Chinese Chemical Letters 34 (2023) 108079

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Thermosensitive injectable hydrogel loaded with hypoxia-induced exosomes maintains chondrocyte phenotype through NDRG3-mediated hypoxic response

Xiongbo Song[a,b], Liling Gu[b], Qiming Yang[b], Jiarui Wu[b], Junrong Chen[b], Xiaobin Tian[c,*], Li Sun[a,b,**], Long Chen[a,b,**]

[a] Medical College, Guizhou University, Guiyang 550000, China
[b] Department of Orthopedics, Guizhou Provincial People's Hospital, Guiyang 550000, China
[c] Department of Orthopaedics, The Affiliated Hospital of Guizhou Medical University, Guiyang 550000, China

ARTICLE INFO

Article history:
Received 31 August 2022
Revised 13 December 2022
Accepted 14 December 2022
Available online 16 December 2022

Keywords:
Osteoarthritis
Injectable thermosensitive hydrogel
Exosomes
Hypoxic response
NDRG3

ABSTRACT

Current clinical treatments cannot effectively delay the progression of osteoarthritis (OA). Consequently, joint replacement surgery is required for late-stage OA when patients cannot tolerate pain and joint dysfunction. Therefore, the prevention of OA progression in the early and middle stages is an urgent clinical problem. In a previous study, we demonstrated that NDRG3-mediated hypoxic response might be closely related to the development and progression of OA. In this study, an injectable thermosensitive hydrogel was established by cross-linking Pluronic F-127 and hyaluronic acid (HA) for the sustained release of hypoxia-induced exosomes (HExos) derived from adipose-derived mesenchymal stem cells. We demonstrated that for OA at the early and middle stages, the HExos-loaded HP hydrogel could maintain the chondrocyte phenotype by enhancing chondrocyte autophagy, reducing chondrocyte apoptosis, and promoting chondrocyte activity and proliferation through the NDRG3-mediated hypoxic response. This novel composite hydrogel, which could activate the NDRG3-mediated hypoxic response, may provide new ideas and a theoretical basis for the treatment of early- and mid-stage OA.

Osteoarthritis (OA) is the most common joint disease in people over 60 years of age and is characterized by the progressive destruction of articular cartilage caused by the imbalance of anabolism and catabolism of chondrocytes [1,2]. The treatment for the early and middle stages of OA is limited to basic treatment, including health education, exercise therapy, physical therapy, mobility support, and drug therapy to relieve OA symptoms and improve joint function [3]. However, these cannot effectively delay the progression of OA, and joint replacement surgery is required for the late stage of OA when patients cannot tolerate pain and dysfunction of the joint. Therefore, the prevention of OA progression in the early and middle stages is an urgent clinical problem.

Normal articular cartilage is an avascular tissue with an oxygen content of approximately 1%–6%, which gradually decreases from the surface of the cartilage to the deep area [4]. Therefore, the articular cartilage of patients with early- and middle-stage OA is relatively hypoxic before the ingrowth of blood vessels caused by inflammatory hyperplasia [5,6]. Studies have shown that NDRG3 is a member of the N-myc downstream regulatory gene family, which can independently regulate the hypoxic response of tumor cells [7]. NDRG3 is hydroxylated by prolyl hydroxylase 2 (PHD2) under normoxic conditions, which in turn is ubiquitinated by von Hippel-Lindau protein (pVHL) and degraded by the proteasomal pathway. Under hypoxic conditions, this degradation process is inhibited, and accumulated lactate interferes with the interaction between NDRG3 and PHD2. Hypoxia and lactate production maintain the stability of NDRG3 under chronic hypoxia, thereby prolonging the hypoxic response of cells during persistent hypoxia. This study also confirmed that the NDRG3-mediated hypoxia response is independent of the hypoxia-inducible factor (HIF) pathway [7]. Moreover, on the basis of the NDRG3-mediated hypoxic response, we surgically collected specimens from normal and OA patients and conducted a variety of histopathological experiments to explore the relationship between NDRG3 and the development and progression of OA. We found that the NDRG3-mediated hypoxic response might be closely related to the development and progression of OA [8].

* Corresponding author.
** Corresponding author at: Medical College, Guizhou University, Guiyang 550000, China.
E-mail addresses: txb6@vip.163.com (X. Tian), lisun@gzu.edu.cn (L. Sun), longchen@gzu.edu.cn (L. Chen).

https://doi.org/10.1016/j.cclet.2022.108079
1001-8417/

Therefore, the NDRG3-mediated hypoxic response is a potentially significant target for the early and middle stages of OA treatment.

Studies have shown that mesenchymal stem cell (MSCs) injection can relieve pain symptoms, improve the quality of life in patients with OA, and delay OA progression in the early and middle stages [9–12]. Accumulating evidence suggests that the therapeutic effects of MSCs are largely attributable to their paracrine mechanisms, which are mainly mediated by exosomes (Exos). At present, there are several studies using Exos isolated from MSCs to treat early and mid-stage OA, but Exos are usually isolated under normoxic conditions, dissolved in PBS or saline, and then injected into the joint [13–16]. However, the aforementioned studies on early and middle stage OA used joint injection of MSCs; after the injected MSCs adhere to the articular cartilage, they are in a relatively hypoxic environment. Therefore, the Exos secreted by the injected MSCs is in a hypoxic state. Moreover, the direct injection of Exos makes it difficult to retain them in the joint for a long time.

Therefore, the isolation of Exos from MSCs under hypoxic conditions and prolonging the residence time of Exos in the joint may ensure a better therapeutic effect. Pluronic is a uniquely heat-sensitive copolymer that exists as a liquid at low temperatures and rapidly forms hydrogels at elevated temperatures [17–19]. In addition, it has porous structures that can delay drug release. However, its low molecular weight and low mechanical strength incur rapid degradation under physiological conditions, causing a sudden release of the drug [20]. Hyaluronic acid (HA) is a natural polysaccharide and one of the largest components of the articular cartilage matrix [21]. According to the characteristics of Pluronic and HA, we intend to combine the acetyl group on HA with the methyl group on Pluronic F-127 to tightly pack the Pluronic F-127 micelles at the gel temperature and improve the retention time of the loaded drug.

In this study, an injectable thermosensitive hydrogel was established by cross-linking Pluronic F-127 and HA for the sustained release of hypoxia-induced Exos derived from adipose-derived mesenchymal stem cells (ADMSCs). Moreover, we investigated whether the injectable thermosensitive hydrogel can maintain the synthesis and decomposition of the cartilage matrix and the stability of energy metabolism of chondrocytes through the NDRG3-mediated hypoxic response.

ADMSCs have the advantages of a wide range of sources, strong proliferation ability, minimal damage to the donor site, easy access, and better cartilage-forming properties; therefore, we chose ADMSCs as the seed cells for Exo extraction [22–25]. The results showed that ADMSCs extracted from C57BL6 mice were typically long fusiform, with high expression of CD29, CD44, and Sca-1, and negative expression of CD31 (Fig. 1A). ADMSCs were cultured under normoxia and hypoxia conditions for 24 h. The cell supernatant was collected, centrifuged at $3000 \times g$ for 10 min at 4 °C, and filtered at 0.22 um. Add Exo concentrate (Umibio) and centrifuge at $10{,}000 \times g$ for 60 min at 4 °C. After resuspending in PBS, add a purification filter and centrifuge at 4 °C, $3000 \times g$ for 10 min to purify Exos. Exos isolated from ADMSCs under normoxic or hypoxic conditions were characterized by transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and western blotting. TEM showed that the Exos derived from ADMSCs under normoxic or hypoxic conditions (normoxia-induced Exos: NExos; hypoxia-induced Exos: HExos) presented a typical transparent disk shape. NTA detection showed that the concentration of NExos or HExos reached 1.0×10^8 particles/mL, and the peak particle size was between 100 and 200 nm (Figs. 1B and C). Western blotting showed that both NExos and HExos were rich in surface markers (CD81 and TSG10) (Fig. 1D). These results indicate that NExos and HExos were successfully isolated from the culture supernatant of ADMSCs under normoxic and hypoxic conditions.

To prepare and characterize the HA-Pluronic F-127 (HP) gel, HA and Pluronic F-127 were dissolved in deionized water and shaken

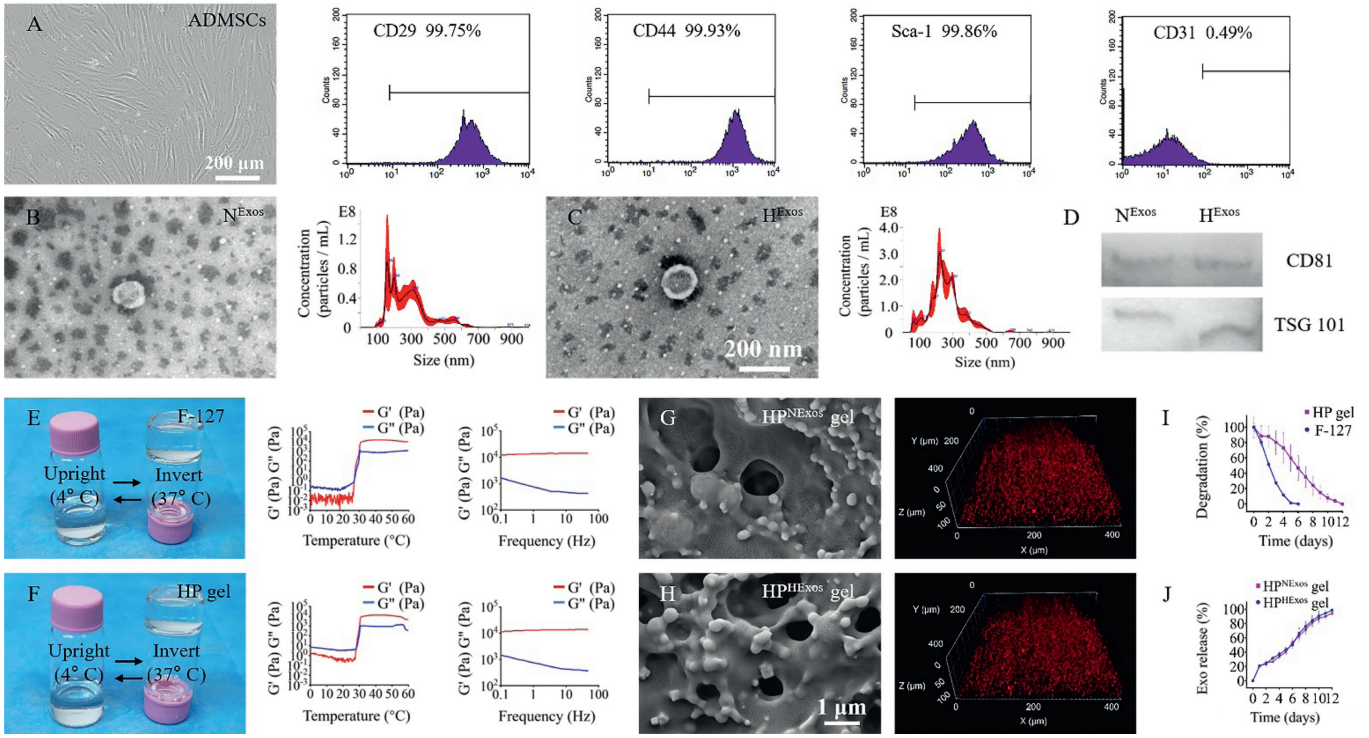

Fig. 1. Preparation and characterization of the adipose-derived mesenchymal stem cells (ADMSCs) derived exosomes (Exos) and Exos-loaded HP hydrogel. (A) Morphology of ADMSCs and identification of surface markers by flow cytometry, scale bar = 200 μm. (B, C) TEM morphology and particle size distribution of normoxia-induced Exos (NExos) and hypoxia-induced Exos (HExos), scale bar = 200 nm. (D) Western blotting for NExos and HExos surface markers. (E, F) The optical images of NExos and HExos-loaded HP hydrogel sol-gel transition, rheogram frequency sweep analysis and temperature curves. (G, H) The internal morphology and distribution of NExos and HExos in HP-Exos hydrogels, scale bar = 1 μm. (I) Degradation rate of the F127 hydrogels and F127-HA hydrogels in PBS at 37 °C. (J) Release profile of NExos and HExos from the F127-HA hydrogels in PBS at 37 °C.

at 4 °C overnight to obtain a completely clear HP solution [26]. Then, Exos were added and shaken at 4 °C overnight to obtain the HP-Exo solution, which was placed in a refrigerator at 4 °C for later use. The HP sol-gel transition was determined using the vial inversion method. The vial was inverted after being placed at 37 °C; the flowing liquid was in the form of a sol, and the non-flowing liquid was in the form of a gel. To further determine the critical temperature of HP sol-gel transition, rheological tests were performed on the HP solutions at 0–60 °C. Subsequently, to observe the internal morphology HP-Exos hydrogels were freeze-dried and examined by scanning electron microscopy (SEM). Exos was labeled with DiR dye, then added to the HP solution, shaken at 4 °C overnight, and three-dimensional (3D) reconstruction by confocal microscopy was performed after gel formation to detect the distribution of Exos. The degradation rate of HP hydrogels was determined using a gravimetric method. To determine the release profile of Exos, the cumulative release of Exos was detected using a BCA protein detection kit.

The inversion of the vial showed that the solution of Pluronic F-127 alone, or the solution of Pluronic F-127 with 1% HA, underwent a sol-gel transition when the temperature was increased from 4 °C to 37 °C. When the temperature dropped to 4 °C, the hydrogel again formed a sol. Rheological results showed that the addition of HA did not change the elastic (G') and viscous (G'') moduli and sol-gel transition temperatures of Pluronic F-127 (Figs. 1E and F). SEM showed that after adding NExo or HExo, the lyophilized HP hydrogel maintained its porous structure, and confocal microscopy 3D reconstruction showed that NExos and HExos were uniformly distributed inside the HP hydrogel (Figs. 1G and H). Moreover, the addition of HA significantly prolonged the degradation of Pluronic F-127 (Fig. 1I). Pluronic F-127, a biocompatible polymer with a reversible sol-gel transition, is widely used for drug delivery. The surface-active function of Pluronic F-127 facilitates uniform dispersion of the drug inside, and it degrades rapidly under physiological conditions, resulting in a sudden release of the loaded drug. Hydrophobic interactions were formed between the acetyl group on the added polymer HA and the methyl group on Pluronic F-127, which densely packed the Pluronic F-127 micelles at the gel temperature and improved the retention time of the loaded drug. The results showed that the HP hydrogel was completely degraded within 12 days, and the release curve indicated that NExos or HExos could be continuously released from the hydrogel for 12 days (Fig. 1J). These properties enable reversible thermoresponsive HP to gel at normal body temperature and remain at the implantation site as a sustained drug-release system.

Regenerative repair after articular cartilage injury for OA at the early and middle stages depends on the ability of the surrounding chondrocytes to survive, proliferate, and synthesize the matrix [27]. For *in vitro* investigation of the effects of Exos-loaded HP hydrogels on chondrocytes during OA at the early and middle stages, chondrocytes were induced with the inflammatory cytokine IL-1β to simulate the inflammatory microenvironment in which chondrocytes live during OA [28–30]. Then, Exos-loaded HP hydrogels were added to examine the chondrocyte activity, proliferation, synthesis, and catabolism under hypoxia. A calcein-AM/PI double stain kit was used to evaluate the viability of chondrocytes. In brief, chondrocytes were incubated with 2 mmol/L calcein-AM and 4.5 mmol/L propidium iodide (PI) for 15 min at 37 °C. The labeled cells were visualized under a fluorescence microscope. Note that green represents live cells, and red represents dead cells. To detect chondrocyte proliferation, the BeyoClick™ EdU Cell Proliferation Kit was used. The acetylene group on EdU can covalently react with fluorescently labeled small-molecule azide probes, catalyzed by monovalent copper ions to form a stable triazole ring. The newly synthesized DNA was labeled with corresponding fluorescent probes so that proliferating cells could be detected using

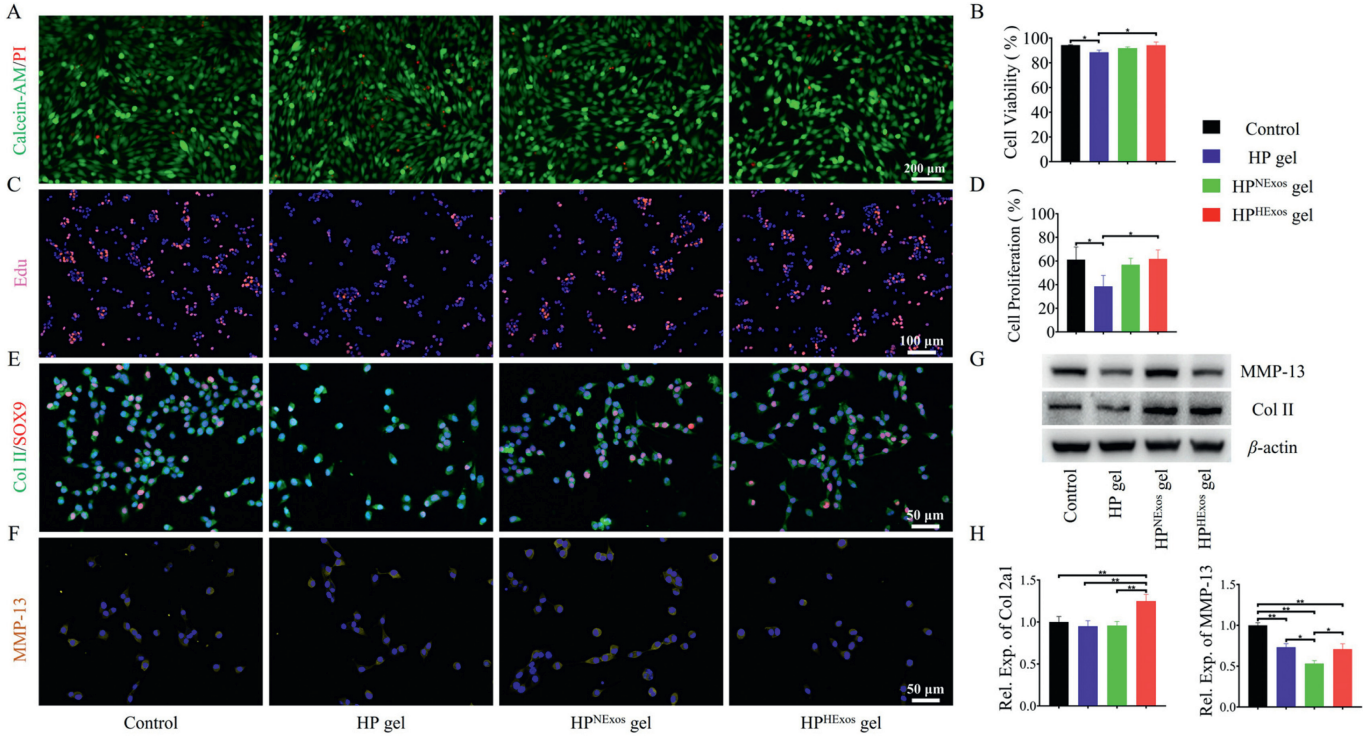

Fig. 2. Effects of NExos and HExos-loaded HP hydrogel for chondrocyte viability, proliferation and expression of related proteins. (A) The live-dead cell staining of chondrocytes, scale bar = 200 μm. (B) The cell viability of chondrocyte, one-way ANOVA, $^{*}P < 0.05$. (C) The Edu staining of chondrocytes, scale bar = 100 μm. (D) The proliferation of chondrocyte, one-way ANOVA, $^{*}P < 0.05$. (E, F) Representative immunofluorescence images of Col II (green), SOX9 (red), and MMP-13 (orange), nucleus was stained with DAPI (blue), scale bars = 50 μm. (G) Western blotting of Col II and MMP-13, β-actin was served as a loading control. (H) Relative mRNA expression of Col2a1 and MMP-13, one-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$.

fluorescent detection equipment. The synthesis and catabolism of chondrocytes were examined by PCR and western blotting. The results showed that both NExos and HExos-loaded HP hydrogels reduced IL-1β-induced chondrocyte death under hypoxic conditions (Figs. 2A and B) while resisting IL-1β-induced chondrocyte proliferation inhibition (Figs. 2C and D). However, HExos-loaded HP hydrogels had a better effect, similar to that of the control group (chondrocytes without IL-1β induction in a hypoxic environment) (Figs. 2B and D). In addition to promoting cell survival and proliferation, promoting chondrocyte matrix synthesis is a key issue in the treatment of OA. The effects on chondrocyte synthesis (Col 2a1 or Col II) and catabolism (MMP-13) were verified at the molecular and protein levels. Immunofluorescence staining and western blot results showed that NExos-loaded HP hydrogels promoted the expression of chondrocyte matrix synthesis proteins such as Col II and SOX9 and increased chondrocyte matrix degradation-related enzymes such as MMP-13 simultaneously; however, HExos-loaded HP hydrogels promoted the expression of Col II and decreased the expression of MMP-13 at the same time (Figs. 2E-G). The PCR results showed that the NExos-loaded HP hydrogel did not promote the expression of Col 2a1 at the mRNA level. However, it reduced the expression of MMP-13, and the HExos-loaded HP hydrogel promoted the expression of Col 2a1 at the mRNA level and decreased the expression of MMP-13 at the same time (Fig. 2H). The above results show that the HExos-loaded HP hydrogel could promote the survival, proliferation, and synthesis of the matrix for chondrocytes during OA at the early and middle stages *in vitro*.

Autophagy is a highly conserved, lysosome-mediated degradation mechanism in eukaryotic cells [31,32]. Autophagy undergoes growth and closure to form autophagosomes, which fuse with lysosomes and are subsequently degraded. Elevated autophagy marker LC3-II and decreased p62 protein levels are generally considered markers of enhanced autophagic activity. Studies have confirmed that autophagy is a protective process that maintains articular cartilage homeostasis. Human OA and surgically induced mouse OA are associated with decreased autophagy in articular chondrocytes [33]. Furthermore, a decrease in key regulators of autophagy is accompanied by an increase in chondrocyte apoptosis [33]. Therefore, we examined the effects of Exos-loaded HP hydrogels on autophagy and apoptosis of chondrocytes induced by IL-1β under hypoxic conditions. To evaluate autophagy in chondrocytes, an autophagy staining assay kit with monodansylcadaverine (MDC) was used. MDC is an eosinophilic fluorescent probe that specifically labels autophagosomes by ion capture and binding to membrane lipids, emitting green fluorescence when excited using fluorescent microscopy equipment. To detect apoptosis in chondrocytes, an Annexin V-FITC/PI double-staining apoptosis detection kit was used. The results showed that both NExos and HExos-loaded HP hydrogels increased the IL-1β-induced reduction of chondrocyte autophagy under hypoxic conditions (Figs. 3A and B) while

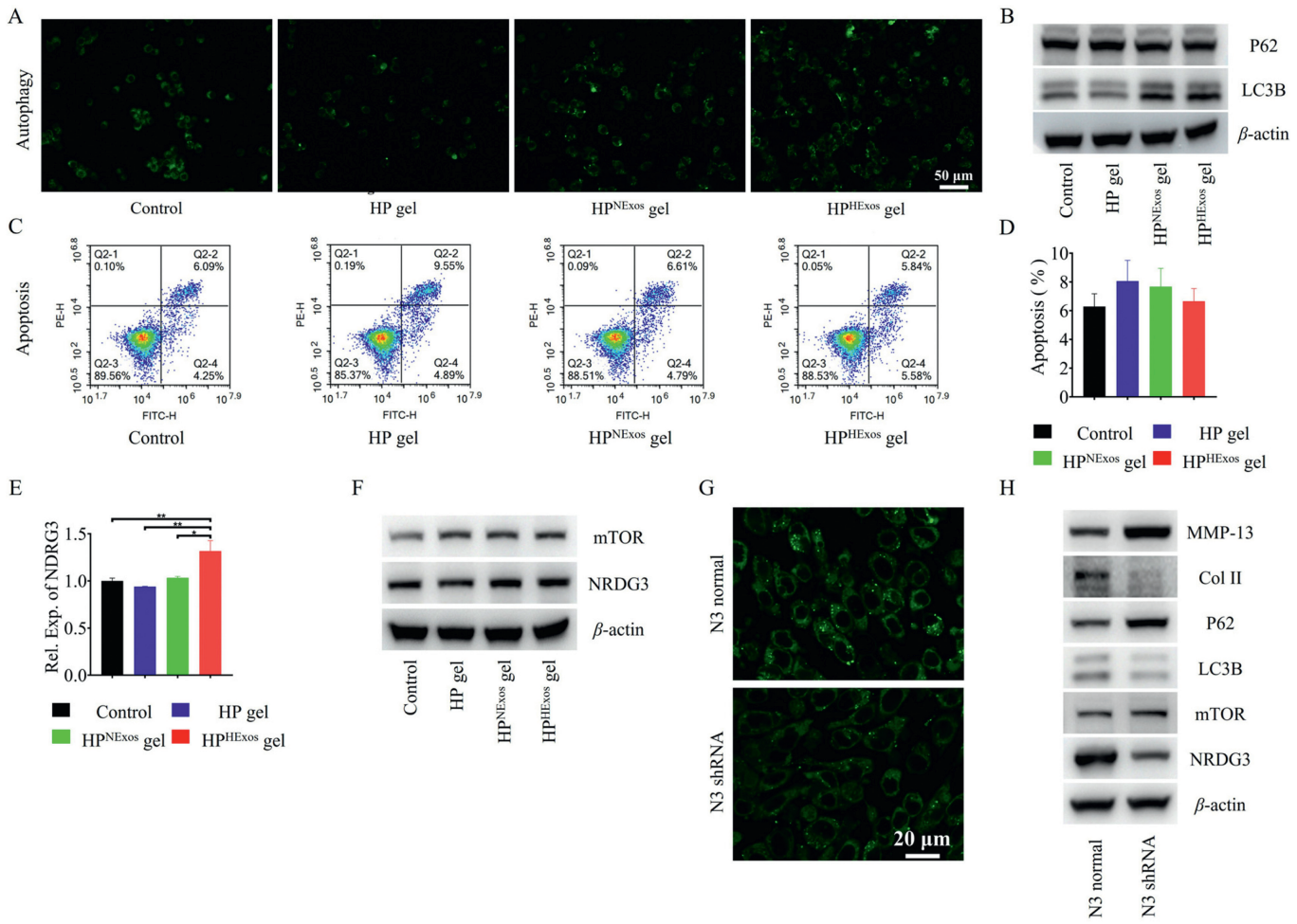

Fig. 3. HExos-loaded HP hydrogel enhanced chondrocyte autophagy and reduced chondrocyte apoptosis by downregulating mTOR expression in response to NDRG3-mediated hypoxia. (A) Representative immunofluorescence images of autophagy, scale bar = 50 μm. (B) Western blotting of LC3B and P62, β-actin was served as a loading control. (C) Representative images of apoptosis. (D) The cell apoptosis of chondrocyte. (E) Relative mRNA expression of NDRG3, one-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$. (F) Western blotting of NDRG3 and mTOR, β-actin was served as a loading control. (G) Representative immunofluorescence images of autophagy, scale bar = 20 μm. (H) Western blotting of NDRG3, mTOR, LC3B, P62, Col II and MMP-13, β-actin was served as a loading control.

resisting IL-1β-induced chondrocyte apoptosis (Figs. 3C and D). However, HExos-loaded HP hydrogels could better maintain autophagy in chondrocytes, as more autophagosomes were labeled with green fluorescence (Fig. 3A). At the same time, the HExos-loaded HP hydrogel could better resist IL-1β-induced chondrocyte apoptosis, and the results were similar to those of the control group (Fig. 3D). These findings suggest that the HExos-loaded HP hydrogel could reduce chondrocyte apoptosis and maintain the normal phenotype of chondrocytes by regulating chondrocyte autophagy better than the NExos-loaded HP hydrogel.

Normal articular cartilage is a hypoxic tissue, and the oxygen content gradually decreases from the surface of the cartilage to depth [4]. Studies have shown that during chronic hypoxia, NDRG3, which is independent of the HIF pathway, is stably expressed and prolongs the hypoxic response of cells during persistent hypoxia [7]. Our previous study showed that the hypoxia response mediated by NDRG3 may be closely related to the occurrence and development of OA [8]. The mammalian target of rapamycin (mTOR) is an evolutionarily conserved kinase that inhibits the transcription of lysosomal biogenesis and lysosomal function by inhibiting the phosphorylation of the ULK1/ATG13/FIP200 complex, thereby preventing autophagosome formation [34]. Our findings show that HExos-loaded HP hydrogels can increase IL-1β-induced chondrocyte autophagy under hypoxic conditions. Other studies have shown that autophagy in chondrocytes is influenced by mTOR [35,36]. Therefore, we hypothesized that in a hypoxic environment, HExos-loaded HP hydrogels might increase autophagy in chondrocytes by inhibiting mTOR expression. To test our hypothesis, chondrocytes were induced with the inflammatory cytokine IL-1β in a hypoxic environment, followed by the addition of Exos-loaded HP hydrogels to examine the expression of NDRG3 and mTOR in chondrocytes. The results showed that, at the mRNA level, HExos-loaded HP hydrogels upregulated the expression of NDRG3 significantly (Fig. 3E). At the protein level, HExos-loaded HP hydrogels upregulated NDRG3 and downregulated mTOR expression (Fig. 3F). To further explore whether NDRG3 could regulate chondrocyte autophagy and maintain chondrocyte phenotype by affecting mTOR expression, we constructed a chondrocyte system with low NDRG3 expression. The expression of NDRG3 in chondrocytes was downregulated by the lentiviral vector, and chondrocytes with normal expression of NDRG3 (N3 normal) and chondrocytes with low expression of NDRG3 (N3 shRNA) were placed in a hypoxic environment, induced by the addition of the inflammatory factor IL-1β, and then HExos-loaded HP hydrogels were added. The results showed that autophagy was reduced in the N3 shRNA group compared to the N3 normal group, as fewer autophagosomes were labeled with green fluorescence (Fig. 3G). Western blotting results showed that compared with the N3 normal group, the N3 shRNA group showed increased expression of mTOR and P62, while the expression of LC3B was decreased (Fig. 3H). Furthermore, compared to the N3 normal group, the N3 shRNA group showed decreased Col II expression and increased MMP-13 expression (Fig. 3H). This suggests that in chondrocytes, the NDRG3-mediated hypoxia response enhanced chondrocyte autophagy, reduced chondrocyte apoptosis, and promoted chondrocyte activity and proliferation by downregulating mTOR expression, thereby maintaining the normal phenotype of chondrocytes.

In conclusion, the addition of HA reduced the degradation of Pluronic F-127 without changing the elastic (G') and viscous (G'') moduli and the sol-gel transition temperature of Pluronic F-127, which is beneficial for the sustained release of loaded Exos. The HP hydrogel loaded with hypoxia-induced ADMSCS-derived Exos maintained the chondrocyte phenotype by enhancing chondrocyte autophagy, reducing chondrocyte apoptosis, and promoting chondrocyte activity and proliferation through the NDRG3-mediated hypoxic response in OA at the early and middle stages.

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.

Acknowledgments

The work was supported by the National Natural Science Foundation of China (Nos. 81960404, 81960401 and 82060308), Guizhou Province Science and Technology Project (Nos. [2019]1429 and [2019]2798), Guizhou Provincial People's Hospital Doctoral Fund (No. GZSYBS[2019]01), Guizhou Provincial People's Hospital Youth Fund (No. GZSYQN[2019]04) and Guizhou Provincial Health Commission Science and Technology Fund (No. gzwkj2021–251).

References

[1] K.D. Allen, L.M. Thoma, Y.M. Golightly, Osteoarthr. Cartil. 30 (2022) 184–195.
[2] L. Zheng, Z. Zhang, P. Sheng, A. Mobasheri, Ageing Res. Rev. 66 (2021) 101249.
[3] A. Krishnamurthy, A.E. Lang, S. Pangarkar, et al., Mayo Clin. Proc. 96 (2021) 2435–2447.
[4] F.J. Zhang, W. Luo, G.H. Lei, Jt. Bone Spine 82 (2015) 144–147.
[5] K. Okada, D. Mori, Y. Makii, et al., Sci. Rep. 10 (2020) 5425.
[6] W. Bouaziz, J. Sigaux, D. Modrowski, et al., Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 5453–5458.
[7] D.C. Lee, H.A. Sohn, Z.Y. Park, et al., Cell 161 (2015) 595–609.
[8] L. Chen, Y. Wang, S. Li, W. Zhou, L. Sun, Arthroplasty 3 (2021) 1.
[9] M. Al-Najar, H. Khalil, J. Al-Ajlouni, et al., J. Orthop. Surg. Res. 12 (2017) 190.
[10] J.M. Lamo-Espinosa, G. Mora, J.F. Blanco, et al., J. Transl. Med. 16 (2018) 213.
[11] D. Garay-Mendoza, L. Villarreal-Martínez, A. Garza-Bedolla, et al., Int. J. Rheum. Dis. 21 (2018) 140–147.
[12] Y. Song, H. Du, C. Dai, et al., Regen. Med. 13 (2018) 295–307.
[13] L. He, T. He, J. Xing, et al., Stem Cell Res. Ther. 11 (2020) 276.
[14] Y. Zhu, Y. Wang, B. Zhao, et al., Stem Cell Res. Ther. 8 (2017) 64.
[15] Y. Wang, D. Yu, Z. Liu, et al., Stem Cell Res. Ther. 8 (2017) 189.
[16] S. Zhang, K.Y.W. Teo, S.J. Chuah, et al., Biomaterials 200 (2019) 35–47.
[17] M.H.M. Nascimento, M.K.K.D. Franco, F. Yokaichyia, et al., Int. J. Biol. Macromol. 111 (2018) 1245–1254.
[18] S. Bhattacharya, A. Ghosh, S. Maiti, et al., J. Control. Release 322 (2020) 357–374.
[19] N. Wei, X. Xu, C. Huang, L. Cao, A.A.P.S. Pharm. Sci. Tech. 21 (2020) 162.
[20] Y.S. Jung, W. Park, H. Park, D.K. Lee, K. Na, Carbohydr. Polym. 156 (2017) 403–408.
[21] Y.S. Kim, F. Guilak, Int. J. Mol. Sci. 23 (2022) 8662.
[22] M. Torres-Torrillas, M. Rubio, E. Damia, et al., Int. J. Mol Sci. 20 (2019) 3105.
[23] A. Hassanshahi, M. Hassanshahi, S. Khabbazi, et al., J. Cell. Physiol. 234 (2019) 7903–7914.
[24] D. Murata, R. Fujimoto, K. Nakayama, Int. J. Mol. Sci. 21 (2020) 3589.
[25] L. Mazini, L. Rochette, M. Amine, G. Malka, Int. J. Mol. Sci. 20 (2019) 2523.
[26] X. Sang, X. Zhao, L. Yan, et al., Tissue Eng. Regen. Med. 19 (2022) 629–642.
[27] H. Katagiri, L.F. Mendes, F.P. Luyten, Osteoarthr. Cartil. 25 (2017) 1531–1540.
[28] X. Ouyang, Y. Ding, L. Yu, et al., Clin. Immunol. 233 (2021) 108889.
[29] R.R. Yammani, R.F. Loeser, Arthr. Rheumatol. 66 (2014) 1266–1271.
[30] G. Han, H. Shao, X. Zhu, et al., Carbohydr. Polym. 89 (2012) 870–875.
[31] M.K. Lotz, B. Caramés, Nat. Rev. Rheumatol. 7 (2011) 579–587.
[32] M.T. Valenti, L. Dalle Carbonare, D. Zipeto, M. Mottes, Int. J. Mol. Sci. 22 (2021) 2700.
[33] B. Caramés, N. Taniguchi, S. Otsuki, F.J. Blanco, M. Lotz, Arthr. Rheum. 62 (2010) 791–801.
[34] N. Deleyto-Seldas, A. Efeyan, Front. Cell. Dev. Biol. 9 (2021) 655731.
[35] J.F. Xue, Z.M. Shi, J. Zou, X.L. Li, Biomed. Pharmacother. 89 (2017) 1252–1261.
[36] K. Sun, J. Luo, J. Guo, et al., Osteoarthr. Cartil. 28 (2020) 400–409.