committee of the Xijing Hospital of Fourth Military Medical University.

Isolation and identity of kidney SP cells

Kidney cells were prepared as described previously (8). To isolate SP cells, 100 μL of kidney cell suspension (1 × 10^6 cells/mL) was incubated in DMEM medium containing 5 μg/mL Hoechst 33342 (Sigma, with or without 50 μM verapamil, for 1 h at 37 °C. After washing, cells were stained with FITC-conjugated anti-Cd45 or anti-c-kit antibody (BD Biosciences, San Jose, Calif) for 20 min on ice. Then, 2 μg/mL of propidium iodide (PI) was added to discriminate live cells from dead cells, followed by analysis in a FACSAria II flow cytometer (BD Biosciences). Cell sorting was carried out via FACS (BD Biosciences) as reported previously (8). For FACs analysis, cells with Hoechst efflux potential were included in SP gate. Cells without efflux potential were defined as non-SP cells.

Cell culture

Primary renal tubule epithelial cells (TECs) were isolated from mice as previously described (18). All cells including SP cells, non-SP cells, and TECs were cultured in DMEM medium containing 10% fetal bovine serum (FBS) and 1% antibiotic solution (Sigma) to initiate reperfusion. Twenty-four hours after surgery, 0.5 mL of SP cells from each mouse was injected into I/R mice through tail vein with a 27-gauge cannula connected to a polyethylene catheter (Braun Melsungen AG, Melsungen, Germany).

Annexin V/PI apoptosis assay

The apoptosis ratio was evaluated using the Annexin V-FITC Apoptosis Detection Kit (Beiyotime, Beijing, China). Briefly, cells (1 × 10^5) were incubated with 500 μL of Binding Buffer containing 5 μL Annexin V and PI under dark. Ten minutes later, the specimens were subjected to flow cytometer. The percentages of apoptotic cells were quantified using BD FACSdiva 6.1.3 software (BD Biosciences).

Analysis of lactate dehydrogenase (LDH) release

The concentration of LDH was measured by a Lactate Dehydrogenase Activity Colorimetric Assay Kit (BioVision, Milpitas, Calif) according to the manufacturers’ instruction. The assay of caspase-3 activity was performed in a fluorometer (model FL600, BioTek Instruments, Inc).

Assay of chemotaxis by transwell system

After hypoxia/reoxygenation, cells (1 × 10^5) under SI/R, or not, were added to the upper chamber in the presence or absence of AMD3100 (50 μg/mL). The culture medium containing 10% FBS, SDF-1 (50 ng/mL), or SI/R-treated TECs (1 × 10^5) was placed in the lower chamber. After 24 h incubation, the non-migrating cells were removed. The migrating cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet (Beiyotime). The number of cells migrating through the membrane was counted from a minimum of five randomly selected visual fields by an inverted microscope.

ELISA analysis

The supernatants from different specimens were harvested and transferred to 96-well plates. The concentrations of SDF-1, vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) were measured using ELISA kit (R&D Systems, Minneapolis, Minn) according to the manufacturers’ protocol.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TRIzol (Sigma) and then reverse transcribed into the first strand cDNA using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, Calif). Approximately 2 μL of cDNA was subjected to real-time PCR assay using the SYBR Green PCR Master Mix (Qiagen, Hilden, Germany). The specific primers were designed as follows: SDF-1, 5'-ATTAGGATATCCCAGAAG-3' (sense), and 5'-GGTCCAATGAGATCCAATG-3' (antisense); CXCR4, 5'-AAAGCTAGCCGACATCCTCA-3' (sense) and 5'-CACATTTAGCTGTGTTTGTGTT-3' (antisense); PCNA, 5'-CAACGTTATATGGCCGAGACCT-3' (sense), and 5'-CCGCTTTTTTCTTATAT-3' (antisense). The mRNA levels of each sample were normalized to GAPDH. All data were calculated as 2^-ΔΔCt.

Western blotting

Total protein concentrations were determined using the BCA protein assay kit (Pierce, Rockford, Ill) and then subjected to 12% SDS-PAGE, followed by transfer to PVDF membrane. For immunoblotting, membranes were incubated for 1 h with primary antibodies against SDF-1 (1:200), CXCR4 (1:250), caspase-3 (1:200), ABCG2 (1:250), Shh (1:100), Gli 1 (1:200), Bax (1:200), and Bcl-2 (1:100). Then, horseradish peroxidase-conjugated secondary antibody was introduced. The binding proteins were detected by ECL reagent (Santa Cruz Biotechnology) and band intensity was quantified using Quantity One (Bio-Rad, Hercules, Calif).

Experiment model of renal I/R injury

After anesthetizing with a ketamine–xylazine mixture, renal ischemia was mimicked in the mice by clamping the bilateral renal pedicles through midline incisions using a microvascular clip. About 30 min later, the clamp was released to initiate reperfusion. Twenty-four hours after surgery, 0.5 mL of SP cells (1 × 10^5 cells/mL) pretreated with or without AMD3100 (50 μg/mL) for 2 h were injected into IR mice through tail vein with a 27-gauge cannula connected to a polyethylene catheter (Braun Melsungen AG, Melsungen, Germany).
Two days after cell transplantation, mice were euthanized by an overdose of pentobarbital. Then, serum and tissue specimens were collected and stored at −80 °C. All efforts were made to minimize suffering.

**Biochemical and histological evaluation**

To evaluate renal function, the levels of blood urea nitrogen (BUN) and serum creatinine (Scr) were determined by the specific assay kits (Arbor Assays, Ann Arbor, Mich) according to the manufacturers’ protocol. For histology analysis, serial 4-μm-thick sections of excised kidneys were acquired and stained with H&E (Sigma). The histological score for kidney (HSK) was assessed in a blind manner by a pathologist with the standard reported previously (19). The total score of each section was counted by the addition of all 10 scores with a maximum possible injury score of 30. Necrotic core areas were assessed based on H&E and quantified by planimetry (Diskus software; Hilgers, Bonn, Germany).

**Immunohistochemical assay**

Sections were incubated with the primary antibody against PCNA (1:200) at 4°C overnight. After washing, the biotinylated goat antirabbit IgG (Abcam) was introduced as the secondary antibody. The specific binding signals were visualized by DAB, followed by the counterstaining with hematoxylin. TUNEL staining was carried out to estimate tubular cell apoptosis (In Situ Cell Death Detection Kit; Roche Diagnostics, Mannheim, Germany). To count the number of PCNA- and TUNEL-positive cells in sections of kidney, 10 fields in high-power fields (×40 magnification) from each section were randomly picked and visualized under light or fluorescence microscope.

**Statistical analysis**

Data were presented as the mean ± SD. All statistical analyses were estimated using the Student t test or ANOVA. SPSS 11.0 was used to analyze all the data. All experiments were performed at least three times. *P < 0.05 was considered statistically significant.

**RESULTS**

**Expression of SDF-1/CXCR4 in postischemic kidneys and SP cells upon SI/R**

As shown in Figure 1A, a dramatic up-regulation of SDF-1 was observed at 24 h in kidneys that had suffered I/R injury, concomitant with a similar increase in CXCR4 protein levels, implying a potential role for renal I/R injury. After staining with Hoechst 33342, we successfully isolated kidney SP cells (Fig. 1B). To mimic I/R injury, SP cells were cultured upon SI/R. Here, SI/R pretreatment saliently increased the concentration of SDF-1 in supernatant into 448.75 ± 8.64 pg/mL (Fig. 1C). Moreover, the mRNA levels of SDF-1 and CXCR4 were notably up-regulated in SP cells after exposure to 8 h hypoxia and 10 h reoxygenation (Fig. 1D). Simultaneously, western blotting analysis demonstrated the higher expression of SDF-1 and CXCR4 protein in SP cells following SI/R injury.
Further quantitative analysis suggested that SI/R treatment induced 2.91-fold and 3.01-fold increases in SDF-1 and CXCR4 protein levels, respectively (Fig. 1E).

### SDF-1/CXCR4 protects SP cells from SI/R-induced injury

The therapeutic potential of SP cells in renal I/R injury has been reported previously (8). To clarify the underlying mechanism, the function of SDF-1/CXCR4 was investigated. As shown in Figure 2A, SP cells exhibited lower SI/R-induced cytotoxicity than non-SP cells. Simultaneously, the higher mRNA levels of proliferate-related marker PCNA were also validated in SP cells compared with non-SP cells (Fig. 2B). Moreover, compared with non-SP cells, SP cells showed less membrane injury as measured by decreasing LDH release (Fig. 2C), apoptotic rate (Fig. 2D), and caspase-3 activity (Fig. 2E). Interestingly, SDF-1 incubation clearly increased SP cell resistance to SI/R-induced cell cytotoxicity, but not in the non-SP cells (Fig. 2, A–E). Treatment with AMD3100 obviously suppressed the expression of CXCR4 (Fig. 2F). Importantly, pretreatment with CXCR4 inhibitor AMD3100 remarkably abrogated SP cell viability (Fig. 2G) under SI/R condition. Additionally, administration with AMD3100 also augmented LDH concentration in SI/R-treated SP cells under SDF-1 stimulation (Fig. 2H). Flow-cytometric analysis revealed that the apoptotic rate of SP cells upon SI/R decreased into 16.01 ± 1.64 % when cells were incubated with SDF-1, which was increased to 30.56 ± 4.02% following AMD3100 pretreatment (Fig. 2I). Therefore, these results indicated a potential protective role of SDF-1/CXCR4 pathway in SI/R-induced SP cell injury.

### SDF-1/CXCR4 axis is responsible for SP cell chemotaxis and paracrine actions

Function assay corroborated that SI/R treatment profoundly triggered SP cell chemotaxis, but this did not occur in the non-SP cells (Fig. 3A). Moreover, SDF-1 further enhanced the

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**Fig. 2.** SDF-1/CXCR4 enhanced SP cell resistance to SI/R-induced injury. Cells were treated with SI/R under SDF-1 stimulation (50 ng/mL), or not. A, Cells viability was determined using MTT assay following treatment with SI/R or SI/R and SDF-1. B, The corresponding mRNA levels of PCNA were detected by qRT-PCR. C, The LDH concentration under SI/R or SI/R and SDF-1 stimulation was evaluated. D, After incubation in SI/R or SI/R + SDF-1, cell apoptosis was measured. E, Caspase-3 activity in cells exposure to SI/R and SI/R + SDF-1. F, The effect of AMD3100 on the expression of CXCR4. G, SP cells upon SI/R were pretreated with AMD3100 prior to SDF-1 stimulation or not. The effect on cell viability was analyzed. H and I, The LDH release and cell apoptosis were also assessed when cells were conducted with AMD3100 prior to SDF-1 stimulation. *P < 0.05. CXCR4 indicates CXC chemokine receptor 4; LDH, lactate dehydrogenase; PCNA, proliferating cell nuclear antigen; SDF-1, stromal cell-derived factor-1; SI/R, simulated ischemia/reperfusion; SP, side population.
chemotaxis of SP cells and the number of migrated cells elevated from 132.24 ± 6.22 to 214.36 ± 3.12 in response to SDF-1 stimulation (Fig. 3, A and B). However, the up-regulation in the amount of migrated cells obviously was reduced following AMD3100 (Fig. 3B). Next, we further assessed the effect of SI/R stimulation on SP cell paracrine actions. As expected, after SI/R exposure, the noticeable increases in VEGF (Fig. 3C) and HGF (Fig. 3D) levels were conferred in SP cells; however, there was little influence in non-SP cells. Notably, ELISA assay corroborated that AMD3100 pretreatment dampened remarkably SI/R-induced VEGF and HGF levels in SP cells treated with SDF-1, or not (Fig. 3E). Together, the data suggested that SDF-1/CXCR4 signaling might elicit pivotal function in SP cell chemotaxis and paracrine actions upon SI/R condition.

The SDF-1/CXCR4 axis is involved in SP cell migration toward SI/R-damaged renal tubule epithelial cells

MSCs can induce the regeneration of renal tubule epithelia and repair renal function (20). SP cells are defined as the enriched stem and progenitor cells; we next set out to examine the effect of SDF-1/CXCR4 on SP cell migration toward I/R-damaged renal tubule epithelial cells. After incubation under SI/R condition, the protein levels of SDF-1 were dramatically up-regulated by western blotting assay (Fig. 4A). Further quantitative analysis substantiated that SI/R triggered approximately 2.32-fold increase of SDF-1 protein expression in relative to control groups (Fig. 4A). Moreover, a notable increase in SP cell chemotaxis toward SI/R-injured TECs was confirmed and the amount of migrated cells increased into 21.45 ± 1.34 (Fig. 4B). However, this reinforced chemotaxis of SP cells was significantly abrogated following CXCR4 antagonist pretreatment as the evidence that AMD3100 treatment restrained the number of migrated cells.

SDF-1 regulates ABCG2 expression in SI/R-treated SP cells by Shh-Gli1 signaling

It is widely accepted that ABCG2 is critical for SP cell-mediated repair of kidney after I/R injury (8). To illustrate the underlying mechanism involved in SP-induced functional repair of damaged kidney, we detected the expression of ABCG2. Here, SI/R treatment dramatically enhanced ABCG2 expression (Fig. 5A). In contrast to control groups, there was approximately 3.42-fold up-regulation in ABCG2 protein levels following SI/R injury, which was further up-regulated following SDF-1 stimulation (Fig. 5A). Furthermore, a profound decrease in ABCG2 expression was observed after AMD3100 pretreatment (Fig. 5B). Further assay confirmed the activation of Shh-Gli 1 signaling upon SI/R condition as the 2.44-fold and 2.48-fold increases in Shh and Gli 1 protein levels, respectively. Moreover, these up-regulations further raised following SDF-1 stimulation (Fig. 5C). Treatment with Shh signal inhibitor of KAAD-cyclopamine noticeably abrogated the protein expression of Shh (Fig. 5D) and Gli 1 (Fig. 5E). Interestingly, KAAD-cyclopamine pretreatment not only suppressed the SI/R-induced elevation of ABCG2 in SP cells.

**Fig. 3.** Effect of SDF-1/CXCR4 on SP cell chemotaxis and paracrine actions upon SI/R. A, Cells were stimulated with SDF-1 under SI/R condition, or not. Transwell assay was performed to detect cell migration ability. B, Following pretreatment with AMD3100, cells were treated with SDF-1 upon SI/R condition. The numbers of migrated cells were determined. C and D, ELISA was used to measure the production of VEGF and HGF from SP cells under SI/R or SI/R+SDF-1 condition. E, After pretreatment with AMD3100, the corresponding effect on VEGF and HGF concentrations was determined upon SI/R or SI/R+SDF-1 administration. *P < 0.05. HGF indicates hepatocyte growth factor; VEGF, vascular endothelial growth factor.
cells, but also in SDF-1-treated groups (Fig. 5F), indicating that SDF-1 might enhance ABCG2 expression under SI/R environment by activating the Shh-Gli 1 pathway.

Blocking CXCR4 signaling attenuates the therapeutic effect of SP cells on renal I/R injury

As shown in Figure 6A and B, the prominent up-regulation of BUN and Scr was detected at 24 h post-I/R injury and reach 191.34 ± 8.21 mg/dI and 2.41 ± 0.33 mg/dI, respectively. Three days after transplantation, administration of SP cells dramatically antagonized the levels of BUN and Scr (Fig. 6, A and B). Upon histological evaluation, I/R groups exhibited the typical microscopic characterizations of acute tubular damage, such as extensive tubular necrosis (Fig. 6C). The HSK (Fig. 6D) and tubular necrosis (Fig. 6E) respectively decreased to 11.56 ± 1.78 and 8.23 ± 1.08 % in I/R injury mice that received SP treatment. Injection of SP cells significantly enhanced the number of PCNA-positive cells (Fig. 6F), accompanied with the decrease in amount of apoptotic cells (Fig. 6G).

Simultaneously, the notable down-regulation of caspase-3 and Bax was observed after SP cell treatment, concomitant with the increase in anti-apoptotic protein expression of Bcl-2 (Fig. 6H). Importantly, SP cells pretreated with AMD3100 strikingly abated the inhibitory effect of SP cells on BUN and Scr concentrations (Fig. 6, A and B). Moreover, the suppressive action of SP cells on HSK, tubular necrosis, and cell number were substantially counteracted after pretreatment with AMD3100 (Fig. 6, D–H).

DISCUSSION

Overall, this present study substantiated the pronounced up-regulation of SDF-1 and CXCR4 in kidney after I/R injury and in SP cells upon SI/R. Intriguingly, SDF-1/CXCR4 protected SP cells from SI/R-induced injury and promoted SP cell paracrine actions and chemotaxis. Additionally, SDF-1 increased ABCG2 expression in SI/R-pretreated SP cells by Shh-Gli 1 signaling. Notably, pretreatment with AMD3100 dramatically counteracted SP cell-triggered repair of renal function in vivo, implying that the SDF-1-CXCR4 axis may be involved in SP cells-mediated protective function in renal I/R injury via Shh/Gli1-ABCG2 pathway.

SP cells isolated from various cells have potential to reconstruct local tissue after injury because of their enrichment and characterization of stem/progenitor cells (7). In dental pulp, the pulp SP cells exhibit higher regenerative potential with the increased expression of CXCR4 in regenerated tissues (21). Recently, our previous study also validated that administration of kidney SP cells saliently improve renal function following I/R injury but not the non-SP cells, implying a good target for clinical renal regenerative therapy (8). Unfortunately, the underlying mechanism remains unclear. Interestingly, this present study confirmed the elevation of SDF-1 and CXCR4 in kidney in response to I/R injury, as well as in kidney SP cells upon SI/R, suggesting a potential relationship between the SDF-1/CXCR4 pathway and SP cells during renal I/R injury.

As a common chemokine, SDF-1 can be highly inducible in certain pathologic conditions, such as ischemia and/or hypoxia (9, 22). SDF-1 acts as an important mediator for tissue regenerative processes by promoting stem cell mobilization and migration to sites of ischemic injury (13, 14). Surprisingly, selective expression of SDF-1 in ischemic tissue is in direct proportion to reduced oxygen tensions (9). Whether SDF-1/CXCR4 signaling is involved in SP cells function to repair renal I/R injury? To elucidate the hypothesis, we constructed the SI/R models by hypoxia/reoxygenation in vitro. As expected, SDF-1 stimulation enhanced SP cell resistance to SI/R injury and cell chemotaxis. It is widely accepted that the regeneration-promoting properties of stem cells largely depend on the secretion and release of trophic factors, such as VEGF and HGF (23). Here, SDF-1 stimulation enhanced SP cell resistance to SI/R injury and cell chemotaxis. It is widely accepted that the regeneration-promoting properties of stem cells largely depend on the secretion and release of trophic factors, such as VEGF and HGF (23). Here, SDF-1 stimulation enhanced SP cell resistance to SI/R injury and cell chemotaxis. It is widely accepted that the regeneration-promoting properties of stem cells largely depend on the secretion and release of trophic factors, such as VEGF and HGF (23). Here, SDF-1 stimulation enhanced SP cell resistance to SI/R injury and cell chemotaxis. It is widely accepted that the regeneration-promoting properties of stem cells largely depend on the secretion and release of trophic factors, such as VEGF and HGF (23). Here, SDF-1 stimulation enhanced SP cell resistance to SI/R injury and cell chemotaxis.

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Fig. 4. SDF-1/CXCR4 axis participated in SP cell migration toward SI/R-damaged renal TECs. A. Following SI/R stimuli, the expression levels of SDF-1 in TECs were detected by western blotting. B. TECs were pretreated with AMD3100, and then were subjected to SI/R. Cell migration ability was evaluated. *P < 0.05. TECs indicates tubule epithelial cells.
the improved renal function attained by the infusion of SP cells was also ameliorated in renal I/R mice when blocking CXCR4 signaling by AMD3100. Therefore, these results indicate that SDF-1/CXCR4 signaling may be involved in SP cells-mediated protective role in the repair of renal I/R injury.

ABCG2 is identified as a pivotal determinant of SP phenotype and is associated with the anti-apoptotic activity of SP cells in contrast to non-SP cells (24). Following I/R injury in kidney, the expression of ABCG2 was increased (25). Suppressing its expression with verapamil remarkably attenuates the improved renal function after SP cell administration, indicating an essential role for kidney SP cells in renal regeneration (8). Considering these results, we further investigated ABCG2 expression. In consistent with our hypothesis, SI/R treatment induced the high expression of ABCG2 in SP cells, which was enhanced by SDF-1 stimulation. Importantly, cessation of CXCR4 signaling with AMD3100 alleviated the elevation in ABCG2 expression, implying that ABCG2 may account for SDF-1/CXCR4-mediated SP cell protective function in renal injury. However, how SDF-1/CXCR4 regulates ABCG2 expression remains unclear.

A recent study has demonstrated that the CXCL12/CXCR4 protein signaling axis can induce activation of sonic hedgehog (SHH) (26). Moreover, ABCG2 is confirmed to be a direct target of Gli 1, an important positive regulator of Hh transcriptional targets (27). Hedgehog (Hh) signaling exerts the critical roles in various pathological and physiological processes, especially classical ligand-dependent sonic hedgehog (SHH) signalling (28). Recently, emerging evidences confirm the activation of Shh signaling in certain ischemic models (29, 30). The Shh pathway has been reported to be involved in the repair of myocardial ischemic I/R injury, indicating a new therapeutic target for ischemic heart disease treatment (26). Surprisingly, enhancement of Shh expression is pivotal to the curative effect of L-Argin kidney ischemia-reperfusion model (31). These findings prompt us to hypothesize that the Shh pathway may be associated with SDF-1/CXCR4-mediated ABCG2 expression during ischemia progression. As expected, SI/R induced the activation of Shh signaling, which was further enhanced by SDF-1 stimuli. Interestingly, blocking this pathway with KAAD-cyclopamine mitigated the up-regulation of ABCG2 induced by SI/R or SI/R-SDF-1.

In conclusion, this present study corroborated that SDF-1/CXCR4 signaling was responsible for SI/R-enhanced SP cell resistance to injury, chemotaxis, and paracrine, which might regulate byShh-Gli1-ABCG2 signaling. Importantly, blocking CXCR4 pathway remarkably attenuated the protective function of SP cells in renal I/R injury. Therefore, this research indicated that SDF-1/CXCR4 may account for SP-mediated kidney

**FIG. 5.** SDF-1 regulates ABCG2 expression in SI/R-treated SP cells by Shh-Gli1 pathway. A, Western blotting was performed to detect the expression of ABCG2 in SP cells under SI/R treatment or SI/R and SDF-1 stimuli. B, SP cells under SI/R were pretreated with AMD3100 prior to SDF-1 treatment or not. The ABCG2 protein levels were detected. C, The expression levels of Shh and Gli 1 in SP cells upon SI/R stimuli or SI/R and SDF-1 treatment. D, Expression of Shh in KAAD-treated SP cells. E, Effect of KAAD on the expression of Gil 1. F, Following pretreatment with KAAD-cyclopamine, the expression levels of ABCG2 were detected in SI/R and SI/R+SDF-1 groups. *P < 0.05.
repair after I/R injury by ABCG2 expression through activating Shh-Gli1 pathway, suggesting a promising therapeutic agent for renal regeneration after I/R injury. However, further study should investigate the above mechanism in vivo.

REFERENCES


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