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Original Article

Platelet-rich plasma promotes MSCs exosomes paracrine to repair acute kidney injury via AKT/Rab27 pathway

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Abstract: Acute kidney injury (AKI) is defined by rapid deterioration of renal function, and is a common complication in hospitalized patients. Among the recent therapeutic options, mesenchymal stem cells (MSCs) are considered a promising therapeutic strategy for damaged tissue repair. Platelet rich plasma (PRP) regulates mesenchymal cells to repair tissue damage through the release of growth factors. In this study, we proposed a possible therapeutic use of MSCs stimulated by platelet-rich plasma (PRP-MSCs) in a glycerin-induced AKI murine model. *In vivo* and *in vitro* studies, showed that PRP-MSCs could significantly attenuate serum blood urea nitrogen and creatinine levels, and reverse the histopathological kidney damage. PRP-MSCs treatment reduced renal tubular cell apoptosis stimulated by glycerin. We confirmed that PRP promoted the proliferation and reinforced the stemness of MSCs by inducing YAP nucleus expression, and that PRP promoted MSCs exosomes in a paracrine manner to repair AKI through an activated AKT/Rab27 pathway. Our results revealed that the PRP stimulated MSCs paracrine pathway could effectively alleviate glycerin-induced AKI. Therefore, PRP pretreatment may be a new method to improve the therapeutic effect of MSCs.

Keywords: Platelet-rich plasma, MSCs, AKT/Rab27, exosomes, AKI

Introduction

Acute kidney injury (AKI) is caused by a variety of factors, including hypoxia, mechanical trauma, surgery, drugs and inflammation, and it induces a decrease in glomerular filtration rate, moreover, it causes the accumulation of blood creatinine, urea nitrogen and other metabolites that characterize a rapid deterioration of renal function [1, 2] and result in the corresponding clinical manifestations of the syndrome, that represent a common clinical emergency [3, 4]. Although the renal tissue exhibits an intrinsic ability to regenerate after injury, full recovery in most cases is not achieved [5]. Therefore, multiple therapeutic approaches for renal regeneration are being considered.

Among new therapeutic options for AKI, mesenchymal stem cells (MSCs) have considerable potential in regenerative medicine for their ability of renewal and differentiation into distinct cell types. Thus, they have received increasing interest for the treatment of kidney injury [6-8]. MSCs can be isolated from the bone marrow, umbilical cord, adipose tissues, and other adult tissues [9]. Our previous studies have shown that hucMSCs could be a favorable candidate for injured tissue repair [10, 11]. Moreover, the efficacy of stem cell-based therapy could be further improved by small-molecule drugs. Small-molecule drugs have an important role in regulating stem cell fate and function and facilitate the development of cell-based therapies [12, 13].

Platelet rich plasma (PRP) is a fraction of blood plasma with a high platelet concentration of 1.2×10^{12} platelets/mL. PRP contains numerous cytokines, including platele-derived growth fac-

tor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF-β), and insulin-like growth factor 1 [14, 15]. Recent studies have reported that PRP could enhance cell clone formation, maintain the adipogenic, chondrogenic and osteogenic differentiation capacity of stem cells, and maintain an immunosuppressive state [16, 17]. On the basis of these biological functions, PRP has been widely investigated in regenerative medicine. It was reported that PRP alleviated multiple-organ damage, particularly in skin injury [18, 19]. However, the effect of PRP on MSCs-based kidney therapy is unclear. Whether PRP-modified MSCs have more efficient reparative ability than MSCs in tissue injury remains unknown.

Intramuscular injection of glycerin, a type of high permeable substance, causes local muscle necrosis and erythrolysis, and glycerin easily enters renal tubules through glomeruli, causing acute renal tubule damage [20]. Therefore, an AKI animal model was established by intramuscular injection of a 50% solution of hypertonic glycerin. Our previous studies have shown that MSCs have certain advantages in tissue damage repair. Furthermore, studies have reported that modified MSCs could play enhanced therapeutic roles in kidney dysfunction diseases. As an endogenous stimulator, PRP may enhance the therapeutic effectiveness of MSCs? However, the mechanism through which MSCs repair injury remains unclear. In addition, for modulating MSCs function, small stimulator compounds have several advantages, including convenient concentration adjustment, working duration, and rapid and reversible working effects.

In the present study, we investigated the effect of PRP-MSCs and MSCs on glycerin-induced AKI. The results showed that PRP could induce YAP nucleus expression to promote proliferation and maintain the stemness of MSCs and that it stimulated the paracrine exosomes of MSCs to inhibit the apoptosis of renal tubular cells through an activated AKT/Rab27 signaling pathway. PRP pretreatment may be a new method to improve the therapeutic effect of MSCs repair for glycerin-induced AKI.

Materials and methods

The study was approved by the Medical Ethics Committee and Ethics Committee for Experimental Animals of Jiangsu University (IRB protocol number: 2020161).

Cells culture

Fresh human umbilical cords were obtained from consenting mothers in the affiliated hospital of Jiangsu University. HucMSCs were isolated and identified as previously described [11] and cultured in serum-free Dulbecco's modified Eagle's medium (DMEM, Gibco, CA) with 10% fetal bovine serum (FBS, Excell), penicillin and streptomycin (Gibco). Platelet-rich plasma (PRP) was provided by the central blood bank of Zhenjiang city, Jiangsu, China. The concentration of PRP used in this study was 1.2×10^{12} platelets/mL. In the following experiments, after incubation with PRP medium that contains 1×10^{8} platelets/mL for 12 h, and then the stimulator (PRP medium) was remove, MSCs were cultured in DMEM medium for 24 h. PRP-MSCs: MSCs were cultured in DMEM for 24 h, MSCs: hucMSCs treated with DMEM as the control. Rat renal tubular epithelial cell lines (NRK-52E) were purchased from Cell Bank (Chinese Academy of Sciences, Shanghai, China) and maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% FBS at 37°C with 5% CO_2. The cells at passage 3 were used for the following studies.

Osteogenic and adipogenic differentiation in vitro

MSCs and PRP-MSCs were seeded in 35-mm plates in an osteogenic differentiation medium (0.1 mM dexamethasone, 10 mM β-glycerophosphate, and 50 mg/L ascorbic acid) or adipogenic differentiation medium (Cyagen Biosciences, USA) for 2 weeks, according to the manufacturer's instructions. After the induction, the osteogenic and adipogenic potential was evaluated through alkaline phosphatase, alizarin red and oil red O staining, respectively.

Isolation and characterization of PRP-MSC-Ex

The supernatant of MSCs stimulated by PRP 12 h was collected, to a certain volume then exosomes were extracted and purified [21]. The protein concentration, as the quantification of exosomes, was determined by using a BCA protein assay kit. The morphology of exosomes was observed by using transmission electron

microscopy (FEITecnai 12, Philips, Netherlands). The sizes of exosomes analyzed the NanoSight LM10 system (nanosight tracking analysis, UK). For *in vivo*, to investigate the roles of PRP-MSCs-Ex in AKI model, MSCs-Ex and PRP-MSCs-Ex (10 mg/kg) were treated in AKI model via tail vein injection. CM-DiR (sigma) labeled exosomes were incubated at 37°C for 30 minutes, and the bio-distribution of exosomes was detected via IVIS Lumina system (Perkin Elmer, USA) *in vivo*.

Glycerin-induced model of AKI

Adult male Sprague-Dawley rats weighing 180-200 g were chosen, the *in vivo* experiments were conducted following the regulatory standards. All the experimental rats were reared in the environment of room temperature of about 26°C, and the room should be kept clean, ventilated and quiet. After feeding for one week, the experimental rats were divided into four groups according to the random number table: control group, glycerin group, MSCs group, PRP-MSCs group, 10 rats in each group. The AKI rat model was established as described previously [20]. As previously described, AKI was performed in rat by intramuscular injection of 50% solution of hypertonic glycerin (10 mL/kg, Sigma) into inferior hindlimbs. On day 1 post injury, rat received a tail vein injection of 1×10^6 MSCs and 1×10^6 PRP-MSCs in 200 mL saline and the same volume of saline as control. All animals were sacrificed at Day 3 after glycerin injection. Renal function (serum BUN, Creatinine), histopathological structure changes and tubular cells apoptosis were evaluated.

Western blotting

Renal tissues or NRK-52E cells were lysed in a radioimmunoprecipitation assay buffer containing proteinase inhibitors. Protein samples were separated by SDS-polyacrylamide gel electrophoresis, transferred to the polyvinylidene difluoride membrane (Millipore), blocked in 5% skim milk, and incubated with primary antibodies and horseradish peroxidase-conjugated secondary antibodies (Invitrogen).

Primary antibodies used in research were as following: Bax (1:200, Bioworld, USA), Activated-caspase3 (1:500, CST, USA), and β-actin (1:800, CST, USA). The secondary antibodies were HRP-conjugated goat anti-rabbit and goat anti-mouse antibodies (1:1500, CWBIO, China). The uneditied gel results for original blot in Figure S2.

TUNEL and immunohistochemistry staining

For histologic analysis, the kidneys were prepared by perfusion of the rat through the left ventricle and slides of the kidney were prepared, fixed in 4% paraformaldehyde, embedded in paraffin and then cut into sections. We detected apoptosis cells by employing terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining according to the manufacturer's protocol (Vazyme, USA). The sections were stained with hematoxylin and eosin (HE) staining, and the histological changes of renal tissues were observed under a microscope (DP73; Olympus, Tokyo, Japan). After inactivating endogenous enzymes by 10% H_2O_2 with 30 minutes, the slices of kidney tissues and cells were incubated with activated-caspase3 antibody (1:100, CST, USA) overnight at 4°C, then incubated with biotinylated sheep anti-rabbit IgG. The signal was observed by DAB staining and hematoxylin counterstaining under microscope (DP73; Olympus, Tokyo, Japan).

Immunofluorescence analysis

MSCs and PRP-MSCs cells slides were placed in 4% paraformaldehyde at 4°C for 12 h. Then permeabilized with PBS solution containing 0.15% Triton X-100 for 30 min and incubated with 5% bovine serum albumin (BSA) for 1 h to block non-specific antibody binding. Primary antibody AKT (1:100, CST), Rab27 (1:100, CST), CD63 (exosomes marker) (1:50, CST) were incubated overnight, followed by incubation with Cy3-labeled (Red) anti-rabbit IgG (1:200, invitrogen), FITC-labeled (Green) anti-rabbit IgG secondary antibody (1:200, invitrogen) at 37°C for 30 min. The nuclei were counterstained with Hoechst 33342 (1:200; Sigma). The slides were visualized with Confocal microscope (DeltaVision Elite, GE, USA).

Statistical analysis

All data were shown as mean ± SD. Statistical analysis between groups was performed by GraphPad Prism 5.0 software (San Diego, USA). Statistical differences between two groups were determined by two-tailed paired Student's t-test. In multiple groups (>2) were determined

by one-way analysis, and variance followed by ANOVA Tukey's post tests. *P<0.05 was considered statistically significant.

Results

PRP enhanced the repairing effect of MSCs on glycerin-induced AKI

To investigate the repairing effect of PRP-MSCs on acute renal injury, we constructed an AKI model by intramuscular injection of 50% hypertonic glycerol solution into the inferior hind limbs of rats (10 mL/kg), and we evaluated the reparative ability of PRP-MSCs in this AKI model (**Figure 1A**). The results showed that serum blood urea nitrogen (BUN) and creatinine levels increased markedly at 3 d and remained at a high level after 50% glycerin injection (Figure S1A and S1B). Compared with MSCs, transplanted PRP-MSCs could reduced BUN and creatinine levels (**Figure 1B** and **1C**). The histological evaluation of kidney sections in the glycerin group revealed the presence of intra-tubular protein casts and tubular epithelial cell necrosis. The number of casts and necrotic tubules was significantly reduced in MSCs and PRP-MSCs-treated rats confirming their beneficial effect, moreover PRP-MSCs were more effective than MSCs in alleviating the vacuoles of renal tubules (**Figure 1D**). Moreover, the histological damage score of the PRP-MSCs group was significantly lower than that of the injury group, and the damage score of the PRP-MSCs group was 40% lower than that of the MSCs group (**Figure 1E**).

We used western blotting to detect the expression of apoptosis-associated proteins in rat kidney tissues. The results showed that transplanting MSCs and PRP-MSCs decreased the expression of Bax and activated-caspase3, and the anti-apoptotic effect of PRP-MSCs was more efficient than that of MSCs (**Figure 1F**). In the pathological section of renal tissue, immunohistochemical staining further confirmed that most activated-caspase3 positive cells were in the glycerin group, whereas the number in the PRP-MSCs groups was significantly lower than that in the MSCs group (**Figure 1G**). Furthermore, results of a TUNEL assay indicated reduced apoptotic cells in both the MSCs and PRP-MSCs groups, and the PRP-MSCs group had fewer apoptotic cells than did the MSCs group (**Figure 1H**). These results indicated that the reparative ability of PRP-MSCs was more efficient than that of MSCs in glycerin-induced kidney injury.

PRP-MSCs attenuated glycerin-induced NRK-52E cells apoptosis

Histopathology showed that AKI mainly damaged renal tubules, therefore *in vitro* experiment, we treated NRK-52E cells with 20% glycerol hypertonic medium. After treatment with glycerin for 12 h, the number of apoptotic NRK-52E cells was significantly increased. Then, we established an *in vitro* Transwell co-culture system to allow the transfer of EVs but preclude direct cell contact, and we evaluated the effects of NRK-52E cells treated with the MSCs/PRP-MSCs for 24 h (**Figure 2A**). Subsequently, lysis and protein extraction of NRK-52E cells and western blot analysis of the expression of apoptosis-associated proteins revealed that Bax and activated-caspase3 significantly increased after glycerin treatment. Nevertheless, the expression levels of Bax and activated-caspase3 in NRK-52E cells were lowest in the PRP-MSCs group (**Figure 2B**). Correspondingly, treatment with PRP-MSCs or MSCs could effectively reverse apoptosis, and the number of apoptotic NRK-52E cells was lowest in the PRP-MSCs group on the basis of flow cytometry (**Figure 2C**). The statistical result is shown in **Figure 2D**. Immunohistochemical and TUNEL staining further confirmed the expression of activated-caspase3 and Bax, and the results were consistent with those found by western blotting. The number of cells with positive results for activated-caspase3 and TUNEL-staining increased after glycerin treatment, and the PRP-MSCs group had fewer such positive cells than did the MSCs group (**Figure 2E** and **2F**). In general, these results showed that the inhibitory effect of PRP-MSCs against glycerin-induced NRK-52E cells apoptosis was significantly stronger than that of MSCs, which confirmed their kidney-protective role.

PRP facilitated the proliferation and differentiation of MSCs

In vivo and *in vitro* experiments, confirmed the reparative effect of PRP-MSCs in the AKI model. Then, we further analyzed the therapeutic mechanism of PRP-MSC. In MSCs treated with 1×10^8 platelets/mL PRP for 12 h, the cell density was 85% higher than that of MSCs treated

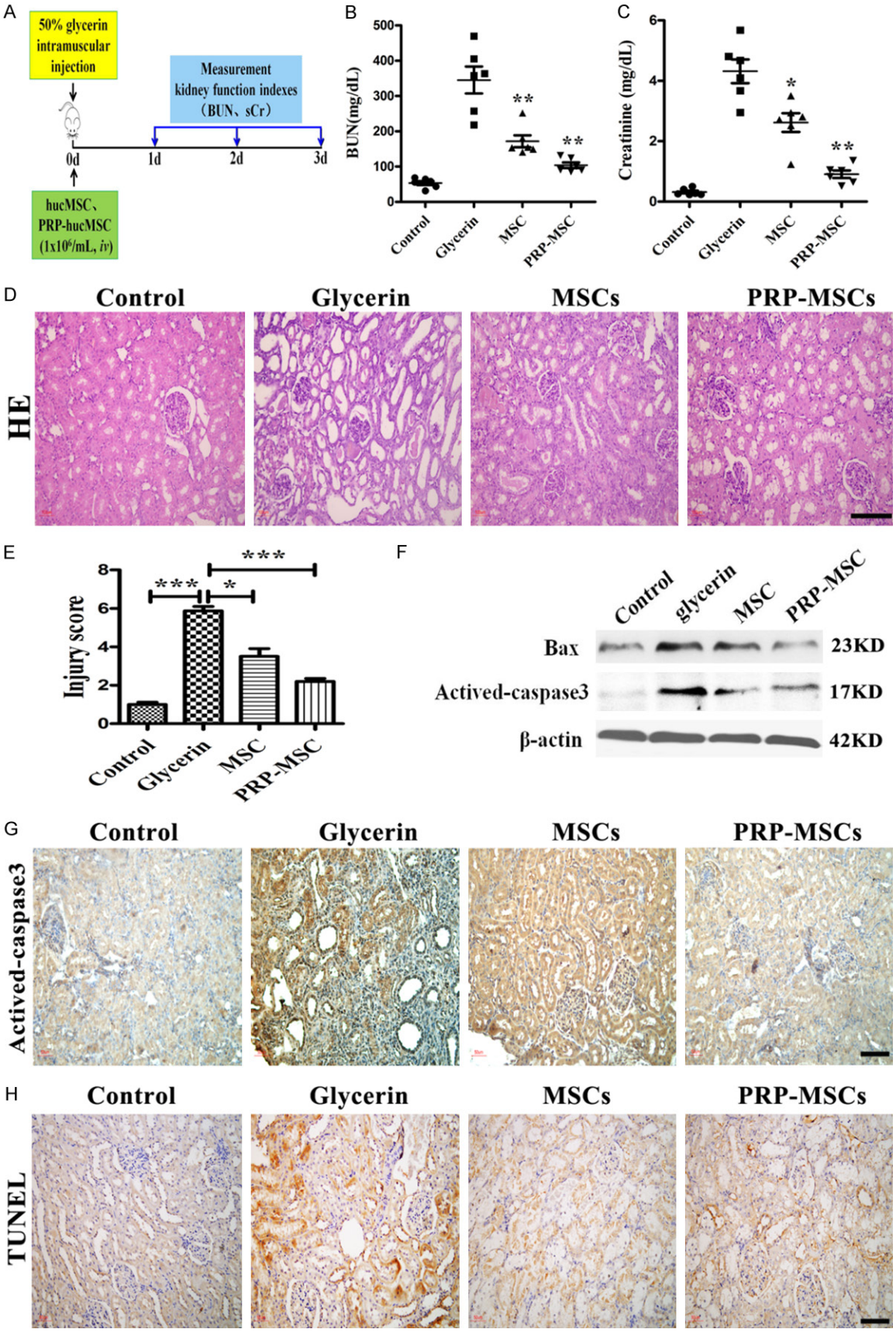
A
50% glycerin intramuscular injection
Measurement kidney function indexes (BUN、sCr)
0d
1d
2d
3d
hucMSC、PRP-hucMSC (1x10⁶/mL, iv)
B
BUN(mg/dL)
Control
Glycerin
MSC
PRP-MSC
C
Creatinine (mg/dL)
D
Control
Glycerin
MSCs
PRP-MSCs
HE
E
Injury score
F
Control
glycerin
MSC
PRP-MSC
Bax
23KD
Actived-caspase3
17KD
β-actin
42KD
G
Actived-caspase3
H
TUNEL

Figure 1. PRP enhanced the reparative effect of MSCs against glycerin-induced AKI. (A) Schematic of the AKI model and MSC/PRP-MSC treatment. AKI rats were administered intravenous injections with MSC/PRP-MSC (1×10^6/injection) on days 1, 2, and 3. The rats were sacrificed on day 4 for subsequent experiments (n=6). (B) Serum BUN and (C) Creatinine levels in the glycerin, MSCs, and PRP-MSCs groups (*P<0.05, **P<0.01, and ***P<0.001). (D) Representative micrographs of renal HE histology at day 3 after glycerin injection in the four groups (Scale bars =100 μm). (E) The degree of tubular damage was scored by choosing 10 non-overlapping fields and calculating the percentage of tubules in the kidney cortex, that exhibited tubular cells necrosis and cast deposition as follows: 0, none; 1, ≤10%; 2, 10-25%; 3, 25-50%; 4, 50-75%; and 5, >75%. The tubular injury score was calculated at day 3 after glycerin injection (*P<0.05, **P<0.01, ***P<0.001). (F) Western blot analysis of Bax, activated-caspase3 in the kidney tissues at day 3 after glycerin injection. (G) Immunohistochemical staining graph of activated-caspase3 in the glycerin-induced injured kidney sections (Scale bars =50 μm). (H) Representative images of TUNEL staining in the kidney sections at day 3 after PRP-MSCs treatment (Scale bars =50 μm).

with Dulbecco's modified Eagle medium (DMEM), which suggested that PRP could significantly promote the proliferation of MSCs (**Figure 3A**). As a key pathway in regeneration progression, YAP signaling controls organ size and regulates tissue cell regeneration [22, 23]. Immunohistochemistry results revealed that PRP induced YAP nucleus expression and promoted cell proliferation in MSCs (**Figure 3B**). We used western blotting and qRT-PCR analysis to determine the stemness-associated proteins expression, at protein and gene levels of Nanog, Sox2, Oct4 and Sall4, all were higher in PRP-MSCs than in MSCs (**Figure 3C** and **3D**). *In vitro* experiments showed that PRP induced adipogenic and osteogenic differentiation of MSCs, and the percentage of alizarin red positive cells in the PRP group was higher than that in the DMEM group, confirming that the PRP could promote multi-directional differentiation of MSCs (**Figure 3E**). Overall, these results suggested that PRP induced YAP nucleus expression and increased stemness-associated genes to promote MSCs proliferation and differentiation.

PRP promoted MSCs exosomes in a paracrine manner through the AKT/Rab27 pathway

We confirmed that PRP could promote MSCs proliferation their ability to repair AKI. Next, we explored the mechanism through which PRP enhanced this repair ability. Recent studies have shown that MSC exosomes (MSC-Ex) are the key paracrine component of MSCs that exert therapeutic effects against tissue injury [24, 25]. Therefore, we focused on the effect of PRP on the exosomes secretion of MSCs. The AKT/Rab27 signaling pathway regulates the synthesis and secretion of exosomes [26]. In this experiment, we found that PRP stimulated AKT to enter the nucleus and that the expression of Rab27 was increased (**Figure 4A** and **4B**). The protein level of PRP-MSCs confirmed this result (**Figure 4C**). Western blot analysis results confirmed the higher expression of exosomal markers CD9, CD63 and CD81 in PRP-MSC-Ex compared with MSC-Ex (**Figure 4D**). PRP stimulated the increase of CD63 in MSCs which was observed using a confocal microscope, indicating that PRP promoted the secretion of exosomes (**Figure 4E**). These results suggested that PRP might affect the secretion of MSC exosomes. We further analyzed, exosomes as cup-like spherical vesicles by using transmission electron microscopy (**Figure 4F**). MSC-Ex was observed as nanoscale membrane vesicles. We used nanoparticle tracking analysis with 1000×dilution (the concentration of MSC-Ex and PRP-MSC-Ex were 2.5×10^9/mL and 5.1×10^9/mL, respectively) to determined the shape and size of exosomes (**Figure 4G**). The size of MSC-Ex was 101.5 ± 24.3 nm, and that of PRP-MSC-Ex was 108.6 ± 33.6 nm (mean ± standard deviation). Therefore, the shape and particle size of were not changed by PRP stimulation, but PRP doubled the secretion of MSC-Ex (**Figure 4H**). These results indicated that PRP promoted the paracrine secretion of MSCs-Ex through activation of the AKT/Rab27 pathway.

PRP-MSC-Ex localization and repair of injured renal tissue

To determine the localization and bio-distribution of PRP-MSCs-Ex within injured renal tissue, exosomes labeled with a fluorescent dye (CM-DiR, 50 μM) were injected intravenously and tracked by optical imaging (PerkinElmer, MA, USA) in AKI mice. After 12 h, an exosomes fluorescence signal was observed in injured kidneys (**Figures 5A** and S1C). Having confirmed the orientation of exosomes in damaged tissues, we analyzed the role of the kidney-protective factor. Compared with MSCs-Ex, PRP-

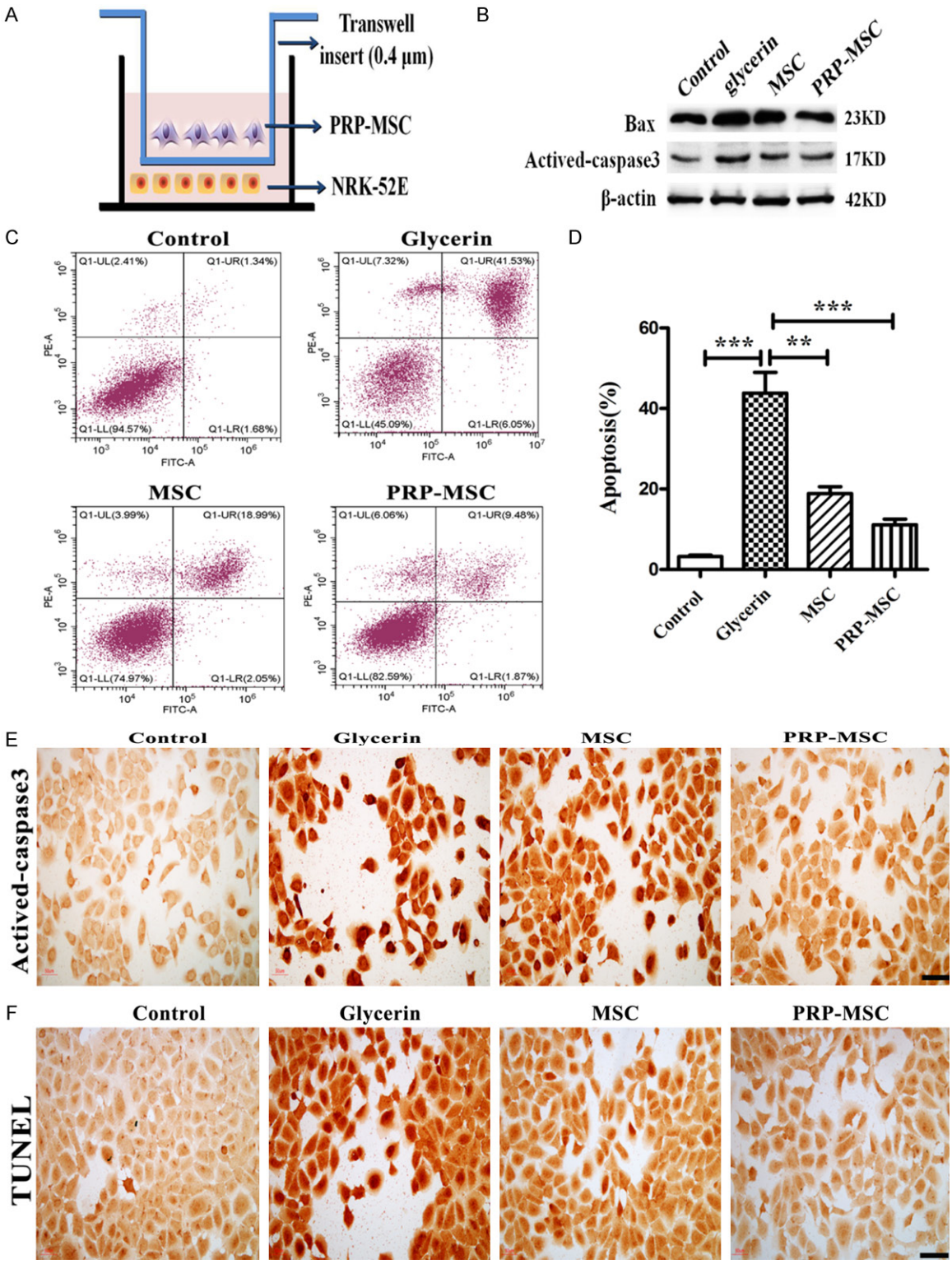

Figure 2. PRP-MSCs attenuated glycerin-induced NRK-52E cells apoptosis. A. Schematic of the Transwell coculture model with PRP-MSCs in the upper chamber and NRK-52E cells in the lower chamber. A porous (0.4 μm) membrane allowed the transfer of exosomes but precluded direct cell contact. B. Western blot analysis for Bax and activated-caspase3 expression in NRK-52E cells. C. Flow cytometry revealed apoptotic NRK-52E cells with PRP-MSCs treatment. D. Statistical result of apoptotic NRK-52E cells (***P*<0.01, ****P*<0.001). E. Immunohistochemical staining of activated-caspase3 in glycerin-induced injured NRK-52E cells with PRP-MSCs treatment (Scale bars =50 μm). F. Representative images of TUNEL staining in NRK-52E cells with glycerin-induced injury treated with PRP-MSCs (Scale bars =50 μm).

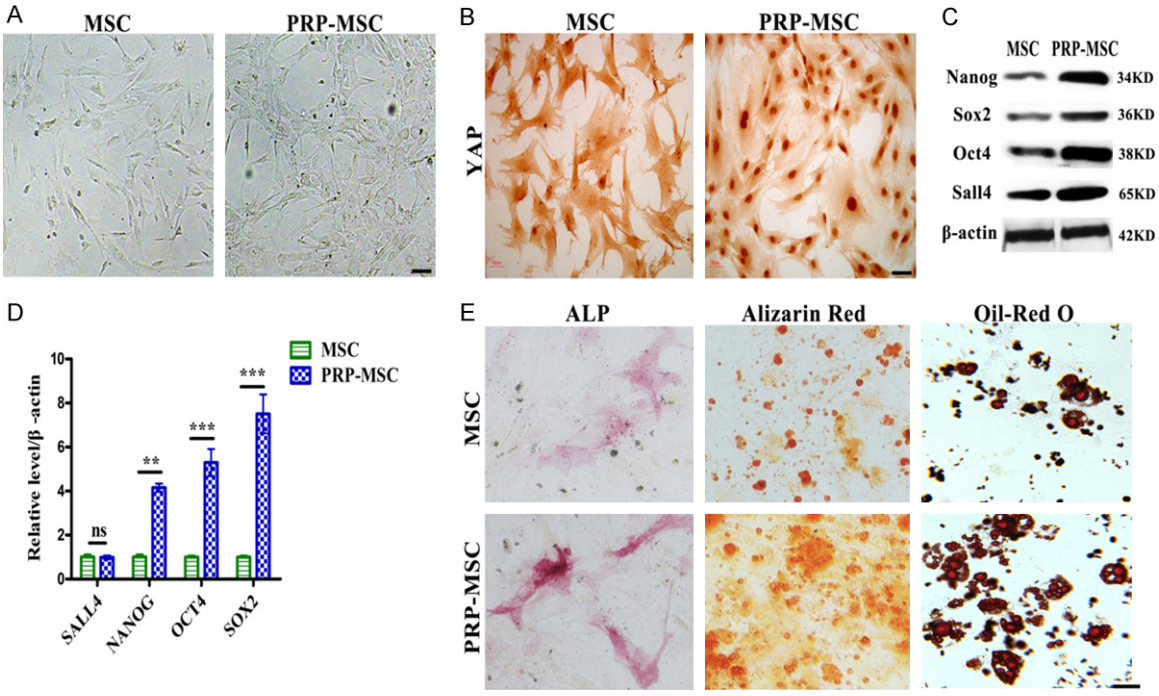

Figure 3. PRP facilitated the proliferation and differentiation of MSCs. A. Microscopic observation of the growth of MSCs 24 h after PRP stimulation (Scale bars =50 μm). B. Immunohistochemical analysis of YAP protein localization in MSCs under PRP stimulation (Scale bars =50 μm). C. Western blot analysis for stemness-associated proteins in MSCs with PRP treatment. D. qRT-PCR detected of the stemness-associated genes of MSCs (**P<0.01, ***P<0.001). E. Adipogenic and osteogenic differentiation capacity after co-culture of MSCs with PRP for 48 h *in vitro* (Scale bars =50 μm).

MSCs-Ex transplant reduced BUN and creatinine levels in the AKI model (Figure S1D and S1E). Kidney histological analysis revealed that the number of casts and necrotic tubules were significantly reduced in rats treated with MSC-Ex and PRP-MSC-Ex (**Figure 5B**). Similarly, TUNEL analysis showed that the numbers of apoptotic cells in the MSC-Ex and PRP-MSC-Ex groups were decreased, and that the number of apoptotic cells in the PRP-MSC-Ex group was significantly lower than that in the glycerin group (**Figure 5C**). The expression of apoptosis-related proteins Bax and activated-caspase3 in renal tissue was inhibited after PRP-MSC-Ex treatment (**Figure 5D**). Statistical analysis showed that the intervention effect of PRP-MSC-Ex was better than that of MSC-Ex (**Figure 5E**).

In vitro experiments and flow cytometry results showed that MSC-Ex could effectively reduce the apoptosis of NRK-52E cells induced by glycerin, and the number of NRK-52E cells in the PRP-MSC-Ex group decreased by 76.3% (**Figure 5F** and **5G**). To further determine this phenomenon, immunohistochemistry was used to detect the expression of cells positive for activated-caspase3 after intervention. We found that the number of positive cells in the PRP-MSC-Ex group was significantly lower than that in the injury group, and that the number of positive cells in the PRP-MSC-Ex group was less than that in the MSC-Ex group (**Figure 5H**). Western blotting results showed that the expression of Bax and activated-caspase3 decreased significantly after MSC-Ex treatment. Moreover, the expression levels of Bax and activated-caspase3 in NRK-52E cells were lowest in PRP-MSC-Ex group (**Figure 5I**). These results confirmed that the PRP-MSC-Ex had a more beneficial effect than PRP-MSC. In general, these results showed that exosomes were the key component of MSCs in the treatment of renal injury, which could significantly inhibit the apoptosis of NRK-52E cells induced by glycerin and this confirmed the mechanism through which PRP stimulated the secretion of MSC exosomes for the repair of AKI.

Discussion

Treatment for AKI has remained a challenging medical problem. Therapeutic methods so far have been function-limited, if the treatment

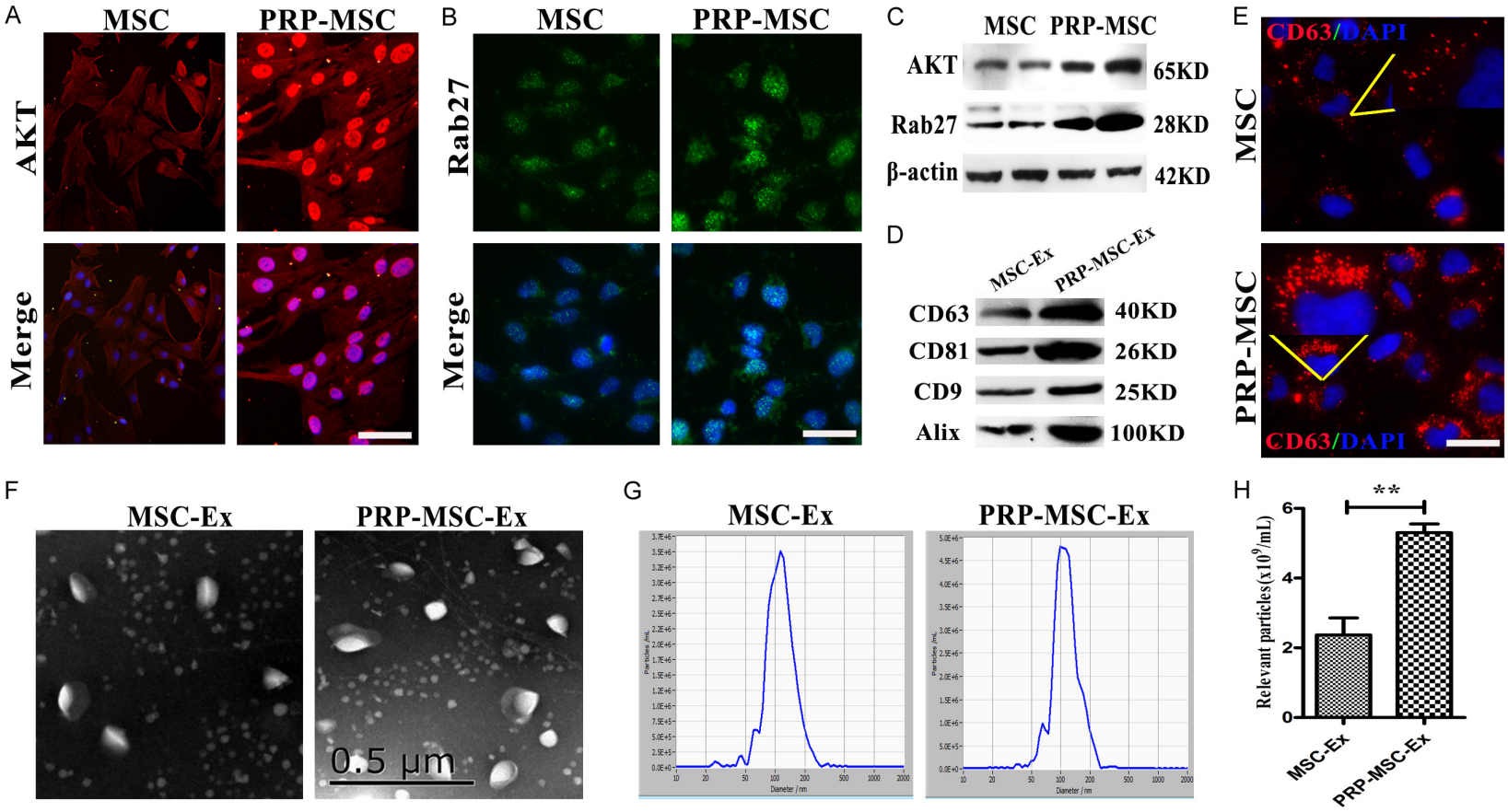

Figure 4. PRP promoted MSCs exosomes in a paracrine manner through the AKT/Rab27 pathway. (A) Immunofluorescence analysis of AKT and (B) Rab27 in MSCs under PRP stimulation (Scale bars =20 μm). (C) Western blot analysis for AKT and Rab27 in MSCs with PRP treatment. (D) Western blotting detected the expression of exosomal markers in MSCs treated with PRP. (E) The expression of exosomal marker CD63 (red) in MSCs with PRP treatment was detected by using a confocal microscope (Scale bars =10 μm). (F) The morphologies of MSC-Ex/PRP-MSC-Ex was identified by TEM. (G) The size and concentration of MSC-Ex/PRP-MSC-Ex were measured by NTA. (H) The quantity difference between MSC-Ex/PRP-MSC-Ex was analyzed (***P*<0.01).

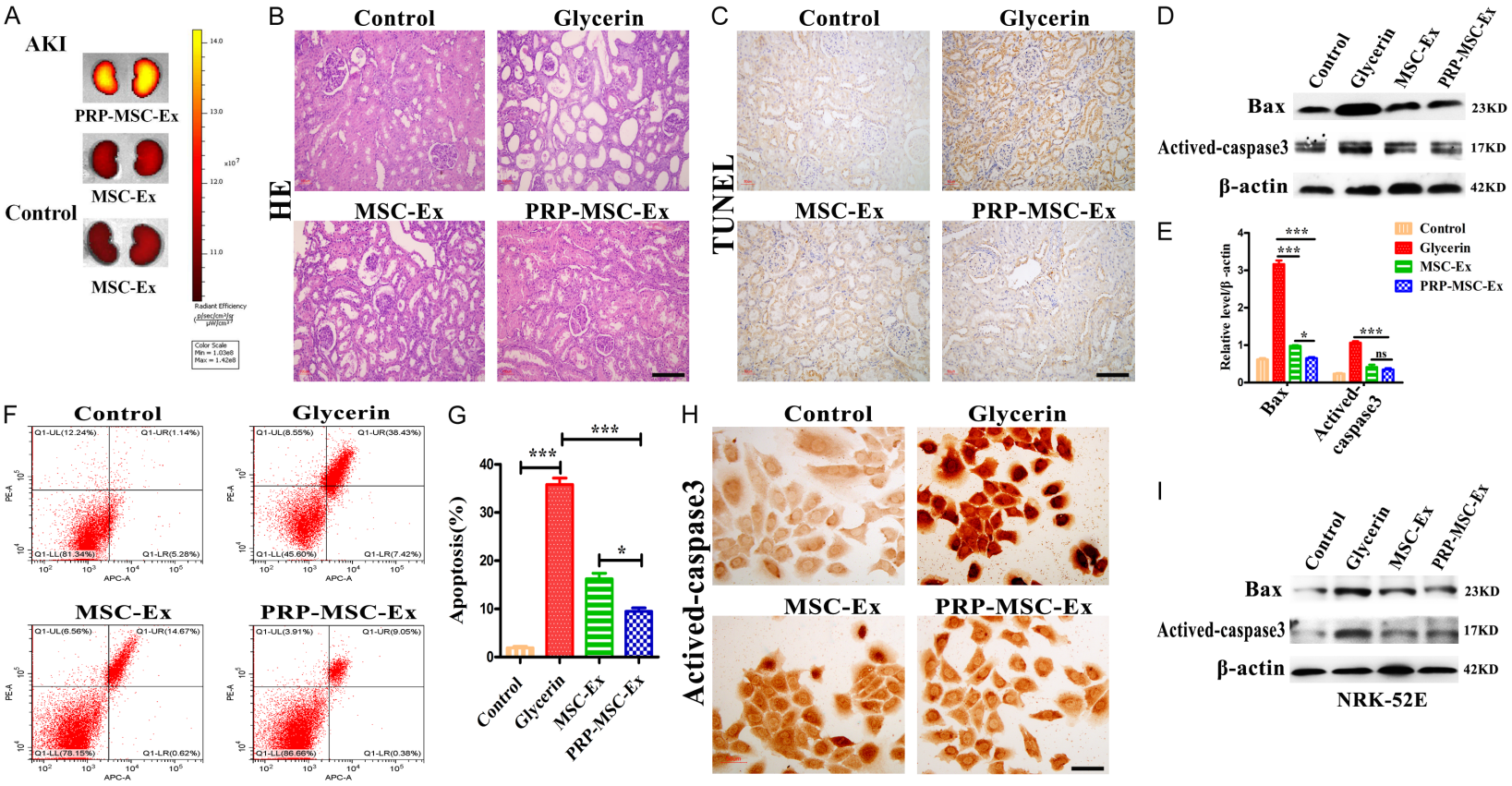

Figure 5. localization and repair ability of PRP-MSC-Ex for injured renal tissue. A. Representative fluorescence images of a rat with AKI that received an injection with DiR-labeled PRP-MSC-Ex and was evaluated 12 h after injection. B. Representative micrographs of renal HE histology after MSC-Ex/PRP-MSC-Ex treatment (Scale bars =100 μm). C. Immunohistochemical staining graph of activated-caspase3 in the kidney sections with glycerin-induced injury (Scale bars =50 μm). D. Western blot analysis for Bax and activated-caspase3 expression in the kidney tissues after MSC-Ex/PRP-MSC-Ex treatment. E. The statistical result of apoptosis-related proteins in NRK-52E cells (*P<0.05, **P<0.01, ***P<0.001). F. Flow cytometry detected apoptotic NRK-52E cells after PRP-MSC-Ex treatment. G. The statistical result of apoptotic NRK-52E cells with PRP-MSC-Ex treatment (*P<0.05, **P<0.01, ***P<0.001). H. Representative immunohistochemical images of activated-caspase3 with PRP-MSC-Ex treatment (Scale bars =50 μm). I. Western blot analysis for Bax and activated-caspase3 expression in NRK-52E cells after PRP-MSC-Ex intervention.

intervention is not timely, the patient's condition could deteriorate to chronic kidney disease, and many patients experience chronic pain for a long time [27]. Cell-based therapies and tissue regeneration are new approaches to overcome the present limitations in the treatment of AKI [28, 29]. MSCs are a promising therapeutic tool in regenerative medicine due to their self-renewal and multi-directional differentiation potency. Recently, numberous studies have reported that MSCs could repair cisplatin/glycerin-induced AKI [30], ischemia/reperfusion-induced acute renal failure [31], and unilateral ureteral obstruction-induced renal interstitial fibrosis [32]. However, transplanted MSCs also have a potential problem in that most of them do not survive for a long time, and only some of them reach the damaged kidney. The lower implantation and survival rates weaken the therapeutic effect of MSCs treatment. Therefore, strategies are required to maximize the therapeutic effect of MSCs.

A common strategy to improve the therapeutic effect of stem cells transplantation is pretreating with small molecule drugs. Our recent studies have demonstrated that 3,3'-diindolylmethane stimulated exosomal Wnt11 autocrine signaling in MSCs to enhance wound healing [11] and that resveratrol improved MSCs repair for cisplatin-induced AKI [33]. These studies have revealed that small-molecule drugs are suitable candidates for stem cell manipulation in regenerative medicine.

The effect of Platelet-rich plasma (PRP) on tissue healing has been attributed mainly to the release of growth factors, such as PDGF, VEGF and TGF-β from platelets, which could promote cell proliferation and migration [34]. Recently, studies have reported that PRP could attract MSCs, after which PRP enhanced cells could promote the initial healing of wounds and this phenomenon is called the chemotactic effect [18]. However, the role of PRP pre-treatment in MSCs-based therapy has not been characterized. In this study, we found that PRP-MSC improved renal function compared with MSCs, as demonstrated by the decrease of serum creatinine and BUN levels. PRP stimulated MSCs could inhibit apoptosis of renal tubular cells and reduce the histological injury score. MSCs primed with PRP exhibited more effective repair than untreated MSCs in glycerin-induced AKI models. These results indicated that PRP pretreatment is safe, efficient and low cost, thus, this approach is expected to emerge as a promising strategy to improve MSC-based therapy. Moreover, we investigated the mechanism through which PRP promoted the ability of MSCs to repair renal injury. YAP is known to play a key role in the control of tissue and cell proliferation and development [22, 35, 36]. In our experiments, PRP stimulated YAP protein to enter the nucleus, which promoted the proliferation of MSCs and the differentiation ability of osteoblasts and adipocytes. These results suggested that an appropriate amount of PRP could enhance the injury repair effect of MSCs.

Exosomes, a type of extracellular vesicles, have potential as mediators of intercellular messaging and play a major role in cell-to-cell communication. The therapeutic effects of human MSC-derived exosomes have been verified in a rodent model [21, 37, 38]. Another study demonstrated that PRP improved the wound healing potential of MSCs through paracrine and metabolism alterations [39]. The AKT/Rab27 pathway is involved in the regulation of exosomes synthesis and secretion [26, 40]. In the present study, we found that PRP doubled the number of MSC exosomes without changing their morphology or particle size. PRP stimulated the paracrine secretion of MSCs exosomes by activating the AKT/Rab27 pathway, and DiR dye-labeled PRP-MSCs-Ex were localized within the injured kidney, thus MSCs exosomes promoted the recovery of renal function and reduced the expression of injury markers.

In the present study, we demonstrate that PRP induced YAP nucleus expression to promote the proliferation and maintain the stemness of MSCs, and stimulated the paracrine secretion of MSCs exosomes to inhibit the apoptosis of renal tubular cells by activating the AKT/Rab27 signaling pathway. We proposed the application of PRP-MSCs as a therapeutic strategy for renal regeneration.

Conclusion

This study revealed that PRP promoted the paracrine secretion of MSCs exosomes to repair glycerin-induced AKI through the AKT/Rab27 pathway. These findings confirm that

PRP pretreatment could improve the therapeutic effect of MSCs against renal injury.

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Disclosure of conflict of interest

None.

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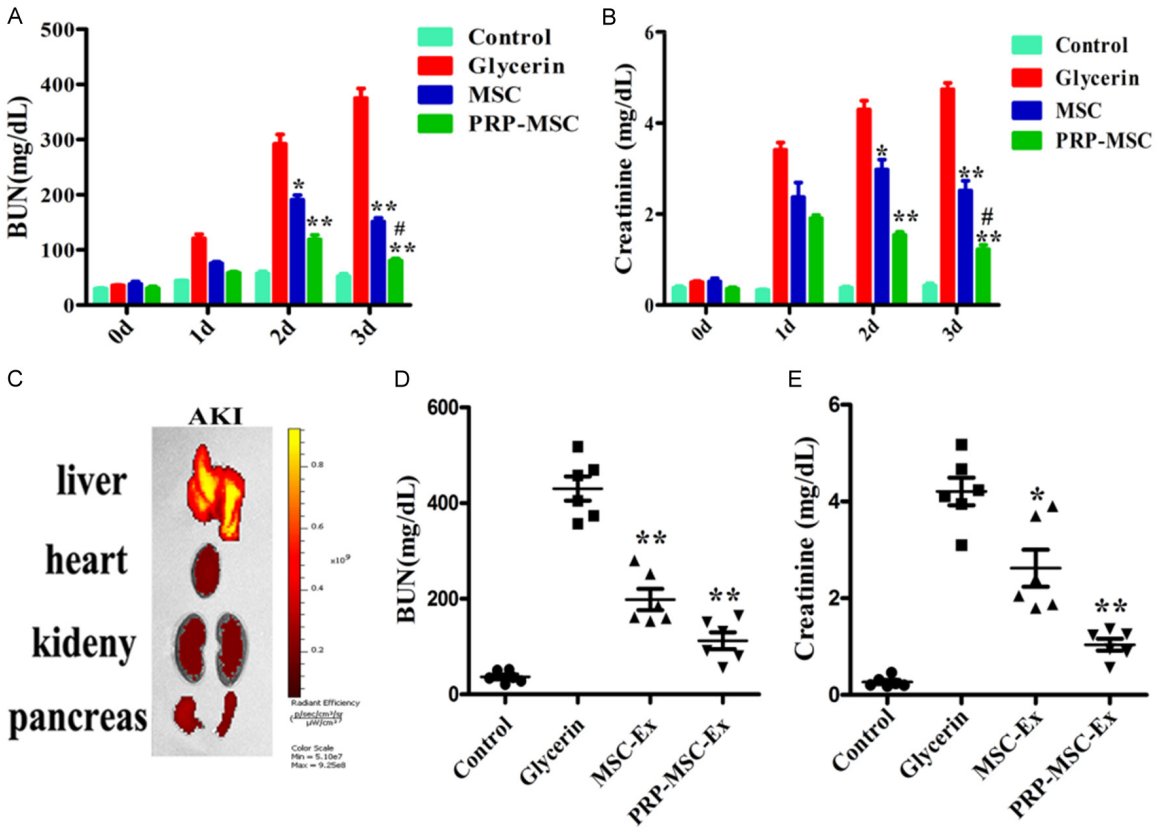

Figure S1. The biochemical indexes of renal function in rat model. (A) Serum BUN and (B) creatinine levels in Control, Glycerin, MSCs, and PRP-MSCs groups (*P<0.05, **P<0.01 PRP-MSCs vs. Glycerin group, #P<0.05 PRP-MSCs vs. MSCs group). (C) Localization and tracing of PRP-MSCs-Ex in injured organs by IVIS imaging system. (D) Serum BUN and (E) creatinine level in AKI rats after PRP-MSCs-Ex treatment (*P<0.05, **P<0.01 vs. Glycerin group).

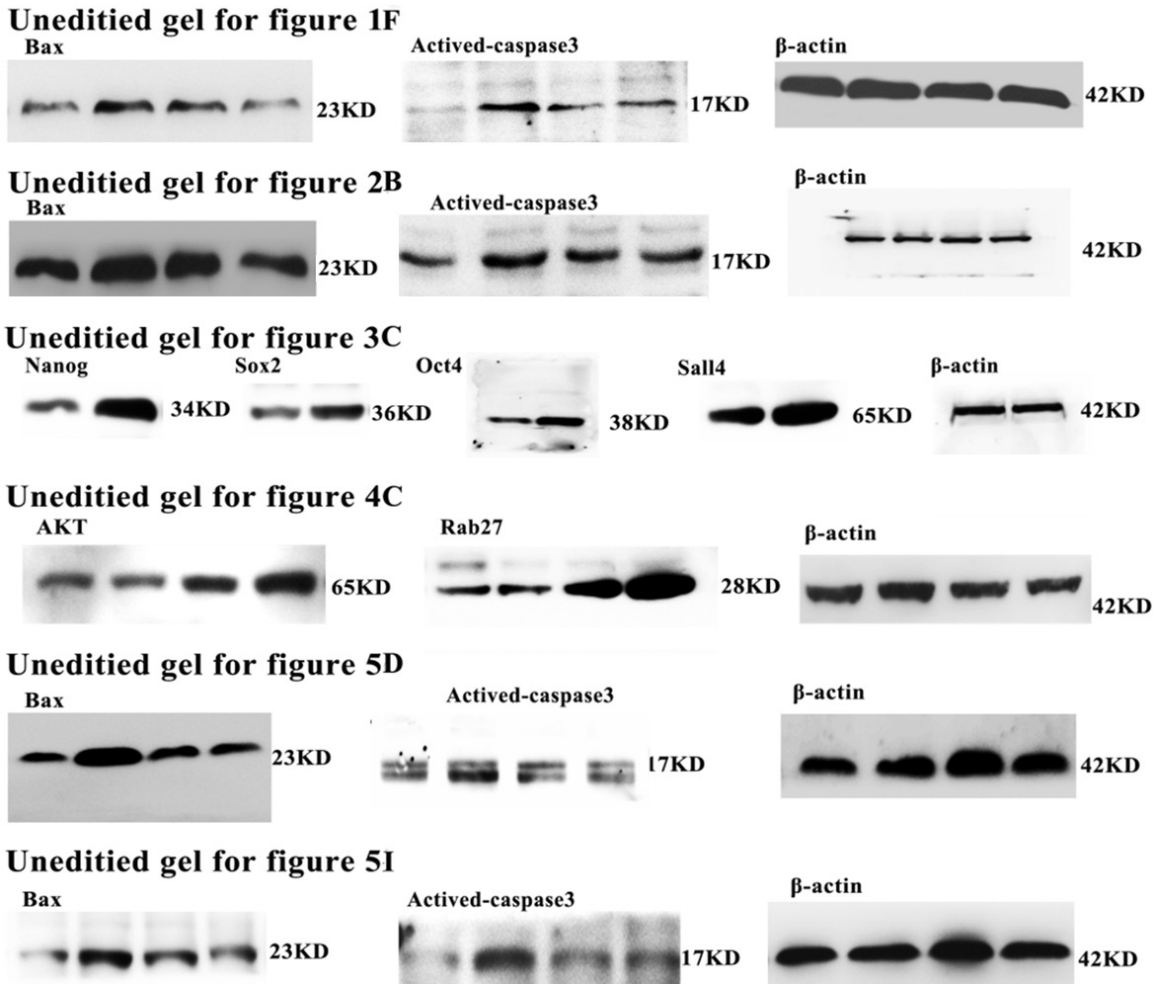

Figure S2. The unedited gel for original blot.