

Protective effect of Bmscs-derived exosomes on testicular ischemia-reperfusion injury in rats

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Research

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Abstract

Background

Testicular Ischemia reperfusion injury(IRI) is a major pathophysiological process of surgical reduction after testicular torsion, and oxidative stress is the main injury factor. However, the role of BMSCs-derived exosomes in testicular IRI and its mechanism have not been reported. In this study, we investigated the protective effect of bone marrow mesenchymal stem cell-derived exosomes against testicular ischemia-reperfusion injury.

Methods

Results: BMSCs were successfully isolated and cultured from rat bone marrow, and exosomes secreted by BMSCs were successfully extracted. In vivo experiment: The testicular torsion rat model was established, and various biochemical indexes of oxidative stress and testicular tissue HE was detected in the sham operation group, testicular torsion group and bone marrow mesenchymal stem cell-derived exosome treatment group. In vitro experiment: H₂O₂ was used to construct TM4 and GC1 oxidative stress models, and various biochemical indexes of oxidative stress and corresponding pathway proteins were detected in the control group, H₂O₂ group and bone marrow mesenchymal stem cell-derived exosome treatment group.

Conclusion

BMSCs-derived exosomes can be absorbed by rat spermatogonia and have antioxidant and anti-inflammatory protective effects against testicular ischemia-reperfusion injury

Introduction

Testicular torsion (TT), is a testicular torsion along the longitudinal axis of the spermatic cord[1], resulting in acute reduction or interruption of testicular blood supply, resulting in testicular tissue ischemia and necrosis. The incidence of testicular torsion is up to 158, which mostly occurs in teenagers under 24 years old[2–6]. Clinically, because it is difficult to distinguish the disease from scrotal inflammation in the early stage[1, 2, 7, 8], domestic data show that the misdiagnosis rate of testicular torsion can be as high as 67.6%. TT is very easy to cause misdiagnosis and torsion testicular ischemia time is too long, tissue necrosis[9], and finally can only carry out surgery to remove necrotic testicles, resulting in testicular loss in patients; this disease is also one of the most common causes of testicular loss in adults, so it should be diagnosed and treated as soon as possible. At present, timely surgical reduction to restore the blood supply of torsional testis is the standard treatment for the disease. however, testicular torsion reduction is a typical (IRI) process of ischemia-reperfusion injury, which can increase testicular germ cell apoptosis[10], lead to varying degrees of testicular atrophy and spermatogenic dysfunction, and even

lead to infertility. Therefore, even if there is timely surgical reduction, testicular ischemia-reperfusion injury is still inevitable. Although most researchers at home and abroad use some drugs to reduce testicular IRI, there is no ideal treatment in clinic[1, 2, 11].

The latest progress in stem cell research brings hope for the prevention and treatment of oxidative stress injury[1, 2, 11]. Because of its multi-directional differentiation potential, immunomodulatory activity, ability to migrate to the injured site, simple expansion in vitro, and a wide range of sources, it has been concerned by more and more researchers. Among them, bone marrow mesenchymal stem cells are easy to separate and culture, which has become a research hotspot in the field of tissue injury and repair. At present, many studies have found that bone marrow mesenchymal stem cells can reduce oxidative stress injury in heart, brain, lung and other parts[14–18], but the mechanism of its effect is unknown. At present, more and more studies have shown that bone marrow mesenchymal stem cells alleviate oxidative stress injury mainly through exosome secreted by bone marrow mesenchymal stem cells[19–23].

Exosomes are extracellular vesicles with lipid bilayer membranes[24]. Their diameters range from 30 to 150nm and can be released by a variety of cells such as immune cells, vascular cells[25], nerve cells, stem cells and tumor cells. Exosomes have rich cellular specificity such as protein, lipid and nucleic acid, and can participate in a variety of physiological and pathological processes, such as antigen presentation[26, 27], RNA transport, tissue repair, tumor metabolism, metabolism and so on. At present, some studies have shown that exosome can alleviate inflammation and oxidative stress injury, but whether exosome derived from bone marrow mesenchymal stem cells can improve testicular ischemia-reperfusion injury has not been reported.

In this study, we explored a new function that exosome derived from bone marrow mesenchymal stem cells can alleviate oxidative stress caused by testicular ischemia-reperfusion injury. We proved that the exosome derived from bone marrow mesenchymal stem cells can pass through the blood-testis barrier to around the seminiferous tubule and reduce the oxidative stress injury caused by testicular ischemia-reperfusion in testicular torsion model rats. These findings reveal the reasons for the resistance of mesenchymal stem cells to oxidative stress injury and provide a new treatment for patients with testicular torsion to prevent and alleviate testicular ischemia-reperfusion after testicular reduction.

Material And Methods

Ethics statement and Animals

This study was approved by the bioethics committees of Southern Medical University and the Third Affiliated Hospital of Southern Medical University, Guangzhou, China. All the rats we need[Male SD: 3 to 4 weeks old] were purchased from Laboratory animal center of southern medical university and kept under specific pathogen-free conditions. All animal experiments were approved by the Ethics Committee of Southern Medical University. Guidelines of the Institutional Animal Ethics Committee were followed when carrying out in vivo experiments. All efforts were made to minimize animal suffering.

Isolation of MSCs from mouse bone marrow

Healthy Male SD rats weighted about 80-120g were used to isolate bone marrow-derived MSCs which were killed by cervical dislocation. Under sterilized conditions The skin was disinfected with 75% alcohol and then The rats were anaesthetized using pentobarbital (50 mg/kg, intraperitoneal injection) to minimize suffering and sacrificed by cervical dislocation without recovery from anesthesia. Tibias and femurs then were removed under sterile conditions. In the laboratory and under sterilized conditions, the femurs and tibias were cleaned off from the remaining muscle tissues with sterile surgical tools and washed few times with normal saline solution. The tip of the bone was inserted into the spiral tip bottom centrifuge tube(15ml)after ends of femurs and tibias the were cut. Bone marrow cell suspension was obtained by flushing marrow cavity using dispensable 1-ml syringe with low-glucose dulbecco's modified eagle medium (DMEM).Then bone marrow cell suspension was filtrated through 200-mesh sieve in order to remove bone debris. the sediment which Contains bone marrow cell was acquired after centrifugation of the filtered suspension at 1000 rpm/min for 5 min. Remove the supernatant, the sediment was suspended with A moderate amount of DMEM .bone marrow cells suspension were shifted into petri dishes On average and incubated at 37°C in a humidified atmosphere containing 5% CO₂,adn 95% air with low-glucose DMEM plus 10% heat-inactivated fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) and 1% penicillin–streptomycin (Beyotime Biotechnology, Shanghai, China).

Culturing and propagation of BMSCs

According to the report(16. Freshney RI. Culture of Animal Cells. A Manual for Basic Technique. 5th ed. New York, NY: John Wiley & Sons; 2005. [Ref list]),The proportion of adhere cells and nonadherent cells would change after overnight incubation with medium. adhere cells was the majority, and the little bit of nonadherent cells were washed out with medium changes in MEM media with 20% FBS.so The remaining nonadherent cells were removed by exchanging the culture medium within every 2-3 days until the cultures become the developing colonies of adherent cells (about 7 days) and then shift to monolayer cells. Cells were subculture after being monolayered using 0.25% trypsin ethylenediaminetetraacetic acid (EDTA) (United States Biological). The morphological changes and growth conditions were observed with inverted phase contrast microscope .The passage one (P1) cells began to proliferate and form a monolayer of cells in 3–5 days (with 3.0× 10⁶).Cells at 4th to 8th passage were utilized for subsequent experiments.

Exosome isolation

In short, the exosomes were isolated using gradient centrifugation.in order to exclude exogenous exosome contamination, BMSCs at 4th to 8th passage was cultured with exosome-free serum and the cell culture medium was collected. the culture supernatants were cleared of cell debris and large vesicles by sequential centrifugation at 300g for 10 min, 1000g for 20 min, and 10,000g for 30 min, followed by filtration using 0.45um and 0.22um sterile filters. Then, the cleared sample was centrifugal at 100,000g for two hour to pellet the exosomes. To get a purer exosome, Re-speeding in the same condition after removing the and the precipitating are suspended with phosphate buffer solution (PBS).The recovery of

exosomes was estimated by measuring the protein concentration using the BCA Protein Assay Kit (Beyotime Biotech, Jiangsu, China). The obtained exosomes fraction was re-suspended in PBS (500 μ L, 1 mg/mL total protein).

Western Blot

The concentrations of all the protein we extracted from cells, exosomes and tissues were determined using a BCA protein assay kit (Beyotime Biotech, Jiangsu, China) according to manufacturer instructions. Subsequently, a certain amount of total protein[20ug-80ug] was heated to 95 °C for 10 min in 1 × DTT-containing sodium dodecyl sulfate (SDS) sample buffer and separated by 10% SDS-polyacrylamide gel electrophoresis, followed by transfer onto polyvinylidene fluoride membranes (BioTrace; Bio-Rad, Hercules, CA, USA). Membranes were blocked with 5% bovine serum albumin for 1 h, and polyclonal antibodies, including anti-CD63,anti-CD81, anti-TSG101,anti-AKT,anti-p-AKT anti-ALIX, anti-HMGB1, anti-Caspase-3,anti-Bcl2 (Abclonal, Woburn, MA, USA), anti-ACTB, anti-GAPDH (Ray Antibody, Beijing, China), and anti-Calnexin (Bioworld, Dublin, OH, USA), were used for immunoblotting at 4 °C overnight. Each specific horseradish peroxidase-conjugated secondary antibody (Ray Antibody) was added accordingly after the membranes were washed in TBS-Tween solution (TBS-T) three times for 30 min, and signals were detected by enhanced chemiluminescence (Pierce, Rockford, IL, USA).

Nanoparticle tracking analysis (NTA)

Particle size and concentration distribution of the isolated exosomes stemmed from BMSCs were measured using NTA (v2.3; Malvern Instruments, Malvern, UK) according to manufacturer's instructions. Briefly, exosomes samples were vortexed and diluted to a final dilution of 1 : 2000 in filtered molecular-grade H₂O. Blank-filtered H₂O was run as a negative control. Each sample analysis was conducted for 60 s and measured three times using Nanosight automatic analysis settings.

Transmission electron microscopy (TEM)

TEM analysis was performed to confirm BMSCs-derived exosomes morphology. Briefly, exosomes samples were diluted with PBS to the appropriate concentration, and ~20–40 μ L of PBS solution containing exosomes was transferred to a copper grid for incubation at room temperature for 5 min. Filter paper was used for absorbing unevaporated solution. Exosomes samples were negatively stained with 4% paraformaldehyde acid solution at room temperature for 5 min and dried at 65 °C for 10 min. Images of exosomes samples were obtained using a Hitachi H-7650 transmission electron microscope (Hitachi, Tokyo, Japan).

BMSCs-derived exosomes uptake by GC-1 cells

Purified BMSC-Exo were labeled with 1 μ M PKH67 (Invitrogen) as previously described. Briefly, BMSC-Exo were mixed with 1 μ M PKH67, and the exosome-dye suspension was incubated for 5 min with regular mixing. Excess dye from the labeled exosomes was removed by ultracentrifugation at 100,000 g for 1 h at 4°C using a 70Ti rotor (Beckman Coulter), and the exosome pellets were washed three times by

resuspending them in PBS. The final pellets were resuspended in PBS. PKH67-labeled exosomes were co-cultured with Spermatogonium cells(GC-1) for 6 h, then GC-1 were washed with PBS, and fixed in 4% paraformaldehyde (PFA).The nucleus of cells were stained using medium containing 4,969-diamidino-2-phenylindole (DAPI; Vector Laboratories, USA). The uptake was observed by fluorescence microscopy.

Animal experiments

Under the condition of Specific pathogen Free(SPF) animal laboratory, Altogether 30 male Sprague - Dawley rats were randomly divided into 3 groups of 10 each :sham-operated control group ,experimental group(I/R) and Treated group(I/R+EXO).In order to induce the ischemia-reperfusion model of testis, rats were anesthetized with xylazine (20 mg/kg) and ketamine (50 mg/kg) after Overnight fasting. In the sham-operated control group, rats underwent left scrotal exploration only with similar surgical procedures without the torsion and detorsion. And the experimental and Treated group were through a sub-inguinal incision the left testis was brought out and was rotated 720°clockwise and then inserted and fixed into the scrotum with a 4/0 silk suture placed through the tunica albuginea and subcutaneous tissue. The incision was primarily closed with a 4/0 silk suture. After 4 hours, by using the same incision line, testis was counter rotated to the natural position and reinserted into the scrotum to induce reperfusion for 2 hours[28].After that ,the two groups ,experimental and Treated group, were respectively injected from the tail vein with the same amount of saline and 100ug/ml exosomes. At the end of the reperfusion period rats were decapitated and testis tissues were taken for biochemical analyses and histological evaluations. Biochemical and histological samples were blindly examined.

Distribution of exosome bodies in vivo (tracking)

According to the manufacturer's instructions, exosomes derived from the BMSCs were isolated as described above were labeled using PKH67 Fluorescent Cell Linker kits (Sigma-Aldrich, St. Louis, MO).The washed exosomes pellets from the 100 ml culture media were resuspended in 700 μ l of Diluent C(exosomes solution). PKH67 dye (1 μ l) was diluted in 250 μ l of Diluent C (PKH67 solution). Then, 250 μ l exosomes solution and 250 μ l PKH67 solution were mixed in a 4.7 ml centrifugation tube. Samples were mixed gently for 4 min, and 4.2 ml of 1% BSA was added to bind the excess PKH67 dye. PKH67-labeled exosomes were ultracentrifuged at 120,000 x g for 3 h at 4°C using the Optima Ultracentrifuge (Beckman Coulter). Exosome pellets were washed three times in PBS by ultracentrifugation. Finally, PKH67-labeled exosomes were resuspended in D-MEM or RPMI-1640 medium. As the negative controls, no PKH67 control and no exosome control were prepared. Exosomes were collected by ultracentrifugation without PKH67 dye, and then D-MEM or RPMI-1640 medium were added to the centrifuged tubes (no PKH67 control). After PKH67 dye was washed by ultracentrifugation without exosomes, the supernatant was discarded and media described above were added to the centrifuged tubes (no exosome control).then the equal amount of exosome was injected into different mice through the tail vein.

Anoikic assay

For anoikic analysis, TM4 and GC1 cells were cultured in 80 μ mol/L H2O2 for 8 h. Cells were then trypsinized and stained with Annexin V- APC/7AAD and analyzed by flow cytometry using an Annexin V- APC/7AAD Apoptosis Detection kit (BD Biosciences) according to the manufacturer's instructions. Data were collected on a BD Franciscan and analyzed using the FlowJo software.

Histological analysis

Testes were removed from the rats and fixed with 10% formaldehyde. Thereafter, the testes were dehydrated with subsequent 70, 90, 96 and 100% ethanol and cleared with toluene. After overnight incubation of paraffin in a 60 °C incubator, testes were embedded and blocked in paraffin at room temperature. Approximately 5 μ m thick paraffin sections in midline area of the testis were stained with hematoxylin and eosin (H&E). In each section at least 30 seminiferous tubules were evaluated microscopically at $\times 200$ magnification. The first seminiferous tubules were selected randomly and the others were taken by sliding the section towards the clockwise.

Biochemical indicators Analysis

An appropriate amount of testicular tissue and aseptic saline were prepared with a ratio of 1:9 to 10% tissue homogenate and then determination of the supernatant after centrifugation for various indicators. The testicular homogenates were assayed for total antioxidant capacity (T-AOC), superoxide dismutase (SOD), nitric oxide synthesis (NOS: tNOS and iNOS) Catalase (CAT), Glutathione (GSH) and malondialdehyde (MDA) by using colorimetry for all the oxidation indicators. The specific determination principle of each index and the preparation method of reagent and the determination method of each indicator protein content were strictly carried out according to the instructions of the kit (Institute of Bioengineering Nanjing Jian Cheng China).

Statistical analysis

For the all experiments, data are presented as the mean \pm S.E.M. Tests for significant differences between the groups were performed using a t-test or one-way ANOVA with multiple comparisons (Fisher's pairwise comparisons) using GraphPad Prism 5.0 (GraphPad software, San Diego, CA, USA). A minimum p value of 0.05 was chosen as the significance level.

Results

Characterization of exosomes

To confirm the presence of BMSCs-derived exosomes, exosome-marker proteins, Including CD63, TSG101, ALIX and Calnexin were validated by western blot (Fig. 1C). In contrast, these exosome-specific proteins were absent from collected exosome-depleted fractions, whereas the endoplasmic reticulum marker Calnexin was detected in supercell lysates. TEM results showed that exosomes contained lipid bilayer-

bound membranes, with size distributions peaking at 105 nm diameter according to NTA (Fig. 1A and 1C).

Exosomes derived from bone marrow mesenchymal stem cells can reduce testicular ischemia-reperfusion injury in rats.

According to the literature, the rat testicular IRI model (Fig. 2A) was successfully constructed. It can be found that compared with the sham operation group, the testicular injury of testicular IRI rats is more serious, showing a large number of exudation and vacuole formation in the lumen. The arrangement of spermatogenic cells is disordered, the cell level is unclear, and the seminiferous epithelium may be absent or exfoliated to some extent (Fig. 2B). On the basis of testicular IRI animal model, we injected BMSCs-derived exosomes and PBS into the tail vein and found that compared with sham-operated group (Fig. 2C), both BMSCs-derived exosome treatment group (Fig. 2D) and PBS treatment group (Fig. 2E) had testicular ischemia-reperfusion injury, while BMSCs-derived exosome treatment group had less testicular tissue injury and less exudation compared with PBS treatment group. There are few vacuoles, the biblical cells are well arranged and the cell layers are lighter. The results showed that BMSCs-derived exosome could reduce testicular ischemia-reperfusion injury in testicular IRI rats.

Exosome derived from bone marrow mesenchymal stem cells can pass through the blood-testis barrier of rats

In order to explore how the exosome bodies derived from bone marrow mesenchymal stem cells play the role of antioxidation, we injected the exosome bodies labeled with green fluorescent dye PKH67 through the tail vein. After a period of time, the testis was removed under aseptic operation and made into frozen sections. PKH67-labeled exosomes can be detected in testicular tissue under confocal microscope. The results show that exosome bodies derived from bone marrow mesenchymal stem cells can indeed reach testicular tissue (Fig. 3A, 3B), which is a key step for bone marrow mesenchymal stem cells to play a protective role.

Exosome derived from bone marrow mesenchymal stem cells can be absorbed by mouse spermatogonia.

Then we explored whether the exosome derived from bone marrow mesenchymal stem cells can be absorbed by rat spermatogonia, so as to protect the fertility of rats with testicular ischemia-reperfusion injury. We co-cultured the exosomes labeled with green fluorescent dye PKH67 with mouse spermatogonia GC1 and fixed them after a period of time. Under microscope, it was observed that some exosome bodies derived from bone marrow mesenchymal stem cells were absorbed by GC1 cells (Fig. 3C, 3D, 3E). The results showed that the exosome bodies derived from bone marrow mesenchymal stem cells could be absorbed by mouse spermatogonia so as to reduce the damage of spermatogonia under oxidative stress and protect the development of spermatozoa.

Exosomes derived from bone marrow mesenchymal stem cells can improve the oxidative stress injury induced by testicular ischemia-reperfusion in rats

Then we detected the indexes of oxidative stress in testicular tissue of rats in three groups. Compared with sham operation group, the contents of T-AOC, SOD and CAT in testicular tissue of rats in BMSCs exosome treatment group and PBS treatment group decreased significantly (Fig. 3D, 3E, 3F), and the contents of T-AOC, SOD and CAT in PBS treatment group decreased significantly. Compared with PBS treatment group, the contents of T-AOC, SOD and CAT in testicular tissue decreased less in BMSCs-derived exosome treatment group, indicating that BMSCs-derived exosome can resist oxidative stress injury caused by testicular ischemia-reperfusion injury in rats. In addition, compared with the sham operation group, the contents of MDA and NOS (including iNOS and TNOS) in testicular tissue in BMSCs-derived exosome treatment group and PBS treatment group increased (Fig. 3A, 3B, 3C), and the contents of MDA and NOS (including iNOS and TNOS) in PBS treatment group increased significantly. Compared with the PBS treatment group, the contents of MDA and NOS (including iNOS and TNOS) in the testicular tissue of the BMSCs-derived exosome group increased less, indicating that the BMSCs-derived exosome can alleviate the oxidative stress injury caused by testicular ischemia-reperfusion injury in rats. In summary, from the perspective of biochemical level of oxidative stress related to testicular tissue, BMSCs-derived exosome can reduce the oxidative stress injury caused by testicular ischemia-reperfusion in testicular IRI rats. < / br > exosome bodies derived from bone marrow mesenchymal stem cells can pass through the blood-testis barrier of rats.

Exosomes derived from bone marrow mesenchymal stem cells can improve oxidative stress injury of TM4 and GC1 cells

Then, TM4 and GC1 cell oxidative stress models were constructed. TM4 and GC1 were treated with different concentrations of H₂O₂ for 8 hours, and 80 mol/L H₂O₂ was selected as the experimental treatment concentration based on their 8-hour IC₅₀ concentration (Fig. 5A and 5B). TM4 and GC1 were divided into four groups: group A and control group; B, 80 mol/L H₂O₂ group; C, 80 mol/L H₂O₂ + PBS group; D, 80 mol/L H₂O₂ + BMSCS-EXO group. After 8 hours of treatment, the expression levels of ROS, MDA and GSH (Figure.5C) and the corresponding proportion of cell apoptosis (Figure.5D and 5E) in each group were detected. The results showed that BMSC-derived exosomes could reduce ROS, MDA and GSH levels in TM4 and GC1 cells treated with 80 mol/L H₂O₂ for 8 h, enhance the ability of anti-oxidative stress injury and reduce the proportion of apoptosis in TM4 and GC1. We further detected the expression levels of p-Akt and Akt in each group, and the results showed that H₂O₂ inhibits phosphorylation of Akt in TM4 and GC1 cells; Bone marrow mesenchymal stem cell-derived exosomes activate phosphorylation of Akt in TM4 and GC1 cells(Figure.6A and 5E).

Discussion

The results of this study showed that the exosome derived from bone marrow mesenchymal stem cells could pass through the blood-testis barrier to the testicular tissue, and it was confirmed by cell experiments that they could be absorbed by spermatogonia. In rat testicular IRI model[28], compared with PBS treatment group and sham operation group after testicular reduction, although exosome derived from bone marrow mesenchymal stem cells could not prevent testicular ischemia-reperfusion injury, it

could significantly improve the degree of testicular injury and protect the integrity and spermatogenic function of testicular tissue to a great extent through the detection of testicular histomorphology and related oxidative stress indexes. It provides a new idea for preventing ischemia-reperfusion injury after testicular torsion reduction.

Testicular torsion, diagnosed mainly in adolescents, is an emergency that requires immediate treatment to save the testicles to prevent loss of testicular function, and about 30% of men with a history of torsion experience adult infertility[29–32]. Although surgical reduction of the affected testis is the main method, the subsequent ischemia-reperfusion injury is still inevitable, and the testicular tissue can still be worsened by ischemia-reperfusion injury. Current clinical experiments and research data show that the degree of ischemia-reperfusion injury after testicular torsion reduction is highly related to the prognosis after testicular torsion reduction. After reduction, too much oxygen is introduced into the tissue, resulting in excessive production of reactive oxygen species. The increase in the number of neutrophils and the excessive production of ROS in the testicular cycle lead to tissue damage by destroying the cell membrane. This process is called "I hammer R damage", which is characterized by oxidative stress, and oxidative stress is the main cause of organ damage[33].

Many previous studies have shown that it is possible to improve the prognosis and protect testicular tissue and spermatogenic function by improving the oxidative stress injury caused by ischemia-reperfusion after testicular torsion reduction[34]. In this study, we confirmed that the exosome derived from bone marrow mesenchymal stem cells can reach around the seminiferous tubule through the blood-testis barrier of rats, and further confirmed that exosome derived from bone marrow mesenchymal stem cells can reduce the degree of oxidative stress injury in testicular tissue and improve testicular ischemia-reperfusion injury by HE staining and the content of related oxidative stress indexes.

At present, the mainstream research is to treat or alleviate testicular ischemia-reperfusion injury through antioxidant stress. Some studies have shown that bone marrow mesenchymal stem cells can reduce the degree of oxidative stress injury caused by testicular ischemia-reperfusion, but the mechanism is unknown[1, 2, 7, 8, 10, 11]. On the basis of the study of bone marrow mesenchymal stem cells, this study further clarified that the exosome secreted by bone marrow mesenchymal stem cells play a major role, and the methods and corresponding indexes used are the same as those of previous studies. It can be fully explained that bone marrow mesenchymal stem cells rely on their secreted exosome bodies to improve testicular ischemia-reperfusion injury.

In this study, a preliminary study was made on the mechanism of exosome. It can reach around the seminiferous tubule through the blood-testis barrier, and the cell uptake experiment confirmed that the exosome body can be absorbed by spermatogonia, suggesting that the exosome body may be absorbed by testicular tissue and play a role. However, the deep mechanism of exosome body in this study is still shallow, and its mechanism can be further explored.

This study focuses on the kinetic energy study of exosome derived from bone marrow mesenchymal stem cells[24, 35], but there is no control group for the effect of bone marrow mesenchymal stem cells in

the experimental design, and the theoretical basis is still lacking, but combined with the results of previous studies and this experiment, to a certain extent, it can be concluded that the exosome derived from bone marrow mesenchymal stem cells has therapeutic effect.

In a word, the exosome derived from bone marrow mesenchymal stem cells can reduce testicular ischemia-reperfusion injury and reduce the level of oxidative stress in testicular tissue through the blood-testis barrier possibly. At the same time, compared with bone marrow mesenchymal stem cells, exosome is more biosafety and has the value of clinical application.

Conclusions

This study shows that exosome bodies derived from bone marrow mesenchyme may play the role of testicular ischemia-reperfusion through the blood-testis barrier, so as to reduce the tissue injury caused by testicular ischemia-reperfusion and protect testicular spermatogenesis. These findings suggest that the exosome derived from bone marrow mesenchymal stem cells can exert the function of antioxidant stress and have more biosafety, which can provide a therapeutic idea for clinical prevention or treatment of ischemia-reperfusion injury after testicular torsion reduction.

Abbreviations

IRI
ischemia reperfusion injury
BMSCs
bone marrow mesenchymal stem cells
NTA
nanoparticle tracking analyzer
TEM
transmission electron microscopy
GSH
glutathione
MDA
malondialdehyde
CAT
catalase
NOS
nitric oxide synthase
SOD
superoxide dismutase
T – AOC
total antioxidant capacity

Declarations

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Availability of data and materials

All materials are commercially available, and data are as presented in this article open for access.

Authors' contributions

LCD and GWB planned the experimental ideas, YC and ZWS conducted experiments including animal experiments, cell separation and processing, confocal microscopy, Western blotting and exosome tracing; ZJH, TH and ZRR measured exosomes; TH conceived the data and wrote manuscripts. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

All experimental procedures were complied with the Guidelines of Animal Care of Southern Medical University, and were approved by the Ethics Committee of Southern Medical University, China. ARRIVE guidelines for reporting animal research was followed.

Acknowledgements

Not applicable

Consent for publication

Not applicable

Competing interests

Not applicable

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Figures

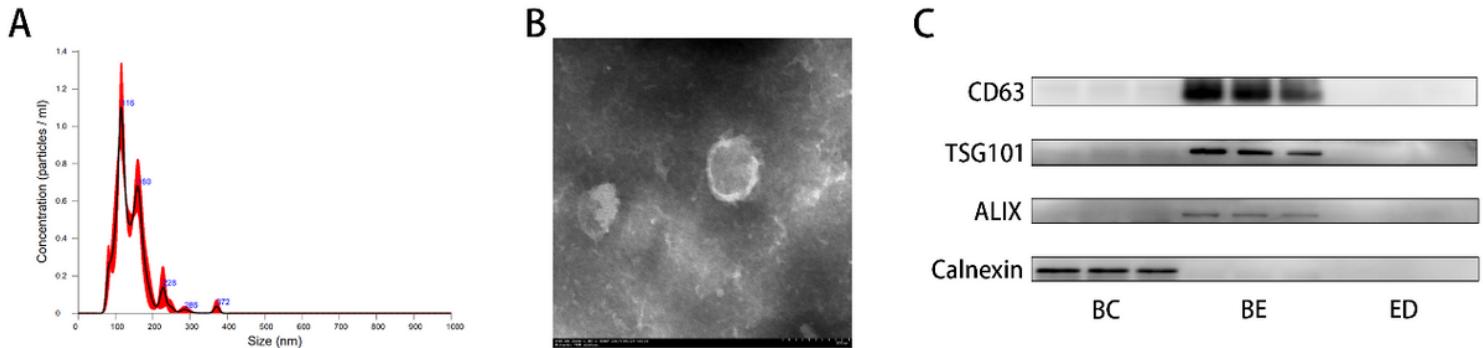


Figure 1

Characterization of exosomes A: Nanoparticle tracking analysis; B: Transmission electron microscopy; C: Western blotting for the markers of the exosomes; BE: BMSCs derived exosomes; BC: BMSCs; ED: Exosome depleted.

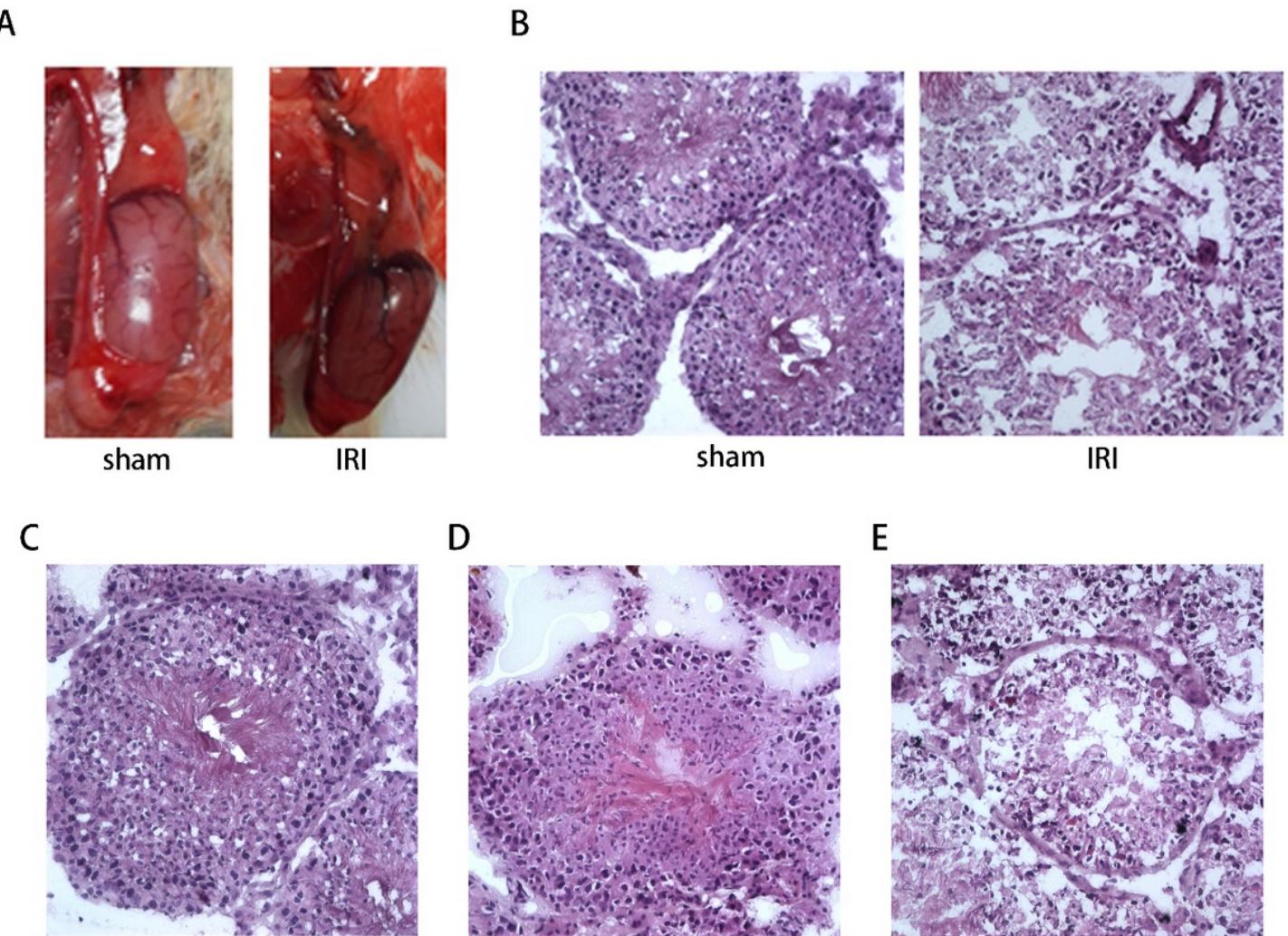


Figure 2

Exosomes derived from bone marrow mesenchymal stem cells can reduce testicular ischemia-reperfusion injury in rats A:IRI animal model of testis; B:Testicular IRI animal model testicular HE staining; C : Sham group; D: I/R + NS group; E: I/R + BMSCs-EXO group.

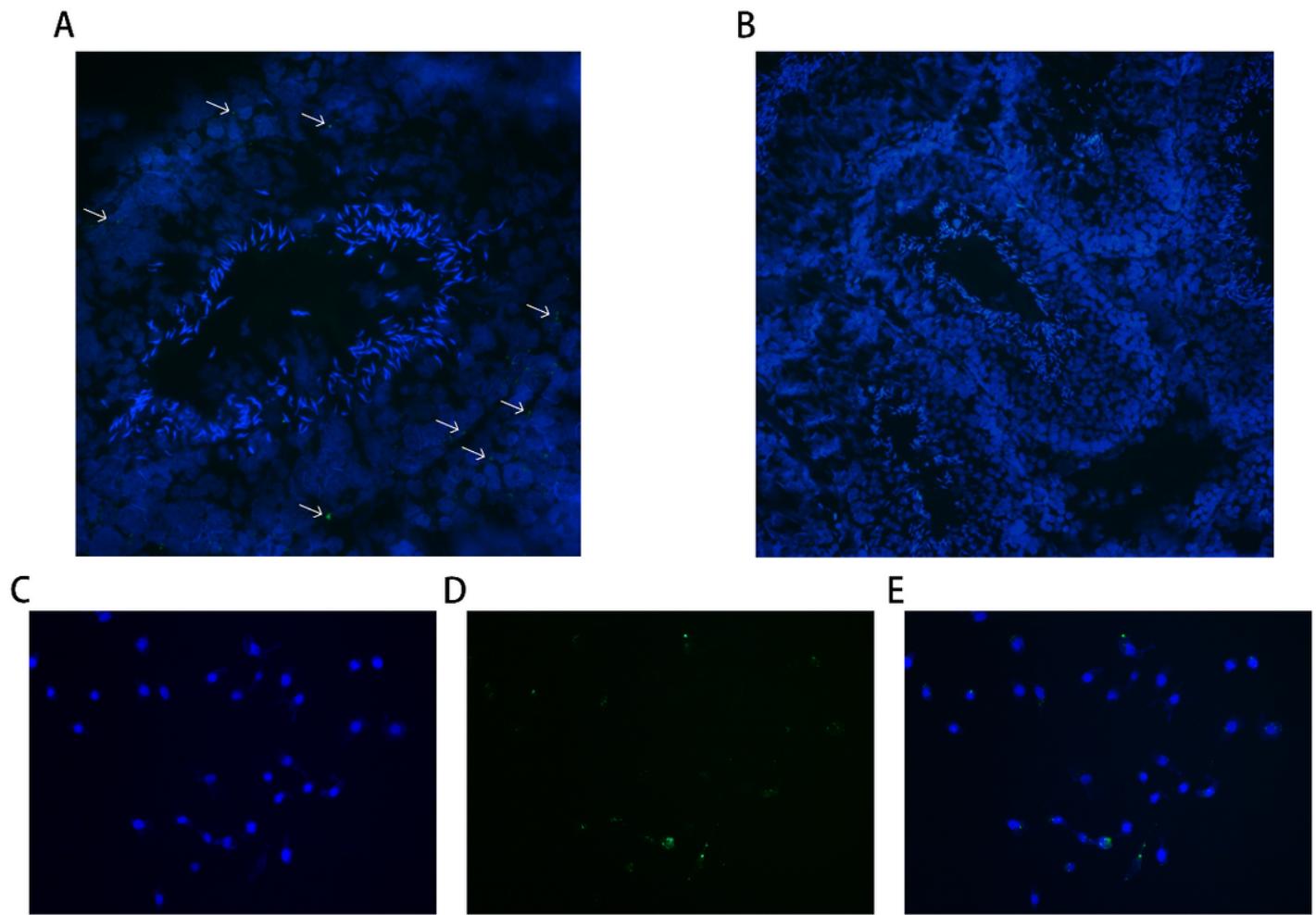


Figure 3

Exosome derived from bone marrow mesenchymal stem cells can pass through the blood-testis barrier of rats and can be absorbed by mouse spermatogonia A&B: Bone marrow mesenchymal stem cell-derived exosomes can penetrate the blood-testosterone barrier C&D&E: Bone marrow mesenchymal stem cell-derived exosomes can be taken up by mouse spermatogonia GC1.

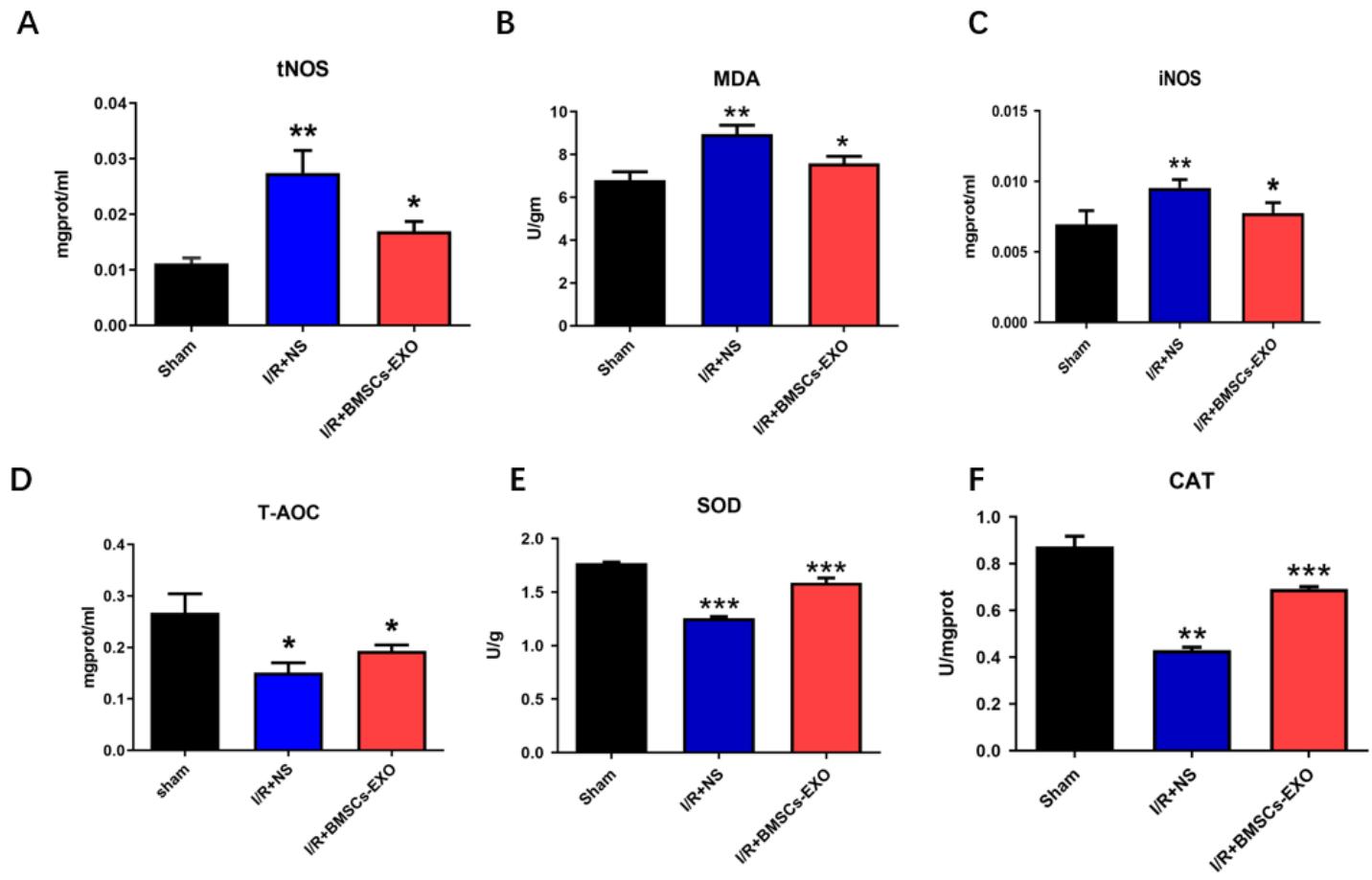


Figure 4

Exosomes derived from bone marrow mesenchymal stem cells can improve the oxidative stress injury induced by testicular ischemia-reperfusion in rats. A-F: The contents of SOD, MDA, TNOS, T-AOC, iNOS and CAT in testes of rats in each group (mean \pm standard deviation, n=4 or 5). **P<0.01, *P<0.05 compared with sham operation group, +. +**P<0.01, *P<0.05 compared with I/R+NS group.

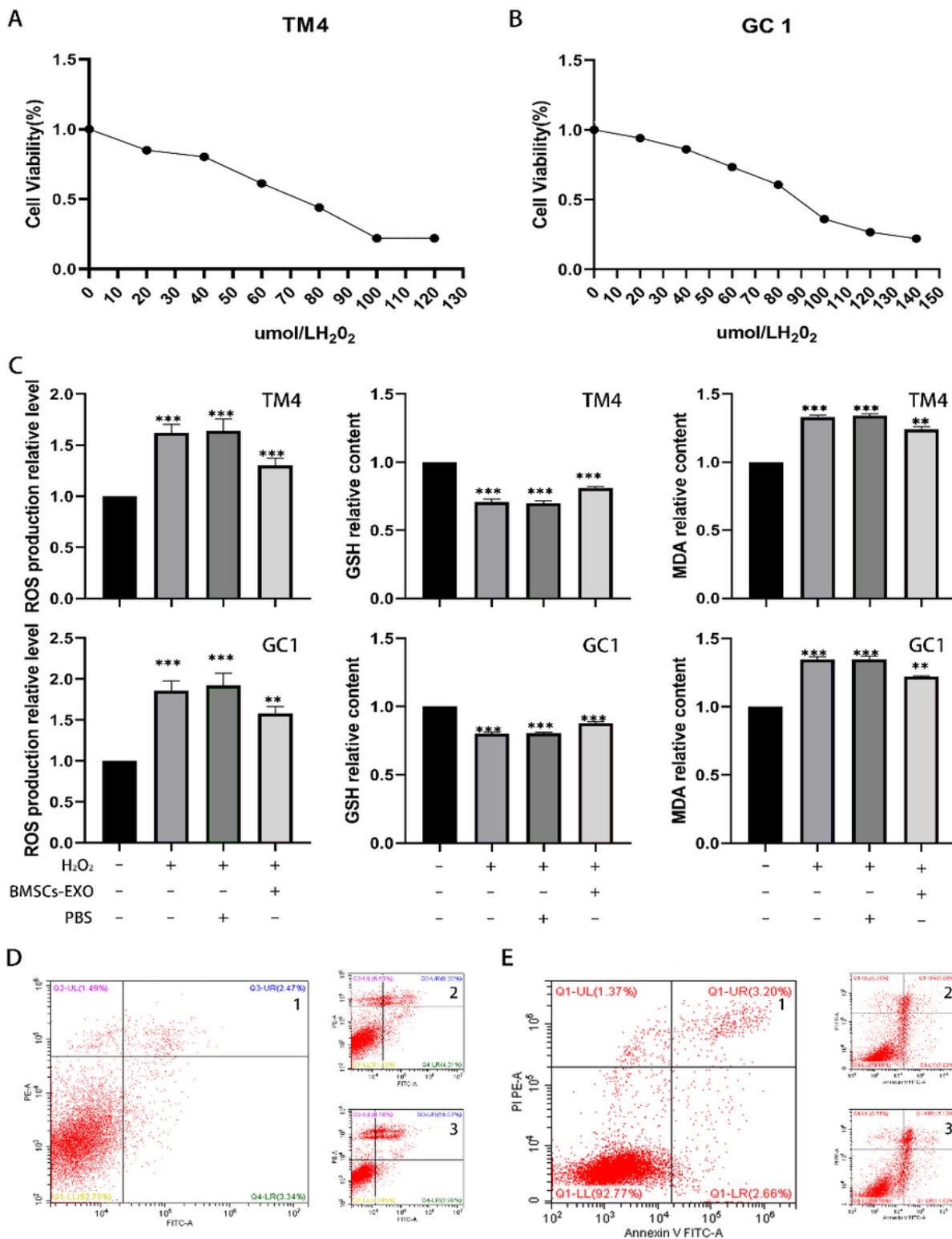
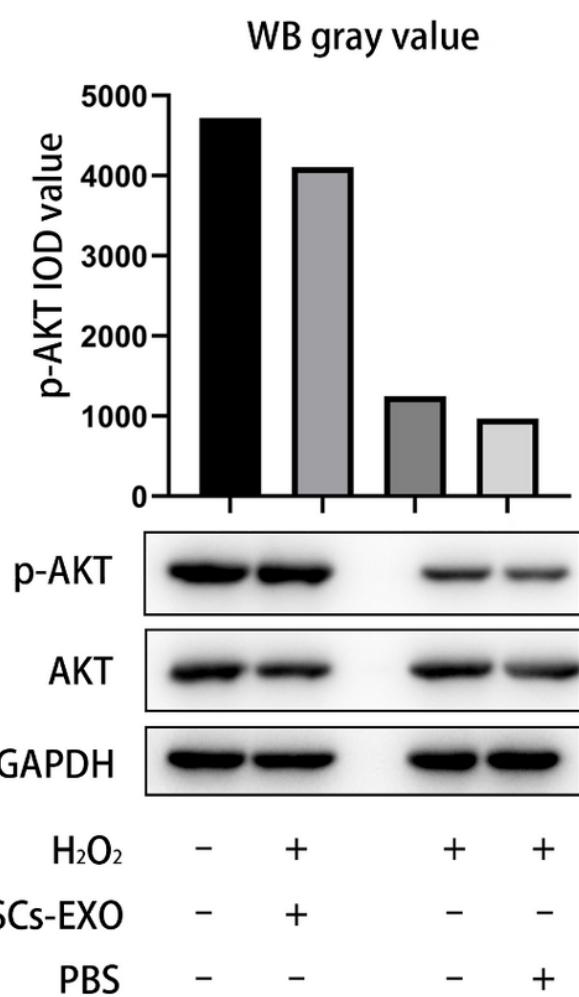


Figure 5

Exosomes derived from bone marrow mesenchymal stem cells can improve oxidative stress injury of TM4 and GC1 cells. A-B: Inhibition of proliferation activity of TM4 and GC1 cells treated with different concentrations of H₂O₂. C: The contents of ROS, MDA, GSH in testes of TM4/GC1 in each group (mean ± standard deviation, n=4 or 5). **P<0.01, *P<0.05 compared with control group, +). +**P<0.01, *P<0.05

compared with H2O₂ group. D-E: Exosomes derived from bone marrow mesenchymal stem cells can reduce apoptosis induced by TM4 and GC1 oxidative stress.

A



B

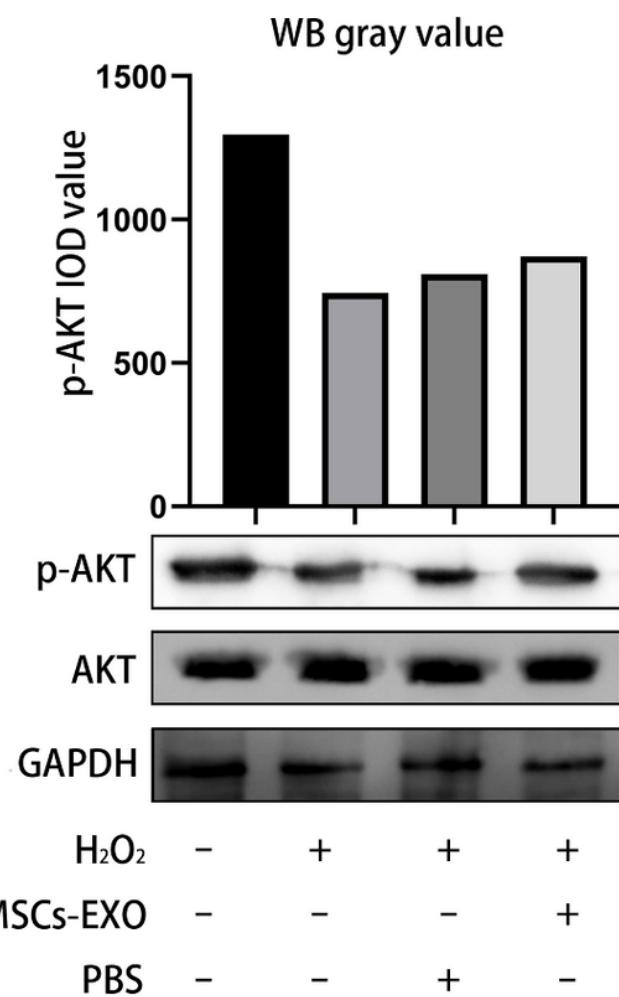


Figure 6

Exosome derived from bone marrow mesenchymal stem cells can activate AKT signal pathway. A-B: H₂O₂ inhibits phosphorylation of Akt in TM4 and GC1 cells; Bone marrow mesenchymal stem cell-derived exosomes activate phosphorylation of Akt in TM4 and GC1 cells