Exosomes Derived from Umbilical Cord Mesenchymal Stem Cells Promote Repair of Damaged Endometrium by Activating PTEN/AKT Pathway

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Research Article

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Abstract

Background: Endometrial injury contributes to impaired endometrial receptivity, and is well recognized as a critical factor in implantation failure. Increasing evidence suggests that the therapeutic effects of mesenchymal stem cells (MSCs) mainly depend on their capacity to secrete paracrine factors and are mediated by MSC-derived exosomes (MSC-Exos). In this study, we investigated the effects of human umbilical cord mesenchymal stem cell-derived exosomes (hUCMSC-Exos) on injured endometrium in the mouse endometrial damage model and the potential mechanisms.

Methods: All female mice were randomly divided into control group, saline group, and exosome (Exo) group. To observe the distribution of exosomes *in vivo*, DiR-labeled hUCMSC-Exos were injected into the tail vein of endometrium-injured mice. HE staining was used to detect changes in endometrial thickness and number of glands. Fertility recovery of the uterus was measured by testing embryo implantation rates. TUNEL staining was used to detect cells apoptosis. The expression of Ki67 and CD31 was examined by immunohistochemistry. Then, western blotting was used to measure the expression of Bcl-2, Bax, Cleaved Caspase-3, PTEN, AKT and p-AKT. The mRNA expression of VEGF and IGF-1 was detected by RT-PCR.

Results: hUCMSC-Exos was able to migrate to the damaged endometrium. After hUCMSC-Exos injection, the endometrial thickness, the number of glands and embryo implantation rate were significantly increased ($P < 0.05$). Compared with the saline group, apoptosis was significantly reduced in the exosome group, and the expression of Ki67 and CD31 was significantly increased ($P < 0.05$). Besides, the expression of Bax, Cleaved Caspase-3 and PTEN was reduced as hUCMSC-Exos addition, and the Bcl-2 and p-AKT expression was increased. The expression of VEGF and IGF-1 was significantly up-regulated in the exosome group compared to the saline group ($P < 0.05$).

Conclusions: hUCMSC-Exos ameliorated the damaged uterus, increased the number of glands and embryo implantation rates, suppressed apoptosis and improved the cell proliferation in the mouse injured endometrium model. Furthermore, we discovered that hUCMSC-Exos could activate the PTEN/AKT signaling pathways and induce the overexpression of VEGF and IGF-1 *in vivo*.

Background

Endometrial injury is commonly caused by various uterine cavity surgery that induces intrauterine adhesions (IUA), fibrosis or thin endometrium. These disorders are characterized by low pregnancy rates, frequent miscarriages, and even infertility [1-3]. Currently, intrauterine implant positioning and high-dose hormone therapy are used in tandem to avoid postoperative recurrence of IUAs and facilitate endometrial repair and regeneration [4, 5]. However, the conventional hormone replacement therapy for endometrium injury generally causes adverse side effects.

Mesenchymal stem cells (MSCs) are a class of adult stem cells with a capacity for self-replication and multi-directional differentiation, which are currently investigated for tissue regeneration [6-8]. Extensive

studies have demonstrated that MSCs can restore damaged endometrium, enhance fertility rate, reduce endometrial fibrosis, increase angiogenesis, and improve conception rates [9-11]. However, the application of MSC may have some disadvantages, such as tumorigenic potential, thrombosis and fever [12-14]. Animal studies indicate that MSCs can promote the growth of tumor cells in mice [12]. The adipose-derived MSCs (AD-MSCs) have pro-coagulant effect in mice, which may form thromboembolism [13]. In addition, in clinical trials of MSCs, some patients have experienced adverse effects such as fever and headache, but no serious adverse effects were found [14]. Considering that paracrine action is thought to be the primary mechanism of action of MSCs in tissue repair, some investigators have explored the role of MSC-secreted exosomes, expect that MSCs could be used in the treatment of uterine injury [15].

MSCs-derived exosome (MSCs-Exo), 30-150 nm in diameter and contains active substances, acts in a paracrine manner for cell-free therapy in many diseases. Many studies have demonstrated MSC-Exos could coordinate the immune system and regulate inflammatory responses in damaged tissues [16, 17]. It showed that transferring MSC-Exos to the injured site could accelerate the re-epithelial formation, minimize scar width and promote collagen maturation [18]. AD-MSCs-derived exosomes (AD-MSCs-Exo) stimulated endometrial regeneration and collagen remodeling and enhanced the expression of integrin-β3, LIF and VEGF [19]. Bone marrow mesenchymal stem cells derived exosomes (BMSC-Exos) could facilitate endometrial repair by the TGF-β1/Smad signaling pathway [20]. Moreover, our previous study found that hUCMSC-Exos preserved human endometrial stromal cells (hEndoSCs) from apoptosis induced by mifepristone and played an active role *in vitro* repair of the damaged hEndoSCs through the PTEN/AKT signaling pathway [21]. However, mechanisms that coordinate hUCMSC-Exos *in vivo* endometrial injury treatment remain uncertain. In this study, the effects of hUCMSC-Exos on injured endometrium in the mouse model and the mechanisms of hUCMSC-Exos on the repair of endometrial injury *in vivo* were explored.

Materials And Methods

Experimental Animals

C57BL/6 female mice were obtained from the Shandong Experimental Animal Center and placed in the Animal Laboratory of Anhui Medical University. Four to five mice per cage were lodged in a room with a cycle time of 12 hours of light and 12 hours of darkness, and provided food and water ad libitum. All animals were treated based on the protocol approved by the Institutional Animal Care and Use Committee of Anhui Medical University.

Establishment of the mouse endometrial injury model

Eight weeks old female mice were used to establish the endometrial injury model with 95% ethanol. More specifically, a longitudinal incision in the abdominal wall was made after administration of 4% chloral hydrate (0.1 mL/10 g) by intraperitoneal injection and the uterus was uncovered. In the single uterine

horn, 0.1 ml ethanol was injected and held for 3 min, then being washed three times with phosphate buffer saline (PBS) solution. We disinfected the uterus and then reset it.

Isolation and characterization of hUCMSC-Exos

hUCMSCs were isolated and characterized as previous study [22]. Briefly, the human umbilical cords were collected from consenting patients and the study was approved by the Ethics Committee of Anhui Medical University. The Wharton's jelly were dissociated from human umbilical cords and cut into small fragments. The cord fragments were properly adherent to cell culture plate (Corning, USA) and cultured for 10 days with the medium consisting of Dulbecco's modified Eagle's medium (DMEM)/F-12 supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco, USA). hUCMSCs migrated out of the Wharton's jelly pieces. The typical markers and the multilineage differentiation potential (adipogenic, osteogenic, and chondrogenic differentiation) were detected to characterize the hUCM-MSCs. The hUCMSC-Exos were isolated from the medium containing no exosomes using an exosome extraction and purification kit (Umibio, Shanghai, China). Exosomes were characterized according to their size and surface marker expression by negative-staining electron microscopy and western blotting. Exosomes were placed into a formvar/carbon-coated grid, negatively stained with 3% aqueous phosphor-tungstic acid for 1 min, and examined by transmission electron microscopy (TEM, FEI, USA) at an accelerating voltage of 120 kV. The distribution of particle sizes for the hUCMSC-Exos was calculated by Nanoparticles Tracking Analysis (NTA, Zeta View PMX 110, Germany). Western blotting has established the typical markers of exosomes, such as CD63 (1: 1000, Abcam, UK), HSPA8 (1: 1000, Abcam, UK), TSG101 (1: 1000, Abcam, UK), PDCD6IP (1: 1000, Abcam, UK), and β-Actin (1: 1000, Abcam, UK).

Intervention of endometrial injury model with hUCMSC-Exos

The mice were randomly divided into three groups: the control group (n=32), the saline group (n=32) and the exosome group (n=32). Intact mice were set as the control group. After the endometrial injury model was developed, exosomes (1.25 mg/ml, 300 μL) were injected via tail vein in the exosome group. And in saline group the endometrial injury mice were injected with the equal normal saline. Some mice were sacrificed and the bilateral uterine horns were removed for histological staining on Day 7 after transplantation.

Ex vivo fluorescence imaging of DIR-labeled exosomes in mice with endometrial injury

As previously described, DIR was used to fluorescently mark the lipid bilayer of exosome [23]. The mice were transplanted with DIR-labelled exosomes after the endometrial injury model was set up. Images of *ex vivo* endometrial damage were collected after 6 h, 12 h, 24 h or 7 day after injection In Vivo Imaging System (IVIS, FMT 2500X, PerkinElmer LifeSciences). The Spectrum was obtained with cooled charge-coupled device (CCD) camera with excitation wavelength at 750 nm and emission wavelength at 780 nm. The fluorescence of the undamaged uterine horn was described as the autofluorescence of the background tissue. The resulting images were analyzed with imaging software by subtracting the background tissue autofluorescence from the activatable fluorescence.

Hematoxylin-eosin (HE) staining

After fixation, dehydration and implantation, isolated uterine tissues in paraffin sections were prepared with a thickness of 4 μm and routine HE staining. The microscope was used to detect morphological variations in the uterus. The scraped endometrium fragments were fixed in 4% paraformaldehyde for 24 hours and then embedded in paraffin. Serial paraffin-embedded parts were collected, sequentially dewaxed for 20 min each in xylene I and xylene II, and rehydrated in a sequence of ethanol solutions with a decreasing concentration (100% for 10 min, 100 percent for 10 min, 95% for 5 min, 90% for 5 min, 80% for 5 min, and 70% for 5 min). The pieces were then rinsed in purified water (three times, 5 min each). As per the manufacturer's orders, the parts were stained with an H&E solution (Servicebio, China). Endometrial morphologic characteristics were observed after staining. And the number of uterine glands and the thickness of endometrium was counted based on five randomly chosen regions.

Fertility testing

As previously described, we assessed the uterine function by testing embryo implantation rates [10]. After the exosomes transplantation for 7 days, mice of the exosome group (n=6) were mated with 8-week-old males at 1:1 ratio for two weeks. The saline group (n=6) and the control group (n=6) were the control. The female mice were sacrificed to assess the fetal number of uterus.

TUNEL staining

To investigate the effects of hUCMSC-Exos on uterus cell apoptosis induced by ethanol, a TUNEL apoptosis assay kit (KeyGen BioTECH Co., Ltd., China) was used to detect endometrial cell apoptosis in each group after one week of exosomes injection according to the manufacturer’s instructions. Apoptotic cells are stained with green fluorescence. Sections were observed and imaged using an optical microscope (LSM800, Zeiss, Germany). The intensity of apoptotic cells were analyzed using the image analysis software (ZEN, Zeiss, Germany).

Immunohistochemistry

The fixed uterine tissues with 4% paraformaldehyde were embedded in paraffin. Sections were fixed for 5 min in neutral buffered formalin, after which endogenous peroxidase activity was quenched by incubating in 3% hydrogen peroxide with methanol for 10 min. The antibody retrieval of each section was treated by microwave two times and washing three times with PBS. Subsequently, rabbit anti-Ki67(1:400; Cell Signaling, USA), rabbit anti-CD 31 (CD 31; 1:100; Cell Signaling, USA) was added and incubated at 4°C overnight. Secondary antibody (ZSGB-BIO, Beijing, China) was then added and incubated at 37°C for 30 min. Color was developed with DAB (Sigma Co., St. Louis, MO, USA) and counterstained with hematoxylin. The sections were observed under an optical microscope (Eclipse E200, Nikon Co., Tokyo, Japan) and photographed. The protein expressions were analyzed with the Image-pro Plus image analysis software (Media Cybernetics, MD, USA).

Western blot

The total protein was collected and the protein concentration was determined by BCA method. Proteins were isolated by 10% SDS-PAGE and transferred electrophoretically from the gels to the transport membranes of polyvinylidene difluoride (PVDF). The membranes were briefly washed in PBS-Tween and incubated at 4℃ overnight with the antibodies Bcl-2 (1:1000, Cell Signaling, UK), Bax (1:1000, Cell Signaling, UK), Cleaved Caspase-3 (1:1000, Cell Signaling, UK), PTEN (1:1000, Cell Signaling, USA), AKT (1:2000, Cell Signaling, USA) and p-AKT (1:2000, Cell Signaling, USA). By using a rotary shaker, the membranes were then washed 3 times in PBS-Tween. Horseradish peroxidase- (HRP-) conjugated anti-rabbit was incubated with the washed membranes for 1 h. In compliance with the guidance of the manufacturer, the membranes were washed again and treated with an ECL detection kit (Biosharp, USA) to image the proteins recognized by the antibodies.

Quantitative real-time PCR (qRT-PCR)

Using Trizol Reagent (Ambion, Carlsbad, CA, United States), total RNA was extracted from each uterine according to the manufacturer's instructions. 1 μg of total RNA was subjected to reverse mRNA transcription using oligo dT as a primer and a total cDNA generation reverse transcription kit (ABclonal, Wuhan, China). RT real-time PCR with SYBRR Premix Ex TapTM (TaKaRa, China) and the ABI 7500 real-time PCR framework measured the expression levels of VEGF and IGF-1 mRNA. Gene amplification reaction conditions were 95 ℃ for 3 min, 95 ℃ for 5 s, and then 60 ℃ for 32s, respectively. In the NCBI database, the unique PCR primers were planned according to DNA sequences (Table 1). The level of expression of the gene was standardized to GAPDH and the reference gene. Each sample was detected in triplicate in each group. The procedure was repeated at least three times.

Statistical analyses

A statistical software SPSS 23.0 was used to analyze the data. Figures were indicated as mean ± standard error (SEM). Comparisons were made between two samples using the independent samples t-test. The condition P value < 0.05 was considered to be statistically significant.

Results

Characterization of hUCMSC-Exos

Using the ExoQick-TC reagent, we isolated exosomes from the hUCMSC culture medium and characterized the hUCMSC-Exos. TEM visualized the exosome morphology and scale (Fig.1A), and the diameters of isolated exosomes as spherical forms were 30-100nm. Particle size distribution at 20-300 nm diameter was measured by NTA (Fig.1B). Western blot confirmed that hUCMSC-Exos expressed CD63, HSPA8, TSG101, and PDCD6IP, exosome-specific markers, but not the β-Actin negative marker autophagosome protein (Fig.1C). The concentration of exosome was determined at 1.25mg/ml by BCA assay.

Fluorescence imaging of DIR-labeled exosomes in mice with endometrial injury

To investigate the influence of endometrial injury on the migration of exosomes to the endometrium, hUCMSC-Exos was pre-labelled with DIR and transplanted into mice with endometrial injury. The right horns of female mice were injured by ethanol, leaving the left horns untreated as self-controls. The mice were intravenously injected with DIR-labeled exosomes. Bilateral horns were collected at 6, 12 and 24 h after injection, and fluorescent signals were detected through IVIS to determine exosomes recruitment. Fluorescence was only detected in injured horns after the exosomes transplantation, while no fluorescent signal was seen in control groups and undamaged horns. Some red-dotted fluorescent signals were found at 6 h after exosome transplantation, and the fluorescent signals increased at 12 h and 24 h (Fig.2). At day 7 after exosome transplantation, no red-dotted fluorescent signal was observed. The findings predominantly revealed that the transplanted hUCMSC-Exos were home to injury endometrium.

hUCMSC-Exos ameliorate the damaged uterus and increase endometrial thickness.

We established a mouse endometrial injury model with 95% ethanol. Compared with the control group, the uterine cavity of mice was atretic after 95% ethanol injury. According to the HE staining results, the endometrium was damaged and the thickness of the endometrium was significantly thinner (Fig.S1). To evaluate the therapeutic efficacy of hUCMSC-Exos on endometrial injury, we assessed the morphological endometrial structure, the endometrial thickness and the number of gland (Fig.3). At Day 7 after transplantation, the endometrial structure of the control group seemed more complete, epithelial cells were arranged closely, and blood vessels and glands were clearly visible. In the saline group, the endometrium was poor and endometrial thinning was serious. The uterus recovered well in the exosome group, and the glands were obvious (Fig.3B). A significant difference in the endometrial thickness and the number of glands was compared between two groups (Fig.3C, D). These results indicated that hUCMSC-Exos promote the restoration of the damaged endometrium effectively.

hUCMSC-Exos ameliorated reproductive function in mouse injured endometrium.

To investigate the fertility restoration of mice after hUCMSC-Exos treatment, three groups of mice were mated with males for two weeks, and then we examined the number of the fetuses. The number of embryos is 9.17 ± 0.41, 0 ± 0 and 2.00 ± 1.55 in control group, saline group and exosome group, respectively (Fig.4). Compared with control group, no embryo was found on the uterus of the female mouse in saline group. Compared with saline group, the number of implanted embryos in the exosome group was significantly increased (P<0.05). These results evidently showed that hUCMSC-Exos ameliorated fertility in mice with endometrial damage.

hUCMSC-Exos suppresses apoptosis and improve the cell proliferation in mouse injured endometrium.

To evaluate the possible roles of hUCMSC-Exos on ethyl alcohol-induced uterus cells apoptosis, TUNEL assays were evaluated by immunofluorescence after hUCMSC-Exos injection for 7 day (Fig.5 A). More apoptotic cells were identified in the endometrium of saline group than in that of the control group (P<0.05). Compared with the saline group, significant difference in apoptosis was observed after hUCMSC-Exos injection (P<0.05) (Fig.5 B). To determine the proliferating cell populations after exosome

therapy, Ki-67 expression of uterine tissue sections was performed by immunohistochemical staining (Fig.6 A). In our mouse model, we found that the expression of Ki-67 in the saline group was significantly downregulated compared to the control group. In the exosome group, it was found that the expression of Ki-67 was significantly up-regulated (Fig.6 B) compared to the model group. These results indicated that hUCMSC-Exos suppressed apoptosis and improved cell proliferation in mouse injured endometrium.

hUCMSC-Exos increase Angiogenesis of the endometrial damaged uterus.

To investigate the proangiogenic effect of hUCMSC-Exos *in vivo*, we detected the enhanced neovascularization of injured tissues at Day 7 by histologic examination. The CD31 immunostaining revealed that microvascular density in the damaged uterus was significantly enhanced by administration of hUCMSC-Exos (Fig.7). These results suggested that the therapeutic based on exosomes dramatically increased the endogenous angiogenesis in the ischemia tissues.

hUCMSC-Exos activates PTEN/AKT signaling pathways, alleviate the apoptosis and improve VEGF and IGF-1 in the endometrial damaged uterus.

To further investigate the mechanisms of hUCMSC-Exos on repairing endometrial injury *in vivo*, the expression of Bcl-2, Bax and Cleaved Caspase-3 protein of uterus from mice were detected by Western blot. The positive expression of Bax and Cleaved Caspase-3 increased after damaged uterus. On the contrary, the Bcl-2 expression was found to be significantly decreased. Whereas, the expression of Bax and Cleaved Caspase-3 was reduced as hUCMSC-Exos addition, and the Bcl-2 expression was increased (Fig.8 A). These findings reveal that hUCMSC-Exos enhance anti-apoptosis, and have a robust protective effect on the damaged uterus. To further investigate the mechanisms of hUCMSC-Exos on repairing endometrial injury *in vivo*, the expression of PTEN, AKT and p-AKT were tested in control group, saline group, exosome group group. Fig.8 B showed that phosphorylated level of AKT increased notably after hUCMSC-Exos treatment. These results demonstrated that hUCMSC-Exos could activate PTEN/AKT signaling pathway through increasing phosphorylated level of AKT, and PTEN/AKT pathway inhibitor could weaken the effect. Furthermore, the vascular endothelial growth factor (VEFG), and insulin-like growth factor (IGF-1) expression of angiogenesis cytokines were detected in the endometria by qRT-PCR. Increased positive expression was found in hUCMSC-Exos transplantation group as expected. After hUCMSC-Exos were injected into the tail vein, there were significant differences in VEGF and IGF-1 between the two transplanted groups (Fig.8 C, D). In addition, when compared with the saline transplantation group, the mRNA expression levels of VEGF and IGF-1 were significantly up-regulated in the hUCMSC-Exos transplantation group ($P < 0.05$), as well as the related cytokines that promote cell proliferation and tissue repair.

Discussion

Cell therapy has been suggested as a new strategy to repair tissue injury. The therapeutic effects of MSC on damaged endometrium have been verified in animal models [9-11, 24]. However, there are some adverse reactions to the application of MSC [12-14]. Thus, it is necessary to investigate an alternative

approach of MSC for the therapy of damaged uterus. It has been shown that MSC-Exos exhibit similar functions to MSCs, with the quality of low immunogenicity and do not produce tumors [25]. Previous studies have demonstrated that hUCMSCs can repair the injured tissue and protect hEndoSCs against apoptosis [21]. Here, our work demonstrated that the thickness of endometrium, the number of glands and embryo implantation rate significantly increased after exosomes treatment in mouse model. Furthermore, the results of these studies indicate that hUCMSC-Exos protected the endometrial cells from alcohol-induced apoptosis, promoted microvascular regeneration, stimulated endometrium regeneration through the PTEN/AKT signaling pathway and activated the AKT to regulate Bcl-2 and Cleaved Caspase-3 expression.

We demonstrated that hUCMSC-Exos can migrate to the damaged endometrium. Our results showed that 24 hours after the administration of hUCMSC-Exos in the tail vein of mice, aggregated hUCMSC-Exos were observed at the site of endometrial damage. This suggests that hUCMSC-Exos can migrate to the area of tissue damage and exert therapeutic effects. This targeted migration function of exosomes not only enables targeted therapy, but also promises to be the next generation of drug delivery systems.

Currently, the mechanisms of endometrial damage repair remain uncertain. Previous studies suggest that it may be primarily related to endometrial epithelial regeneration, angiogenesis and inflammatory response [26]. Besides, it was demonstrated that hUCMSC-Exos could promote the proliferation and migration of wound epithelial cells in a burn model [27]. Consistent with this, our results indicated that in mouse endometrial injury model, hUCMSC-Exos may repair the damaged endometrium by inhibiting apoptosis, increasing cell proliferation and promoting angiogenesis.

Our results showed that *in vivo* hUCMSC-Exos can also activate the PTEN/AKT pathway by inhibiting PTEN expression, which is consistent with our previous in vitro experimental findings [21]. Moreover, there is ample evidence that AKT can facilitate cell survival by specifically inhibiting pro-apoptotic signals such as pro-apoptotic regulators Bad and Forkhead family transcription factors. And AKT plays an important role in both physiological and pathological angiogenesis by affecting endothelial cells and angiogenic signal producing cells [28]. Besides, the pathophysiology of the endometrium in patients with thin endometrium was characterized by a significant increase in the uterine artery resistance index (RA-RI), a remarkable decrease in the area of the glandular epithelium, the number of blood vessels and the expression of angiogenic factor (VEGF) [29]. AKT activation of endothelial cells contributes to the expression and eventual secretion of VEGF and other angiogenic factors, thus promoting angiogenesis by both autocrine and paracrine signaling [28]. Our studies have shown hUCMSC-Exos could stimulate PTEN/AKT pathway and promote angiogenesis cytokines expression, such as VEGF and IGF-1.

In our mouse endometrial injury model, hUCMSC-Exos may promote cell proliferation and angiogenesis, inhibit apoptosis, and thus repair the damaged endometrium by activating the PTEN/AKT pathway. Our work showed that hUCMSC-Exos improved the reconstruction of endometrium through activation PTEN/AKT pathway which has a broad range of effects on restoration. In our future works, we will examine the mechanism of exosomes on the PTEN /AKT pathway in-depth in endometrial repair and the

role of exosomes in endometrial receptivity. More experimental studies are required to estimate the basic mechanism of exosomes in the endometrial injury model and for the clinical application of exosomes.

Conclusion

In summary, our results showed that hUCMSC-Exos could improve the repair of damaged endometrium and activate the PTEN/AKT signaling pathway in the mouse model. hUCMSC-Exos may up-regulate the expression of Bcl-2 and down-regulate the expression of Cleaved Caspase-3 and facilitate cell proliferation. Furthermore, our study suggested that hUCMSC-Exos had the potential to be a novel approach for the clinical investigation therapy of endometrial injury.

Abbreviations

AD-MSCs: adipose-derived MSCs; AD-MSCs-Exo: AD-MSCs-derived exosomes; BMSC-Exos: bone marrow mesenchymal stem cells derived exosomes; CCD: cooled charge-coupled device; DMEM: Dulbecco's modified Eagle's medium; Exo: exosome; HE: hematoxylin-eosin; HRP: horseradish peroxidase; hEndoSCs: human endometrial stromal cells; hUCMSC-Exos: human umbilical cord mesenchymal stem cell-derived exosomes; IGF-1: insulin-like growth factor-1; IUA: intrauterine adhesions; IVIS: In Vivo Imaging System; MSCs: mesenchymal stem cells; MSC-Exos: MSC-derived exosomes; NTA: Nanoparticles Tracking Analysis; PBS: phosphate buffer saline; PVDF: polyvinylidene difluoride; qRT-PCR: quantitative real-time PCR; SEM: standard error; TEM: transmission electron microscopy; VEFG: vascular endothelial growth factor;

Declarations

Acknowledgements

Not applicable.

Authors' Contributions

XJ and LD contributed equally to this work. ZW and JW designed and supervised the study. XJ, LD and XX made the animal models. XJ, XX and TL collected materials, performed the experiments, analyzed the data, and wrote the manuscript. ZL, QX and ZY performed the cell culture and exosome isolation. All authors wrote parts of the manuscript, read and approved the final manuscript.

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Availability of data and materials

Literature search results are available from the authors on reasonable request.

Ethics approval and consent to participate

The study was approved by the the Institutional Animal Care and Use Committee of Anhui Medical University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Tables

Table 1 is not available with this version

Figures

Figure 1

Identification of hUCMSC-Exos.

(A) Morphology of hUCMSC-Exos obtained by electron microscopy of propagation. (B) Particle size distribution histogram for the hUCMSC-Exos sample by NTA. (C) CD63, HSPA8, PDCD6IP, TSG101 and β-Actin expression of hUCMSC-Exos have been observed by western blotting.

Figure 2

DiR-labeled hUCMSC-Exos were recruited to the endometrium in response to injury.

After the left uteri were injured by electrocoagulation, hUCMSC-Exos were injected into the endometrial injury mice via tail vein injection. Fluorescent signals were captured by in vivo imaging system at 6 h, 12 h, 24 h and 7 day after exosomes injection.

Figure 3

hUCMSC-Exos ameliorated the damaged uterus and increased endometrial thickness.

(A) Differences in the appearance of the endometrium in the three groups, with alcohol damage on the left and no damage on the right. (B) Morphological observations of injured endometrium by HE staining (200× Scale bar: 50μm, 400× Scale bar: 20μm). (C) The changes in the histology of uterus were analyzed using HE staining (n=20). (D) Statistical data on the number of glands in the endometrium of three groups of mice (n=20). Groups were compared by independent samples t-test. *, $P<0.05$.

Figure 4

hUCMSC-Exos ameliorated reproductive function in mouse injured endometrium.

(A)Embryos implanted in the uterus in control, saline and exosome groups. (B) The number of embryos in control, saline and exosome groups (n=6). Groups were compared by independent samples t-test. *, $P<0.05$.

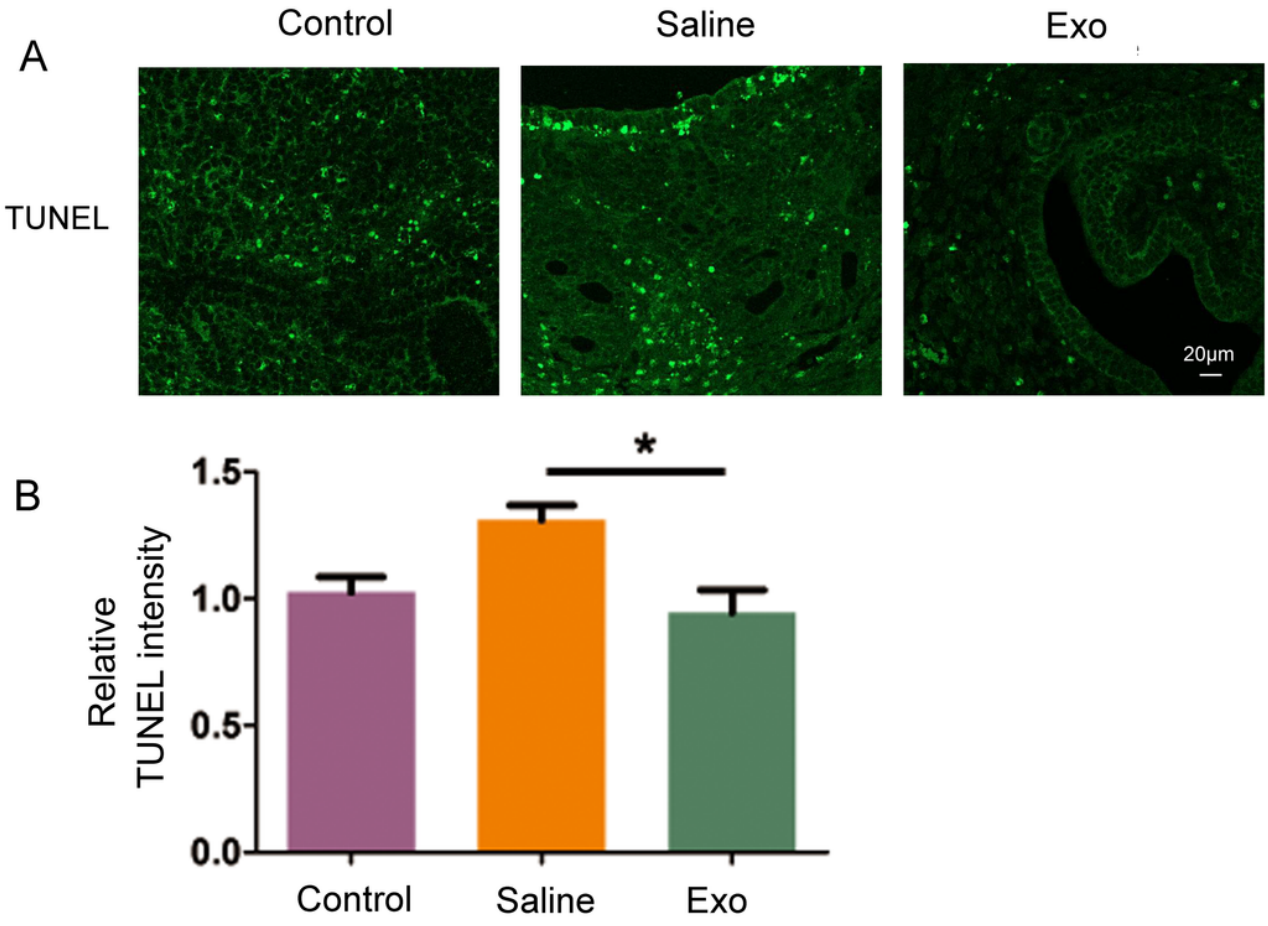

Figure 5

hUCMSC-Exos decreased apoptosis expression in damaged uterus.

(A) Representative micrographs of TUNEL staining for apoptosis in the mouse endometrium (400× Scale bar: 20μm). TUNEL-positive apoptotic cell with green fluorescence. (B) The number of TUNEL-positive cells in each group (n=6). Groups were compared by independent samples t-test. *, $P<0.05$.

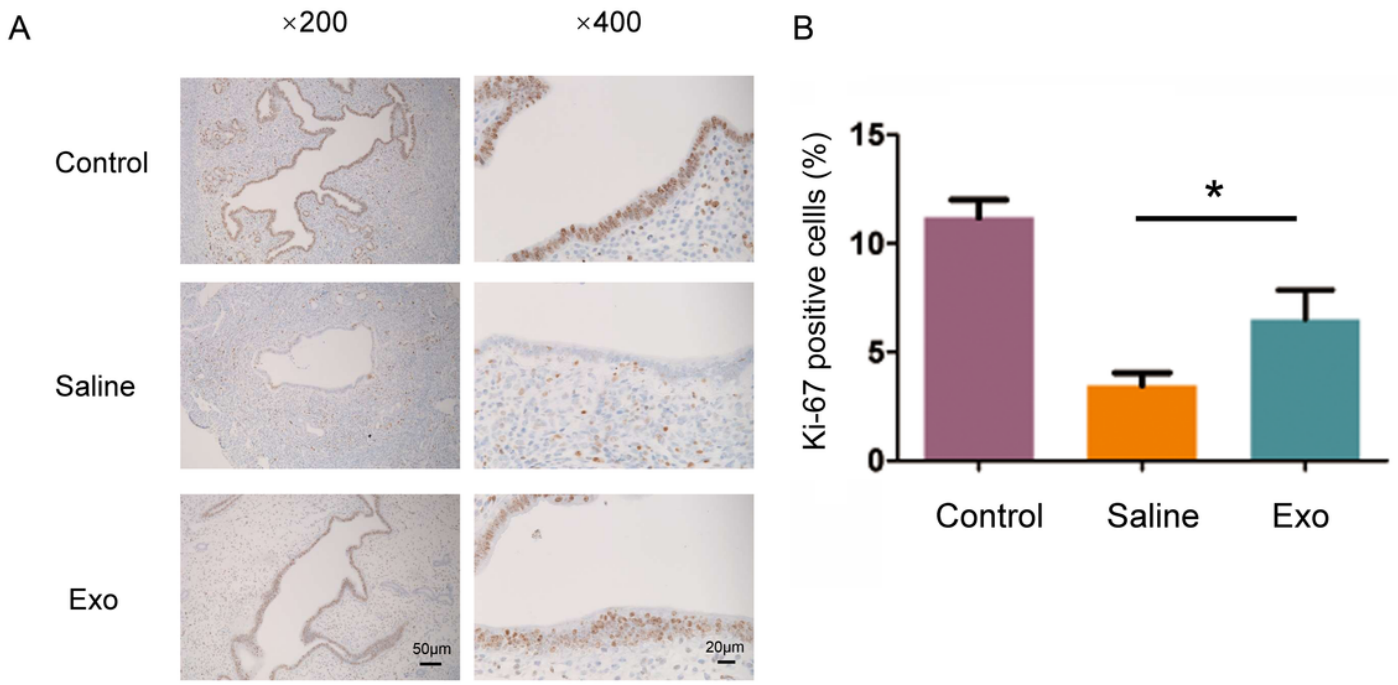

Figure 6

hUCMSC-Exos increased Ki-67 expression in damaged uterus.

(A) Immunohistochemical identification of Ki-67 in the mouse endometrium. Expression of Ki-67 in mouse endometrium in three group (control group, saline group, exosome group, 200× Scale bar: 50μm, 400× Scale bar: 20μm). (B) Statistical results of Ki-67 expression (n=6). Groups were compared by independent samples t-test. *, $P<0.05$.

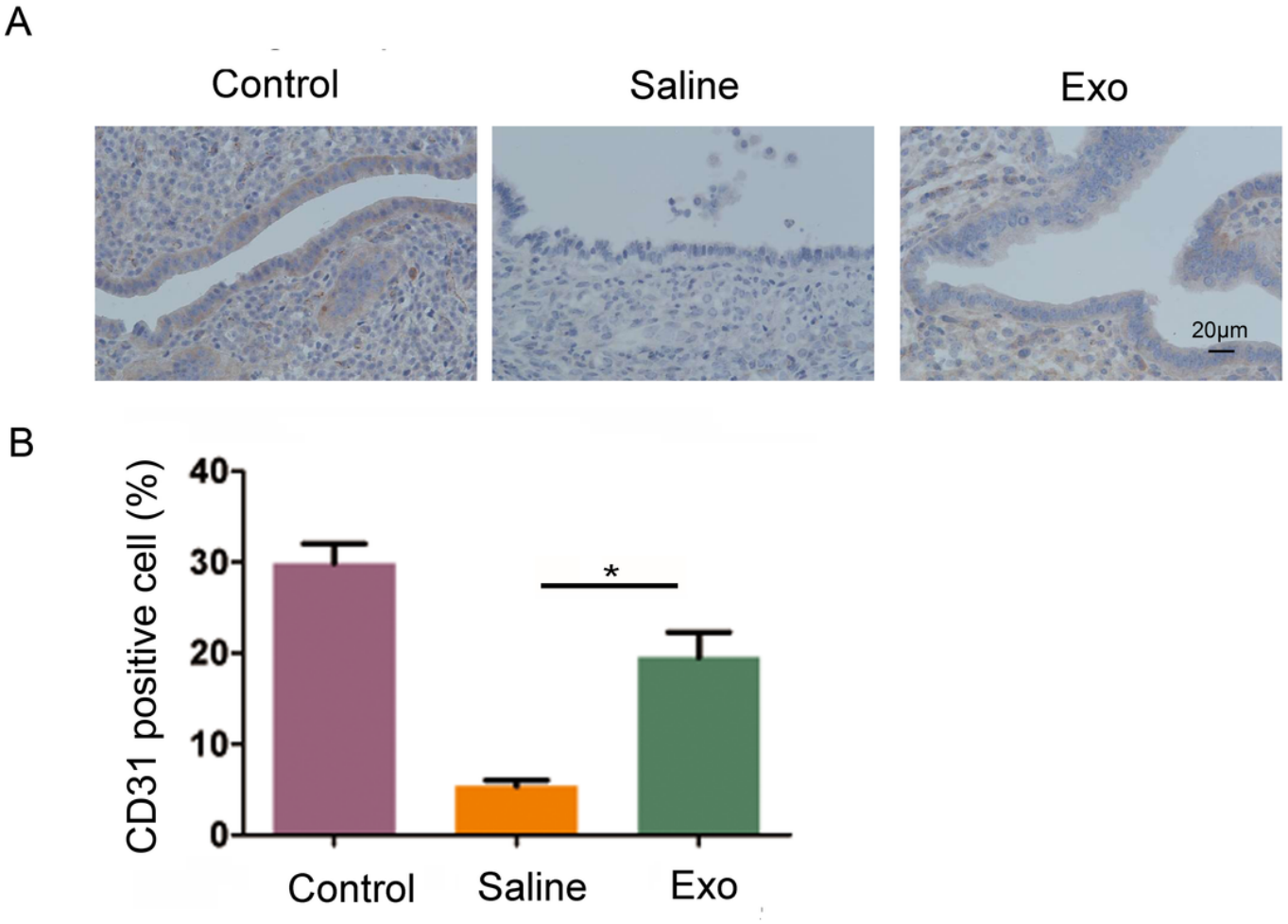

Figure 7

hUCMSC-Exos improved the CD31 expression of damaged uterus.

(A) Immunohistochemistry of endometrial CD31 in mice. Expression of CD31 in mouse endometrium in three group (control group, saline group, exosome group, 200× Scale bar: 50μm, 400× Scale bar: 20μm). (B) Statistical results of CD31 expression (n=3). Groups were compared by independent samples t-test. *, $P<0.05$.

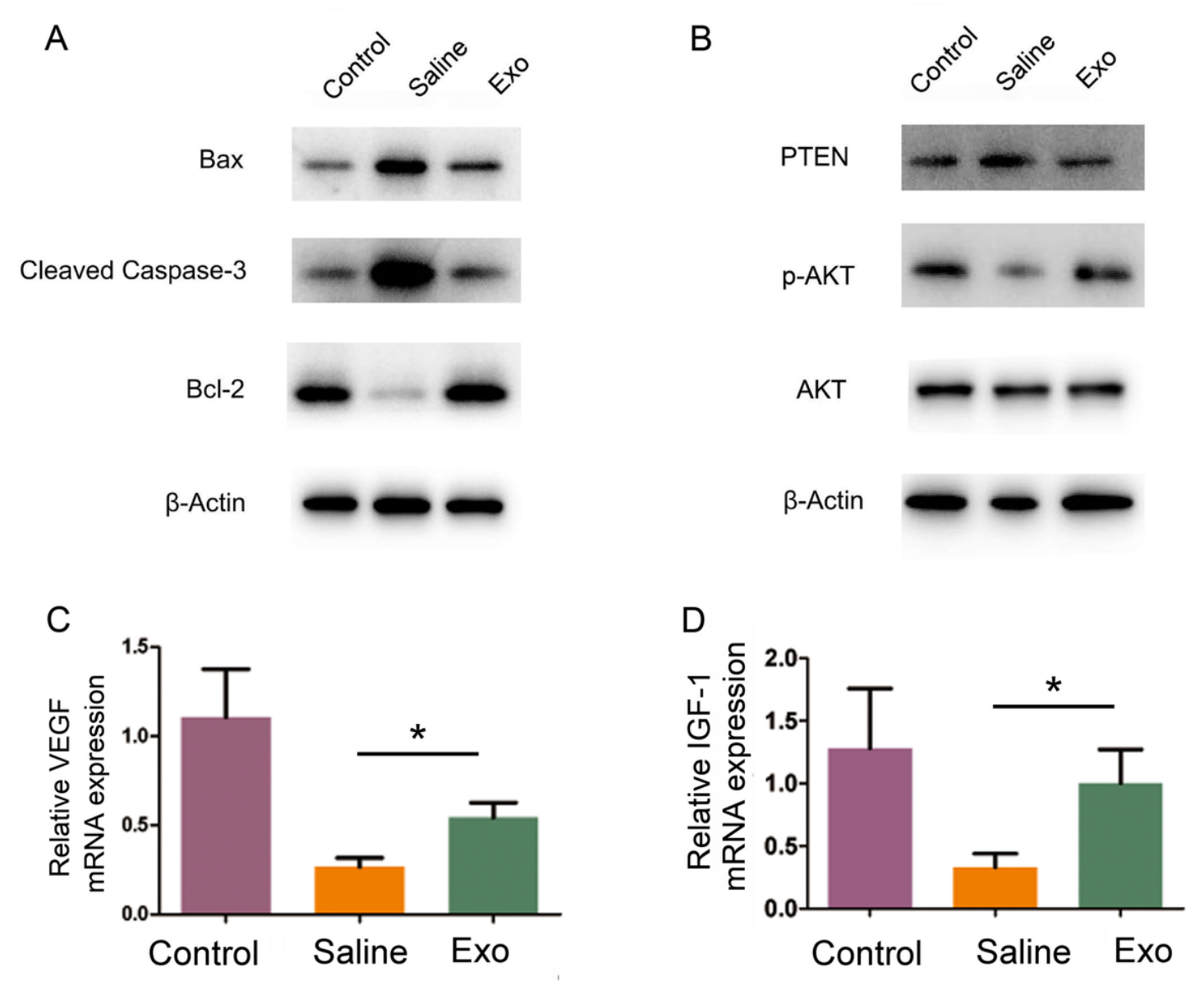

Figure 8

hUCMSC-Exos activated AKT signaling pathways in the endometrial damaged uterus.

(A) Expression of Bcl-2, Bax, Cleaved Capase-3 protein of uterus from mice in three groups were detected. (B) Expression of PTEN, AKT and p-AKT protein of uterus from mice were detected. (C) The mRNA expression of VEGF of uterus from mice in three groups were analyzed by quantitative RT-PCR. (D) The mRNA expression of IGF-1 of uterus from mice in three groups were analyzed by quantitative RT-PCR (n=3). Groups were compared by independent samples t-test. *, $P<0.05$.

Supplementary Files

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