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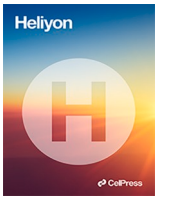

BMP-7 modified exosomes derived from synovial mesenchymal stem cells attenuate osteoarthritis by M2 polarization of macrophages

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ABSTRACT

Background: Although the exosomes derived from mesenchymal stem cells (MSCs) display a therapeutic effect on inflammatory diseases, its application on OA has great limitations due to lack of specificity and targeting. The current study aimed to elucidate the potential therapeutic role of bone morphogenetic proteins-7(BMP-7) modified synovial mesenchymal stem cells-derived exosomes (SMSCs-exo) on OA and mechanism.

Methods: For in vitro experiments, LPS-treated macrophages RAW264.7 were treated with SMSCs-exo (exo) or BMP-7 modified SMSCs-exos (BMP-7-exo). The levels of inflammatory factors were assessed by ELISA. Also, the proportion of iNOS and CD206 positive cells were quantified by flow cytometry. Chondrocytes and RAW264.7 were co-culture to evaluate the effects of macrophage polarization on chondrocytes cellular behaviors. This effect on KOA was verified by an experiment in vivo. HE staining and Safranin fast green staining were used to observe the damage of articular cartilage. Immunohistochemistry was used to determine the expression of collagen II and aggrecan in articular cartilage, as well as the expression of iNOS and CD206 in synovial tissues.

Results: Our in vitro results showed that BMP-7-exo treatment promoted LPS-induced proliferation of macrophages and chondrocytes, and showed a better ability to reduce inflammation by promoting macrophages M2 polarization. After co-culture with LPS treated macrophages, the proliferation rate and migration of chondrocytes were significantly decreased, while the apoptosis was significantly increased. The macrophages treated with BMP-7-exo and exo partially reversed these changes. The chondrocytes in BMP-7-exo group had higher proliferation rate and migration, as well as lower apoptosis compared with the exo group. Also, the in vivo results showed BMP-7-exo treatment improved the pathological changes of KOA and promoted synovial macrophages M2 polarization.

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Conclusions: Our results demonstrated that BMP-7-exo attenuated KOA inflammation and cartilage injury by synovial macrophages M2 polarization, suggesting that BMP-7-exo carry much therapeutic potential for OA.

1. Introduction

Osteoarthritis (OA), the most common chronic osteoarthropathy around the world, is mainly characterized by progressive degradation of articular cartilage, synovial tissue inflammation, subchondral bone remodeling and osteophyte formation [1,2]. It may elicit joint pains and deformities even severe disabilities and loss of motor function for patients [3]. At present, clinical nondrug therapy and drug therapy for OA are both mainly merely aimed at remission of symptom, and there is presently no practical treatments used to rescue OA progress.

In recent years, a new mesenchymal stem cell- (MSC-) based OA therapy has attracted increasing attention [4,5]. MSCs, as endogenous stem cells derived from multifarious mesenchymal tissues, can be directed to differentiate into neurocytes, adipocytes, osteoblasts and chondrocytes [6]. Due to the chondrogenic potential and the ability to generate extracellular matrix, MSCs is emphasized to exhibit great potential for OA treatment [7]. However, accumulating evidence has suggested the efficacy of many MSC-based therapies should be attributed to the paracrine secretion of trophic factors, particularly exosomes [8,9]. Exosomes are cell-secreted nanovesicles (40–150 nm in diameter) involved in intercellular communication with the capability of transferring cargo molecules including protein, lipids and microRNAs (miRNAs) [10,11]. Exosomes play regulatory roles in the immune system, inflammatory response inhibition and tissue damage repair [12]. Duo to their lower immunogenicity than cell therapy, exosomes has been used as an alternative therapy for a variety of diseases [13]. It is reported that exosomes can protect cartilage against damage and mitigate knee OA pain in a rat model [8]. In addition, they were recognized as a good vector and widely used in genetic and pharmaceutical fields [14].

Bone morphogenetic proteins (BMPs), the important growth factors belonging to the transforming growth factor beta (TGF-β) superfamily, is related to cartilage homeostasis and repair [15]. BMP-7, a member of BMPs subfamily, was proved to implicate in chondrocyte metabolism by stimulating the synthesis, renewal, and retention of matrix molecules, and have been reported to be chondroprotective in animal models of OA [16–18]. However, the use of conventional recombinant BMP-7 as therapeutic agents has been limited due to its instability [19]. Therefore, BMP-7 is generally considered as a potential disease modifying agent for the treatment of Knee OA(KOA). However, it remains unknown whether synovial mesenchymal stem cells-derived exosomes (SMSCs-exo) by BMP-7 modify from could act as an effective treatment option for OA. In this study, we transfected BMP-7 overexpressed plasmid into SMSCs to acquire BMP-7 overexpressed SMSCs-exo and explored their role on macrophage polarization. Further, the effects of macrophage polarization on chondrocyte were analyzed. Rat KOA model was established to verify the role of BMP-7 overexpressed SMSCs-exo on OA. We demonstrated that BMP-7 modified SMSCs-exos alleviated KOA by promoting macrophage M2 polarization. We believe this will provide a new possibility for the therapy of OA.

2. Methods

2.1. Cell culture

Mouse SMSCs, macrophage cell line RAW264.7 and chondrocytes were purchased from Procell Life Science & Technology Co., Ltd (Wuhan, China). The cells were incubated in low-glucose Dulbecco's modified Eagle's medium (DMEM, Gibco Life Technologies, USA) containing 10% fetal bovine serum (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA). The cultures were maintained in a humidified incubator at 37 °C with an atmosphere containing 95% air and 5% CO_2.

2.2. Isolation and characterization of exosomes from SMSCs

Exosomes were extracted from the 3rd passages of SMSCs. Briefly, SMSCs were cultured in exosome-free medium for 48 h. After that, culture supernatant was collected and centrifuged at 300 g for 10 min, 2000 g for 10 min, 10,000 g for 30 min and 100,000 for 70 min. The final pellets were resuspended in PBS and stored at −80 °C. Subsequently, the particle distribution was tested by NanoSight NS300 system, (Malvern Instruments, Malvern, UK). The morphology was observed by transmission electron microscopy (TEM) (H-7000FA, Hitachi Co. Ltd., Tokyo, Japan). Antibodies against CD63 (ab134045, Abcam, UK), CD81 (ab109201, K) and CD9 (ab213090, Abcam, UK) were utilized to identify the expression of exosomal surface marker proteins by Western blot assay.

2.3. SMSCs grouping and transfection

SMSCs were assigned into three groups: BMP-7 group (transfected with BMP-7 overexpressed plasmid), NC group (transfected with empty overexpressed vectors) and control group (untreated). BMP-7 overexpressed plasmid and empty overexpressed vectors was purchased from Shanghai Shengbo Biomedical Co., LTD (Shengbo Biomedical Co., LTD, China). Cell transfection was performed using Lipofectamine 3000 (Invitrogen) following the manufacturer's instructions. After transfection, the medium was changed to exosome-free medium and the cells were cultured for 48 h. After that, the cell supernatant was collected to acquire exosomes. The exosomes

extracted from control group named as exo, and from BMP-7 group named as BMP-7-exo. The expression of BMP-7 in exo and BMP-7-exo were detected by Western blot assay.

2.4. SMSCs grouping and treatment

The macrophages RAW264.7 and chondrocytes were respectively divided into four groups: Control group, LPS group, LPS + exo group and LPS + BMP-7-exo group. The cells were seeded into the 6-well plates. When cell confluence reached 70%–80%, the cells in LPS, LPS + exo and LPS + BMP-7-exo groups were treated with 50 ng/mL lipopolysaccharide (LPS) (Invitrogen, USA) to create macrophage inflammation model in vitro. After 24 h, the cells in LPS + exo and LPS + BMP-7-exo groups were treated with 17.2×10^7 particles of exo or BMP-7-exo, respectively. The cells in control group were cultured without any treatment. After 48 h of treatment, cells were collected for subsequent analyses.

2.5. Western blot assay

Total protein was extracted from RAW264.7 cells using RIPA Lysis buffer (Beyotime, China) with 1 mM of PMSF (Thermo, USA) following the manufacturer's instructions. The extracted proteins (50 μg) were quantified using bicinchoninic acid (BCA) kit. Equal amounts of protein were separated by 10~12% SDS-PAGE gels and then transferred onto PVDF membranes (Millipore, USA), and incubated overnight at 4 °C with appropriate primary antibody BMP-7 (ab129156, Abcam, UK) followed by blocking with 5% bovine serum albumin (BSA). Membranes were then incubated for 120 min at room temperature with the secondary antibody (Santa Cruz, Colombia). At last, the blots were determined by enhanced chemiluminescence reagents (Beyotime, China) and Tanon 5200 Multi imager (Tanon Science & Technology, China).

2.6. Proliferation assay

Cells were seeded in a 96-well plate (1×10^4 cells) and cultured for 24 h. 10 μl CCK-8 (Dojindo, Japan) solution was added to each well, and incubated for 4 h. The optical density at a wavelength of 450 nm were detected using a microplate reader (SpectraMax M5, USA).

2.7. In vitro detection of pro- and anti-inflammatory cytokine using Enzyme linked immunosorbent assay (ELISA)

The levels of interleukin 1β (IL-1β), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), interleukin 10 (IL-10) and transforming growth factor beta (TGF-β) in the culture media of RAW264.7 cells were detected using ELISA. Briefly, following treatment, the culture media was isolated from each group, centrifuged, and the supernatant collected. Then, the ELISA kits (R&D Systems, Minneapolis, MN, USA) were added and ELISAs were performed as per manufacturer's instructions.

2.8. Co-culture of RAW264.7 cells and chondrocytes

The co-culture system of RAW264.7 cells and chondrocytes were co-cultured established using Transwell insert (4 mm pore size, Corning, USA). RAW264.7 cells collected from different groups were plated in the upper chamber at a density of 2×10^5 cells/chamber with 1 mL medium. Chondrocytes were plated in the lower chamber at a density of 2×10^5 cells/chamber with 1 mL medium. The Transwell chamber inoculated with chondrocytes was then inserted into the plate inoculated with RAW264.7 cells. After 72 h of co-culture, chondrocytes were collected for further analysis.

2.9. Migration assay

To evaluate migration, the obtained chondrocytes (2×10^5 cells) were cultured in the upper chamber of Transwell inserts with an 8 μm pore size (Corning, USA) for 12 h. The invaded cells were fixed with 4% paraformaldehyde for 15 min, and then stained with crystal violet (Sangon Biotech, China). Cell migration were observed by a fluorescence microscope (Olympus IX71, Japan).

2.10. Flow cytometry

The obtained chondrocytes were collected by trypsin and centrifuged at 1000 g for 5 min. After the supernatant was removed, cells were washed twice by PBS and then resuspended in PBS. The suspended cells were incubated with antibodies, anti-CD206 (ab270647, Abcam, UK) and anti-iNOS (ab283655, Abcam, UK) at 4 °C kept for 15 min in the dark. The proportion of iNOS and CD206 positive cell were detected by flow cytometry system (Becton Dickinson FACS Vantage SE, Sanjose).

Flow cytometry was also used to detected the apoptosis. The same method was used in the preparing of suspended cells. Then, cells were incubated with 5 μl Annexin V-FITC/PI for 15 min in the dark. The apoptotic cells were detected.

2.11. Animal grouping and establishment of KOA

Forty SD male rats (10 weeks old) were provided by Wuhan Institute of Biotechnology (Wuhan, China). All rats were divided

randomly into 4 groups comprised of 10: Sham group, KOA group, exo group and BMP-7-exo group. The model of KOA was produced by unilateral intraarticular injection with 8% sodium iodoacetate (Sangon Biotech, China). Three weeks later, BMP-7 exo (1×10^{11} exosome particles/mL) was intraarticular injected for the rats in BMP-7-exo group, 100 μl every time, once every three days, and same volume and concentration of exo was used in exo group. The rats in sham group were performed with joint puncture only and intraarticular injection of 100 μl PBS. At 8 weeks after operation, the rats were sacrificed and synovial fluid were extracted from the joint cavity. The joint samples were subjected to pathological analysis.

All animal care protocols and experiments were carried out in strict accordance with the guidelines of the China Council on Animal Care and Use and approved by the ethics committee of Yantai Yuhuangding Hospital Affiliated to Qingdao University.

2.12. Histological staining

Joint samples were fixed in 10% formalin, embedded in paraffin. 4 μm sections were prepared and respectively stained with hematoxylin and eosin (HE) and Safranin-O-Fast-Green kit. Morphological changes were observed using a microscope (Olympus BX51, Japan). The therapeutic effect of OA cartilage tissue was assessed according to the Osteoarthritis Research Society International (OARSI) scoring system [20].

2.13. Detection of pro- and anti-inflammatory cytokine in synovial fluid using ELISA

Synovial fluid was extracted from the joint cavity. Firstly, 500 μL Dulbecco's PBS (DPBS) was absorbed into a 2 ml syringe injector and injected into the knee joint along the joint space. Then, the mixture of DPBS and synovial fluid was extracted back. After repeated suction for 3 times, the extracted liquid was collected and stored at −80 °C. The levels of IL-1β, IL-6, TNF-α, IL-10 and TGF-β were detected using ELISA as per manufacturer's instructions.

2.14. Immunohistochemistry (IHC) staining

The deparaffinized joint samples were rehydrated in a 3% hydrogen peroxide solution and blocked with 3% BSA. The sections were incubated with collagen II (ab34712, Abcam, UK), aggrecan (ab3778, Abcam, UK), iNOS antibody (ab178945, Abcam, UK) and CD206 antibody (ab270647, Abcam, UK) overnight at 4 °C. The sections were then incubated with secondary antibodies (Vector Laboratories, USA) for 1 h at room temperature. The sections were then developed with 3,3′-diaminobenzidine (DAB)-peroxidase substrate and counterstained with hematoxylin. Images were captured using a microscope (Olympus BX7I, Japan).

2.15. Statistical analysis

Statistical analyses were conducted using SPSS20.0 (IBM, New York, USA) for Windows. Statistical comparisons between groups

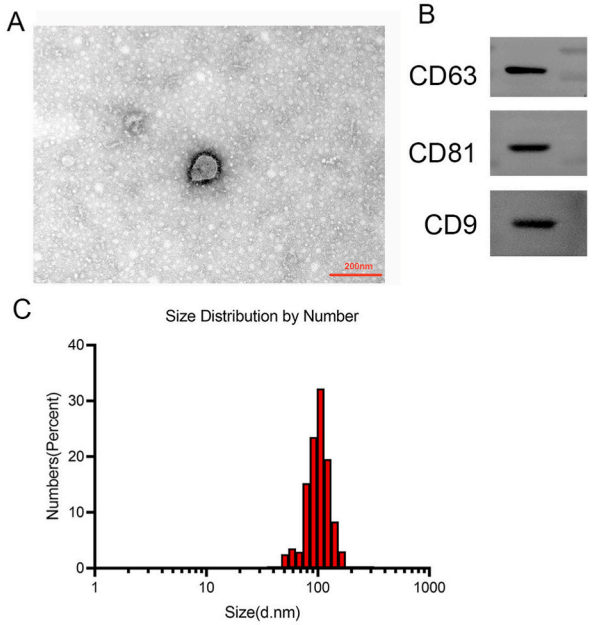

Fig. 1. Identification and characterization of SMSCs-exos. A: Representative TEM image (scale bar = 200 nm). B: The particle size of SMSCs-derived exosomes was assessed by NTA. C: The typical surface markers of MSCs-exos including CD63, CD81 and CD9 was detected by Western blot.

were performed using one-way analysis of variance (ANOVA). Multiple comparisons were performed using the Duncan's test. All data are reported as mean ± SD, and the level of statistical significance was set at $P < 0.05$. All trials were performed three times.

3. Results

3.1. Identification and characterization of exosomes

To identify and characterize exosomes, morphology analysis through TEM, particle size distribution by Nanoparticle Tracking Analysis (NTA) and exosomal markers expression by Western blot were applied. The exosomes presented the classic morphology as a vacuolar cup surrounded by a double membrane (Fig. 1A), and had a diameter of 50–160 nm (Fig. 1B). Western blot analysis revealed that exosomal surface markers CD63, CD81 and CD9 were observed in exos (Fig. 1C).

3.2. The expression of BMP-7 in exosomes

In order to prepare exosomes with high expression of BMP-7, SMSCs were transfected with BMP-7 overexpressed plasmid. The expression of BMP-7 in SMSCs was determined by Western blot. Fristly, BMP-7 expression in transfected SMSCs was detected. The results showed that the expression of BMP-7 in BMP-7group was higher than control and NC groups (Fig. 2A). Subsequently, exosomes were isolated from transfected SMSCs. Western blot assay showed the expression of BMP-7 in BMP-7 group was higher than exo groups (Fig. 2B). These results indicated that exosomes with high expression of BMP-7 were produced by SMSCs transfected with BMP-7 overexpressed plasmid.

3.3. BMP-7-exo promoted LPS-induced proliferation of macrophages and chondrocytes

As shown in Fig. 3A-B, compared with the control group, LPS stimulate significantly decreased the proliferation of macrophages and chondrocytes. However, BMP-7-exo and exo treatment partially reversed this change. Compared with the exo group, proliferation rate was higher in the BMP-7-exo group. These results indicated that BMP-7-exos promoted LPS-induced proliferation of macrophages and chondrocytes.

3.4. BMP-7-exo ameliorates inflammation by induced macrophages polarization to M2

Accumulating evidence has confirmed that two phenotypes of macrophages are in a dynamic balance in inflammation occurrence and development, and its polarization phenotype are decisive for the final outcome of inflammation [21–23]. Therefore, we wonder whether BMP-7-exo could change macrophage phenotype to ameliorate inflammatory and alleviate OA. LPS-treated RAW264.7 cells were treated with BMP-7-exo or exo. Proportion of positive cells of M1-associted marker iNOS and M2-assocaited marker CD206 in RAW264.7 cells were measured by flow cytometry. As shown in Fig. 4A-B, compared with the control group, a marked increase in iNOS-positive macrophages was observed in LPS group. While, the proportion of iNOS-positive macrophage tend to be lower and CD206-positive macrophage significantly increased in exo group and BMP-7-exo group. Moreover, the proportion of iNOS-positive

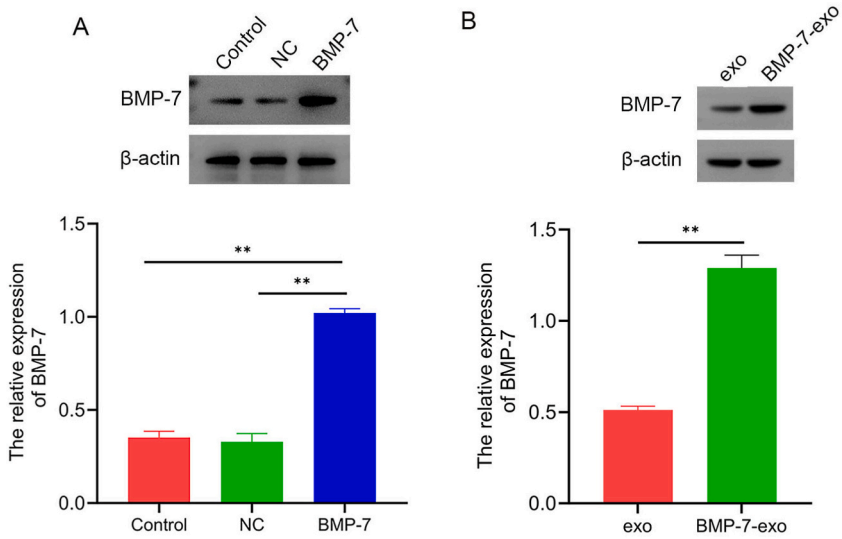

Fig. 2. The expression of BMP-7 in the transfected SMSCs and SMSCs-exos. Western blot was used to detect the expression of BMP-7 in the transfected SMSCs(A) and SMSCs-exos(B). **p < 0.01.

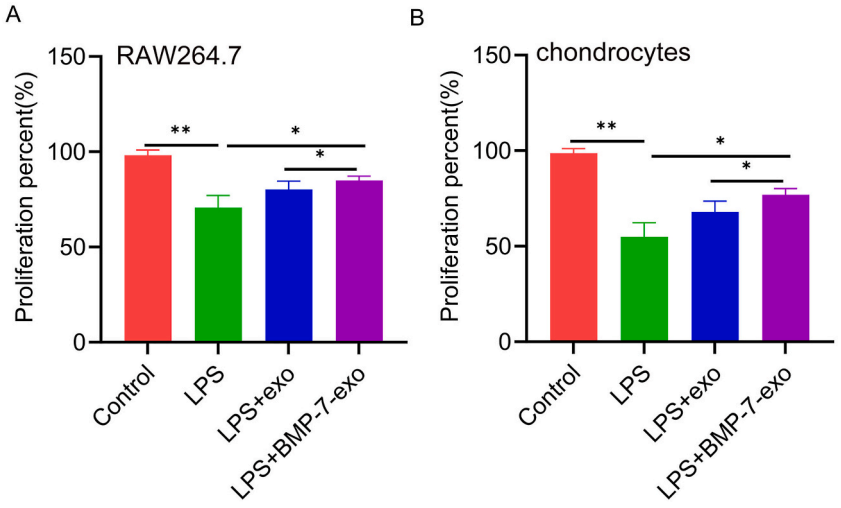

Fig. 3. BMP-7-exo promoted LPS-induced proliferation of macrophages and chondrocytes. A. The proliferation of RAW264.7 was detected by CCK-8 assay. B. The proliferation of chondrocytes was detected by CCK-8 assay. *P < 0.05, **p < 0.01.

macrophage was lower and CD206-positive macrophage was higher in BMP-7-exo group than those in exo group. Compared with the exo group, the iNOS/CD206 ratio was lower in BMP-7-exo group (Fig. 4C).

To evaluate the anti-inflammatory function of BMP-7-exo via macrophages polarization, we measured the concentration of inflammatory cytokines which were partially produced by M1 and M2 macrophages. The ELISA results showed that BMP-7-exo and exo alone reversed the increase of the pro-inflammatory cytokines secreted by M1 macrophages (TNF-α, IL-1β and IL-6) and decrease of the anti-inflammatory cytokines secreted by M2 macrophages (IL-10 and TGF-β) induced by LPS (Fig. 4D). These results indicated that BMP-7-exos attenuated macrophages inflammation by shifting macrophage polarization from M1 to M2.

3.5. Macrophage M2 polarization promotes proliferation and migration of chondrocytes

To explore the functional significance of macrophage M2 polarization on chondrocytes, we cocultured macrophages with chondrocytes for 72 h. Transwell assay and CCK-8 assay were conducted to access the proliferation and migration of chondrocytes. The results in Fig. 5A–C showed that compared with the control group, chondrocytes proliferation and migration ability in the LPS group were significantly decreased. Compared with the LPS group, chondrocytes proliferation and migration ability in the exo and BMP-7-exo group were significantly increased. Whereas, compared with the exo group, chondrocytes proliferation and migration ability were increased in BMP-7-exo group. Taken together, these results indicated that BMP-7-exo promoted chondrocytes proliferation and migration by macrophage M2 polarization.

3.6. Macrophage M2 polarization inhibits apoptosis of chondrocytes

Apoptosis is actively involved in the pathogenesis of KOA. It is reported that LPS can induce apoptosis through the autocrine secretion of TNF-α andnitric oxide [24]. Flow cytometry was performed to detected the apoptosis rate. As shown in Fig. 6A-B, compared with the control group, the apoptosis of chondrocytes in LPS group was significantly increased. While, macrophage treated with BMP-7-exo and exo partially reversed this change. Compared with the exo group, apoptosis rate was decreased in the BMP-7-exo group. These results indicated that BMP-7-exo inhibited apoptosis of chondrocytes by macrophage M2 polarization.

3.7. Histological alterations of knee joint induced by BMP-7 modified SMSCs-exos

HE and Safranin-O-Fast-Green were conducted to evaluate the articular cartilage injury. According to HE staining (Fig. 7A), the articular cartilage in sham group displayed smooth and intact surface. In KOA group, cartilage layer was thinner with irregular arrangement of cells. Disrupted and discontinuous cartilage appeared. Exos and BMP-7-exo treatment obviously weakened the pathological damage of cartilage. While in BMP-7-exo group, articular cartilage exhibited more clear hierarchical structure and fewer fractures. Similarly, the Safranin-O-Fast-Green staining (Fig. 7B) showed that extensive proteoglycan loss was observed in the OA Group, which was notably reversed by exo and BMP-7-exos treatment. In addition, we assessed the KOA severity by the OARSI score. The score in the BMP-7-exo group were lower than that in exo group (Fig. 7C). Interestingly, immunohistochemical results showed the production of collagen II and aggrecan in BMP-7-exo group was greater than the exo group (Fig. 7D–E). The results suggested that BMP-7-exos could prevent articular cartilage joints from degradation, alleviate the progression of KOA.

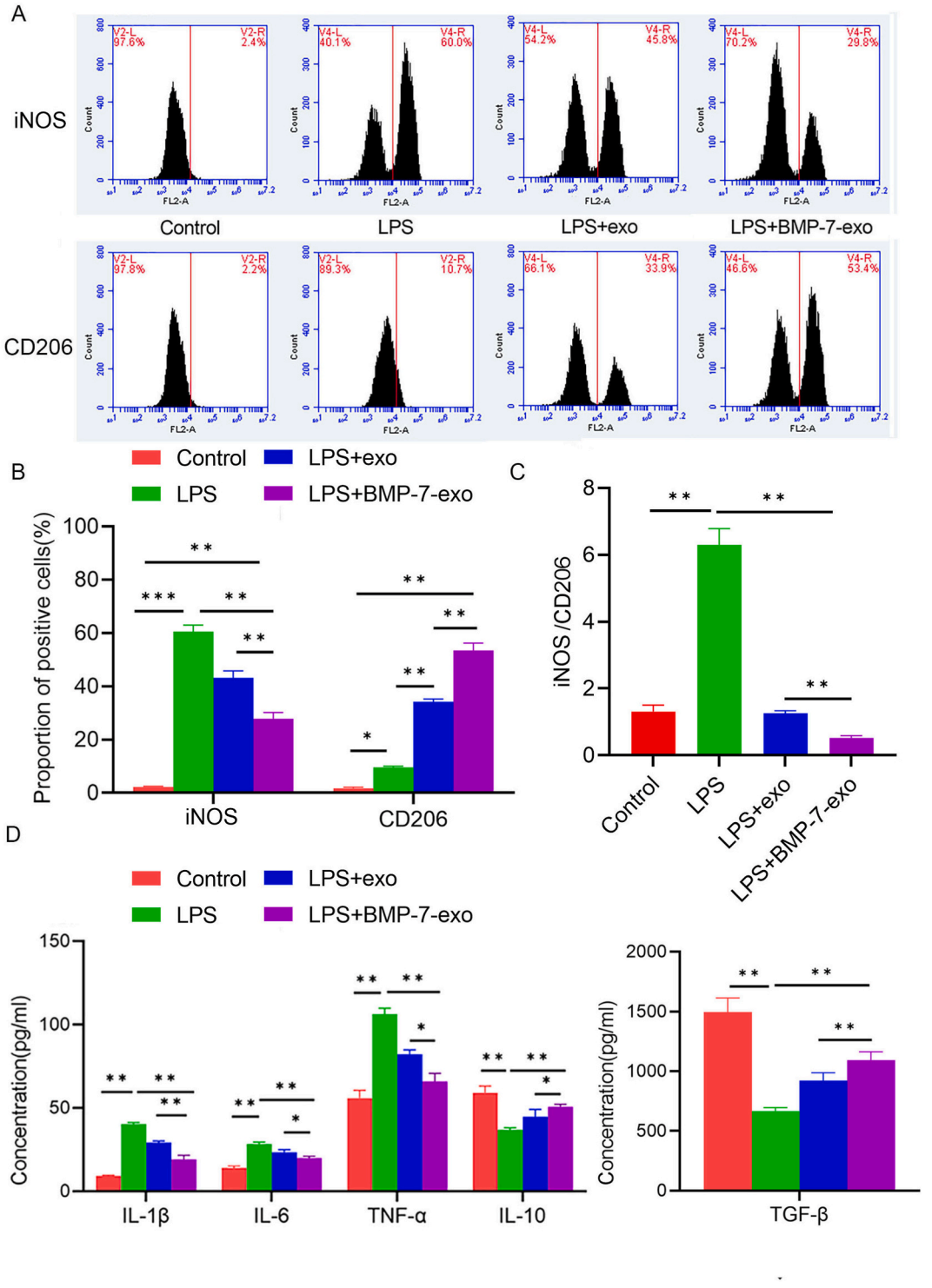

Fig. 4. BMP-7-modified SMSCs-exos induces M2 polarization of macrophages RAW264.7. A and B: The proportion of iNOS and CD206 positive cells in macrophages RAW264.7 were detected by flow cytometry. C: The ratio of iNOS-positive cells/CD206-positive cells. D: The levels of inflammatory related factors including IL-β, IL-6, TNF-α, IL-10 and TGF-β in macrophages RAW264.7 were detected by ELISA. *P < 0.05, **p < 0.01, ***p < 0.001.

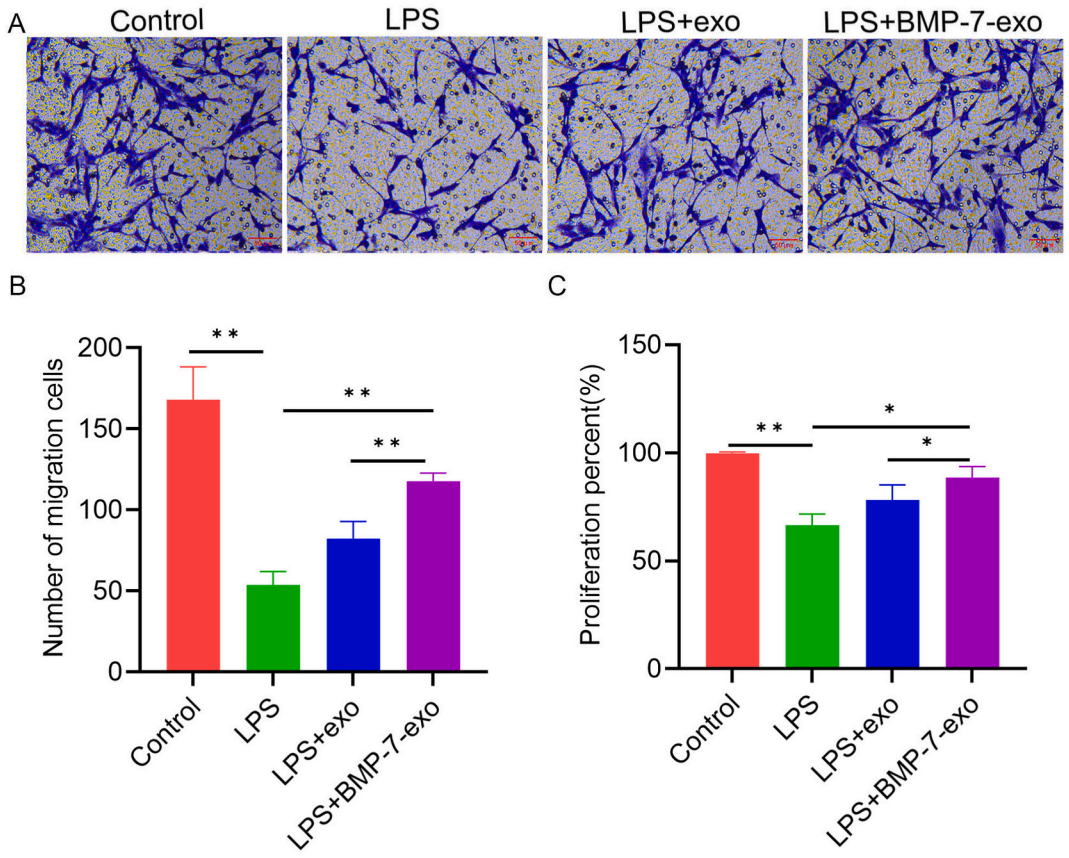

Fig. 5. Macrophage M2 polarization promotes proliferation and migration of chondrocytes. A. The migration of chondrocytes was detected by transwell assay (scale bar = 50 μm) B. The number of migration cells. C. The proliferation of chondrocytes was detected by CCK-8 assay. *P < 0.05, **p < 0.01.

3.8. BMP-7 modified SMSCs-exos induces macrophages M2 polarization in synovial tissues

To verify the protective effect of BMP-7-exo on KOA and the mechanism, we performed immunohistochemistry on synovial tissues to detected distribution of M1 and M2 macrophages. As shown in Fig. 8A–C, KOA significantly increased positive rate of iNOS. Exo and BMP-7-exo treatment upregulated positive rate of CD206, which is M2 macrophages marker. Also, the levels of pro-inflammation-related factors in synovial fluid were determined by ELISA. As shown in Fig. 8D, exos and BMP-exo decreased the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 and increased the anti-inflammatory cytokines IL-10 and TGF-β in synovial fluid. And the levels of TNF-α, IL-1β and IL-6 in BMP-7-exo group were lower, the levels of IL-10 and TGF-β were higher than those in exo group. These data indicated that BMP-7-exo had a significant anti-inflammatory effect on KOA by shifting macrophage polarization from M1 to M2 phenotype.

4. Discussion

Recently, exo is believed to be a potential therapeutic tool for the treatment of inflammatory diseases and tissue injury [21]. Previous studies have shown that exosomes can carry key genetic information to participate in cellular bioactivities [22,25,26,27]. In our study, the results showed that exo benefit LPS induced chondrocyte injury via promoting proliferation and migration, diminishing apoptosis and reducing inflammation [28]. In order to improve the ability of exosomes for treatment, a purposeful modification is very necessary. Studies have revealed that TGFβ1 and miR-486-5p modified exosomes show positive effect on OA treatment [29,30]. In this study, we validated that BMP-7-exo had a better effect on promoting chondrocyte proliferation and migration, as well as attenuating apoptosis compared with exo. SMSC-derived exosomes with BMP-7 overexpression also attenuate the inflammatory by M2 polarization of macrophages in vivo and in vitro. These findings suggested that MSC-derived exosomes with BMP-7 overexpression may be a new therapy for OA treatment.

It is well known that inflammation is a prominent feature of OA pathology [31]. In the process of OA occurrence and development, chondrocyte apoptosis and matrix degradation are mainly due to secretion of the inflammation-related factors IL-6, TNF-α, and IL-1β, While, infiltration of macrophages was demonstrated to be the primary source of raised levels of inflammation-related factors (TNF-α,

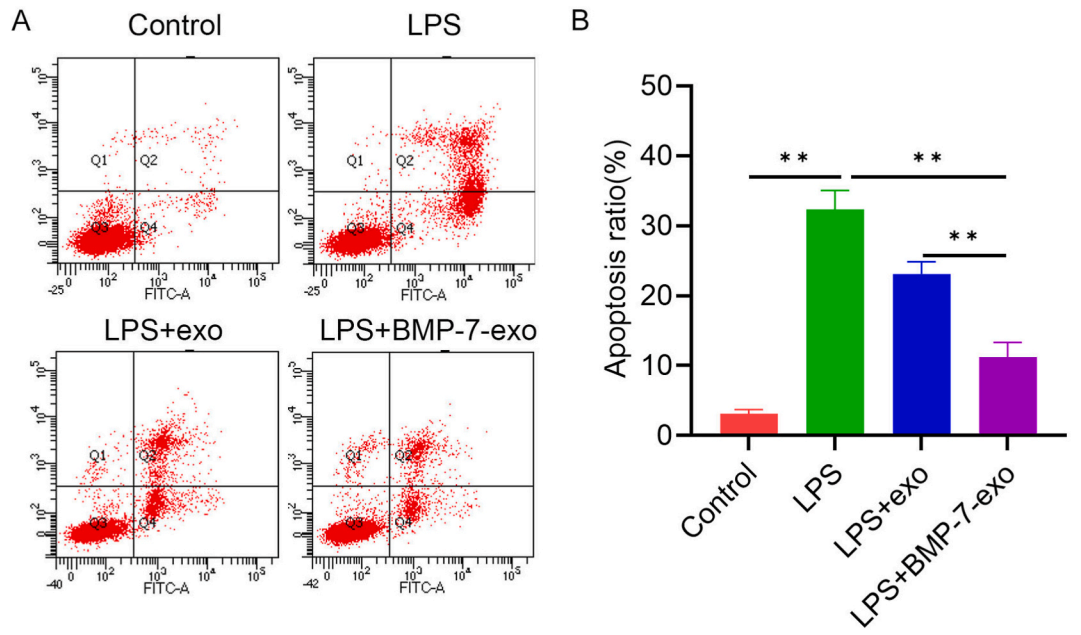

Fig. 6. Macrophage M2 polarization promotes chondrocytes apoptosis. A. The representative diagram of apoptosis detected by flow cytometry. B. The apoptosis ratio of chondrocytes. The apoptosis ratio is the sum of the upper right quadrant and the lower right quadrant. **p < 0.01.

IL-6, IL-1β) in OA mouse models [31]. A recent study found that macrophages were activated throughout OA progression, in particular during early disease stages in a rat model by SPECT-CT imaging technology [32]. Increasing evidences have shown that macrophages play an important role in regulating inflammation and the severity of osteoarthritis via inflammatory cytokine secretion [23,33]. Macrophages are a significant type of plastic cell and can be divided into typically activated M1 macrophages or selectively activated M2 macrophage [34]. An imbalance of M1/M2 macrophage polarization is identified as a critical role in OA inflammation [35]. It is reported that synovitis is the main e etiological factor in OA, and the aggregation of synovial macrophages is the main pathological feature of synovitis. Therefore, orchestrating synovial macrophage M2 polarization might be an effective strategy for OA suppression.

Growing evidences indicated that MSCs might release a mass of exosomes with superior regulatory and regenerative abilities for the balance of macrophages and the resolution of chronic inflammation after LPS treatment [36,37]. We also found that treatment macrophages using LPS-pretreated exosome promoted the release of more anti-inflammatory and less pro-inflammatory cytokine. Exosomes have a certain effect on improving OA, but still not ideal. The anti-inflammatory effect of BMP-7 on OA has attracted increasing attention. It has been reported that BMP-7 treatment attenuated cardiomyocyte apoptosis, fibrosis, and improved cardiac function in prediabetic cardiomyopathy by activating infiltrated monocytes into anti-inflammatory M2 macrophages [38]. Also, BMP-7 can modulate macrophage polarization in hepatocellular carcinoma and atherosclerosis [39,40]. We speculated that BMP-7 would reduce inflammation to prevent osteoarthritis progression by macrophage into M2 polarization. The results in vitro demonstrated that SMSC-derived exosomes with BMP-7 overexpression mimic promoted M2 macrophage polarization., and M2 macrophage polarization promoted chondrocytes proliferation and migration, suppressed apoptosis. The results in vivo showed that SMSC-derived exos with BMP-7 overexpression reduced infiltration of inflammatory cells, alleviated the pathological damage and also increased the expression of M2 macrophage marker. Those results demonstrate that BMP-7 modified SMSC-exos attenuates the KOA by inducting M2 macrophage polarization.

In conclusion, M2 macrophage exerts promotive role on chondrocytes proliferation and migration, and inhibitory effect on apoptosis, and BMP-7-exos could attenuate inflammation by promoting M2 polarization of macrophage. The above findings suggested that BMP-7-exo could be a promising therapeutic method for OA.

Ethics approval

All animal care protocols and experiments were carried out in strict accordance with the guidelines of the China Council on Animal Care and Use and approved by the ethics committee of Yantai Yuhuangding Hospital Affiliated to Qingdao University.

Author contribution statement

Weixue Sun, Shaozheng Qu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mingxia Ji: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

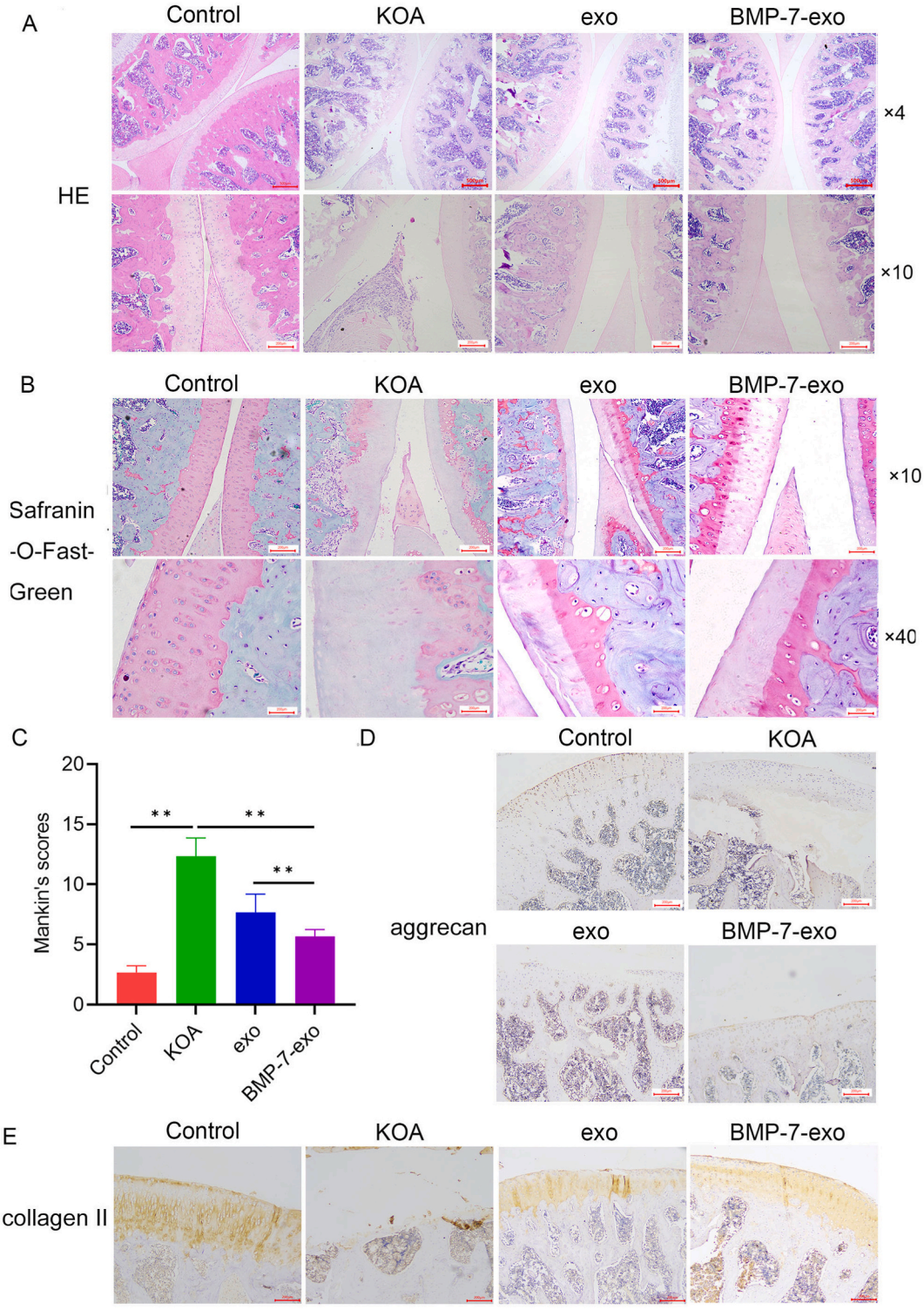

Fig. 7. BMP-7 modified SMSCs-exos alleviate histological injury of KOA. A. HE staining. Safranin-O Fast green staining. C. Histological score. **p < 0.01. D. The expression of aggrecan were detected by immunohistochemistry (scale bar = 200 μm). E. The expression of collagen II was detected by immunohistochemistry (scale bar = 200 μm).

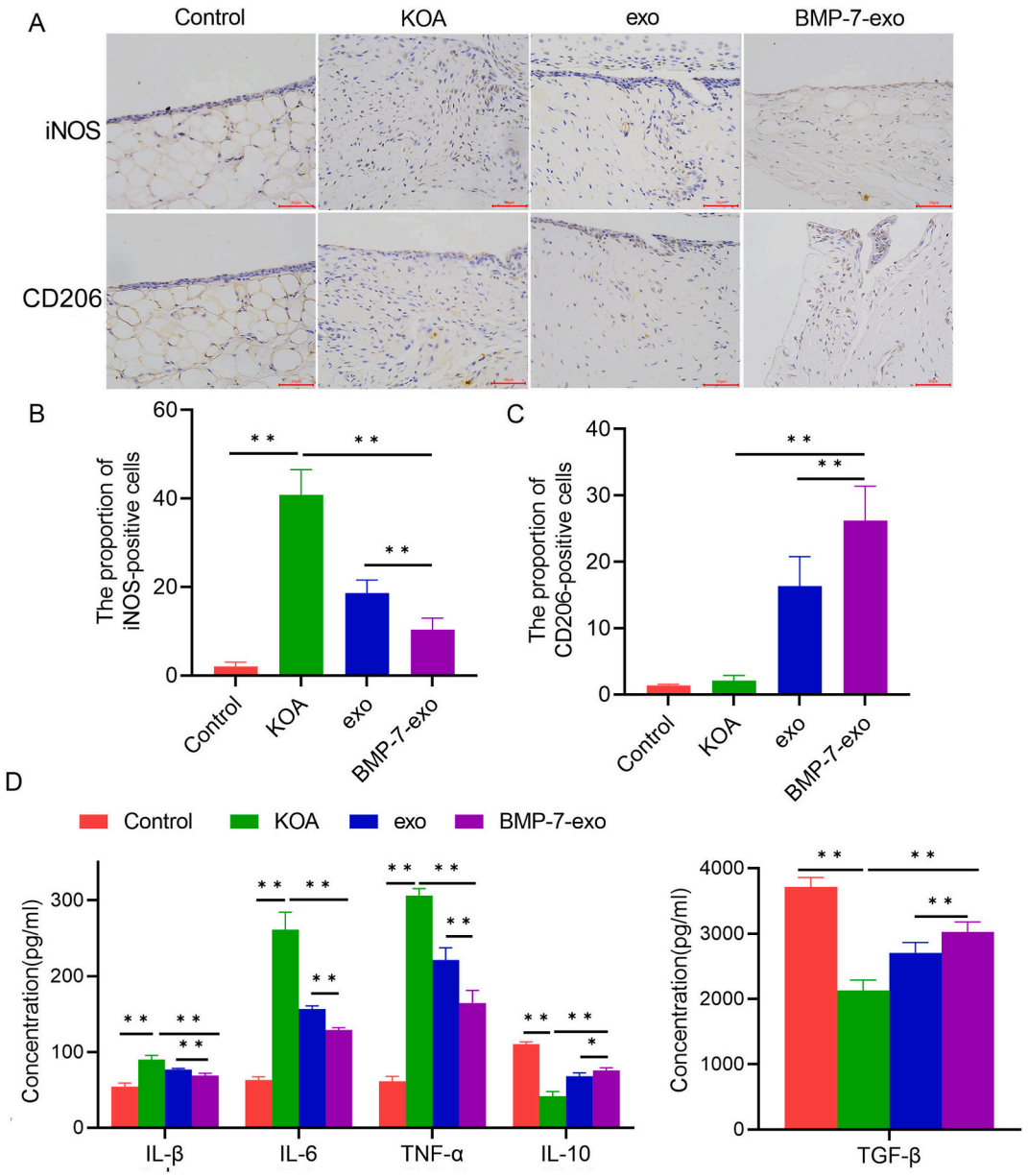

Fig. 8. BMP-7-modified SMSCs-exos reduces KOA inflammation by induced synovial macrophages M2 polarization in synovial tissues. A. The expression of iNOS and CD206 in synovial tissues were detected by immunohistochemistry (scale bar = 50 μm). B. The proportion of iNOS positive cells in synovial tissues. C. The proportion of CD206 positive cells in synovial tissues. D. The levels of inflammatory related factors including IL-β, IL-6, TNF-α, IL-10 and TGF-β in synovial tissues were detected by ELISA. *P < 0.05, **p < 0.01.

Yanli Sun, Baiqiang Hu: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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