 Acta Chir Orthop Traumatol Cech. 90, 2023, No. 4, p. 267–276
DOI: 10.55095/achot2023/032

ORIGINAL PAPER
PŮVODNÍ PRÁCE

The Anti-Inflammatory Effect of miR-140-3p in BMSCs-Exosomes on Osteoarthritis

Protizánětlivý efekt miR-140-3p v BMSCs-exosomech na osteoartrózu

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ABSTRACT

PURPOSE OF THE STUDY

Articular cartilage injury is a common disease in daily life, with a high incidence. The aim of this study was to investigate the effect and mechanism of miRNA-140-3p in bone mesenchymal stem cells (BMSCs)-derived exosomes under hypoxia on inflammatory articular chondrocytes.

MATERIAL AND METHODS

To simulate the pathological status of arthritis, rat chondrocytes were used to establish the osteoarthritis (OA) model by IL-1β (10 μg/ml) as a modulating *in vitro*, and exosomes were isolated by differential ultra-high speed centrifugation. The cell counting kit-8, wound healing and flow cytometry assays were utilized to assess proliferation, migration and apoptosis of chondrocytes, respectively. Lipogenic and chondrogenic differentiation of chondrocytes were detected by oil red O staining and toluidine blue staining individually. The expressions of miR-140-3p and chondrocyte-specific gene mRNA were investigated using qRT–PCR. Western blot was applied to assess chondrocyte associated proteins and BMSC-Exo surface protein markers, and immunohistochemistry was adopted to detect the staining of collagen I and II.

RESULTS

Under scanning electronic microscope, the shape of exosomes was almost round. Exosome treatment prominently impaired the inhibition of chondrocytes' proliferative and migrative ability by IL-1β. It was found hypoxia had a more marked impact on proliferation, expression of collagen II and apoptosis in OA chondrocytes than normoxia, as well as a stronger effect on weakening adipose differentiation and enhancing chondrogenic differentiation in inflammatory chondrocytes. Furthermore, incubation with BMSC-Exo overexpressing miR-140-3p can remarkably increase the survival rate and migration in inflammatory chondrocytes. In addition, overexpression of miR-140-3p was found to enhance the chondrogenic differentiation of inflammatory chondrocytes. Furthermore, we found that the healing effect of exosomes on inflammatory chondrocytes under hypoxic conditions was produced by a rise in miR-140-3p expression within them and that hypoxia-mediated upregulation of miR-140-3p expression occurred through HIF-1α.

CONCLUSIONS

Under hypoxia, BMSC-Exo enhanced the chondrogenic phenotype, increased the viability of inflammatory chondrocytes. The overexpression of miR-140-3p in BMSC-Exo is beneficial to protect joints and delaying the pathogenesis in OA.

Key words: HIF-1α, apoptosis, lipogenic differentiation, chondrogenic differentiation.

This work was supported by the Zhejiang Medical and Health Science and Technology Program (grant number 2019KY107)

INTRODUCTION

Osteoarthritis (OA) is one of the leading causes of pain and disability worldwide and it affects more than 250 million people worldwide (19). Osteoarthritis of the fingers, hip and kneecap are all considered to be the major burden of the disease, which has a serious impact on people's activities of daily living (12). Although the molecular mechanism underlying the pathogenesis of OA is still unclear, studies revealed that both chondrocytes and inflammation play essential roles. There are few cures for OA, including painkillers and NSAIDs (8). Nonetheless, these therapies are symptomatic and do not provide a cure, and the focus is on reducing physical disability and managing pain (6). In view of this, a new mesenchymal stem cell- (MSC-) based OA therapy has attracted increasing attention.

MSCs, with the potential of self-renewal and directed differentiation, can repair cartilage tissue and inhibit the secretion of inflammatory factors by chondrocytes (29). Bone marrow mesenchymal stem cells (BMSCs) is one kind of the MSCs, and their acquisition methods are very convenient and common. Compared with extraskeletal MSCs, BMSCs have advantages in phenotype, morphology, function, and potential therapeutic applications (4). Activation of endogenous or exoge-

nous BMSCs can repair long bone and vertebrae fractures caused by osteoporosis or trauma and BMSCs can be used in preclinical and clinical Settings to treat bone-related diseases such as osteogenesis imperfecta (1). However, the risk of tumor formation, ethical issues, and transplant rejection remain obstacles to the further clinical application of stem cells.

There is growing evidence that the released exosomes (Exos) are responsible for many of the regenerative properties traditionally attributed to MSCs (3). Exos are a subset of extracellular vesicles that play a key role in normal and disease physiology (21). Exos contain mRNAs, miRNAs, enzymes, and lipids that, in addition to their structural roles and their function as vectors, play specific roles in this mode of cellular communication (25). Studies have found that MSC-derived exosomes (MSC-Exo) can promote the proliferation of chondrocytes, inhibit cell apoptosis, enhance chondrogenesis, inhibit cartilage degradation, promote cartilage repair and alleviate osteoarthritis by balancing the synthesis and degradation of chondrocytes ECM (11, 18). A recent study showed that BMSC-Exo could protect cartilage damage and alleviate knee pain in osteoarthritis model rats (7). In addition, BMSC-Exo from congenital polydactyly tissues alleviate osteoarthritis by promoting chondrocyte proliferation (29). However, the specific mechanism of BMSC-Exo against osteoarthritis remains unclear.

Hypoxic microenvironment is an important pathological feature of OA. Under the condition of pathological hypoxia, the body produces the hypoxia-inducible factor-1 alpha (HIF-1α) that affects cartilage synovial bone metabolism (9). Zhou et al. found that hypoxia can induce the expression of catabolic factors in fibrocystic-like synovial cells and enhance the role of inflammatory factors, which are involved in the occurrence and development of OA (28). Thus, understanding and exploring the hypoxia environment is of great value for revealing OA pathogenesis and clinical diagnosis.

MicroRNAs (miRNAs) are endogenous, non-coding, single-stranded small RNAs that downregulate the expression of related proteins by degrading or blocking their target mRNAs translation. MiRNAs are involved in a variety of cellular processes, including OA pathogenesis, ranging from cell fate determination to signal transduction (2). A recent study reported the chondrocyte-targeting Exos as vehicles for the delivery of miR-140-3p into chondrocytes as a new treatment for OA (14). In this study, we will explore the effect of miR-140-3p in BMSC-Exo on articular chondrocytes under hypoxic condition and the potential mechanism, and discuss the possibility of BMSC-Exo in the treatment of OA.

MATERIAL AND METHODS

Cell extraction and cell culture

Ethics statement. All procedures were performed with the approval of the Experimental Animal Ethics Committee of Zhejiang Haikang Biological Products Co. (approval number HKSYDWLL2021004), in accordance with the National Research Council‘s Guide for the Care and Use of Laboratory Animals.

Culture of chondrocytes. Rat chondrocytes were isolated from 1-week-old male Sprague-Dawley (SD) rats’ ribs provided by the SPF (Beijing) biotechnology co., LTD (n = 2) according to the previous literature (5). The resultant cells were grown in a DMEM/F-12 medium with 10% FBS, 100 U/ml streptomycin, and 100 U/ml penicillin (Gibco, USA). Every 3 days, the medium was replaced. In all experiments mentioned, the monolayer-cultured chondrocytes were used between 2 and 3 generations. Chondrocytes was stimulated by 10 ng/mL interleukin-1β (IL-1β) for 24 h to construct the osteoarthritis model as previously described (7).

Isolation of rat BMSCs. Rat BMSCs were isolated from 2-week-old male Sprague-Dawley rats’ femurs provided by the SPF (Beijing) biotechnology co., LTD (n = 2) according to the previous literature (13). DMEM supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) containing bone marrow cells was cultured at 37 °C, 5% CO_2 cell culture incubator. The medium was replaced for the first time after 24–48 h and was replaced every 3 days afterward. Passage 3–5 cells were used for subsequent experiments.

Extraction and identification of exosomes

The cultured BMSCs grew to about 90% of the surface area of the culture dish. The culture medium was discarded, washed with PBS for 3 times, and the serum-free medium containing 1% penicillomycin DMEM was added. After 24 hours of culture, the conditioned culture medium was collected and stored at 4 °C. Cell conditioned culture medium was collected to 300 ml and centrifuged by supernatant centrifuge. After supernatant was removed, the cell conditioned culture medium was cleaned and suspended by PBS. 10 μl Exo suspension was dropped onto the copper wire, and the samples were dried at room temperature. Morphological characteristics of Exo were observed under transmission electron microscope. The Exo added in this experiment were all Exo-100 μg/ml.

Cell Counting Kit-8 (CCK-8)

CCK-8 was performed to test cell viability. The chondrocytes were inoculated on a 96-well plate, and the cells were treated in groups after adherence, and cultured for 48 h. Then CCK-8 reagent was added and incubated for another 4 h. The absorbance value (OD value) of the cells was measured at 560 nm of the microplate.

Wound healing assay

The chondrocytes were cultured in 6-well plates to 70–80% confluency and were wounded with a 200-μl sterile pipette tip. After washing with PBS, the cells were cultured in serum-free medium. Images were acquired at each time point (0 and 24 h).

Flow cytometry

The apoptosis rates were determined by using Pharmingen annexin V-FITC Apoptosis Detection Kit I (BD, USA) according to the manufacturer's instructions. Chondrocytes were inoculated with 6-well plates. When the cell density was about 80%, corresponding complete culture medium was added to each group, and cells were collected after 24 and 48 hours of culture. FITC and PI staining were performed according to the instructions of the kit, and the cell death was analyzed and quantified by flow cytometry within 1 hour (BD Biosciences, USA).

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using Trizol reagent (Thermo Fisher, USA) and reversely transcribed into cDNA according to manufacturer's instructions (Takara Biological, Japan). Real-time PCR with gene-specific primers was performed on the resulting cDNA using a Step One Plus real-time PCR system (Applied Biosystems) in the presence of SYBR Green PCR Master Mix (Applied Biosystems). The relative expression level of mRNA was calculated using $2^{-\Delta\Delta Ct}$ method.

Oil red O staining

The saturated solution of oil red O (O8010; Solarbio, China) isopropyl alcohol was mixed with distilled water in a ratio of 3:2 to prepare the dyeing solution and soaked for 20 minutes. The treated cells were washed twice by PBS and fixed with 4% paraformaldehyde at room temperature for 30 min. For lipid identification, cells were stained with the prepared solution for 30 minutes and the absorbance was measured at 490 nm.

Toluidine blue staining of chondrocytes

The slides were sterilized, treated with polylysine, placed on plates and inoculated into 6-well plates with low density chondrocytes. The cells were placed in

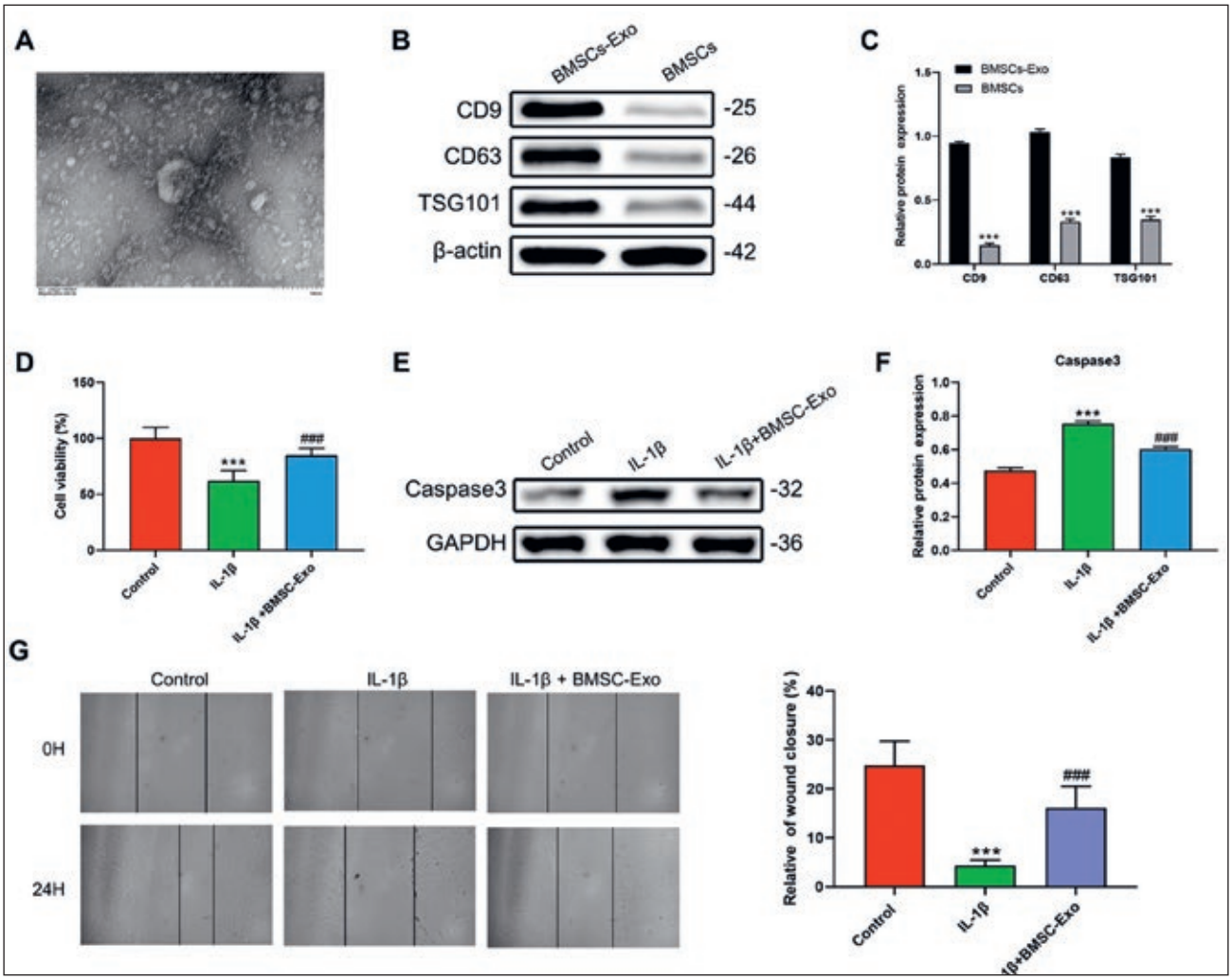

*Fig. 1. Morphology of BMSC-Exo and effects of BMSC-Exo and BMSC on chondrocytes. (A) Morphology of BMSC-Exo under transmission electron microscopy. (B) Western blot results and (C) its quantitative analysis of CD9, CD63 and TSG101 protein levels in BMSCs-Exo and BMSCs. β-actin was used as a loading control. P < 0.001 compared to BMSCs-Exo group. (D) Chondrocyte survival detected by CCK-8 experiment. (E) Western blotting results of Caspase3 protein levels under IL-1β and IL-1β+BMSCs-Exo conditions and (F) its quantitative analysis. GAPDH was used as loading control. (G) Results of wound healing and quantitative analysis. ***P<0.001 compared to the Control group, ###P<0.001 compared to the IL-1β group.*

a 5% CO_2 37 °C incubator and then fixed with 4% paraformaldehyde for 30 minutes. The fixate was rinsed with double steam water and dyed with 0.04% toluidine blue (Solarbio, Beijing, China) for 2 h. The dye was removed with anhydrous ethanol and the cells were rinsed 3 times with PBS, followed by xylene cleaning and sealing. The staining results were observed and photographed under a light microscope.

Cell transfection of miR-140-3p and HIF-1α

BMSCs were transfected with miR-140-3p mimics (5'-UACCACAGGGUAGAACCACGG dTdT-3') for overexpression the miR-140-3p level, miRNA mimics control (mimics NC), miR-140-3p inhibitor (5'-AUGGUGUCCCAUCUUGGUGCC dTdT-3') for knockdown the miR-140-3p level, and miRNA inhibitor control (inhibitor NC) (GenePharma, China). HIF-1α was

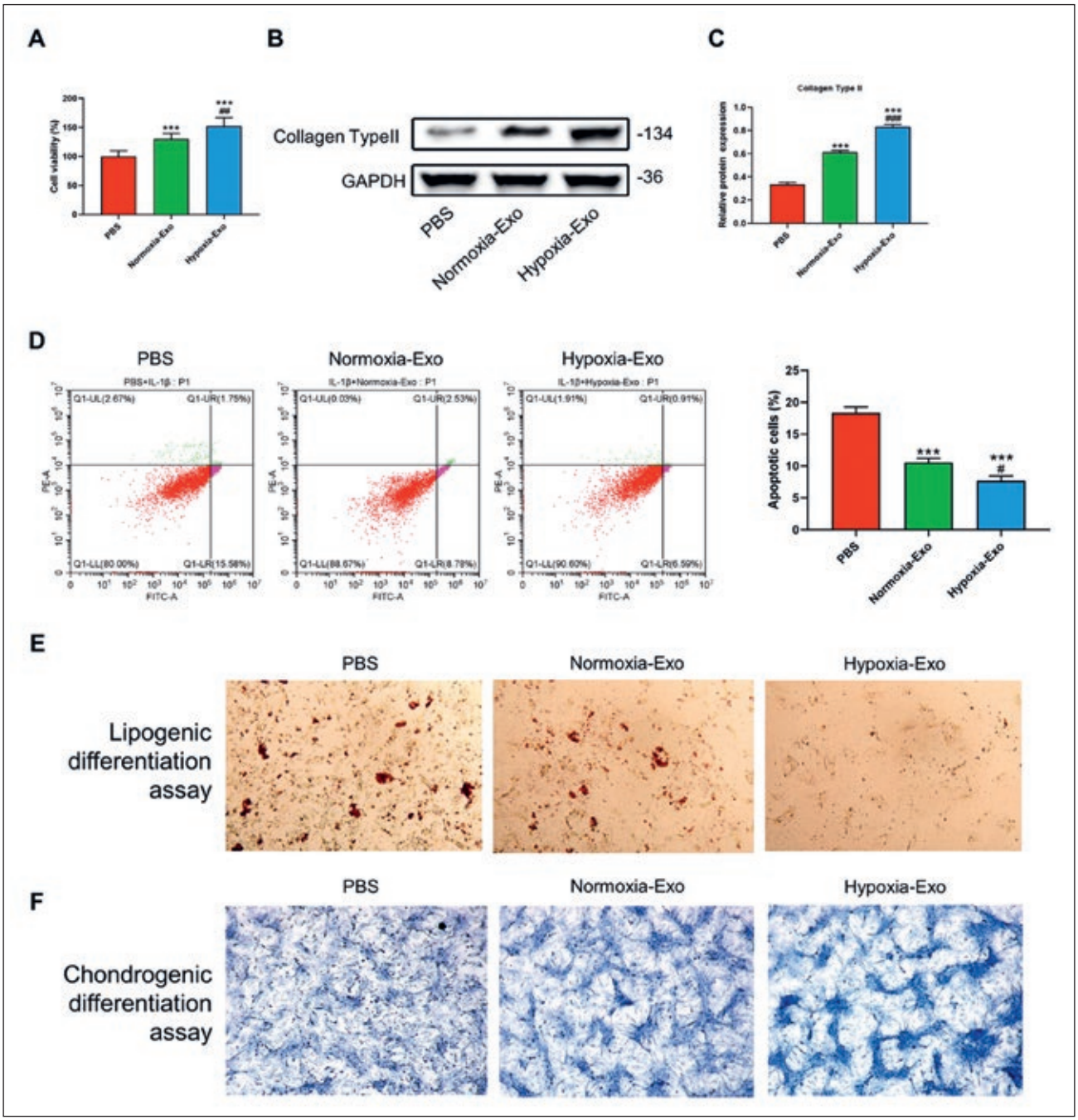

*Fig. 2. Effect of oxygen conditions on inflammatory chondrocytes. (A) The inflammatory chondrocyte cells viability under the condition of PBS, Normoxia-Exo and Hypoxia-Exo. (B) Western blot result and (C) its quantitative analysis of collagen type II protein expression. GAPDH functioned as loading control. (D) Flow cytometry result and its quantitative analysis of inflammatory chondrocyte cells. (E) The lipid differentiation result of inflammatory chondrocyte cells under the condition of PBS, Normoxia-Exo and Hypoxia-Exo. (F) The chondrogenic differentiation result of inflammatory chondrocyte cells under the condition of PBS, Normoxia-Exo and Hypoxia-Exo. ***P<0.001 compared to the PBS group, #P<0.05, ##P<0.01, ###P<0.001 compared to the Normoxia-Exo group.*

silenced by HIF-1α specific siRNAs (5'-TGGATTCTTCGCTTCTGTGdTdT-3') or negative controls (NC), which purchased from GenePharma and were used to transfect chondrocytes. Cells (3×10^5) were inoculated into 6-well plates after 60-80% cell fusion for cell transfection using transfection reagent Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions.

Western blot

Chondrocytes were cultured in 6-well plates, treated with inflammatory factors and Exos, and proteins were extracted from cell lysates RIPA buffer (Thermo Fisher, USA). BCA protein assay kit (P1511-1) (Applygen Technologies Inc. China) was applied to measure the protein concentration in lysates according to the manufacturer's instructions. Electrophoresis was performed by sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and then transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, USA). Then, the primary antibody was added and incubated overnight at 4 °C, and the membrane was washed 3 times every other day. The second antibody was added and incubated for 1 h at room temperature, and then the membrane was washed for 3 times before exposure. β-actin and GAPDH were used as internal reference in the detection of proteins on Exos and chondrocytes individually. Image J software was adopted to measure the gray value of protein bands.

Immunohistochemistry (IHC)

Paraformaldehyde fixed cells were stained by immunohistochemistry. The fixed cells were infiltrated by blocking solution (0.1% Triton-x), then incubated with primary antibody overnight at 4 °C, and washed with PBS for 3 times before incubating with secondary antibody. The photos were collected with a digital camera (Nikon).

Statistical analysis

All data was analyzed by SPSS 20.0 and GraphPad Prism 6.0 (GraphPad Software, San Diego, CA). The experimental results are presented as the means ± standard deviation (SD). Student's t-test and one-way analysis of variance were used to assess differences between groups. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of BMSC on inflammatory articular chondrocytes and extraction and identification of exosomes

Under transmission electronic microscope, BMSC-Exo were round or oval in shape and had a complete membrane structure (Fig. 1A). Besides, the expressions of surface specific markers CD9, CD63 and TSG101 were proved to be positive in the BMSCs-Exo group by western blot ($P<0.001$, Fig. 1B-C). Therefore, we proved that the extraction of exosomes was successful.

CCK-8 experimental results showed that compared with the control group, the chondrocyte survival was significantly reduced under IL-1β inflammatory condition ($P<0.001$), while the addition of BMSC-Exo alleviated the IL-1β-induced reduction in cell viability ($P<0.001$, Fig. 1D). Moreover, Western blot was also used to detect the changes of apoptosis indicator Caspase3. The content of Caspase3 in IL-1β inflammatory condition was significantly higher than that in the control group, while Caspase3 levels in the IL-1β+BMSC-Exo group were lower than in the IL-1β group ($P<0.001$, Fig. 1E–F). In addition, we also detected the effect of BMSC-Exo on chondrocyte migration by wound heal-

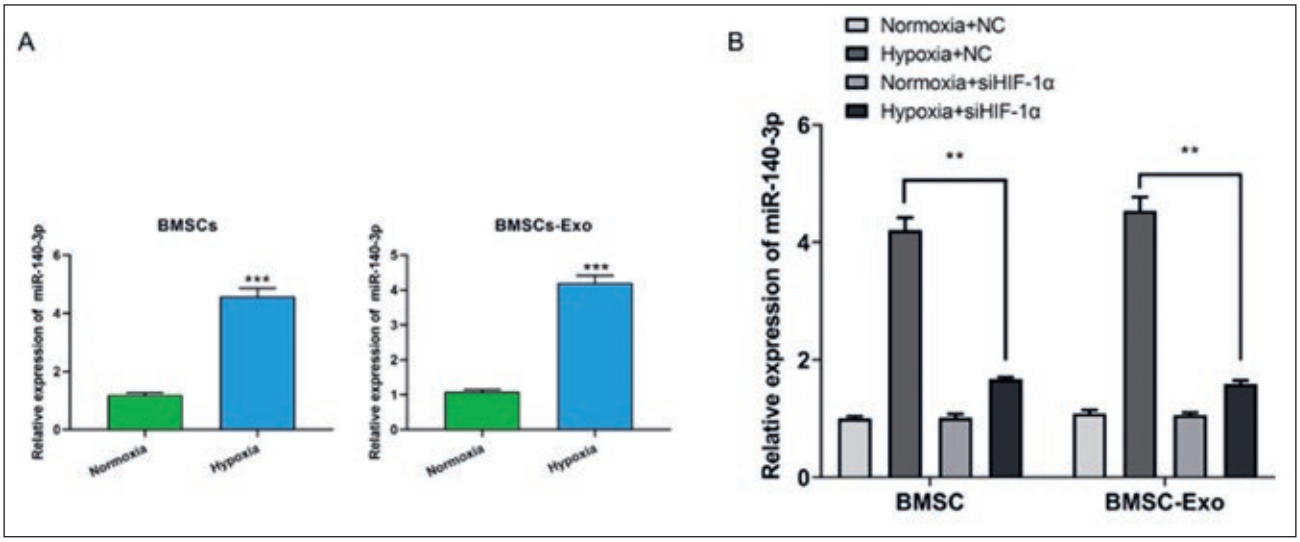

*Fig. 3. Effect of oxygen conditions and siHIF-1α on BMSC-Exos. (A) Relative expression of miR-140-3p in BMSCs and BMSCs-Exo under hypoxia and normoxia. (B) Relative expression of miR-140-3p in BMSCs and BMSCs-Exo after knockdown of HIF-1α under hypoxia and normoxia. ***P<0.001 compared to the Normoxia group; **P<0.01, compared to the Hypoxia+NC group.*

ing assay. Compared with the control group, the cell migration was inhibited in the IL-1β group (P<0.001) and was significantly increased when BMSC-Exo was added (P<0.001, Fig. 1G). These results both suggested that BMSC can alleviate adverse symptoms of chondrocytes induced by IL-1β.

Effect of oxygen conditions on inflammatory chondrocytes

The result of CCK-8 showed that both normoxia and hypoxic conditions of Exo can lead to increased inflammatory chondrocyte viability compared to the PBS group (P<0.001), with a better effect in the hypoxia-Exo group (P<0.01, Fig. 2A). Identically, the expression levels of collagen type II on the surface of inflammatory chondrocytes also showed the same trend compared to the PBS group, that is, the collagen type II expression increased significantly following Exo was added (P<0.001), and the Hypoxia-Exo group had a markedly higher collagen type II expression than the Normoxia-Exo group (P<0.001, Fig. 2B-C). Furthermore, flow cytometry showed that the apoptosis rate of chondrocytes in the both two Exo groups were lower than that in the PBS group (P<0.001), while the apoptosis rate of chondrocytes in the Hypoxia-Exo group was even lower than that in the Normoxia-Exo group (P<0.05, Fig. 2D). In addition, lipid differentiation ability and chondrogenic differentiation ability of chondrocytes were detected by oil red O staining and toluidine blue staining respectively. Compared with the PBS group, the two Exo groups significantly reduced lipid differentiation and promoted chondrogenic differentia-

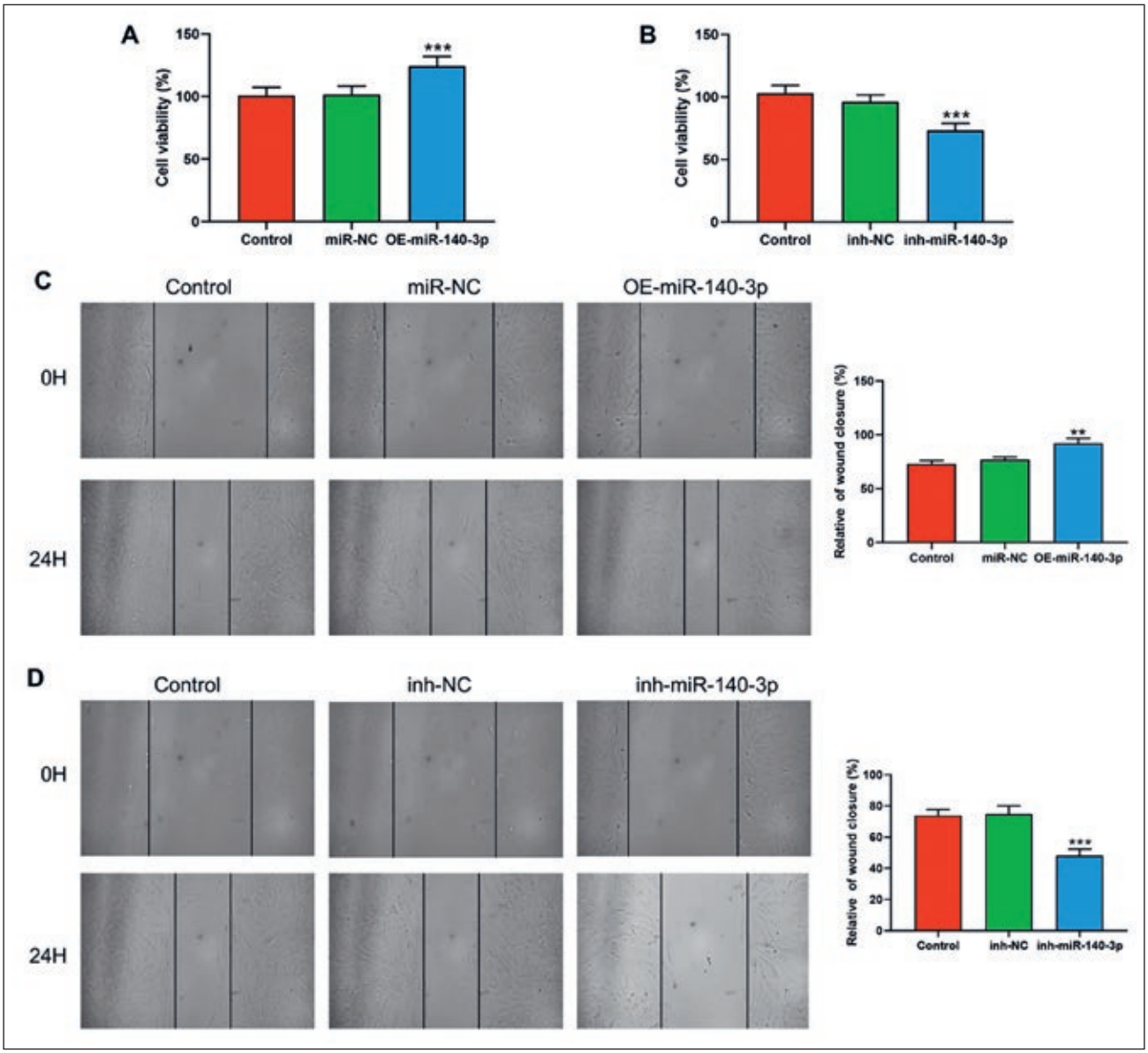

*Fig. 4. Effect of miR-140-3p in BMSC-Exo on proliferation and migration of chondrocytes under hypoxic condition. (A) The chondrocyte cells viability when miR-140-3p was overexpressed or (B) inhibited. (C) The wound healing result and its quantitative analysis of different wound closure when miR-140-3p was overexpressed or (D) inhibited. **P<0.01, ***P<0.001 compared to the NC group.*

tion of chondrocytes, and the effect of Hypoxia-Exo group was more significant (Fig. 2E–F).

Effects of oxygen conditions and siHIF-1α on BMSC-Exos

To further investigate the effect of hypoxia on chondrocytes, we evaluated miR-140-3p expression in BMSC and BMSC-exo under normoxic and hypoxic conditions, respectively. The experiments showed that the relative expression of miR-140-3p was much higher in hypoxia than in normoxia both in BMSC and BMSC-Exo (P<0.001, Fig. 3A). In addition, we knocked down the HIF-1α gene in chondrocytes for experiments, and the results showed that the relative expression of miR-140-3p decreased dramatically in both hypoxia groups after knocking down the HIF-1α gene (P<0.01), while the normoxia group showed essentially no change (Fig. 3B). Thus, Hypoxia-mediated upregulation of miR-140-3p expression occurs through HIF-1α, and the following studies will investigate the effect of miR-140-3p on inflammatory chondrocytes under hypoxic condition.

Effect of miR-140-3p in BMSC-Exo on chondrocytes under hypoxic condition

To further investigate the effect of miR-140-3p on chondrocytes under hypoxic condition, OE-miR-140-3p and inh-miR-140-3p were constructed, respectively.

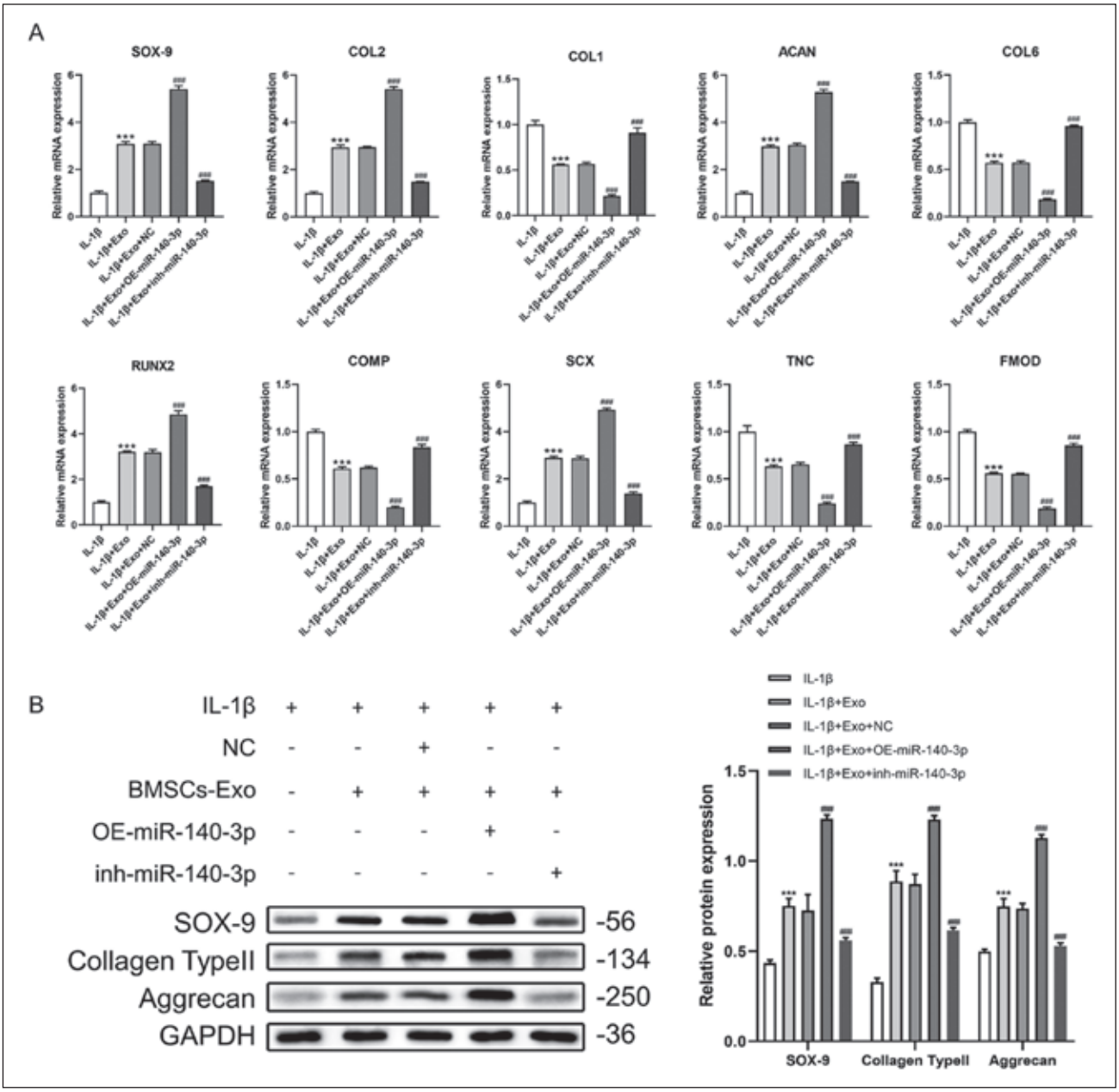

*Fig. 5. Effects of miR-140-3p in BMSC-Exo on mRNA and related protein expression of chondrocyte specific genes under hypoxic condition. (A) Relative mRNA and (B) protein expression of chondrocyte specific genes upon overexpression and inhibition of miR-140-3p. ***P<0.001 compared to the IL-1β+Exo group; ###P<0.001 compared to the IL-1β+Exo+NC group.*

As shown in Fig. 4A-D, overexpression of miR-140-3p greatly increased chondrocyte viability and migration ability compared to the corresponding NC group, whereas inhibition of miR-140-3p significantly decreased chondrocyte viability and migration ability ($P<0.01$, $P<0.001$). Moreover, qRT-PCR results showed that overexpression of miR-140-3p increased the mRNA of SOX-9, COL2, ACAN, RUNX2, SCX, and decreased the mRNA of COL1, COL6, COMP, TNC, FMOD, while inhibiting miR-140-3p did the opposite ($P<0.001$, Fig. 5A). Western blot experiments revealed that the protein expression of SOX-9, collagen type II, aggrecan were strongest at miR-140-3p overexpression ($P<0.001$, Fig. 5B). In addition, the result of toluidine blue staining revealed that miR-140-3p overexpression enhanced chondrogenic differentiation of chondrocytes, while miR-140-3p inhibition weakened its effect. Consistently, the immunohistochemical results showed that miR-140-3p overexpression led to a decrease in collagen type I and an increase in collagen type II, while inhibition of miR-140-3p reversed the effect as well (Fig. 6).

DISCUSSION

To mimic the microenvironment of OA *in vitro*, this study treated cultured rat normal chondrocytes with IL-1β. We found that BMSC-Exo enhanced the chondrogenic phenotype, increased the viability and migration ability of inflammatory chondrocytes, and the effect of Exo under hypoxic condition was better than that under normoxic condition. Moreover, hypoxia-mediated upregulation of miR-140-3p expression occurs through HIF-1α, the overexpression of miR-140-3p in BMSC-Exo is beneficial to protect joints and delaying the pathogenesis in OA.

The most important feature of OA is articular cartilage degeneration, which leads to the inhibition of cell proliferation, migration, and matrix production (16). In recent years, many studies have shown that exosomes can arrest the progression of cartilage destruction in OA (15). Exos regulate various biological processes by transferring microRNAs, proteins, and other nucleic acids to other cells. Evidence has been provided that BMSC-Exo treatment significantly attenuated the inhibitory effect of IL-1β on the proliferation and migration of chondrocytes and IL-1β-induced downregulation of Col-II and upregulation of MMP-13 (7). The anti-inflammatory and pro-growth effects of BMSC-Exo have also been demonstrated, and it can promote the regeneration of OA articular cartilage (10). These effects of BMSC-Exo on chondrocytes are compatible with our findings. More importantly, we discovered that hypoxic condition has a more marked effect on the proliferation, production of collagen type II, apoptosis, lipid differentiation and chondrogenic differentiation than Normoxic condition in OA chondrocytes.

A previous paper revealed that miRNAs were associated with OA progression and severity. Thus, gene therapy strategies based on miRNA are promising candidates for treating OA through fine-tuning or ensuring homeostatic control of some of the cellular processes that are altered in OA (22). Earlier research reported that cartilage had abundantly expressed miR-140-3p, implying miR-140-3p's potential function in OA (24). Moreover, former studies demonstrated that miR-140-3p was downregulated in OA and negatively correlated with the severity of OA; miR-140-3p re-expression mitigated the progression of OA (20, 26). The present work confirmed significantly increased miR-140-3p expression both in BMSCs and BMSCs-Exo in chondrocytes under hypoxia induced by IL-1β.

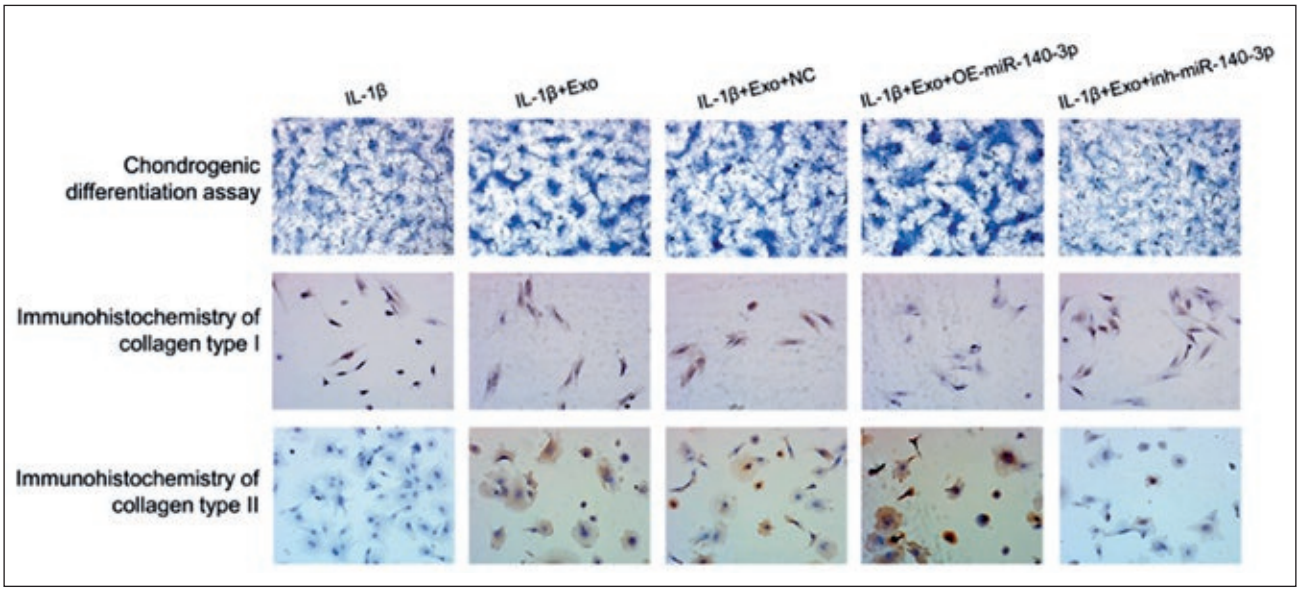

Fig. 6. Effects of miR-140-3p in BMSC-Exo on chondrogenic differentiation, collagen type I and collagen type II of chondrocytes under hypoxic condition.

This led us to question the mechanism and link between the hypoxic environment and miR-140-3p expression. HIF-1α is a subunit of HIF-1, which can mediate hypoxia signal and regulate a series of compensatory responses to hypoxia through different signaling pathways with multiple upstream and downstream proteins, and play an important role in the growth and development of the body as well as physiological and pathological processes. HIF-1α can induce BMSCs to differentiate into chondrocytes, which can treat early cartilage defects caused by osteoarthritis (23). Recent studies have shown that BMSC-Exo-HIF-1α stimulates BMSC proliferation and osteogenesis, HIF-1α plays a crucial role in the growth and differentiation of chondrocytes, and when cartilage is deficient in HIF-1α protein, a large number of cartilage cells will die (27). As expected, we found that miR-140-3p expression was unaffected by HIF-1α knockdown under normoxic condition, while miR-140-3p expressions in siHIF-1α group were significantly reduced in both BMSC and BMSC-Exo groups under hypoxic condition compared to the NC group, so we conclude that hypoxia-mediated upregulation of miR-140-3p expression occurs through HIF-1α.

Deeply, the present experiment confirmed that overexpressed miR-140-3p could promote chondrocyte proliferation and migration by CCK-8 assay and wound healing assay, and further examined chondrocyte differentiation-related markers including type II collagen, SOX-9 and aggrecan as well as chondrocyte-related gene expression by PCR and Western blot assay, while SOX-9, COL2, ACAN and RUNX2 were important osteogenic and chondrogenic markers, upregulation of SOX-9 and COL2 expression was also found to inhibit IL-1β-induced inflammatory responses in chondrocytes, It is thus clear that chondrocyte recovery is best facilitated when miR-140-3p is overexpressed. Pauline Po-Yee Lui et al. found that a high percentage of collagen type I may be responsible for poor matrix organization in calcified tendinopathy (17), type II collagen, on the other hand, plays an indispensable role in chondrocytes. Thus, we performed toluidine blue staining and immunohistochemical experiments for type I and type II collagen, through our experiments, we found that miR-140-3p has an important role in the recovery of articular chondrocyte activity and its adverse symptoms.

CONCLUSIONS

In summary, we found that the significant effect of BMSC-Exo on articular chondrocytes under hypoxic condition was closely related to the expression of miR-140-3p, and the hypoxia-mediated upregulation of miR-140-3p expression occurred through HIF-1α. Scientists have never-ending research related to articular chondrocytes, and we hope that this experiment can provide some reference for the therapeutic aspects of arthritis.

Abbreviations

BMSCs – bone marrow mesenchymal stem cells;
CCK-8 – Cell Counting Kit-8;
HIF-1α – hypoxia-inducible factor-1α;
Hypoxia-Exo – exosomes in the hypoxia state;
MSCs –mesenchymal stem cells;
Normoxia-Exo – exosomes in the normoxia state;
qRT-PCR – quantitative real-time PCR.

Declarations

The study was approved by the Experimental Animal Ethics Committee of Zhejiang Haikang Biological Products Co. (approval number HKSYDWLL2021004).

The authors declare that they have no conflicts of interest to report regarding the present study.

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