

# Platelet-rich plasma enhanced MSCs repair for glycerin-induced acute kidney injury via AKT/Rab27 paracrine pathway

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## Research

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# Abstract

**Background:** Acute kidney injury (AKI) was defined by rapid deterioration of renal function, as a common complication in hospitalized patients. Among the recent therapeutic options, mesenchymal stem cells (MSCs) were considered a promising therapeutic strategy for damaged tissues repair. Platelet rich plasma (PRP) regulates stromal cells to repair tissue damage through the release of growth factors. Here we proposed a possible therapeutic use of human umbilical cord mesenchymal stem cells stimulated by platelet-rich plasma (PRP-MSCs) in glycerin-induced acute kidney injury murine model.

**Methods:** In vivo, we constructed glycerin-induced acute kidney injury rat models. On day 1 post injury, rat received a tail vein injection of  $1 \times 10^6$  MSCs and  $1 \times 10^6$  PRP-MSCs. All animals were sacrificed at Day 3 after glycerin injection. In vitro, NRK-52E cells were damaged by 20% glycerol for 12 hours, after that NRK-52E incubated with MSCs and PRP-MSCs for 24 hours in transwell co-culture system, DMEM as a negative control. NRK-52E cells were harvested for apoptosis assay, Western blot, and quantitative real-time polymerase chain reaction (qRT-PCR). Then the number of MSCs exosomes stimulated by PRP was detected by confocal microscopy and Nanosight tracking analysis (NTA), PRP-MSCs-Ex (10mg/kg) and MSCs-Ex (10mg/kg) were injected intravenously, saline as control. The therapeutic effect of PRP-MSCs was evaluated by analyzing renal function (serum BUN, Creatinine), histopathological structure changes and apoptosis and proliferation of renal tubular cells.

**Results:** In vivo and vitro studies confirmed that the PRP induced YAP nucleus expression to promoting the proliferation and reinforces the stemness of MSCs, and PRP could promoted the paracrine secretion of exosomes by MSCs to repair AKI though AKT/Rab27 pathway.

**Conclusions:** Our results revealed that PRP stimulated MSCs paracrine pathway could effectively alleviate glycerin-induced acute kidney injury. Therefore, RPP pretreatment may be a new method to improve the therapeutic effect of MSCs.

## Background

Acute renal failure was caused by a variety of factors, including hypoxia, mechanical trauma, surgery, drugs and inflammation, induced the decrease of glomerular filtration rate, the accumulation of blood creatinine, urea nitrogen and other metabolites which was characterized by a rapid deterioration of renal function [1, 2]. And resulted in the corresponding clinical manifestations of the syndrome, which was a common clinical critical emergency [3, 4]. Although the renal tissue exhibited an intrinsic ability to regenerate after injury, full recovery in most cases was not achieved [5]. So that, multiple therapy approaches for renal regeneration have being considered.

Mesenchymal stem cells (MSCs) have considerable potential in regenerative medicine for their ability of renewal and differentiation into distinct cell types, among the new therapeutic options for AKI, and MSCs were gaining increasing interest for the treatment of kidney injury [6-8]. MSCs could be isolated from the

bone marrow, umbilical cord, adipose tissues, and other adult tissues<sup>[9]</sup>. Our previous studies have showed hucMSCs could be favourable candidate for injured tissue repair<sup>[10, 11]</sup>. Moreover, the efficacy of stem cell-based therapy could be further improved by small-molecule. Small-molecule drugs have an important role in regulating stem cell fate and function, and facilitate the development of cell-based therapies<sup>[12, 13]</sup>.

Platelet rich plasma (PRP) are fractions of blood plasma with platelet concentrations and the platelet content was  $1.2 \times 10^{12}$  platelets/ml. PRP contains a lot of cytokines, for instance PDGF, VEGF, TGF- $\beta$ , IGF-1 and so on<sup>[14, 15]</sup>. Recently studies reported that PRP could enhance cell clone formation, maintain the adipogenic, chondrogenic and osteogenic differentiation capacity of stem cells, and maintain an immunosuppressive state<sup>[16, 17]</sup>. Based on these biologic functions, PRP have been widely investigated in regenerative medicine. It was reported that PRP alleviated multiple organs damage, particularly in the skin injury<sup>[18, 19]</sup>. However, the effect of PRP on MSCs-based kidney therapy had not been cleared. It remains unknown whether PRP-modified MSCs could showed a more efficient repairing ability than did MSCs in tissue injury.

As we known glycerin was a kind of high permeable substance and intramuscular injection of glycerin causes local muscle necrosis and erytholysis, and it was easy to enter renal tubules through glomeruli, caused acute renal tubule damage<sup>[20]</sup>. Therefore, AKI animal model was established by intramuscular injection of 50% solution of hypertonic glycerin. Our previous studies have shown that MSCs have certain advantages in tissue damage repair. Furthermore, there were reported that modified MSCs could play enhanced therapeutic roles in kidney dysfunction diseases. As an endogenous stimulator, PRP could enhance the therapeutic effective of MSCs? What was the mechanism of MSCs repairing injury? In addition, for modulating MSCs function, small stimulator compounds have several advantages, including the convenience in modifying their concentration, working duration, and rapid and reversible working effects.

Here we investigated the effect of PRP-MSCs and MSCs on glycerin-induced AKI. Our results showed that PRP induced YAP nucleus expression to promoting proliferation and maintain the stemness of MSCs, and stimulated the paracrine exosomes of MSCs though activated AKT/Rab27 signaling pathway to inhibiting the apoptosis of renal tubular cells. RPP 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 ethical committee of Jiangsu University (2014280).

### 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<sup>[11]</sup> 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 primary concentration was  $1.2 \times 10^{12}$  platelets/mL. In the following experiments, hucMSCs treated with  $1 \times 10^8$  platelets/mL PRP 12 h, and then the stimulator (PRP medium) was removed, MSCs were cultured in DMEM for 24 hours and collected cells as PRP-MSCs, 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<sub>2</sub>. 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  $\beta$ -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 hours 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 (FEITecnaei 12, Philips, Netherlands). The size of exosomes was 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 (10mg/kg) were treatment for AKI model via tail vein injected. 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 180-200g (provided by the experimental animal center, JiangSu University, Permit Number: 2014280) were selected, 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 (10mL/kg, Sigma) into inferior hindlimbs. On day 1 post injury, rat received a tail vein injection of  $1 \times 10^6$  MSCs and  $1 \times 10^6$  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  $\beta$ -actin (1:800, CST, USA). The secondary antibodies were HRP-conjugated goat anti-rabbit and goat anti-mouse antibodies (1:1500, CWBIO, China).

### **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<sub>2</sub>O<sub>2</sub> 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 slide were placed in 4% paraformaldehyde at 4°C for 12 h. Then permeabilized with PBS solution containing 0.15% Triton X-100 for 30min and incubated with 5% bovine serum albumin (BSA) for 1h 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  $\pm$  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

## Platelet-rich plasma enhanced the repairing effect of MSCs on glycerin-induced acute kidney injury

In order to investigate the repairing effect of PRP-MSCs on acute renal injury, we constructed the AKI model by intramuscular injection of 50% hypertonic glycerol solution into the inferior hind limbs of rats (10 mL/kg), and to evaluating the repairing ability of PRP-MSCs in AKI model (Fig. 1a). Results showed that serum BUN and Creatinine levels increased markedly at Day 3 and remained at a high level after 50% glycerin injection. Compared with MSCs, PRP-MSCs transplantation reduced Creatinine levels (Fig. 1b and Fig. 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 rat confirming their beneficial effect, and PRP-MSCs was more effective than MSCs in alleviating the vacuoles of renal tubules (Fig. 1d). Moreover, the histological damage score of PRP-MSCs group was significantly lower than that of glycerin injury group, and the damage score of PRP-MSCs group was 40% lower than MSCs group (Fig. 1e). In rat kidney tissues, we used western blotting to detecting the expression of apoptosis-associated proteins. The results showed that though transplanting MSCs and PRP-MSCs decreased the expression of Bax and activated-caspase3, and the anti-apoptosis effect of PRP-MSCs group was more efficiency than MSCs group (Fig. 1f). In the pathological section of renal tissue, immunohistochemical staining further confirmed the most activated-caspase3 positive cells were glycerin groups, while the number of PRP-MSCs groups was significantly lower than that of MSCs group (Fig. 1g). Furthermore, TUNEL assay indicated that a reduced number of apoptotic cells in both MSCs and PRP-MSCs groups, and the PRP-MSCs group had fewer apoptotic cells than did MSCs group (Fig. 1h). These results indicated that PRP-MSCs showed more efficient repair ability than 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 used 20% glycerol hypertonic medium-treated NRK-52E cells. After treating with glycerin for 12 h, there were a significantly increased number of apoptotic NRK-52E cells. Then, we established an in vitro transwell co-culture system to allow the transfer of EVs but to preclude direct cell contact and evaluated the effects of NRK-52E cells treated with the MSCs/PRP-MSCs for 24 hours (Fig. 2a). Then lysis and extraction protein in NRK-52E cells, western blotting analysis the expression of apoptosis-associated proteins and the results showed that Bax and activated-caspase3 significantly increased after glycerin treatment. Nevertheless, in the PRP-MSCs group, the expression levels of Bax and activated-caspase3 were lowest in NRK-52E cell (Fig. 2d). Corresponding to these, treatment with PRP-MSCs or MSCs could effectively reverse cells apoptosis and the number of apoptotic NRK-52E cells was least in the PRP-MSCs group by flow cytometry (Fig. 2c). The statistical result was shown in Fig. 2d. Immunohistochemical and TUNEL staining further confirmed the expression of activated-caspase3 and Bax the results were consistent with that by western blotting. Respectively, the number of activated-caspase3 and TUNEL-positive cells obviously increased after glycerin treatment, PRP-MSCs group had fewer positive cells than did MSCs

group (Fig. 2e and 2f). In general, these results showed that PRP-MSCs could significantly inhibit glycerin-induced NRK-52E cells apoptosis compared with MSCs, which confirmed the role of the reno-protective.

### **PRP promoted the proliferation and differentiation of MSCs**

In vivo and in vitro experiments, we confirmed the repair effect of PRP-MSCs in AKI model. Then, MSCs treated with  $1 \times 10^8$  platelets/mL PRP 12 h, we observed that PRP could significantly promote the proliferation of MSCs (Fig. 3a). Being a key pathway involved in regeneration progression, YAP signaling controls organ size and regulates tissue cell regeneration [22, 23]. Immunohistochemistry showed that PRP induced YAP nucleus expression and promoted cell proliferation in MSCs (Fig. 3b). Then, we used western blotting and qRT-PCR analysis for stemness-associated proteins expression, at protein and gene level of Nanog, Sox2, Oct4 and Sall4 were increased in MSCs with PRP treatment (Fig. 3c and 3d). In vitro experiments, PRP induced adipogenic and osteogenic differentiation of MSCs, and the percentage of alizarin red positive cells in PRP group was higher than DMEM group, confirmed PRP could promote multi-directional differentiation of MSCs (Fig. 3e). Overall, these results suggest that PRP induced YAP nucleus expression and increased stemness-associated gene to promoting MSCs proliferation and differentiation.

### **PRP promoted the paracrine secretion of MSCs exosomes through AKT/Rab27 pathway**

It is confirmed that PRP could promoted the proliferation and repair AKI of MSCs. Next, we explored the mechanism of PRP enhanced the repair of MSCs, recently research have reported that MSC exosomes (MSC-Ex) were the key paracrine component of MSCs, and shown to exert therapeutic effects on tissue injury [24, 25]. Therefore, we focused on the effect of PRP on the exosomes secretion of MSCs. AKT/Rab27 signaling pathway regulated the synthesis and secretion of exosomes [26], in this experiment, our found that PRP stimulated AKT to enter the nucleus and the expression of Rab27 was increased (Fig. 4a and 4b). In the other side, the protein level of PRP-MSCs confirmed this result (Fig. 4c). Western blot results confirmed the higher expression of exosomal marker CD9, CD63 and CD81 in PRP-MSC-Ex (Fig. 4d). PRP stimulated the increase of CD63 in MSCs was observed with confocal microscope, which indicated that PRP promoted the secretion of exosomes (Fig. 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 (TEM) (Fig. 4f), MSC-Ex as nanoscale membrane vesicles, the size was determined to be  $101.5 \pm 24.3$ nm and PRP-MSC-Ex was  $108.6 \pm 33.6$ nm (mean  $\pm$  SD) by nanoparticle tracking analysis (NTA), diluted 1000 times when measuring, so the concentration of MSC-Ex was  $2.5 \times 10^9$ /ml and PRP-MSC-Ex was  $5.1 \times 10^9$ /ml (Fig. 4g), and we found the shape and particle size of exosomes not changed by PRP stimulation, while PRP could increased the secretion of MSC-Ex twice (Fig. 4h). These results indicated that PRP promoted the paracrine secretion exosomes of MSCs through activated AKT/Rab27 pathway.

### **PRP-MSC-Ex localization and repaired for renal injured tissue**

To determine the localization and bio-distribution of PRP-MSCs-Ex within injured renal tissue, exosomes were labeled with a fluorescent dye (DIR, 50 $\mu$ M) were injected intravenously and tracked by optical imaging (PerkinElmer, USA) in AKI mice. The performed 12 h after the administration of exosomes, revealed the presence of exosomes fluorescence signal in injury kidneys (Fig. 5a and Fig. S2a). Previously, we confirmed the orientation of exosomes in damaged tissues, next step we analyzed the role of the reno-protective factor. Compared with MSCs-Ex, PRP-MSCs-Ex transplantation reduced Bun and Creatinine levels in AKI model (Fig. S1c and Fig. S1d). Kidney histological revealed that the number of casts and necrotic tubules was significantly reduced in MSC-Ex and PRP-MSC-Ex treated rats (Fig. 5b). Similarly, the TUNEL analysis showed that the number of apoptotic cells in MSC-Ex group and PRP-MSC-Ex group were decreased, and the number of apoptotic cells in PRP-MSC-Ex group was significantly less than that in glycerin group (Fig. 5c). The expression of apoptosis-related proteins Bax and Activated-caspase3 in renal tissue were inhibited after PRP-MSC-Ex treatment (Fig. 5d). And the statistical analysis of the intervention effect of PRP-MSC-Ex was better than that of MSC-Ex group (Fig. 5e). In vitro experiments, 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 PRP-MSC-Ex group decreased by 76.3% (Fig. 5f and 5g). In order to further determine this phenomenon, immunohistochemistry was used to detected the expression of activated-caspase3 positive cells after intervention, the number of positive cells in PRP-MSC-Ex group was significantly less than in injury group, and the positive cells were less than the MSC-Ex group (Fig. 5h). Western blotting results showed that the expression of Bax and activated-caspase3 decreased significantly after MSC-Ex treatment. Moreover, in PRP-MSC-Ex group, Bax and activated-caspase3 had the lowest expression level in NRK-52E cells (Fig. 5i). These results confirmed that the PRP-MSC-Ex had more beneficial effect compare with PRP-MSC. In general, these results showed that exosomes were the key component of MSC in the treatment of renal injury which could significantly inhibited the apoptosis of NRK-52E cells induced by glycerin, and confirmed the mechanism of PRP stimulated the secretion exosomes of MSC in the repair of acute renal injury.

## Discussion

Kidney injury treatments have always been the intractable medical problem. The therapeutic methods so far were function-limited and if the treatment intervention was not timely, could deteriorate into chronic kidney disease, and many patients suffer from chronic pain for a long time<sup>[27]</sup>. Cell-based therapies and tissue regeneration are new approach to overcome the present limitations of kidney injury healing<sup>[28, 29]</sup>. MSCs were a promising therapeutic tool in regenerative medicine due to their self-renewal and multi-directional differentiation potency. Recently have a large number of studies showed that MSCs could repair cisplatin/glycerin-induced acute kidney injury<sup>[30]</sup>, ischemia/reperfusion-induced acute renal failure<sup>[31]</sup>, and unilateral ureteral obstruction-induced renal interstitial fibrosis<sup>[32]</sup>. Nevertheless, the transplanted MSCs also existed potential problem, most of them could not survive for a long time, only a few of them exist in the damaged kidney. The lower implantation and survival rates weakened the therapeutic effect of MSCs treatment. Therefore, in order to maximize the clinical application value of MSCs, we must take corresponding strategies to improve their therapeutic effect.

One of the common strategies that improved the therapeutic effects of stem cell transplantation was pretreatment stem cells with small molecule drugs. Our recently studies showed that 3,3'-diindolylmethane stimulated exosomal Wnt11 autocrine signaling in MSCs to enhance wound healing<sup>[11]</sup> and resveratrol improved MSCs repair for cisplatin-induced AKI<sup>[33]</sup>. These studies proved that small-molecule drugs were potential candidates for stem cell manipulation in regenerative medicine.

The effect of Platelet-rich plasma (PRP) on tissue healing has been mainly attributed to release of growth factors from platelets, such as PDGF, VEGF and TGF- $\beta$  could promote cell proliferation and migration<sup>[34]</sup>. Recently, studies have reported that PRP could attract MSCs, then PRP enhanced cells repair and promote the initial healing of wounds and this phenomenon called the chemotactic effect<sup>[18]</sup>. 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 MSC, as demonstrated by the decrease of serum creatinine and BUN levels, PRP stimulated MSC could inhibit apoptosis of renal tubular cells and as well as the decrease of histological injury score. MSC primed with PRP exhibited more effective repair effects than untreated MSC in glycerin-induced AKI models. These results indicated that PRP pre-treatment was safe, efficient and low cost, which was expected to emerge as a promising strategy to improve MSC-based therapy. The next step was to thinking about the mechanism of PRP promoting MSC to repair renal injury. As we know, YAP plays a key role in the control of tissue and cell proliferation and development<sup>[22, 35, 36]</sup>. Through the experiments PRP stimulated YAP protein to enter the nucleus which promoted the proliferation of MSC and the differentiation ability of osteoblasts and adipocytes. These information were suggested that appropriate amount of PRP could enhance the injury repair effective of MSC.

Exosomes, one form of extracellular vesicles, have potential as mediators of intercellular message and play a major role in cell-to-cell communication, while therapeutic effects of human MSC-derived exosomes have been verified using a rodent model<sup>[21, 37, 38]</sup>. And then research team finding platelet-rich plasma improved the wound healing potential of mesenchymal stem cells through paracrine and metabolism alterations<sup>[39]</sup>. AKT/Rab27 pathway was involved in the regulation of exosomes synthesis and secretion<sup>[26, 40]</sup>. In this experiment, we found that PRP could increase two times number of MSC exosomes, without changing its morphology and particle size. And PRP stimulated paracrine secretion of MSCs exosomes by activating AKT/Rab27 pathway, DiR dye-labeled PRP-MSCs-Ex localized within the injured kidney and promoted the recovery of renal function, reduced the expression of injury markers.

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

## Conclusion

This study reveals that PRP promoted the paracrine secretion of exosomes by MSCs to repair glycerin-induced AKI though AKT/Rab27 pathway. These findings confirm that RPP pretreatment could improved

the therapeutic effect of MSCs on renal injury.

## **Declarations**

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### **Availability of data and materials**

Not applicable.

### **Authors' contributions**

CJ: conception and design, collection and assembly of data, and manuscript writing. JZ: conception and design, data analysis and interpretation, and manuscript writing. XZ: conception and design, data analysis. HS: collection and/or assembly of data and data analysis. WL: collection and/or assembly of data and data analysis. FS: data analysis and interpretation. CZ: provision of study material and interpretation. LZ: data analysis and interpretation. XZ: data analysis and interpretation. HQ: study design, manuscript writing, and final approval of manuscript.

All authors read and approved the final manuscript.

### **Conflict of interest**

The authors declare that they have no conflict of interest.

### **Consent for publication**

Not applicable.

### **Ethical approval and consent to participate**

This study was approved by the ethical committee of Jiangsu University (2014280).

## **Abbreviations**

Platelet-rich plasma (PRP)

Mesenchymal stem cells (MSCs)

Acute kidney injury (AKI)

Mesenchymal stem cells-exosomes (MSCs-Ex)

Quantitative real-time polymerase chain reaction (qRT-PCR)

Nanoparticle tracking analysis (NTA)

Immunohistochemistry (IHC)

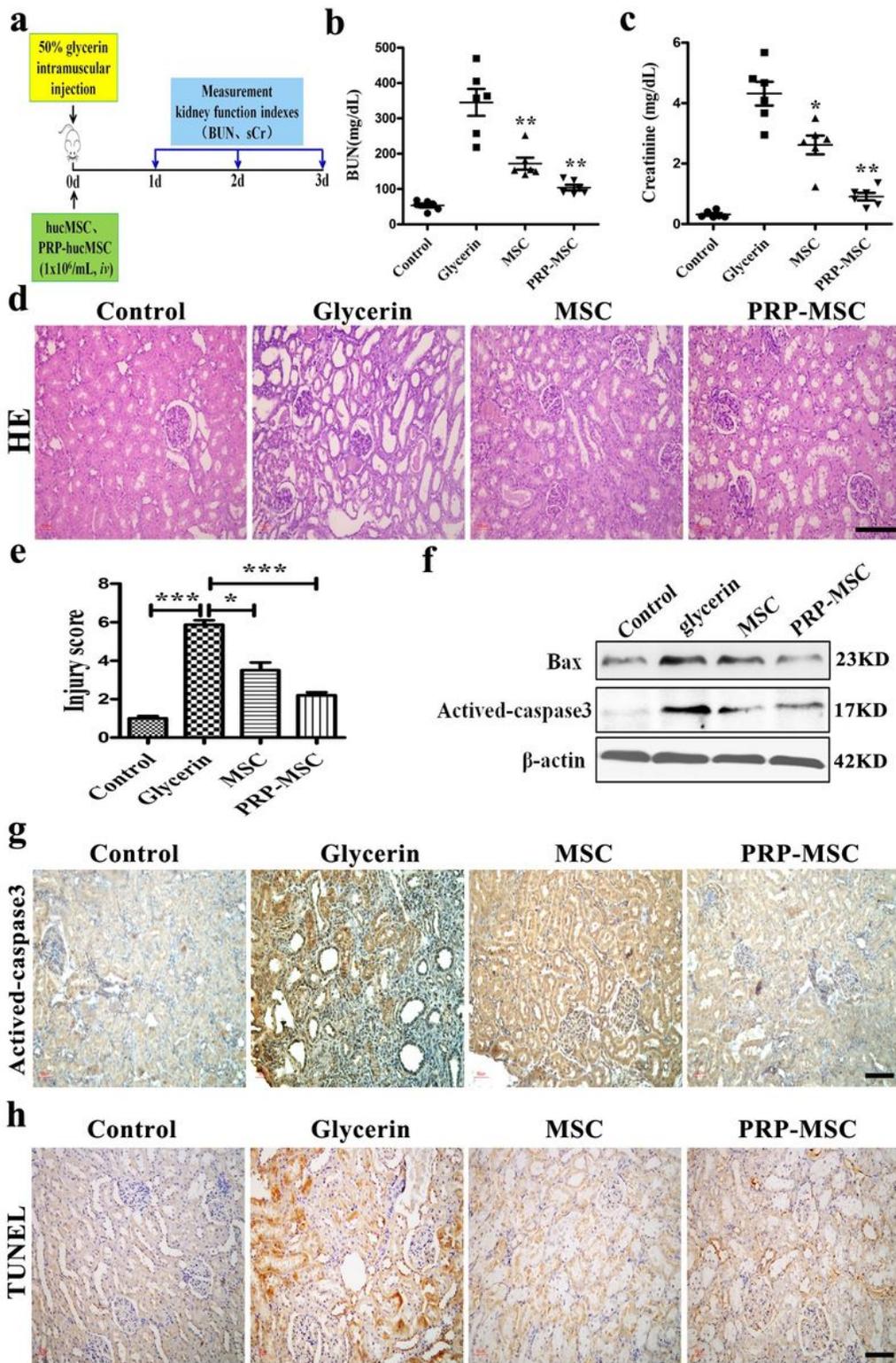
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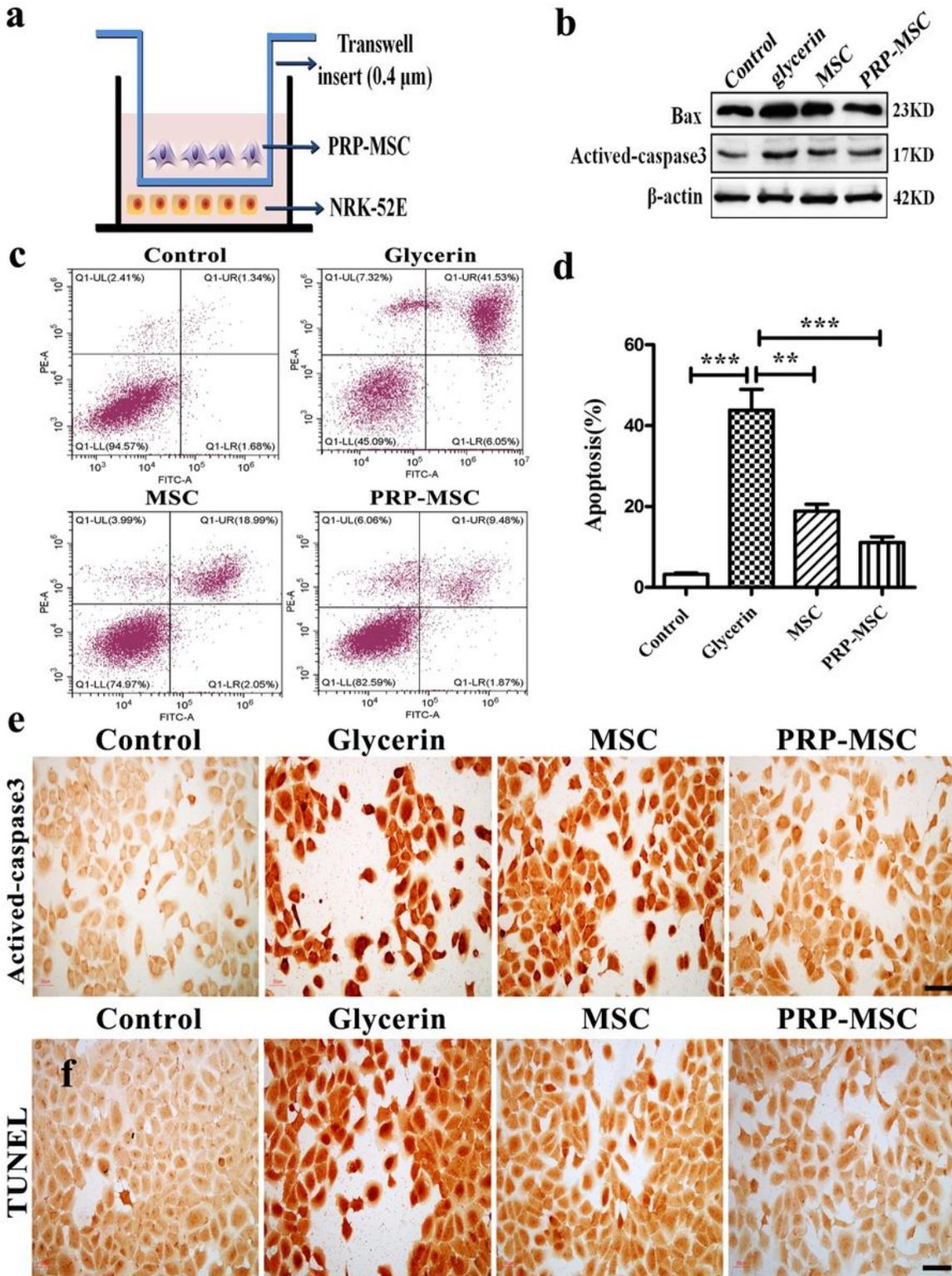
## Figures



**Figure 1**

Platelet-rich plasma enhanced the repairing effect of MSCs on glycerin-induced acute kidney injury a. Schematic of AKI model and MSC/PRP-MSC treatment. AKI rats were intravenously injected with MSC/PRP-MSC ( $1 \times 10^6$ /injection) on days 1 and 2, 3. The rats were sacrificed on day 4 for subsequent experiments ( $n=6$ ). b. Serum BUN and c.) Creatinine levels in 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

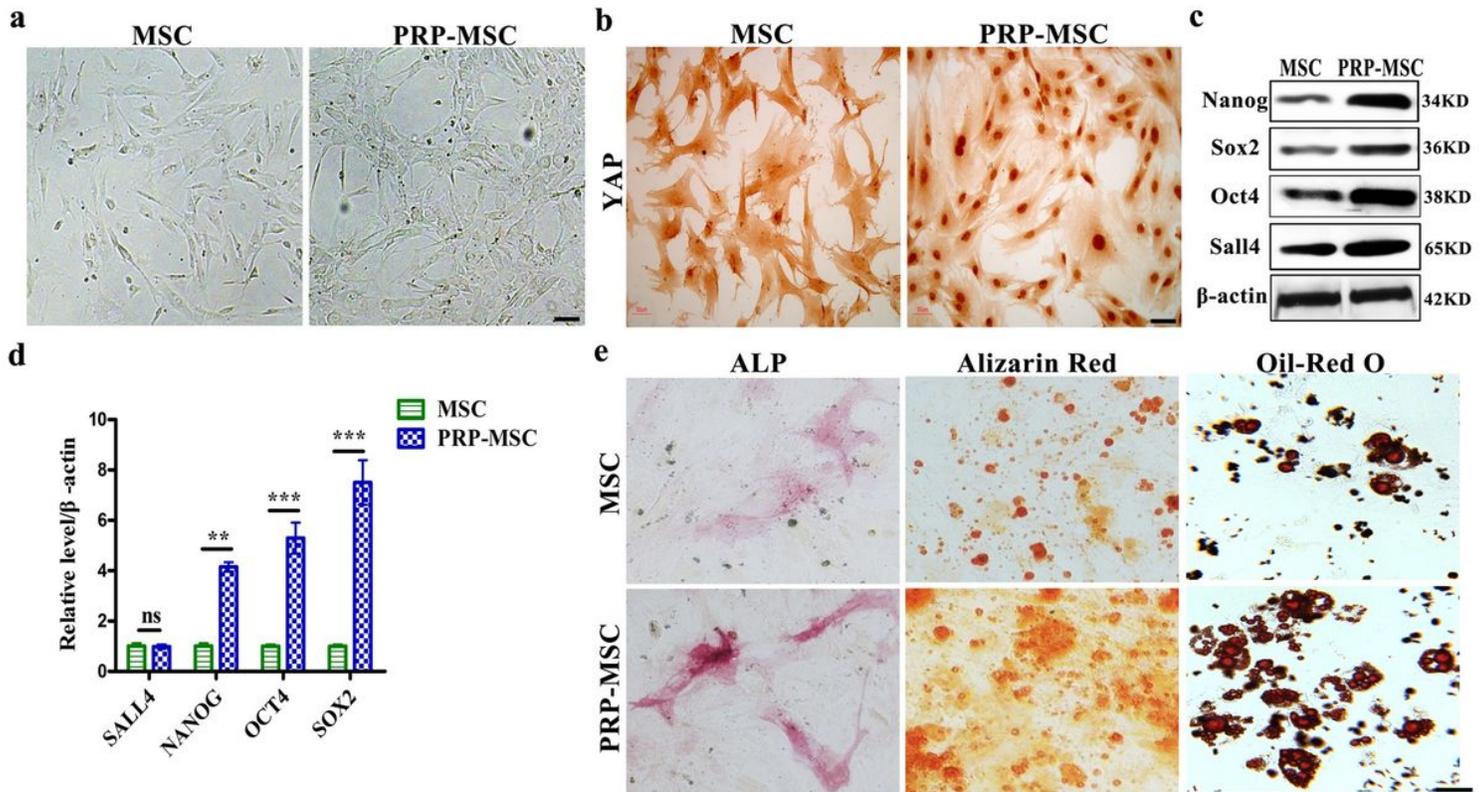
glycerin injection in the four groups (Scale bars =100 $\mu$ m). e. The degree of tubular damage was scored by choosing 10 non-overlapping fields and calculating the percentage of tubules in the kidney cortex, which exhibited tubular cells necrosis and cast deposition as follows: 0, none; 1,  $\leq$  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 blotting 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 $\mu$ m). h. Representative images of TUNEL staining in the kidney sections at Day 3 after PRP-MSCs treatment (Scale bars = 50 $\mu$ m).



**Figure 2**

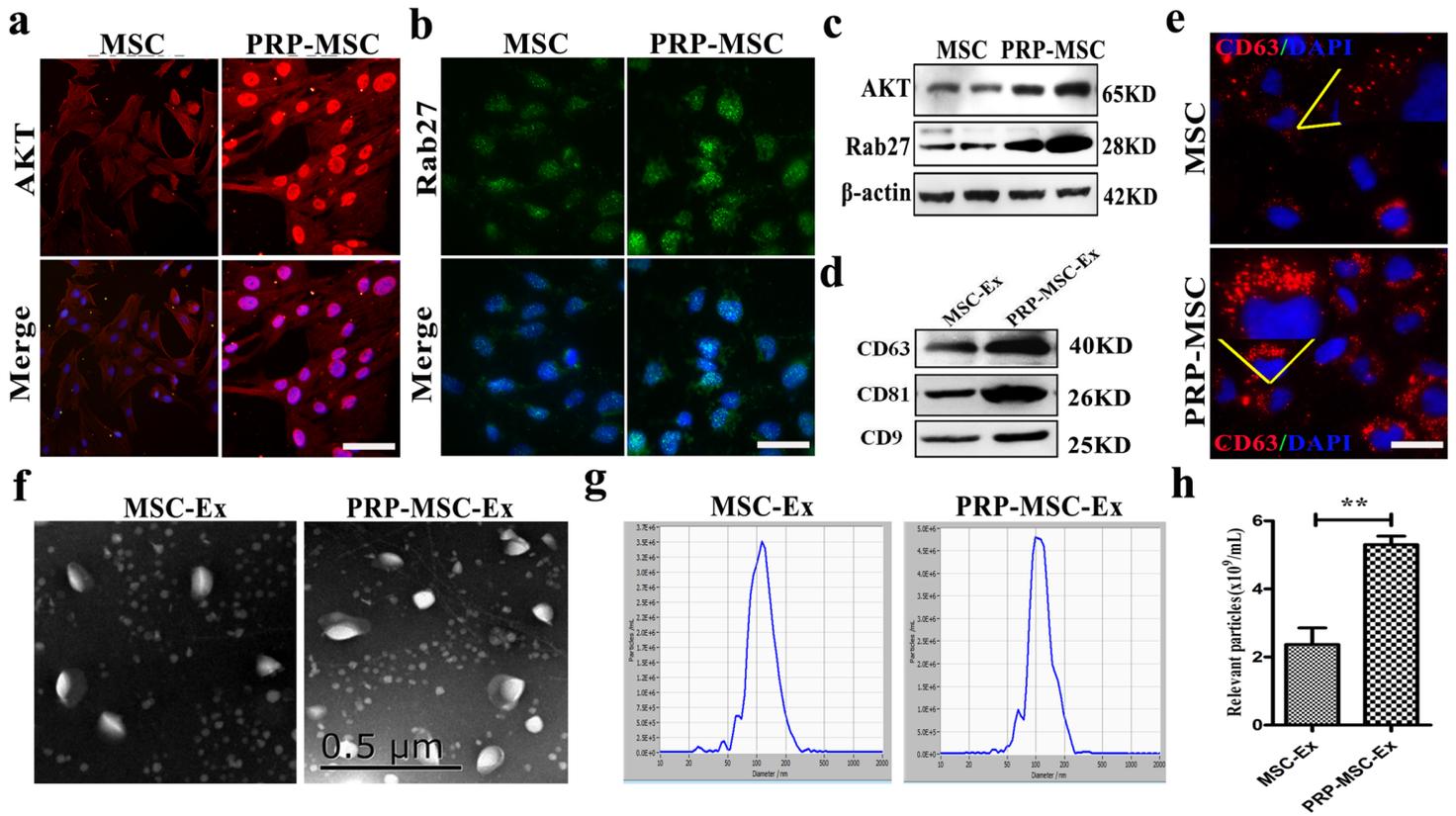
PRP-MSCs attenuated glycerin-induced NRK-52E cells apoptosis a. A schematic of the Transwell coculture model with PRP-MSCs in the upper chamber and NRK-52E cells in the lower chamber of the well. A porous (0.4  $\mu\text{m}$ ) membrane allows the transfer of exosomes but precludes direct cell contact. b. Western blotting analysis for Bax, active-caspase3 expression in NRK-52E cells. c. Flow cytometry detected apoptotic NRK-52E cells with PRP-MSCs treatment. d. The 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 = 50µm). f. Representative images of TUNEL staining in glycerin-induced injured NRK-52E cells with PRP-MSCs treatment (Scale bars = 50µm).



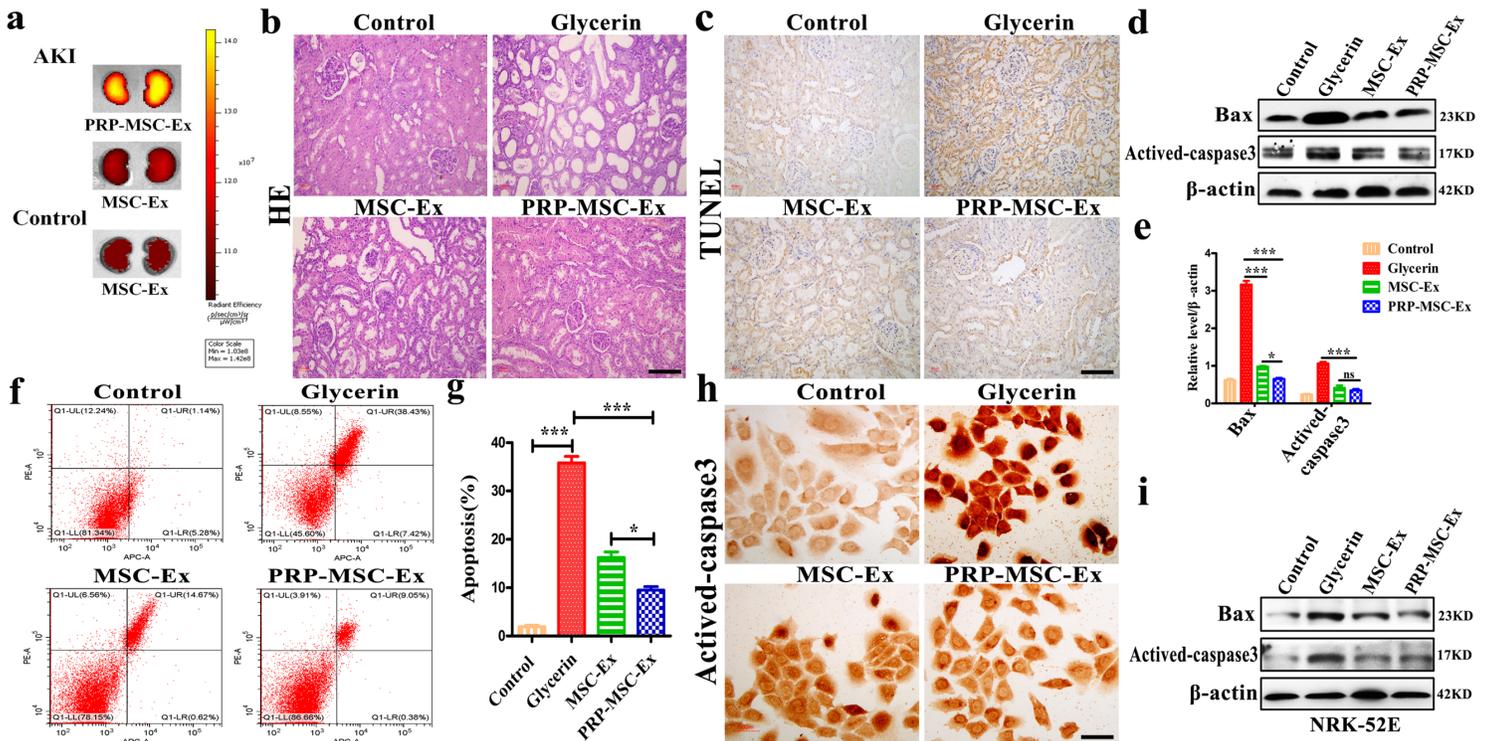
**Figure 3**

PRP promoted the proliferation and differentiation of MSCs a. Microscope observed the growth of MSC 24 h after PRP stimulation (Scale bars=50µm). b. Immunohistochemical analysis of YAP protein localization in MSCs under PRP stimulation (Scale bars=50µm). c. Western blotting analysis for stemness-associated proteins in MSCs with PRP treatment. d. qRT-PCR analysis 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 = 50µm).



**Figure 4**

PRP promoted the paracrine secretion of MSCs exosomes through AKT/Rab27 pathway a. Immunofluorescence analysis of AKT and b) Rab27 in MSCs under PRP stimulation (Scale bars = 20 $\mu$ m). c. Western blotting analysis for AKT and Rab27 in MSCs with PRP treatment. d. Western blotting detected the expression of exosomal markers in MSCs treatment with PRP. e. The expression of exosomal marker CD63 (red) in MSCs with PRP treatment was detected by confocal microscope (Scale bars = 10 $\mu$ m). f. The morphology 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**

PRP-MSC-Ex localization and repaired for renal injured tissue a. Representative fluorescence images of AKI rat injected with DiR-labeled PRP-MSC-Ex, evaluated 12 h after injection. b. Representative micrographs of renal HE histology after MSC-Ex/PRP-MSC-Ex treatment (Scale bars =100µm). c. Immunohistochemical staining graph of active-caspase3 in the glycerin-induced injured kidney sections (Scale bars = 50µm). d. Western blotting analysis for Bax, active-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 with PRP-MSC-Ex treatment. g. The statistical result of apoptotic NRK-52E cells with MSC-Ex/PRP-MSC-Ex treatment (\*P<0.05, \*\*P<0.01, \*\*\*P<0.001). h. Representative immunohistochemical images of active-caspase3 with PRP-MSC-Ex treatment (Scale bars = 50µm). i. Western blotting analysis for Bax, active-caspase3 expression in NRK-52E cells after PRP-MSC-Ex interence.

## Supplementary Files

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