The effect and mechanism of miRNA-140 in exosomes of hypoxic bone marrow mesenchymal stem cells on articular chondrocytes

Yuezheng Hu
Yuying Children's Hospital of Wenzhou Medical University
HaiXiao Liu
Yuying Children's Hospital of Wenzhou Medical University
Daoliang Xu
Yuying Children's Hospital of Wenzhou Medical University
Xinhe Xue
Yuying Children's Hospital of Wenzhou Medical University
Xinxian Xu (a379806041@163.com)
Yuying Children's Hospital of Wenzhou Medical University

Research Article

Keywords: exosome, HIF-1α, articular chondrocyte, miR-140, bone marrow mesenchymal stem cell

Posted Date: June 22nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1763964/v1

Abstract

This study aimed to investigate the effects and mechanisms of miRNA-140 in exosomes of hypoxic bone marrow mesenchymal stem cells on articular chondrocytes. Articular chondrocytes were stimulated with IL-1β (10 μg/ml) to give them an inflammatory state. Exosomes were extracted by differential ultra-high speed centrifugation, and their morphology was identified by transmission electron microscopy. The Cell Counting Kit-8 (CCK-8) assay, cell scratch assay and flow cytometry method were utilized to assess the proliferation, migration and apoptosis of chondrocytes respectively. Expressions of miR-140, HIF-1α mRNA and mRNA associated with chondrocytes were investigated using quantitative reverse transcription PCR (qRT-PCR). Western blot was applied to assess the chondrocyte associated proteins Caspase3, Collagen II, HIF-1α, SOX-9 and BMSC-Exo surface protein markers CD9, CD63, TSG101. To observe effects of inflammatory chondrocytes, immunohistochemistry was adopted to detect the staining of Collagen I and Collagen II. Eventually, exosomes' shape was almost round, and the scratch healing was significantly increased in BMSC-Exo treated groups compared with the IL-1β group ($P < 0.001$). Additionally, it was found that exosomes in the hypoxic state (Hypoxia-Exo) resulted in higher cellular activity, less apoptosis and enhanced protein expression in inflammatory chondrocytes compared to the normoxic state (Normoxia-Exo), while weakening adipose differentiation and enhancing chondrogenic differentiation. Furthermore, the healing effect of exosomes on inflammatory chondrocytes under hypoxic conditions was produced by a rise in miR-140 expression within them. In conclusion, under hypoxia, miRNA-140 in bone marrow mesenchymal stem cell exosomes promotes proliferation and migration of chondrocytes and reduces apoptosis of chondrocytes.

Introduction

Osteoarthritis (OA) is one of the leading causes of pain and disability worldwide and it affects more than 250 million people worldwide[1, 2]. 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[3, 4]. There are few cures for osteoarthritis and the focus is on reducing physical disability and managing pain[5–7].

Mesenchymal stem cells (MSCs), with the potential of self-renewal and directed differentiation, can repair cartilage tissue and inhibit the secretion of inflammatory factors by chondrocytes[8]. Bone marrow mesenchymal stem cells (BMSCs) is one kind of the MSCs and BMSCs are superior in phenotype, morphology, function, and potential therapeutic applications compared with extraskeletal MSCs[9]. The method of obtaining BMSCs is very convenient and common, and they have the ability to repair bone tissue damage[10]. Activation of endogenous or exogenous 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[11, 12].

Exosomes are a subset of extracellular vesicles that play a key role in normal and disease physiology, but exosomes are so small that it is difficult to do investigations[13]. Exosomes 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[14–16]. Studies have proved that normal cells and cancer cells shed exosomes contain a large number of related proteins, nucleic acids, can be analyzed by these substances to determine the type of cancer[17]. Moreover, exosomes improve heart function, reduce myocardial fibrosis, improve vascular remodeling, and are a new player in diabetes and cardiomyopathy[18–20]. Additionally, BMSC-derived exosomes alleviate osteoarthritis by promoting chondrocyte[8], which suggested that BMSCs-Exo have similar biological functions to BMSCs, that is, promoting bone regeneration. Therefore, this study will focus on the effect of BMSC-EXO on articular chondrocytes and preliminarily explore the mechanism of its effect.

Hypoxia-inducible factor-1α (HIF-1α) is a subunit of hypoxia-inducible factor-1 (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[21, 22]. HIF-1α can induce BMSCs to differentiate into chondrocytes, which can treat early cartilage defects caused by osteoarthritis[23]. However, the mechanism and process of miRNA-140's action with HIF-1α has not been further investigated. In this study, we explored the effect of BMSC-Exo on articular chondrocytes under hypoxic conditions and attempted to investigate whether hypoxia-mediated upregulation of miR-140 expression occurs in dependence on HIF-1α, thus further elucidating the mechanism of miR-140 effect on chondrocytes.

Materials And Methods

Animals

4-week-old male SD rats with SPF grade, body weight about 100g, purchased from Changzhou Cavens Experimental Animal Co., Ltd. The rats were free to move in the cage and eat normally at (25 ± 2) ℃.

Cell extraction and cell culture

Culture of chondrocytes. The rats were killed by neck removal, and the knee joint of the rats was separated aseptic. The connective tissue of cartilage surface was removed, and hyaloid cartilage was taken, washed with PBS (Thermo Fisher Scientific), and the tissue was cut into 1 mm^3 pieces. 0.25% trypsin was predigested at 37℃ for 30 minutes, and the discard solution was added with 0.2% type II collagenase for digestion at 37℃ for 4h. Chondrocytes collected from digestion were suspended in DMEM medium (Thermo Fisher Scientific) containing 10% PBS and cultured at 37℃ and 5% CO_2 for 8-10 days.[24, 25].

Culture of BMSCs. The rats were sacrificed by neck removal, disinfected with 75% alcohol and rinsed with PBS. The right tibia and femur of the rats were taken, the bone ends were removed, and the bone marrow cavity was exposed. The bone marrow cavity was fully rinsed with DMEM medium and the bone marrow

was collected, centrifuged at 1500 r/min for 10 minutes. Cells were collected and suspended again, and cultured in DMEM medium containing 15% FBS.

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℃. Cell conditioned culture medium was collected to 300ml and centrifuged by supernatant centrifuge. After supernatant was removed, the cell conditioned culture medium was cleaned and suspended by PBS. 10μl exosome suspension was dropped onto the copper wire, and the samples were dried at room temperature. Morphological characteristics of exosome were observed under transmission electron microscope. The exosomes added in this experiment were all Exo-100 μg/ml.

CCK-8

Cell Counting Kit-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 4h. 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-ΔΔCt 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

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 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 for 2h. The dye was removed with anhydrous ethanol and the cells were rinsed 3 times with PBS, followed by xylene cleaning and sealing. Tissue is viewed and photographed under a light microscope.

Expression and transfection of HIF-1α

The third generation rat BMSC with cell density of 1×10^6/ml were transfected with 5 ml human medium and adenovirus vector carrying HIF-1α, and the medium was changed every 24 h after transfection. The transfection efficiency was observed by fluorescence microscope 3 days after transfection.

Western blot

Chondrocytes were cultured in 6-well plates, treated with inflammatory factors and exosomes, 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 1h 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 exosomes and chondrocytes individually. Image J software was adopted to measure the gray value of protein bands.

Immunohistochemistry (IHC)

Formalin fixed tissue, paraffin-embedded tissue or paraformaldehyde fixed cells were stained by immunohistochemistry. The fixed tissues or 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 (Figure 1A). Besides, the expressions of surface specific markers CD9, CD63 and TSG101 were proved to be positive by Western blot (Figure 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 cell viability was significantly lower under IL-1β inflammatory condition, while the survival of the BMSC-Exo group was higher than that of the IL-1β group (Figure 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 BMSC-Exo group were lower than in the IL-1β group (Figure 1E-F). These results both suggested that BMSC can alleviate adverse symptoms of chondrocytes induced by IL-1β. In addition, we also detected the effect of BMSC-Exo on chondrocyte migration by wound healing assay. Compared with the control group, the cell migration was inhibited in the IL-1β group and was significantly increased when Exo was added.(Figure 1G).

Effect of Hypoxia-Exo on inflammatory chondrocytes

By CCK-8, we found that both normoxic and hypoxic conditions of Exo can lead to increased chondrocyte viability compared with PBS + IL-1β group, in which the IL-1β+hypoxia-Exo group had a better effect. (Figure 2A). Identically, the expression levels of Collagen Type II on the surface of inflammatory chondrocytes also showed the same trend. The protein expression in the IL-1β + Hypoxia-Exo group was significantly higher than that in the IL-1β + Normoxia-Exo group compared with the PBS + IL-1β group (Figure 2B-C). Furthermore, flow cytometry showed that the apoptosis rate of chondrocytes in the IL-1β + Normoxia-Exo group was lower than that in the PBS + IL-1β group, while the apoptosis rate of chondrocytes in the IL-1β+ Hypoxia-Exo group was even lower than that in the IL-1β+ Normoxia-Exo group (Figure 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 IL-1β group, the IL-1β+Exo group significantly reduced lipid differentiation and promoted chondrogenic differentiation of chondrocytes, and the effect of IL-1β+Hypoxia-Exo group was more significant. (Figure 2E-F).

Effects of HIF-1α on BMSC-Exos

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

Effect of miRNA-140 in BMSC-Exos on chondrocytes

To further investigate the effect of miR-140 on chondrocytes, we constructed OE-miR-140 mimics and Inh-miR-140 mimics respectively. As shown in Figure 4A, overexpression of miR-140 greatly increased chondrocyte viability compared to the NC group, whereas inhibition of miR-140 significantly decreased chondrocyte viability (Figure 4B). Overexpression of miR-140 significantly increased cell migration ability compared to the NC group (Figure 4C-D), while inhibition of miR-140 had the opposite effect (Figure 4E-F). As shown in Figure 5A, qRT-PCR results showed that overexpression of HIF-1α increased the mRNA of SOX-9, COL2, ACAN, RUNX2, SCX, and decreased the mRNA of COL1, COL6, COMP, TNC, FMOD. and Western blot experiments revealed that the protein expression of SOX-9, Collagen Type II, aggrecan aggrecan's protein expression was strongest at the overexpression of HIF-1α(5B). In addition, toluidine blue staining and immunohistochemistry revealed that miR-140 overexpression enhanced chondrogenic differentiation of chondrocytes, while miR-140 inhibition weakened its effect. Besides, the immunohistochemical results showed that miR-140 overexpression led to a decrease in collagen I and an increase in collagen II, while inhibition of miR-140 reversed the effect as well (Figure 6).

Discussion

It has been reported in the literature, Co-culture of BMSCs with chondrocytes significantly promotes cell proliferation and differentiation, while exosomes, as an important component of the paracrine component of stem cells, can play a role in tissue repair instead of stem cells[26]. The anti-inflammatory and pro-growth effects of BMSC-Exo have also been demonstrated, and it can promote the regeneration of OA articular cartilage[27]. Chondrocytes are the only cellular component in articular cartilage, and there are few studies on the effect of BMSC-Exo on chondrocytes. In this experiment, we demonstrated that BMSC-Exo can alleviate the symptoms caused by IL-1β through relevant experiments, and the differences in the effects of exosomes in different oxygen environments were further investigated.

Through the assay of collagen type II protein, lipogenic differentiation assay and chondrogenic differentiation assay, we found that the therapeutic effect of exosomes was more significant and chondrocyte activity was stronger in hypoxic conditions compared to normoxic environment. In addition, some researchers found that miR-29a of BMSC-Exo can promote angiogenesis and osteogenesis [28], and we further investigated miR-140, another gene of BMSC-Exo, in depth and found that miR-140 of

BMSC-Exo was significantly increased in expression under hypoxia. This led us to question the mechanism and link between the hypoxic environment and miR-140 expression, while recent studies have shown that BMSC-Exo-HIF-1α stimulates BMSC proliferation and osteogenesis[29],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[30]. and found that miR-140 expression was unaffected by HIF-1α knockdown under normoxic conditions, while miR-140 expression was significantly reduced in both si-HIF group BMSC and BMSC-Exo under hypoxic conditions compared to si-NC, so we conclude that hypoxia-mediated upregulation of miR-140 expression occurs through HIF-1α.

Previous studies have demonstrated that overexpression of miR-140-3p inhibits the proliferation, migration and invasion of breast cancer cells[31], The present experiment confirmed that overexpressed miR-140 could promote chondrocyte proliferation and migration by CCK-8 assay and scratch 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[32], upregulation of SOX-9 and COL2 expression was also found to inhibit IL-1β-induced inflammatory responses in chondrocytes [33], It is thus clear that chondrocyte recovery is best facilitated when miR-140 is overexpressed. Pauline Po-Yee Lui et al. found that a high percentage of type I collagen may be responsible for poor matrix organization in calcified tendinopathy[34],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 miRNA-140 has an important role in the recovery of articular chondrocyte activity and its adverse symptoms.

Conclusion

we found that the significant effect of BMSC-Exo on articular chondrocytes under hypoxic conditions was closely related to the expression of miRNA-140, and the hypoxia-mediated upregulation of miR-140 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

CCK-8, Cell Counting Kit-8; qRT-PCR, quantitative reverse transcription PCR; Hypoxia-Exo, exosomes in the hypoxic state; Normoxia-Exo, exosomes in the normoxia state; MSCs, Mesenchymal stem cells; BMSCs, Bone marrow mesenchymal stem cells; HIF-1α, Hypoxia-inducible factor-1α; HIF-1, αhypoxia-inducible factor-1

Declarations

Acknowledgements Not applicable.

Authors' contributions YZH conceived and designed the experiments; HXL and XXX performed the experiments; DLX and XHX done statistical analysis; YZH wrote the paper; all authors read and approved the final manuscript.

Funding Not applicable.

Availability of data and materials The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

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

Consent for publication Approve.

Competing interests The authors declare that they have no competing interests.

References

1. O'Neill TW and Felson DT (2018) Mechanisms of Osteoarthritis (OA) Pain. Curr Osteoporos Rep 16:611-616. https://doi.org/10.1007/s11914-018-0477-1
2. Murphy L, Schwartz TA, Helmick CG, Renner JB, Tudor G, Koch G, Dragomir A, Kalsbeek WD, Luta G and Jordan JM (2008) Lifetime risk of symptomatic knee osteoarthritis. Arthritis Rheum 59:1207-13. https://doi.org/10.1002/art.24021
3. Everhart JS, Abouljoud MM, Poland SG and Flanigan DC (2019) Medial compartment defects progress at a more rapid rate than lateral cartilage defects in older adults with minimal to moderate knee osteoarthritis (OA): data from the OA initiative. Knee Surg Sports Traumatol Arthrosc 27:2401-2409. https://doi.org/10.1007/s00167-018-5202-1
4. King LK, Epstein J, Cross M, Buzzi M, Buttel T, Cembalo SM, Spitz E, Adams CL, Adebajo A, Bennell K, Blanco B, Courage U, Decary S, Gill M, Gill TK, Hajji R, Hinman RS, Jones A, Li LC, Mather K, Mani R, Nasef SI, Oo WM, Osteras N, Otobo TM, Ramiro S, Sharma S, April KT, Touma Z, Whittaker JL, Wluka AE, Grosskleg S, Hunter DJ, Shea B, Hawker GA, Callahan LF, March L and Guillemin F (2021) Endorsement of the domains of knee and hip osteoarthritis (OA) flare: A report from the OMERACT 2020 inaugural virtual consensus vote from the flares in OA working group. Semin Arthritis Rheum 51:618-622. https://doi.org/10.1016/j.semarthrit.2021.03.010
5. Runhaar J and Zhang Y (2018) Can we prevent OA? Epidemiology and public health insights and implications. Rheumatology (Oxford) 57:iv3-iv9. https://doi.org/10.1093/rheumatology/key014
6. Hawker GA and Lohmander LS (2021) What an earlier recognition of osteoarthritis can do for OA prevention. Osteoarthritis Cartilage 29:1632-1634. https://doi.org/10.1016/j.joca.2021.08.007

7. Huesa C, Ortiz AC, Dunning L, McGavin L, Bennett L, McIntosh K, Crilly A, Kurowska-Stolarska M, Plevin R, van 't Hof RJ, Rowan AD, McInnes IB, Goodyear CS, Lockhart JC and Ferrell WR (2016) Proteinase-activated receptor 2 modulates OA-related pain, cartilage and bone pathology. Ann Rheum Dis 75:1989-1997. https://doi.org/10.1136/annrheumdis-2015-208268
8. Zhou X, Liang H, Hu X, An J, Ding S, Yu S, Liu C, Li F and Xu Y (2020) BMSC-derived exosomes from congenital polydactyly tissue alleviate osteoarthritis by promoting chondrocyte proliferation. Cell Death Discov 6:142. https://doi.org/10.1038/s41420-020-00374-z
9. Garcia-Garcia A, de Castillejo CL and Mendez-Ferrer S (2015) BMSCs and hematopoiesis. Immunol Lett 168:129-35. https://doi.org/10.1016/j.imlet.2015.06.020
10. Cetin Z, Saygili EI, Gorgisen G and Sokullu E (2021) Preclinical Experimental Applications of miRNA Loaded BMSC Extracellular Vesicles. Stem Cell Rev Rep 17:471-501. https://doi.org/10.1007/s12015-020-10082-x
11. Arthur A and Gronthos S (2020) Clinical Application of Bone Marrow Mesenchymal Stem/Stromal Cells to Repair Skeletal Tissue. Int J Mol Sci 21. https://doi.org/10.3390/ijms21249759
12. Voss PJ, Matsumoto A, Alvarado E, Schmelzeisen R, Duttenhofer F and Poxleitner P (2017) Treatment of stage II medication-related osteonecrosis of the jaw with necrosectomy and autologous bone marrow mesenchymal stem cells. Odontology 105:484-493. https://doi.org/10.1007/s10266-017-0295-4
13. Strack R (2019) Improved exosome detection. Nat Methods 16:286. https://doi.org/10.1038/s41592-019-0386-y
14. Egea-Jimenez AL and Zimmermann P (2020) Lipids in Exosome Biology. Handb Exp Pharmacol 259:309-336. https://doi.org/10.1007/164_2019_220
15. Jeppesen DK, Fenix AM, Franklin JL, Higginbotham JN, Zhang Q, Zimmerman LJ, Liebler DC, Ping J, Liu Q, Evans R, Fissell WH, Patton JG, Rome LH, Burnette DT and Coffey RJ (2019) Reassessment of Exosome Composition. Cell 177:428-445 e18. https://doi.org/10.1016/j.cell.2019.02.029
16. Yang D, Zhang W, Zhang H, Zhang F, Chen L, Ma L, Larcher LM, Chen S, Liu N, Zhao Q, Tran PHL, Chen C, Veedu RN and Wang T (2020) Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. Theranostics 10:3684-3707. https://doi.org/10.7150/thno.41580
17. Exosome Profiling Pinpoints Cancer Type. Cancer Discov 10:1619. https://doi.org/10.1158/2159-8290.CD-NB2020-083
18. Robson A (2021) Exosome-derived microRNAs improve cardiac function. Nat Rev Cardiol 18:150-151. https://doi.org/10.1038/s41569-020-00498-w
19. Lim GB (2021) Exosome-eluting stents improve vascular remodelling. Nat Rev Cardiol 18:386. https://doi.org/10.1038/s41569-021-00557-w
20. Tao L, Shi J, Yang X, Yang L and Hua F (2019) The Exosome: a New Player in Diabetic Cardiomyopathy. J Cardiovasc Transl Res 12:62-67. https://doi.org/10.1007/s12265-018-9825-x

21. Borsi E, Perrone G, Terragna C, Martello M, Zamagni E, Tacchetti P, Pantani L, Brioli A, Dico AF, Zannetti BA, Rocchi S and Cavo M (2014) HIF-1alpha inhibition blocks the cross talk between multiple myeloma plasma cells and tumor microenvironment. Exp Cell Res 328:444-55. https://doi.org/10.1016/j.yexcr.2014.09.018
22. Sheu SY, Ho SR, Sun JS, Chen CY and Ke CJ (2015) Arthropod steroid hormone (20-Hydroxyecdysone) suppresses IL-1beta-induced catabolic gene expression in cartilage. BMC Complement Altern Med 15:1. https://doi.org/10.1186/s12906-015-0520-z
23. Weinstein SL (2016) The Burden of Musculoskeletal Conditions. J Bone Joint Surg Am 98:1331. https://doi.org/10.2106/JBJS.16.00595
24. Fei J, Liang B, Jiang C, Ni H and Wang L (2019) Luteolin inhibits IL-1beta-induced in fl ammation in rat chondrocytes and attenuates osteoarthritis progression in a rat model. Biomed Pharmacother 109:1586-1592. https://doi.org/10.1016/j.biopha.2018.09.161
25. Sun FF, Hu PF, Xiong Y, Bao JP, Qian J and Wu LD (2019) Tricetin Protects Rat Chondrocytes against IL-1beta-Induced Inflammation and Apoptosis. Oxid Med Cell Longev 2019:4695381. https://doi.org/10.1155/2019/4695381
26. Yamaguchi T, Izumi Y, Nakamura Y, Yamazaki T, Shiota M, Sano S, Tanaka M, Osada-Oka M, Shimada K, Miura K, Yoshiyama M and Iwao H (2015) Repeated remote ischemic conditioning attenuates left ventricular remodeling via exosome-mediated intercellular communication on chronic heart failure after myocardial infarction. Int J Cardiol 178:239-46. https://doi.org/10.1016/j.ijcard.2014.10.144
27. Jiang Y, Zhang J, Li Z and Jia G (2020) Bone Marrow Mesenchymal Stem Cell-Derived Exosomal miR-25 Regulates the Ubiquitination and Degradation of Runx2 by SMURF1 to Promote Fracture Healing in Mice. Front Med (Lausanne) 7:577578. https://doi.org/10.3389/fmed.2020.577578
28. Lu GD, Cheng P, Liu T and Wang Z (2020) BMSC-Derived Exosomal miR-29a Promotes Angiogenesis and Osteogenesis. Front Cell Dev Biol 8:608521. https://doi.org/10.3389/fcell.2020.608521
29. Ying C, Wang R, Wang Z, Tao J, Yin W, Zhang J, Yi C, Qi X and Han D (2020) BMSC-Exosomes Carry Mutant HIF-1alpha for Improving Angiogenesis and Osteogenesis in Critical-Sized Calvarial Defects. Front Bioeng Biotechnol 8:565561. https://doi.org/10.3389/fbioe.2020.565561
30. Aro E, Khatri R, Gerard-O'Riley R, Mangiavini L, Myllyharju J and Schipani E (2012) Hypoxia-inducible factor-1 (HIF-1) but not HIF-2 is essential for hypoxic induction of collagen prolyl 4-hydroxylases in primary newborn mouse epiphyseal growth plate chondrocytes. J Biol Chem 287:37134-44. https://doi.org/10.1074/jbc.M112.352872
31. Zhou Y, Wang B, Wang Y, Chen G, Lian Q and Wang H (2019) miR-140-3p inhibits breast cancer proliferation and migration by directly regulating the expression of tripartite motif 28. Oncol Lett 17:3835-3841. https://doi.org/10.3892/ol.2019.10038
32. Horner CB, Hirota K, Liu J, Maldonado M, Hyle Park B and Nam J (2018) Magnitude-dependent and inversely-related osteogenic/chondrogenic differentiation of human mesenchymal stem cells under

dynamic compressive strain. J Tissue Eng Regen Med 12:e637-e647. https://doi.org/10.1002/term.2332

33. Sun Q, Wu G, Chen H, Chen L, Chen H, Zhu G and Zhao H (2017) Hyperbaric oxygen protects type II collagen in interleukin-1beta-induced mandibular condylar chondrocyte via inhibiting the JNK/c-Jun signaling pathway. Oncotarget 8:60312-60323. https://doi.org/10.18632/oncotarget.19294
34. Lui PP, Chan LS, Lee YW, Fu SC and Chan KM (2010) Sustained expression of proteoglycans and collagen type III/type I ratio in a calcified tendinopathy model. Rheumatology (Oxford) 49:231-9. https://doi.org/10.1093/rheumatology/kep384

Figures

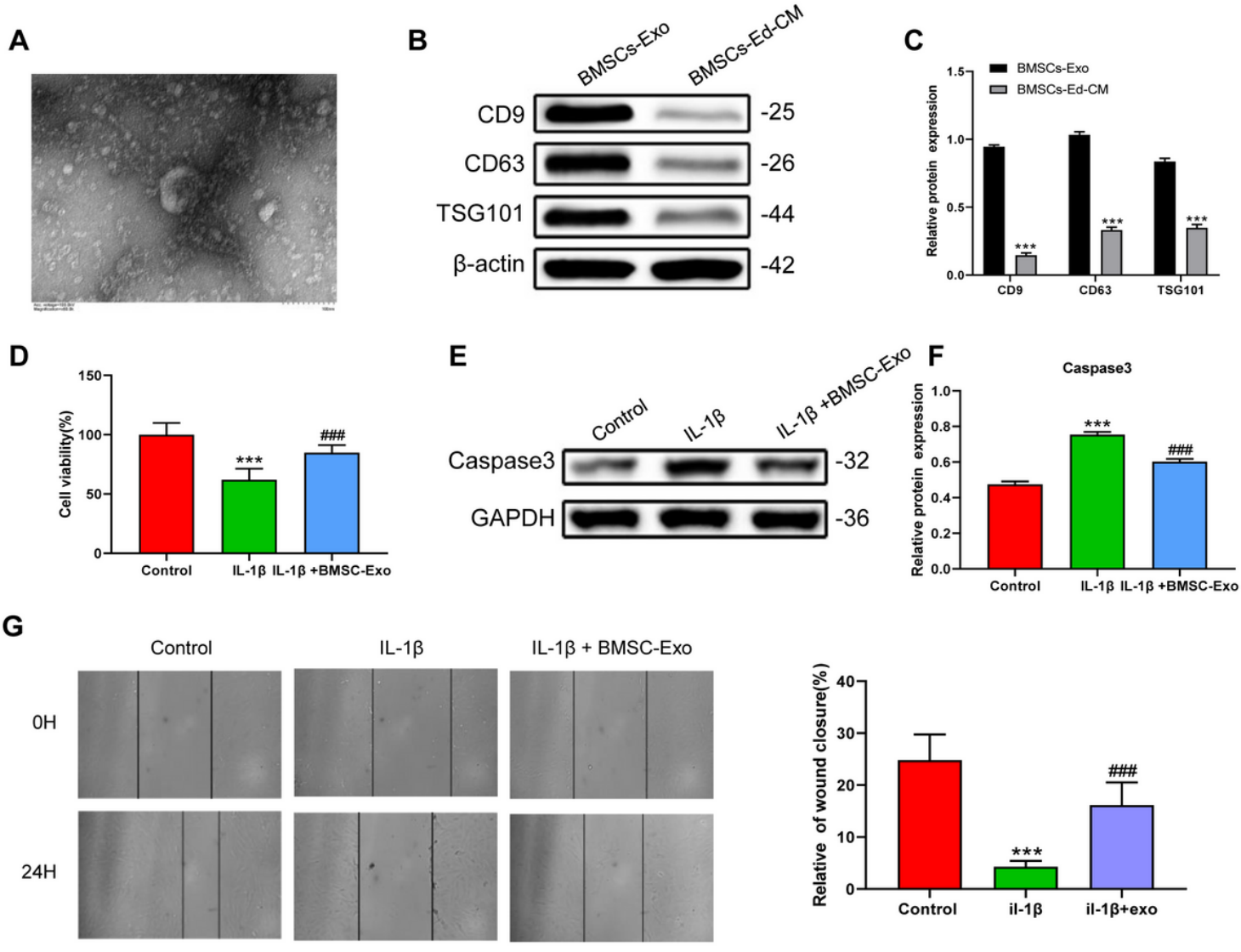

Figure 1

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. (E) Western blotting results of Caspase3 protein levels in IL-1β and IL-1β+BMSCs conditions and (F) its quantitative analysis. GAPDH was used as loading control. ***P < 0.001 compared to control and ##P < 0.001 compared to IL-1β group. (D) Chondrocyte survival under IL-1β and IL-1β+BMSCs conditions. ***P < 0.001 compared to control and ##P < 0.001 compared to IL-1β group. (E) Western blotting results of Caspase3 protein levels under IL-1β and IL-1β+BMSCs conditions and (F) its quantitative analysis. GAPDH was used as loading control. ***P < 0.001 compared to control and ##P < 0.001 compared to IL-1β group. (G) Results of wound healing and quantitative analysis of different wound closures. ***P < 0.001 compared to control or IL-1β group; ##P < 0.001 compared to Exo group. ***, compared to control; ##P < 0.001 compared to IL-1β

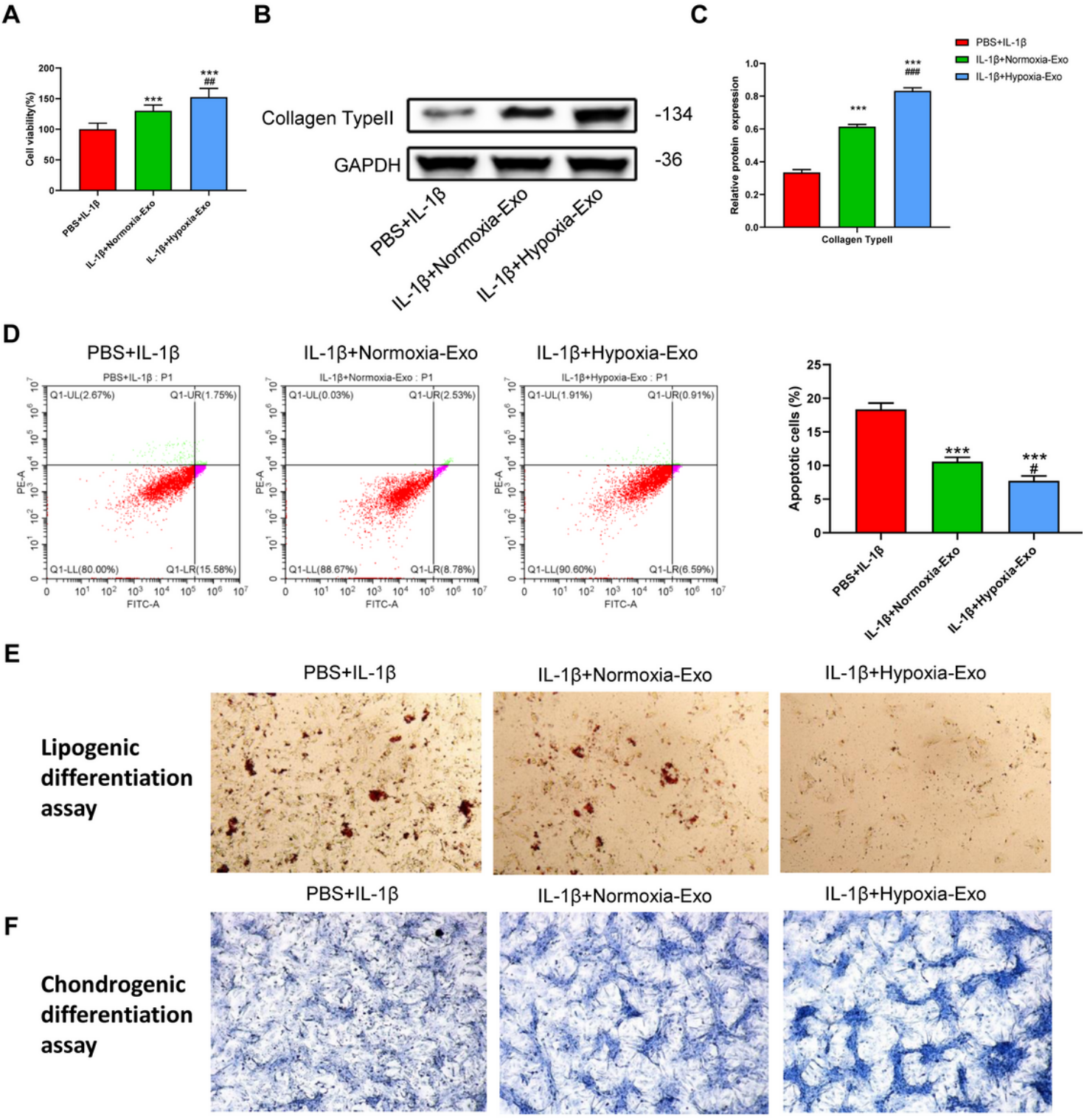

Figure 2

Effect of Hypoxia-Exo 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 and HIF-1α protein level. GAPDH functioned as loading control. (D) Flow cytometry result and its quantitative analysis of

inflammatory chondrocyte cells. Compared with the PBS + IL-1β group, ***$P < 0.001$, compared with the IL-1β group + Normoxia-Exo, $^{###}P < 0.001$, $^{##}P < 0.01$, $^{#}P < 0.05$. (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.

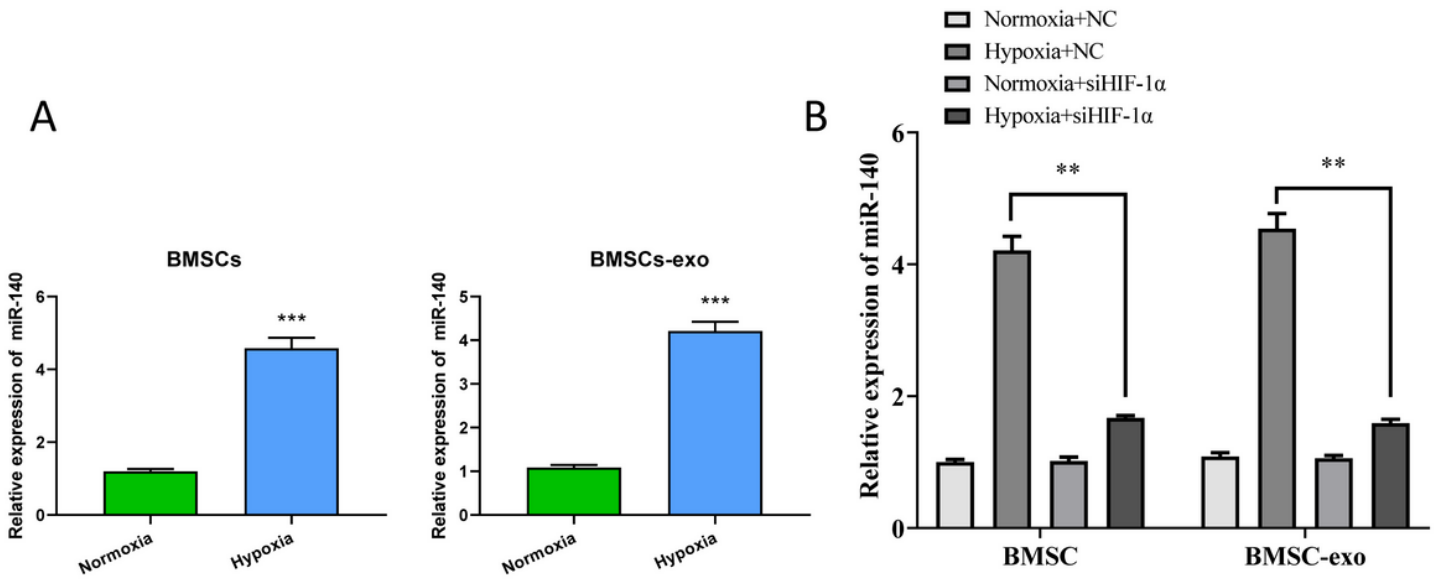

Figure 3

Effect of oxygen conditions on BMSC-Exos

(A) Relative expression of mRNA-140 in BMSCs and Exo during hypoxia and normoxia. (B) Relative expression of mRNA-140 in BMSCs and Exo after knockdown of HIF-1α at hypoxia and normoxia. **P < 0.01.

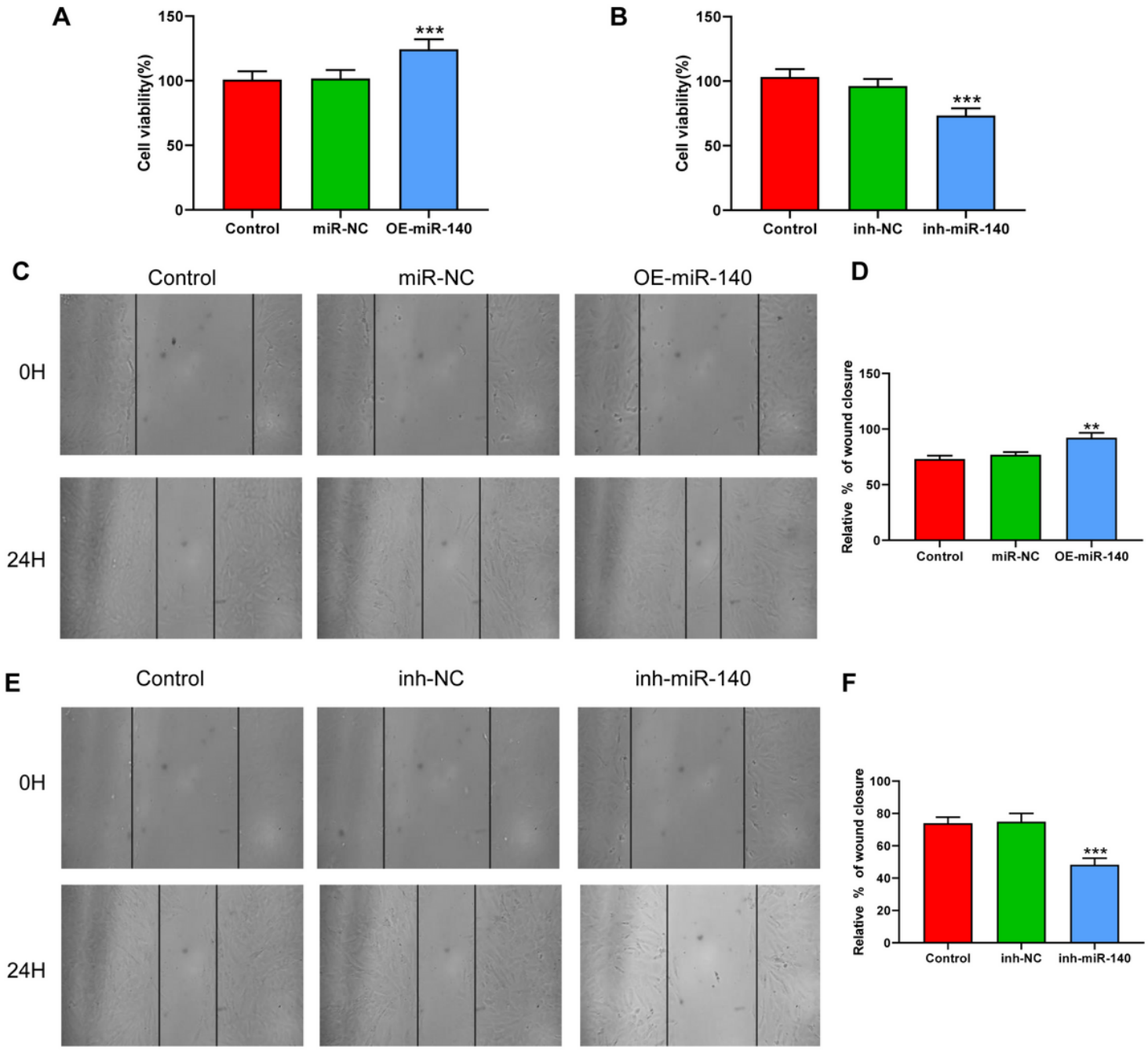

Figure 4

Effect of miR-140 in BMSC-Exo on proliferation and migration of chondrocytes

(A) The chondrocyte cells viability when miR-140 was overexpressed. Compared with the control group or the miR-NC group, ***$P < 0.001$. (B) The chondrocyte cells viability when miR-140 was inhibited. Compared with the control group or the Inh-NC group, ***$P < 0.001$. (C) The wound healing result and its quantitative analysis (D) of different wound closure when miR-140 was overexpressed. Compared with the control group or the miR-NC group, **$P < 0.01$. (E) The wound healing result and its quantitative

analysis (F) of different wound closure when miR-140 was inhibited. Compared with the control group or the Inh-NC group, ***$P < 0.001$.

Figure 5

Effects of miR-140 in BMSC-Exo on mRNA and related protein expression in chondrocytes

Relative mRNA and protein expression of inflammatory chondrocytes upon overexpression and inhibition of miR-140. P < 0.001 compared with IL-1β group; ##P < 0.001 compared with IL-1β+exo+NC group; $$P < 0.001 compared with IL-1β+exo+inh-miR-140 group.

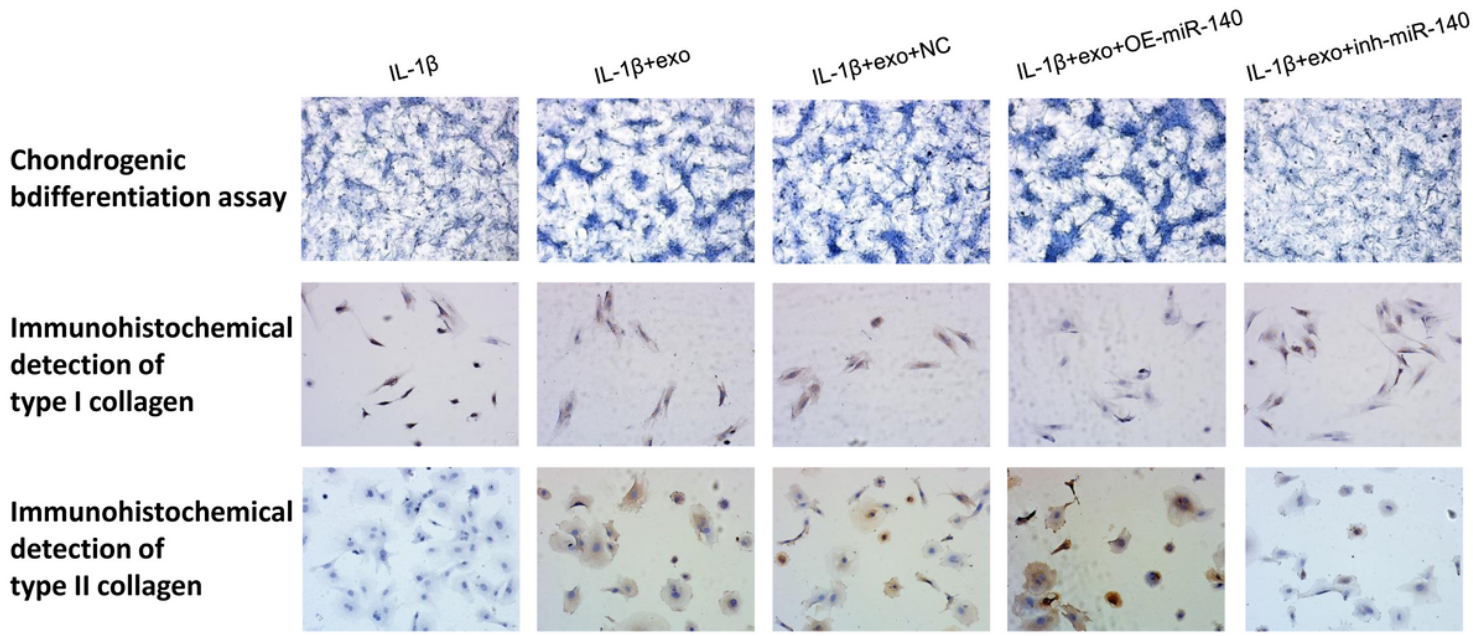

Figure 6

Effects of miR-140 in BMSC-Exo on chondrogenic differentiation, collagen I and collagen II of chondrocytes

Morphology of inflammatory chondrocytes upon overexpression and inhibition of miR-140, collagen I and collagen II content.