Mechanism study of BMSC-exosomes combined with hyaluronic acid gel in regulating oxidative stress in the treatment of posttraumatic osteoarthritis

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Research Article

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Abstract

Objective

To explore the efficacy and mechanism of exosomes derived from bone marrow mesenchymal stem cells (BMSC-EXOs) combined with hyaluronic acid (HA) gel in treating post-traumatic osteoarthritis (PTOA) by regulating injury caused by mitochondrial reactive oxygen species (ROS)-induced oxidative stress.

Methods

This study utilized a combination of in vitro and in vivo experiments to investigate the potential benefits of BMSC-EXOs in the treatment of post-traumatic osteoarthritis (PTOA). The in vitro experiment involved the isolation and characterization of BMSC-EXOs from rats, which were then labeled with Dil. Then the primary chondrocytes of rats were isolated, and a cell model of PTOA was established. The cells were assigned into control group, model group, BMSC-EXOs group, HA group, BMSC-EXOs + HA group, BMSC-EXOs + 740Y-P group, and BMSC-EXOs + HA + 740Y-P group. Oxidative stress levels and cartilage matrix function were measured in each group. In the in vivo experiment, the rat model of PTOA was constructed via anterior cruciate ligament resection alone. The rats were divided into the same aforementioned groups and evaluated for oxidative stress levels, cartilage matrix function, and joint recovery.

Results

According to in vivo and in vitro experimental results, BMSC-EXOs + HA gel could effectively lower the level of oxidative stress of chondrocytes and rat PTOA models, and improve the mechanical function of the cartilage, exhibiting superior effects to those of BMSC-EXOs alone.

Conclusion

BMSC-EXOs + HA gel can be adopted to treat PTOA by regulating injury caused by mitochondrial ROS-induced oxidative stress.

1 Introduction

Osteoarthritis (OA) is the most common chronic disease of the joint, with prevalence rising with age. It arises in joints in most cases, including knees, hips and hands, which is primarily manifested as chronic pain, joint instability, ankylosis and joint deformity in clinic. Post-traumatic OA (PTOA) is a subtype of OA, which occurs after intra-articular fracture, ligament injury or other joint injuries. PTOA cases take up 12% of OA cases, and most of them are young people. Risk factors for PTOA mainly include anterior cruciate ligament injury, meniscus tear, glenohumeral instability, patella, and dislocation.
intervention measures can prevent the progression of PTOA when the underlying pathogenesis is known. Articular chondrocytes, specifically the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, can normally produce low-level reactive oxygen species (ROS), which participate in intracellular signal transduction, maintain cartilage homeostasis, and regulate the proliferation of chondrocytes and extracellular matrix (ECM) synthesis [6]. It has been previously confirmed that deoxyribonucleic acid (DNA) damage induced by OA chondrocyte-produced ROS is evidently severer than that induced by normal chondrocyte-produced ROS [7]. In chondrocytes, the NADPH oxidase is the predominant enzyme producing ROS in synovial fluid, which can aggravate oxidative stress in joints and mediate the progressive cartilage degradation in the course of OA [8]. Moreover, excessively produced ROS can serve as second messengers to directly degrade matrix components by suppressing matrix synthesis, cell migration and the activity of growth factors and facilitate cartilage degradation by activating matrix metalloproteinases (MMPs) and triggering cell death, thus ultimately aggravating OA [9–10].

Exosomes (EXOs), tiny vesicles secreted by all cells [11], are deemed to be vital factors that regulate intercellular communication and participate in multiple physiological and pathological processes [12]. Bone marrow mesenchymal stem cells (BMSCs), as non-hematopoietic stem cells in the bone marrow, are able to differentiate into different types of cells [13]. In OA cases, the functions of BMSC-EXOs include an inhibitory effect on mitochondrial dysfunction, induction of chondrocyte apoptosis and relief of the pain caused by OA of the lumbar facet joints [14]. In addition, micro ribonucleic acid (miR)-206 in BMSC-EXOs can reduce ELF3 to facilitate the proliferation and differentiation of osteoblasts in OA, thus influencing the development of OA [15]. As an integral component of normal synovial fluid, hyaluronic acid (HA) contributes to the prevention of wear and tear. HA cannot be produced in all OA patients, so it can be exogenously supplemented to alleviate pain and reduce the friction between joints by suppressing the release of inflammatory cytokines and apoptosis and controlling fibrosis [16–17]. Exosome therapy is regarded as a highly promising cell-free therapeutic strategy for tissue regeneration and repair. However, the rapid clearance of EXOs from body fluids and circulation poses a significant challenge for their sustained release at targeted sites. In this regard, hydrogels garnered considerable attention due to their excellent biocompatibility and loose, porous structure, which allows them to function as ideal carriers for EXOs, thereby facilitating the prolonged release of EXOs. Hence, HA has become a preparation frequently used for OA symptom relief.

In this study, PTOA was treated by BMSC-EXOs + HA, and the potential mechanism of this scheme was investigated.

2 Materials and methods

2.1 Isolation, culture, and identification of BMSCs

The whole bone marrow adherent method was employed for BMSC extraction. In detail, Sprague-Dawley rats aged 2 weeks old (n = 10/group, Beijing HFK Bioscience Co., Ltd.) were killed by overdose anesthetics
and soaked in 75% alcohol for 5 min. Later, bilateral femurs were dissected under aseptic conditions. After the blood stains were washed away by Dulbecco's phosphate-buffered saline (D-PBS) (D1040, Solarbio), Dulbecco's Modified Eagle Medium (DMEM) (Biological Industries: 06-1055-57-1ACS) was aspirated using a 5 mL sterile syringe, and the bone marrow tissues in the femur were washed into a sterile culture dish and gently blown into a single cell suspension with a disposable sterile straw. Next, red blood cells were lysed with red blood cell lysis buffer (Solarbio: R1010), and the remaining tissue pieces were filtered out with a 200-mesh sieve. After 5 min of centrifugation at 1200 rpm, the supernatant was removed, the single-cell suspension was inoculated into a sterile culture T75 flask (cells were inoculated at an area of 75 cm²/rat), and 15 mL of complete culture medium containing 10% exosome-depleted fetal bovine serum (Exo-FBS, ViVaCell: C38010050) was added. Then the mixture well mixed by shaking was cultured in a cell incubator with 5% CO₂ at 37ºC, and the original culture medium was replaced with a fresh culture medium after 48 h. After the cells overgrew, they were passaged every three days. Passaged to the 3rd -5th generation, the cells were identified, and the identified cells were utilized for subsequent experiments.

2.2 Isolation, identification, and tracing staining of BMSC-EXOs

EXOs were extracted from BMSC culture medium using Wayen Exosome Isolation kit (H-Wayen: EIQ3-04001). The BMSC culture medium was centrifuged at 4ºC and 3000×g for 15 min to collect the supernatant. Then 5 mL of extraction reagent was evenly mixed with every 10 mL of supernatant samples by shaking up and down. After standing overnight at 4ºC, the mixture was centrifuged at 4ºC and 3000×g for 1 h, and the supernatant was discarded. Subsequently, the cells on the bottom and side wall of the tube were repeatedly pipetted with 1 mL of D-PBS, and the resuspension was centrifuged at 4ºC and 10,000×g for 10 min. After the supernatant was discarded, 50–100 µL of D-PBS was utilized to resuspend the precipitate, and the resuspension was centrifuged for 5 min again at 4ºC and 10,000×g. The supernatant was collected and identified as the PBS resuspension of EXOs. After the identification, the tracer Dil (Beyotime Biotechnology: C1991S) was employed to label the EXO membrane with red fluorescence.

2.3 Primary culture of rat chondrocytes and construction of an OA model

Chondrocytes were digested and harvested with trypsin-collagenase. Specifically, Sprague-Dawley rats (n = 10) aged 4 weeks old were killed by overdose anesthetics and soaked in 75% alcohol for 5 min. Then the knee cartilage and costal cartilage of the rats were taken out under aseptic conditions. Next, tissues were cut into 1 mm³ pieces, and then digested with 10 mL of 0.25% trypsin (Solarbio: 9002-07-7) solution by shaking in a constant temperature water bath kettle at 37ºC for 15–20 min. After standing for 5 min, the supernatant of the resulting mixture was discarded. Later, 0.2% collagenase II (Gibco: 17101-015) was added for digestion by shaking in the constant temperature water bath kettle at 37ºC for 30–60 min. This step was repeated until the tissue pieces disappeared. Subsequently, the resulting mixture was repeatedly
pipetted and filtered by a 200-mesh sieve. Then the filtrate was centrifuged at 1200 rpm for 5 min. After the removal of the supernatant, the growth medium composed of 88% DMEM + 10% EXO-FBS + 1% ITS (Meilun: PWL083) + 1% PS (Gibco: 15140122) was inoculated into a T25 flask for cell culture at 37°C and 5% CO₂. The cultured cells were treated with 10 ng/mL interleukin-1β (IL-1β) (MedChemExpress: HY-P73900) to construct the OA model. According to different treatments, these cells were assigned into control group, model group, BMSC-EXOs group, HA group, BMSC-EXOs + HA (MedChemExpress: HY-B0633) group, BMSC-EXOs + 740Y-P group, and BMSC-EXOs + HA + 740Y-P group. BMSC-EXOs + 740Y-P group and BMSC-EXOs + HA + 740Y-P group were administered an injection of 50 µg/mL of 740Y-P based on the experimental conditions in BMSC-EXOs group and BMSC-EXOs + HA group. In the treatment, the concentration of BMSC-EXOs was 40 µg/mL, and that of HA was 300 µg/mL. In this study, the animal treatment and experimental procedures were approved by the Animal Ethics Committee of Beichen District Hospital of Traditional Chinese Medicine, which were in line with the Guide to the Care and Use of Experimental Animals of the National Health Research Institutes.

2.4 Construction of rat model of PTOA by anterior cruciate ligament resection alone

Sprague-Dawley rats (n = 56) aged 4 weeks old were randomly assigned into control group, model group, BMSC-EXOs group, HA group, BMSC-EXOs + HA group, BMSC-EXOs + 740Y-P group, and BMSC-EXOs + HA + 740Y-P group. After anesthesia with isoflurane (VETISO: 235180101), the cruciate ligament of knee joints was resected under aseptic conditions to change local stress in joints. Then a PTOA model was established. In control group, the skin was cut open and sutured, but the cruciate ligament was not resected. At 1 week after the operation, the rats were driven twice a day (30 min/day). 4 weeks later, model group received an injection of 100 µL of normal saline into the joint cavity, and BMSC-EXOs group was injected with 100 µL of normal saline containing 40 µg of BMSC-EXOs. In the HA group, a single injection of 100 µL of 2% HA was administered, while in the BMSC-EXOs + HA group, 100 µL of 2% HA containing 40 µg of BMSC-EXOs was delivered via injection. The BMSC-EXOs + 740Y-P group and BMSC-EXOs + HA + 740Y-P group received weekly injections of 10 mg/kg of 740Y-P for 12 weeks, based on the experimental conditions of the BMSC-EXOs group and BMSC-EXOs + HA group. Joint damage was assessed using the Osteoarthritis Research Society International (OARSI) scoring system.

2.5 Coverslip immunofluorescence assay

After the trypsinization of chondrocytes in each group, 1,000 chondrocytes were inoculated on a coverslip with an area of 10 mm² and incubated overnight. Next, the chondrocytes were fixed with 10% neutral formalin solution, the membrane was perforated for 15 min using 0.1% Triton-100 (Solarbio: 9002-93-1), and 3% hydrogen peroxide (H₂O₂) was used to inactivate the catalase in the chondrocytes. Later, the chondrocytes were fixed with 2% bovine serum albumin (BSA, Solarbio: A8020) for 2 h, and incubated with the working solution of primary antibodies against tubulin (diluted at 1: 400, Abcam: AB7291) and collagen II (diluted at 1: 200, Abcam: AB34712) overnight at 4°C. On the next day, the chondrocytes were incubated with pre-adsorption goat anti-rabbit immunoglobulin G (IgG) H&L (Alexa Fluor® 488) (diluted
at 1: 500, Abcam: ab150081) and goat anti-mouse IgG H&L (Alexa Fluor® 594) (diluted at 1: 500, Abcam: ab150116) secondary antibodies at room temperature for 1 h. After mounting by antifade mounting medium containing DAPI (Solarbio: S2110), the chondrocytes were observed under laser confocal microscope (Nikon A1HD25).

2.6 HE staining

The paraffin sections were subjected to routine deparaffinization with a graded series of alcohol to water. Subsequently, the sections were immersed in a hematoxylin solution (Beso: ba4097) for 4 min and washed thoroughly with running water. To remove excessive staining, the sections were treated with 1% hydrochloric acid alcohol for several seconds and then exposed to 1% ammonia to restore the blue hue. After further washing with running water, the sections were treated with eosin solution (Beso: ba4095) for 30 s. Following this, the sections were rinsed with water and routinely dehydrated and transparentized, after which they were sealed with neutral gum and observed under a microscope (LECAI: DM750).

2.7 Western blotting assay

The total proteins were extracted from cells and tissues in each group, and the protein concentration in each group was determined using bicinchoninic acid (BCA) protein assay kit (Solarbio: PC0020). 5×gel loading buffer was added in proportion, and the proteins were subjected to a metal bath (100ºC) for 10 min. Then the proteins were loaded at 30 µg/well and underwent sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (staking gel: 80 V/40 min, and separating gel: 110 V/60 min). After the SDS-PAGE, the separated proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane (300 mA). Later, the membrane was sealed with 5% skim milk powder (OXOID: LP0031B) for 2 h, incubated with primary antibodies at 4ºC overnight, and washed. Afterwards, the membrane was incubated with secondary antibodies (diluted at 1: 5000, Abcam: ab97080) at room temperature for 1 h, followed by washing. Finally, ECL kit was employed for image development, and statistical analysis was conducted.

2.8 Statistical analysis

Prism 9.0 software was used for data analysis, the data were expressed as x ± s, the differences between groups were analyzed by t-test, and the comparison between groups was analyzed by one-way ANOVA. P < 0.05 considered the difference statistically significant.

3 Results

3.1 Identification results of BMSC-EXOs

Firstly, the morphology of EXOs extracted from the rat BMSC culture medium was observed the morphology under the electron microscope (Fig. 1A), and it was found that EXOs appeared cup-shaped with a diameter of about 100 nm or bi-concave (Fig. 1B) with obvious membrane boundaries. According to the results of Western blotting assay, in EXOs, CD63, TSG101 and ALIX were highly expressed, but no expression of Calnexin was detected (Fig. 1C). Western blotting assay results demonstrated that the
expressions levels of both ALIX and CD63 were considerably high in EXOs and HA (Fig. 1D), indicating that the substances isolated were BMSC-EXOs.

3.2 Effects of BMSC-EXOs + HA on synthesis and metabolism of cartilage matrix in OA

The membrane of the EXOs obtained by extraction was labeled with the tracer Dil, and the EXOs were co-cultured with chondrocytes. The results revealed that chondrocytes could phagocytize EXOs, and the quantities of EXOs phagocytized by chondrocytes were increased over culture time (8 h, 16 h, 24 h) (Fig. 2A). Collagen II and proteoglycan (PG) are the main components of the chondrocyte-derived ECM of chondrocytes \[18\], and matrix metalloproteinase 13 (MMP13) is a vital substance involved in the degradation of chondrocyte-derived ECM \[19\]. In this study, an OA model was established by stimulation of chondrocytes with 10 ng/mL IL-1\(\beta\) \[20\], and the results manifested that the expression level of collagen II was markedly reduced, while that of cellular metabolic protein MMP13 was elevated in chondrocytes activated by IL-1\(\beta\). The observed trends were subsequently reversed after treatment with BMSC-EXOs and HA, with the reversion effect being superior in BMSC-EXOs + HA group to that in BMSC-EXOs group and HA group. Notably, there were no statistically significant differences in the expressions of Collagen II and MMP13 between BMSC-EXOs + 740Y-P group and BMSC-EXOs + HA + 740Y-P group (Fig. 2B).

3.3 Effect of BMSC-EXOs + HA on ROS level in osteoarthritic chondrocytes

Some studies have shown that ROS are crucial for cartilage degeneration, and high-level ROS often trigger the inflammation and apoptosis of articular chondrocytes \[21\]. The NADPH oxidase is a pivotal indicator reflecting the level of ROS in cells, including P40phox, P47phox and P67phox in the cytoplasm, and P22phox and GP91phox in the membrane. Malondialdehyde (MDA) is the end-product of lipid oxidation, which influences mitochondrial respiratory chain complex and key enzyme activities in mitochondria, and aggravates membrane damage. Hence, the level of MDA can reflect the degree of lipid peroxidation and indirectly reflect the severity of cell damage. Superoxide dismutase (SOD), glutathione (GSH) and other corresponding antioxidant systems can scavenge various ROS to maintain the redox environment in cells \[8\]. According to the results of this study, the levels of P47phox, P22phox, GP91phox and MDA in chondrocytes stimulated by IL-1\(\beta\) rose remarkably, but the stimulation by IL-1\(\beta\) was weakened by treatment with BMSC-EXOs. Besides, the weakening effect in BMSC-EXOs + HA group was more evident than that in BMSC-EXOs group and HA group. The expression levels of P47phox, P22phox, and GP91phox were not significantly different between BMSC-EXOs + 740Y-P group and BMSC-EXOs + HA + 740Y-P group (Fig. 3A). As for the levels of the corresponding antioxidant systems, the levels of SOD and GSH were notably reduced after stimulation by IL-1\(\beta\), which were effectively restored by BMSC-EXOs treatment. Moreover, BMSC-EXOs + HA group exhibited an obviously superior restoring effect to BMSC-EXOs group and HA group. There were no significant differences in the expression levels of MDA, SOD, and GSH between BMSC-EXOs + 740Y-P group and BMSC-EXOs + HA + 740Y-P group (Fig. 3B).
3.4 Effect of BMSC-EXOs + HA on the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) signaling pathway in IL-1β-induced OA

It has been previously demonstrated that IL-1β can destruct the homeostasis of OA cells by activating the PI3K/AKT pathway [22]. This finding was also confirmed by this study. It was uncovered that BMSC-EXOs treatment can dramatically down-regulate the phosphorylation level of PI3K/AKT, thus protecting the damaged cells, and this protection was more effective in BMSC-EXOs + HA group and HA group than that in BMSC-EXOs group. Following the addition of the PI3K agonist 740Y-P to the BMSC-EXOs group and BMSC-EXOs + HA groups, the previously observed significant difference between the two groups was eliminated (Fig. 4). The above results suggested that OA can be treated by BMSC-EXOs + HA, potentially through their regulation of the PI3K/AKT signaling pathway.

3.5 BMSC-EXOs + HA reduced the level of ROS in the PTOA model

The rat model of PTOA was established by anterior cruciate ligament resection alone, and BMSC-EXOs and BMSC-EXOs + HA were injected into the joint cavity to explore and analyze the effect of BMSC-EXOs + HA on the ROS level in the PTOA model. The results uncovered that the level of MDA in model group evidently rose, while the levels of SOD and GSH dropped significantly after BMSC-EXOs treatment. The changes in BMSC-EXOs + HA group and HA group were more remarkable than those in BMSC-EXOs group. There was no significant difference in the expression of MDA, SOD and GSH in BMSC-EXOs + 740Y-P group and BMSC-EXOs + HA + 740Y-P group (Fig. 5). The above results indicated that BMSC-EXOs + HA can reduce the level of ROS in the PTOA model.

3.6 BMSC-EXOs + HA promoted the synthesis of chondrocyte-derived ECM and suppressed its degradation

HE staining was used to evaluate the recovery of the PTOA rat model. Control group exhibited a smooth and complete cartilage surface on the tibial plateau, with cells in good condition and obvious stratification. Conversely, model group showed obvious cartilage damage with atrophic and necrotic chondrocytes and disordered arrangement. BMSC-EXOs group exhibited incomplete chondrocyte structure, small cracks, shallow matrix red staining, and a small number of chondrocytes. In contrast, BMSC-EXOs + HA group demonstrated a relatively complete cartilage surface with significantly increased chondrocytes and a small number of vacuolized and hypertrophic cells. Furthermore, the OARSI score was used to evaluate articular cartilage injury, which revealed that BMSC-EXOs + HA effectively improved the proliferation of chondrocytes (Fig. 6A). In addition, we evaluated cartilage extracellular matrix synthesis and degradation inhibition by the Western blotting assay. Compared with that in control group, the expression level of MMP13 in model group was raised remarkably, but it notably declined after BMSC-EXOs treatment and BMSC-EXOs + HA treatment. The similar results of the expressions of collagen II and
MMP13 were obtained from the Western blotting assay (Fig. 6B). It could be concluded that BMSC-EXOs + HA effectively facilitate the collagen synthesis and suppresses its degradation by chondrocytes.

4 Discussion

In the present study, the effect of BMSC-EXOs + HA on chondrocyte repair in PTOA was investigated. It was revealed by the in vitro experiment that BMSC-EXOs were endocytosed into the cytoplasm of chondrocytes. Through the PI3K/AKT signaling pathway, BMSC-EXOs markedly decreased the level of IL-1β-induced ROS in chondrocytes, and stimulated the production of collagen and inhibited its degradation in the ECM. In the rat model of PTOA, the level of ROS in the body dropped, the production of collagen II in the ECM was increased, and the degradation of collagen II was inhibited after the rats were treated with BMSC-EXOs or BMSC-EXOs + HA. The effect of BMSC-EXOs + HA was better than that of BMSC-EXOs alone, which suggested that BMSC-EXOs + HA evidently facilitate the repair of chondrocytes and the synthesis of ECM, so it has become a potential treatment scheme for PTOA.

BMSC-EXOs, 30–150 nm in diameter, are small lipid membrane vesicles secreted by BMSCs, which are able to mediate cell-to-cell communication. Released by one cell, they will be captured by neighboring cells through ligand receptors or directly bind to neighboring cells, so as to achieve information transmission [23]. As osteoprogenitor cells of the cartilage, BMSCs can be substitutes of damaged chondrocytes or stimulate the repair of endogenous chondrocytes, so they were chosen as objects for cartilage tissue repair. It has been shown by numerous studies that BMSCs secrete multiple nutritional factors to modulate the damaged tissue environment and coordinate cell proliferation, cell differentiation, matrix synthesis and other cell regeneration processes [24]. In addition, BMSCs can mediate TGF-β, MMPs, TIMPs, etc. to reduce tissue damage, stimulate ECM synthesis and suppress ECM degradation and remodeling [25]. As such, BMSCs are promising in the improvement of cartilage lesions and the treatment of OA. Furthermore, BMSCs exhibit the highest yield of EXOs. BMSC-EXOs are similar to BMSCs in biological functions, but BMSC-EXOs are easy-to-store small vesicles that are capable of penetrating biofilms and possess low immunogenicity [26]. Hence, EXOs are present with many unique advantages compared with MSCs, and EXOs do not proliferate, which eliminates the possibility of tumorigenesis [27]. In this study, BMSC-EXOs mediated the PI3K/AKT signaling pathway to promote the production and suppress the degradation of collagen II, the most important component in the articular chondrocytes-derived ECM, and they also evidently decreased the level of ROS in vivo, which lays a theoretical foundation for the function of BMSC-EXOs in the treatment of OA.

HA, a long polysaccharide chain, is the predominant constituent of the cartilage and synovial fluid, which is hydrophilic. It serves as a lubricant and shock absorber to prevent articular cartilage and joint structure from compression and shear force, thus alleviating the wear of articular cartilage [28]. In OA, the rheological properties of synovial fluid will weaken with the decrease of HA concentration, so the supplemented HA can restore rheology to enhance the mechanical function of joints. It has been shown that HA performs biological functions, including functions in anti-inflammation, analgesia, and
anabolism, by suppressing MMPs and interleukins [29]. In a clinical trial, autologous BMSCs and HA were injected to treat knee OA, and the patients were followed up for a long term. The follow-up data revealed that the symptoms of patients treated with BMSCs and HA were alleviated, suggesting that BMSCs and HA are safe and feasible in the treatment. According to the results of this study, BMSC-EXOs + HA effectively facilitates the production of collagen II, the predominant constituent in the ECM derived from articular chondrocytes, suppressed its degradation, and dramatically decreased the level of ROS in the body. Besides, the effect of BMSC-EXOs + HA was superior to that of BMSC-EXOs alone. This phenomenon may be attributed to the fact that HA serves as a lubricant and shock absorber for joints and provides a good microenvironment for BMSC-EXOs. Meanwhile, it assists in the ECM repair by BMSC-EXOs, and down-regulates the level of ROS in the body to repair damaged chondrocytes, thus playing a role in the treatment of PTOA. In addition, in comparison with previous related studies, our investigation revealed that EXOs and HA could play a protective role against OA by regulating endocytosis or polarized cells. This finding supports the effectiveness of EXOs and HA on OA. Moreover, our research expands upon prior studies by demonstrating that the combined use of EXOs and HA regulates OA through the PI3K/AKT signaling pathway, which represents a novel research direction and underscores our contribution to the field of OA research [30, 31].

To sum up, BMSC-EXOs + HA can evidently promote the repair of the cartilage and the synthesis of the ECM, thus alleviating the PTOA-induced damage to articular chondrocytes, which provides a new treatment option for OA patients. However, limitations still exist in this study. Despite the ability of BMSC-EXOs + HA to relieve articular cartilage damage in OA rats, the upstream signal molecules were not studied, which needs further in-depth exploration.

Declarations

Authors’ Contributions

X.Q.L contributed to the conception and design of the study, interpretation, data analysis and interpretation, and writing of the manuscript. Y.S.C contributed to data analysis and interpretation, and manuscript revision. T.Z contributed to data analysis and interpretation, data collection, animal models, designed experiments, writing and revising the manuscript. All authors read and approved the final manuscript.

Ethics approval

The animal use protocol for this study has been reviewed and approved by Beichen District Chinese Medicine Hospital Animal Experiment Ethics Committee.

Conflict of interest statement

The authors declare that they have no conflicts of interest.
Acknowledgments

None

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References


Figures
Characterization of BMSC-EXOs.

A: BMSCs display a dense, spindle-shaped growth pattern under an inverted microscope. B: Transmission electron microscopy reveals that the EXOs exhibited a characteristic tea tray or hemispherical depression shape on one side. C: Western blotting analysis demonstrates significant expression of exosomal
markers CD63, TSG101, and Alix, while the endoplasmic reticulum marker calnexin is absent. D: Western blotting analysis confirms the elevated expressions of CD63 and Alix following BMSC-EXOs + HA treatment. *p < 0.05; ** p < 0.01; ns p > 0.05. **Figure 2:** BMSC-EXOs + HA attenuates IL-1β.

**Figure 2**

**A:** Phagocytosis of BMSC-EXOs by chondrocytes is evaluated using laser confocal microscopy (magnification: 600×) at different time points (8 h, 16 h, and 24 h); **B:** BMSC-EXOs+ HA promotes the synthesis of extracellular matrix (collagen) and inhibited its degradation (MMP13). Three repeated experiments were performed for each group. * p < 0.05; ** p < 0.01; ns p > 0.05.
Figure 3

BMSC-EXOs + HA attenuates IL-1β-induced ROS levels in chondrocytes induced by OA.

A: The expression levels of p47phox, p22phox, and gp91phox in cells are detected using Western blotting analysis. B: The expression levels of SOD, GSH, and MDA in cells are assessed. Three independent replicates were performed for each group. * p < 0.05; ** p < 0.01; ns p > 0.05
Figure 4

BMSC-EXOs + HA inhibits the activation of the PI3K/AKT signaling pathway in OA.

A: BMSC-EXOs + HA reduces IL-1β and the phosphorylation level of PI3K/Akt signaling pathway in OA. B: Statistical charts of Western blotting bands. The experiment was repeated thrice for each group. * p < 0.05; ** p < 0.01; ns p > 0.05

Figure 5

A: MDA

B: GSH

C: SOD
BMSC-EXOs + HA reduces ROS levels in the PTOA model. The rat model of PTOA is established by anterior cruciate ligament resection, and BMSC-EXOs + HA was injected into the articular cavity to investigate ROS levels in the model of PTOA. A: The expression levels of MDA in each group. B: The expression level of GSH in each group. C: The expression level of SOD in each group. BMSC-EXOs + HA can reduce ROS levels in the serum of the model of PTOA rats. n = 8 in each group. * p < 0.05; ** p < 0.01; ns p > 0.05

Figure 6

BMSC-EXOs + HA promotes the synthesis and inhibits the degradation of chondrocyte extracellular matrix in the PTOA rat model.

A: HE staining reveals the recovery of the PTOA rat model and OARSI score assessment. B: Western blotting analysis demonstrates that BMSC-EXOs + HA promotes the synthesis of cartilage extracellular matrix (collagen) and inhibited its degradation (MMP13) in the PTOA model. n = 8 in each group. * p < 0.05; ** p < 0.01; ns p > 0.05
BMSC-EXOs + HA can evidently promote the repair of the cartilage and the synthesis of the ECM, thus alleviating the PTOA-induced damage to articular chondrocytes.