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ZEB2 knockdown inhibits interleukin-1β-induced cartilage degradation and inflammatory response through the Wnt/β-catenin pathway in human chondrocytes

ZB Li[1], YZ Li[2], ZP Sun[1], WX Li[1], Z Xiao[1], F Wang[1]

[1]Trauma Ward 2, The Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang, Shaanxi, PR China
[2]Department of Orthopedics, Shangluo Chinese Medicine Hospital, Shangluo, Shaanxi, PR China

Objective: Osteoarthritis (OA) is a degenerative disease of the joints characterized by inflammation and cartilage degeneration. Zinc finger E-box binding homeobox 2 (*ZEB2*) contains various function domains that interact with multiple transcription factors involved in various cellular functions. However, the function of *ZEB2* in OA has not been clearly illustrated.

Method: Interleukin-1β (IL-1β) was used to establish an OA model in vitro. We quantified the *ZEB2* expression in cartilage tissues from OA patients and IL-1β-induced chondrocytes through reverse transcription–quantitative polymerase chain reaction and Western blot. We then used functional assays to explore the function of *ZEB2* during OA progression.

Results: *ZEB2* expression was increased in OA cartilage tissues and chondrocytes. The silencing of *ZEB2* increased aggrecan and collagen II levels, and reduced the content of matrix metalloproteinase-3 (MMP-3), MMP-9, and MMP-13. *ZEB2* knockdown inhibited the effects of IL-1β on the production of nitric oxide and prostaglandin E_2, and the expression of inducible nitric oxide synthase and cyclooxygenase-2. *ZEB2* inhibition also suppressed the levels of IL-6 and tumour necrosis factor-α, and increased the IL-10 level in IL-1β-treated cells. Mechanically, *ZEB2* knockdown blocked the activation of the Wnt/β-catenin pathway in chondrocytes.

Conclusion: Knockdown of *ZEB2* alleviated IL-1β-induced cartilage degradation and the inflammatory response through the Wnt/β-catenin pathway in chondrocytes.

Osteoarthritis (OA) is a degenerative disease of the joints and the most common cause of musculoskeletal dysfunction (1, 2). A 2023 study indicated that with the increased severity of OA, treatment for OA patients will have a poorer outcome (3). Knee OA is the most common type of OA, with clinical symptoms such as hyperosteogeny and local inflammation of the subchondral bone plate and joint edge (4). The aetiology of OA is unknown. However, there is evidence demonstrating that inflammatory cytokines are engaged in the development and progression of OA (5, 6). Among these cytokines, interleukin-1β (IL-1β) has a significant effect on OA initiation (7). The release of IL-1β results in lower expression of aggrecan and collagen II, the phenotypic markers of chondrocytes (8). Stimulation of IL-1β enhances the production and release of inflammatory cytokines and factors, contributing to cell dysfunction (9–11). The expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) can cause the increased production of prostaglandin E_2 (PGE_2) and nitric oxide (NO) in chondrocytes (12). The action of PGE_2 has been confirmed in joint pain and bone resorption (13). In addition, iNOS induces the production of NO, which leads to the increased release of matrix metalloproteinases (MMPs) in OA (14, 15). Cytokines result in the degradation of cartilage matrix via increasing MMP expression (16).

As a transcriptional factor, zinc finger E-box binding homeobox 2 (*ZEB2*) contains various function domains that interact with multiple transcription factors. Hypoxia signals, transforming growth factor-β (*TGF*-β), tumour necrosis factor-α (*TNF*-α), and *IL-1* have been shown to be engaged in regulating *ZEB2* expression (17). Previous studies have reported that *ZEB2* regulates the inflammatory response in many diseases. *ZEB2* was found to be upregulated in the lung tissues of mice with pulmonary emphysema compared with normal mice, and *ZEB2* overexpression promoted cellular ageing and inflammation in vivo (18). Katsura et al reported that the knockdown of *ZEB2* resulted in the downregulation of genes encoding inflammatory

F Wang, Trauma Ward 2, The Affiliated Hospital of Shaanxi University of Chinese Medicine, No. 2, Weiyang West Road, Qindu District, Xianyang, Shaanxi 712000, PR China.
E-mail: fengkingf@126.com

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cytokines, including *IL-6* and *IL-8*, and led to poor prognosis in breast cancer patients (19). In addition, the downregulation of *ZEB2* inhibited the occurrence and development of osteosarcoma (20, 21). Previous research has characterized that *ZEB2* was a more highly expressed gene in hip chondrocytes in OA (22). Another study also indicated that *ZEB2* is an upregulated gene during OA and investigated the effect of *ZEB2* on alleviating OA (23). However, more validation of the role and regulatory mechanism of *ZEB2* in OA is needed. This study investigated the function of *ZEB2* on articular cartilage degradation and inflammation in chondrocytes treated with IL-1β.

In the present study, the expression of *ZEB2* was increased in IL-1β-induced chondrocytes. *ZEB2* inhibition reduced cartilage degradation and the occurrence of inflammation by blocking Wnt/β-catenin activation in OA in an in vitro model. The results demonstrate that *ZEB2* could be a new molecular target for OA therapy.

Method

Human cartilage tissue

Human OA cartilage tissue was obtained from 16 patients with OA (age range 46–68 years, mean age 57.3 years; seven males and nine females) who presented with serious joint movement limitation and pain in the knee, but with no complications, and had been diagnosed by three professional rheumatologists to meet the criteria for surgery. Normal human articular cartilage was obtained from 16 healthy participants (11 males and five females) who had experienced traumatic fractures with no symptoms or history of OA.

Informed consent was obtained from all participants. The study was carried out in accordance with the Declaration of Helsinki and approved by the ethics committee of the Affiliated Hospital of Shaanxi University of Chinese Medicine (number SZFYIEC-PJ-2022[90]). All clinical cartilage tissue specimens were collected immediately after the participants had undergone total hip arthroplasty, and were frozen and stored at −80°C. Before the following procedures, such as reverse transcription–quantitative polymerase chain reaction (RT-qPCR) and Western blotting, the tissues were fragmented into small chippings and then digested using 0.25% trypsin (Merck, Darmstadt, Germany) for 60 min.

Cell culture and building the OA in vitro model

A human cartilage cell line, CHON-001, was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal bovine serum (FBS) and 1% penicillin–streptomycin was used to culture cells at 37°C with 5% CO_2. IL-1β reagent was purchased from Sigma Aldrich (St Louis, MO, USA) and used to stimulate chondrocytes for building the OA in vitro model at a series of concentrations (1, 5, 10, and 20 ng/mL).

Cell transfection and treatment

The short hairpin RNA (shRNA) against *ZEB2* (sh-ZEB2) and negative control shRNA (sh-NC) were synthesized by GenePharma (Shanghai, China), with the following sequence: sh-ZEB2 (5'-CCCACCATGAATAGTAATTTA-3'). Then, the shRNAs were subcloned on to pLenti vectors and subsequently transfected into 293 T cells using Lipofectamine® 3000 Invitrogen (Carlsbad, CA, USA). The transfected cells were incubated for 48 h in medium containing 5% FBS. The harvested supernatant was stored at −80°C. Then, incubation was carried out for 8 h using chondrocytes at 50% confluence in the obtained lentivirus medium; and finally, cells were cultured in DMEM (10% FBS) supplemented with 10 μg/mL puromycin for over 24 h to obtain stably transfected cells.

The pcDNA3.1-ZEB2 and pcDNA3.1 vectors were obtained from RiboBio Co. (Guangzhou, China). When chondrocytes had grown to about 80% confluence in 24-well plates, the pcDNA3.1 and pcDNA3.1-ZEB2 vectors were transfected into chondrocytes using Lipofectamine 3000 (Invitrogen).

To investigate the role of the Wnt pathway in IL-1β-induced OA progression, a Wnt agonist, LiCl (10 ng/mL), was used to treat the transfected chondrocytes for another 24 h before IL-1β stimulation to perform the rescue assay for *ZEB2* silencing. The treatment procedure is presented in Figure 1.

Cell viability assay

Transfected or untransfected cartilage cells were grown in 24-well plates (1×10^5 cells/well) and then exposed to IL-1β (10 ng/mL) for 24 h. Cell viability was assessed using a Cell Counting Kit-8 (CCK-8) (Dojindo Molecular Technologies, Shanghai, China), under a microplate reader (Applied Biosystems, Shanghai, China) at 450 nm wavelength.

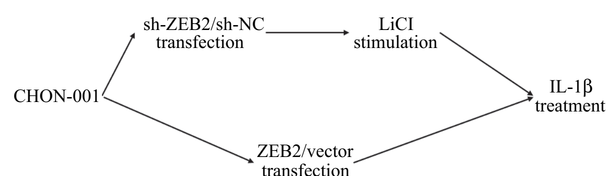

Figure 1. Illustration of the treatment procedure in the in vitro experiments.

Cell apoptosis evaluation

Flow cytometry was used to evaluate the cell apoptosis of cartilage cells in different groups. First, the cells were resuspended using the binding buffer (500 µL) after treatments and stained using fluorescein isothiocyanate (FITC) Annexin V (5 µL) along with 10 µL propidium iodide (PI) solution. The incubation was conducted at room temperature in the dark for 15 min. Subsequently, a Moflo XDP flow cytometer purchased from Beckman Coulter (Miami, FL, USA) was used to test the samples. The final apoptotic data were shown as percentages.

RT-qPCR

TRIzol reagent (Invitrogen, USA) was used to extract total RNA from cartilage tissues and cells, according to the protocol. Reverse transcription of *ZEB2, MMP-3, MMP-9, MMP-13, IL-6, TNF*-α, and *IL-10* was performed using a Prime-Script RT reagent kit (TaKaRa, Dalian, China). Gene expression was quantified using a TaqMan quantitation kit (Applied Biosystems, Foster City, CA, USA) on a Light Cycler instrument Bio-Rad (Hercules, CA, USA). The final expression data for the target genes were calculated using the $2^{-\Delta\Delta CT}$ method and normalized to *GAPDH* (24).

Western blot analysis

Total protein was extracted with RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) after the specimens had been separated from the cultured systems and washed. The protein content was measured using a BCA assay kit (Abcam, Shanghai, China). Next, 10–15% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) gels were used to separate equal amounts (50 ng) of proteins; then, the protein was transferred to polyvinylidene difluoride (PVDF) membranes (GE Healthcare, Freiburg, Germany) and blocked with 5% non-fat milk. Primary antibodies against ZEB2 (ab223688), collagen II (ab34712), aggrecan (ab3778), iNOS (ab178945), COX-2 (ab179800), Wnt1 (ab15251), β-catenin (ab16051), c-Myc (ab32072), and GAPDH (ab9485) were purchased from Abcam (Cambridge, MA, USA) and used for primary incubation at 4°C overnight. Then, membranes were incubated with horseradish peroxidase (HRP)-conjugated species-specific secondary antibody, followed by an enhanced chemiluminescence assay (Pierce Chemical Company, Rockford, IL, USA). Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used as the loading control for the detected proteins.

Enzyme-linked immunosorbent assay (ELISA)

After the transfection, chondrocytes were cultured in 24-well plates (density 1×10^5 cells/well) and then exposed to 10 ng/mL IL-1β for 24 h. The level of PGE_2, and the concentrations of aggrecan, MMPs (MMP-3, MMP-9, and MMP-13), and inflammatory cytokines (IL-6, TNF-α, and IL-10) in the supernatant of the cultured system were detected by specific ELISA kits (Abebio Science Co., Wuhan, China: MMP-3, cat no. AE33262HU; MMP-9, cat no. AE63046HU; MMP-13, cat no. AE63045HU; IL-6, cat no. AE62763 HU; TNF-α, cat no. AE13959HU; IL-10, cat no. AE38536HU; aggrecan, cat no. AE23321HU; and BIOESN tech, Shanghai, China: PGE_2, cat no. BES0753K).

Griess reaction

The Griess reaction was performed to evaluate the concentration of NO. In brief, the cultured cells were isolated after the treatments to collect the supernatants for analysis. The concentration of NO production was measured as indicated by NO_2^- accumulation, which was detected using the Griess reaction according to a previous study (25).

Statistical analysis

SPSS version 19.0 (IBM Corp., Armonk, NY, USA) was used to analyse the data. Statistically significant differences among three groups were analysed using a one-way analysis of variance (ANOVA) test. Differences between two groups were identified by Student's t-test. All data are shown as the mean ± sem. A p-value less than 0.05 was considered statistically significant.

Results

ZEB2 expression was increased in cartilage tissues of OA patients and IL-1β-induced chondrocytes

The expression of *ZEB2* was first determined in OA cartilage tissues and IL-1β-induced chondrocytes. As indicated in Figure 2(A), the protein level of ZEB2 was significantly increased in OA cartilage tissues compared with normal cartilage tissues using Western blotting. Furthermore, IL-1β was used to stimulate CHON-001 cells to build an OA model in vitro. Cell viability was suppressed by IL-1β treatment in a dose-dependent manner (Figure 2B). RT-qPCR and Western blotting indicated that the expression of *ZEB2* was also significantly upregulated in IL-1β-treated chondrocytes in a dose-dependent manner (Figure 2C, D).

Suppression of *ZEB2* decreased the effect of IL-1β on cartilage degradation

To further investigate the role of *ZEB2* in vitro, the knockdown and overexpression plasmids of *ZEB2* were transfected into chondrocytes. With the transfection of

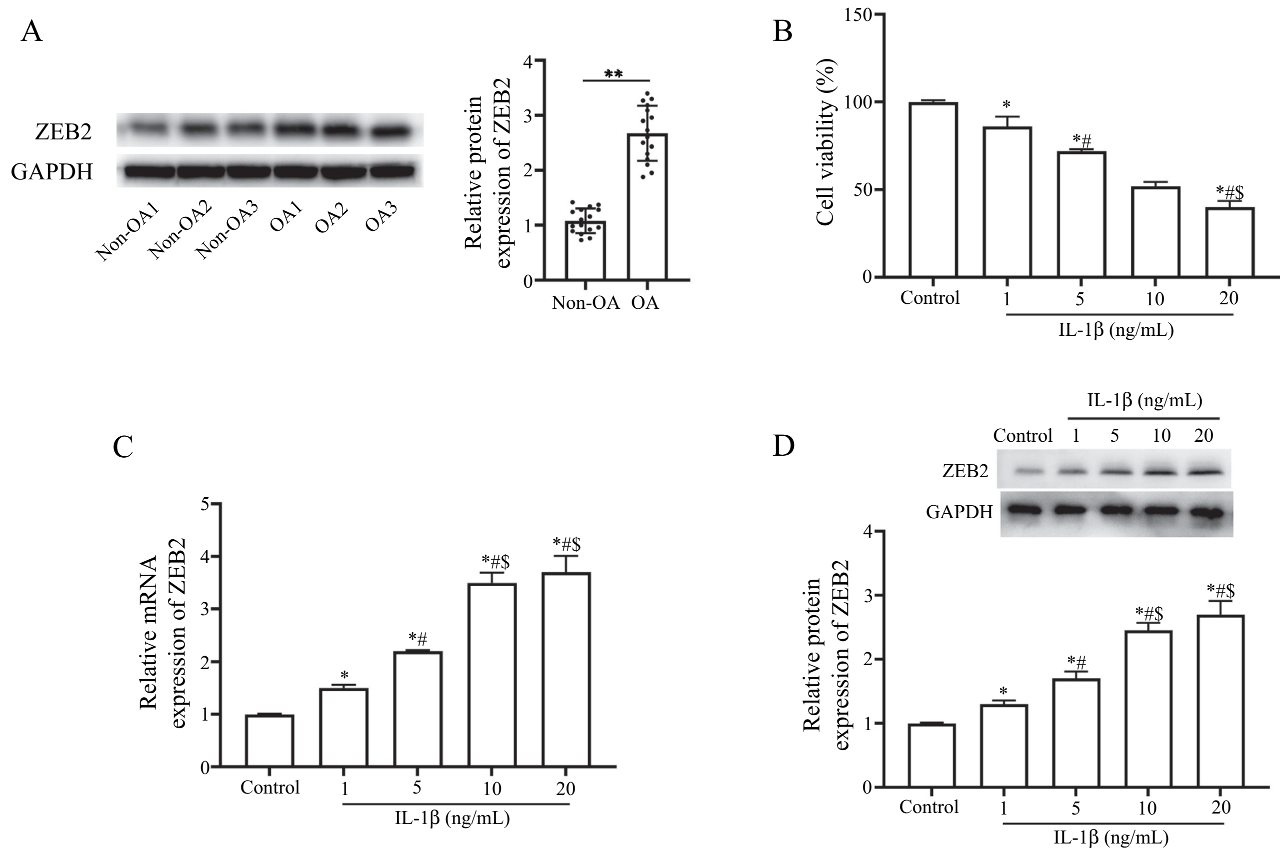

Figure 2. Expression of ZEB2 was increased in cartilage tissues of osteoarthritis (OA) patients and interleukin-1β (IL-1β)-induced chondrocytes. (A) The protein level of ZEB2 in cartilage tissues of OA patients was detected by Western blot. The columns are presented as the mean ± sd (n = 16). **p < 0.01 compared with Non-OA group. (B) The CCK-8 assay was used to measure cell viability. Cells were exposed to a series of concentrations of IL-1β (1, 5, 10, and 20 ng/mL) for 24 h. (C) The mRNA expression of ZEB2 was measured by reverse transcription–quantitiative polymerase chain reaction. (D) ZEB2 protein expression was detected by Western blot. *p < 0.05 compared with control group; #p < 0.05 compared with 1 ng/mL IL-1β group; $p < 0.05 compared with 5 ng/mL IL-1β group. Columns are presented as the mean ± sem obtained from three repeat experiments.

ZEB2 knockdown plasmid, the expression of *ZEB2* was significantly decreased. After pcDNA3.1-ZEB2 was transfected into chondrocytes, the expression of *ZEB2* was significantly upregulated (Figure 3A). IL-1β treatment significantly inhibited cell viability and the protein expression of aggrecan and collagen II, which was reversed by *ZEB2* suppression, while *ZEB2* upregulation aggravated the inhibitory effect of IL-1β on cell viability, aggrecan, and collagen II in chondrocytes (Figure 3B–G). In addition, the inhibition of *ZEB2* attenuated the promoted effects of IL-1β on cell apoptosis and the mRNA expressions and content of *MMP-3, MMP-9*, and *MMP-13*, while their increases induced by IL-1β were further aggravated by *ZEB2* upregulation (Figure 3H–M).

Suppression of *ZEB2* alleviated the IL-1β-induced inflammatory response in chondrocytes

Next, we assessed the effects of *ZEB2* on the production of NO and PGE_2. The results showed that the suppression of *ZEB2* significantly decreased the IL-1β-induced increases in the content of NO and PGE_2 in the supernatant, while *ZEB2* overexpression further promoted the production of NO and PGE_2 (Figure 4A, B). With the suppression of *ZEB2*, the protein expression levels of iNOS and COX-2 were significantly downregulated, and the upregulation of *ZEB2* exerted the opposite effects (Figure 4C–E). Subsequently, we detected the expression and secretion of IL-6, TNF-α, and IL-10. As shown in Figure 4(F), the results proved that the IL-1β-stimulated mRNA expression of *IL-6* and *TNF*-α in chondrocytes was significantly suppressed by *ZEB2* knockdown and increased by the overexpression of *ZEB2*. The downregulation of IL-10 in IL-1β-induced cells was markedly elevated by *ZEB2* silencing, and further decreased by *ZEB2* overexpression. In addition, the effects of *ZEB2* inhibition and overexpression on the secretion of IL-6, TNF-α, and IL-10 in cultured medium were similar to the mRNA expression of *IL-6* and *TNF*-α in chondrocytes (Figure 4G–I).

Suppression of *ZEB2* inhibited the Wnt/β-catenin pathway

Western blot analysis demonstrated that the protein expression levels of Wnt1, β-catenin, and c-Myc, key proteins in

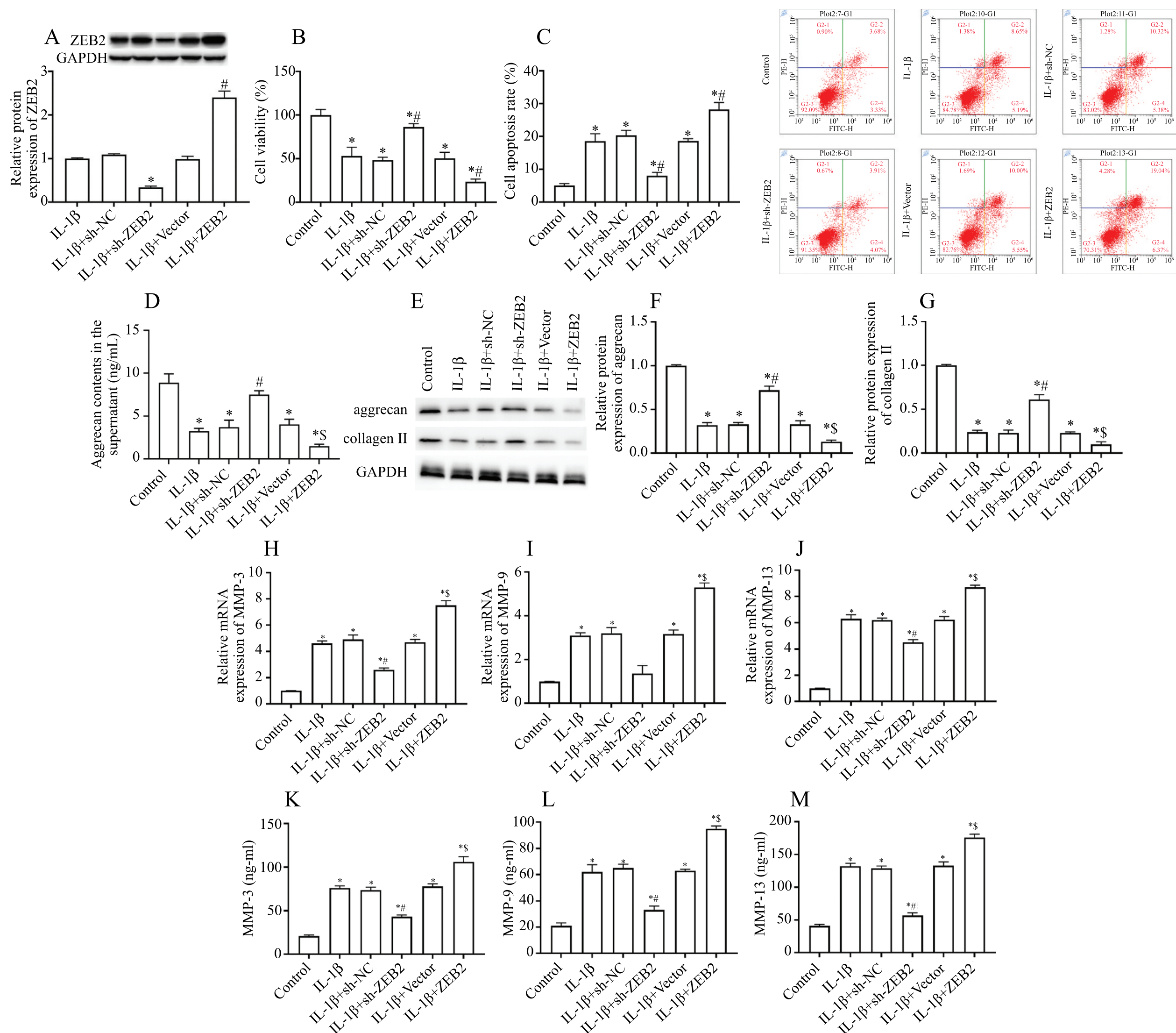

Figure 3. ZEB2 inhibition reduced cartilage degradation induced by interleukin-1β (IL-1β) in chondrocytes. Cells were transfected with ZEB2 knockdown or overexpression plasmid, and then cultured in IL-1β (10 ng/mL) for 24 h. (A) The transfection effect was assessed by Western blot in chondrocytes. *p < 0.05 compared with IL-1β + sh-NC group; #p < 0.05 compared with IL-1β + vector group. (B, C) Cell viability and apoptosis were measured using CCK-8 and flow cytometry assays. (D–F) Expression of aggrecan and collagen II protein was measured by Western blot in chondrocytes. (G–J) mRNA levels of matrix metalloproteinases (MMPs) were assessed by reverse transcription–quantitiative polymerase chain reaction in chondrocytes. (D, K–M) The release of aggrecan and MMPs was examined by enzyme-linked immunosorbent assay. *p < 0.05 compared with control group; #p < 0.05 compared with IL-1β + sh-RNA group; $p < 0.05 compared with IL-1β + vector group. Columns are presented as the mean ± sem (n ≥ 3).

the Wnt/β-catenin signalling pathway, were significantly increased by the treatment with IL-1β. However, their expression was significantly lower in the IL-1β + sh-ZEB2 group than in the IL-1β + sh-NC group (Figure 5).

Activation of the Wnt/β-catenin pathway reversed the effects of *ZEB2* knockdown on cartilage degradation

To investigate whether *ZEB2* knockdown suppressed IL-1β-induced cartilage degradation through the Wnt/β-catenin signalling pathway, the Wnt/β-catenin signalling pathway was activated by a Wnt agonist, LiCl. As shown in Figure 6(A)–(E), the protein levels of Wnt1, β-catenin, and c-Myc reduced by the silencing of *ZEB2* were significantly increased in IL-1β-stimulated cells after LiCl treatment. The upregulation of aggrecan and collagen II caused by *ZEB2* knockdown was decreased by the Wnt agonist. In addition, the content of MMP-3, MMP-9, and MMP-13 in supernatants in the IL-1β + sh-ZEB2 + LiCl group was significantly higher than in the IL-1β + sh-ZEB2 group (Figure 6F–H).

Wnt agonist reversed the effect of *ZEB2* knockdown on inflammatory responses

Next, we investigated the involvement of the Wnt/β-catenin signalling pathway in the suppressive effect

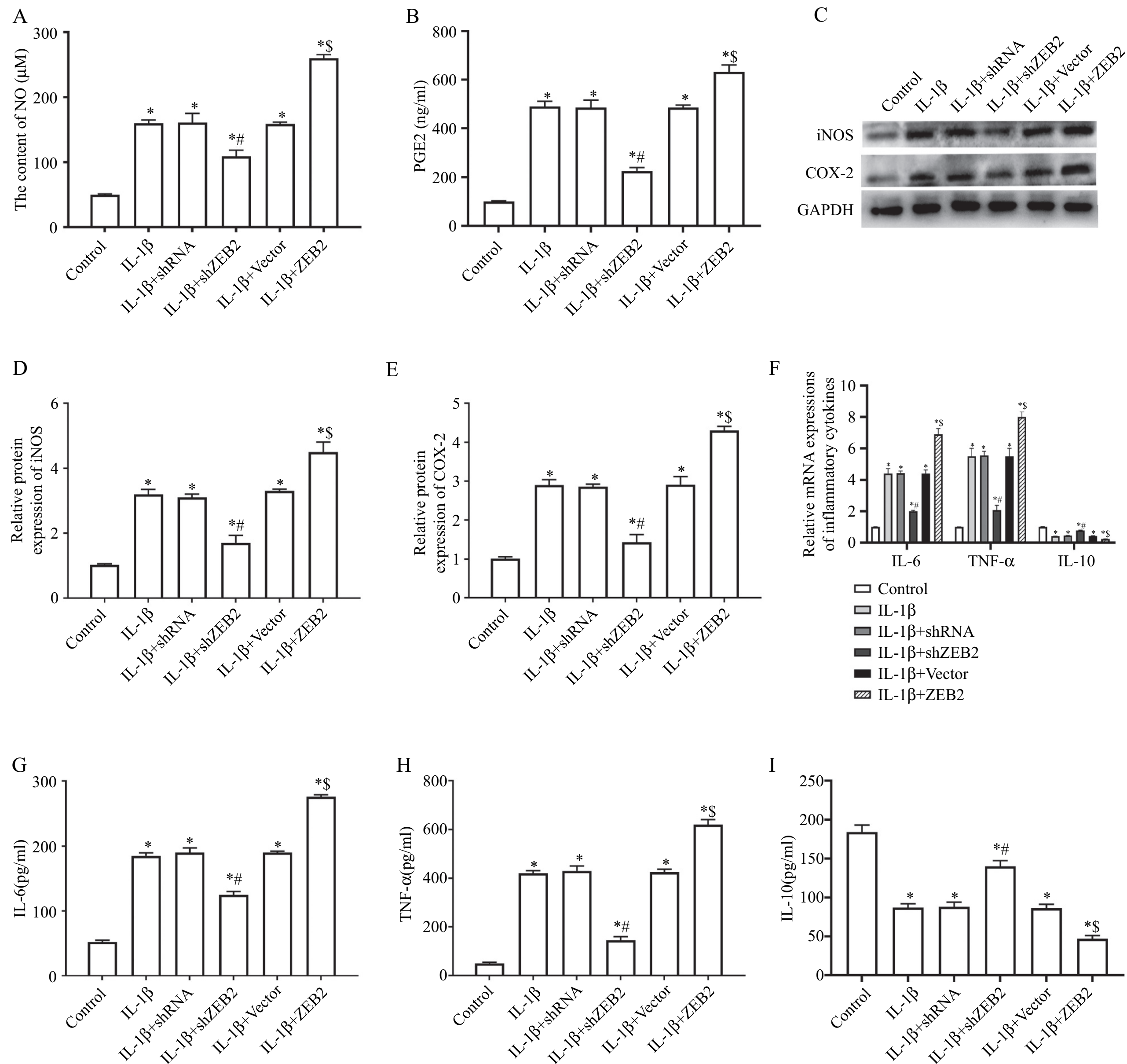

Figure 4. ZEB2 regulated the interleukin-1β (IL-1β)-induced production of inflammatory mediators and cytokines in chondrocytes. Cells were transfected with ZEB2 knockdown or overexpression plasmid, and then cultured in IL-1β (10 ng/mL) for 24 h. (A) The Griess method was used to assess the level of nitric oxide (NO). (B) Production of prostaglandin E_2 (PGE_2) in supernatants was assessed by enzyme-linked immunosorbent assay (ELISA). (C–E) Protein expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) was examined by Western blot in chondrocytes. (F) mRNA expression of IL-6, tumour necrosis factor-α (TNF-α), and IL-10 in chondrocytes was assessed using reverse transcription–quantitiative polymerase chain reaction. (G–I) The content of IL-6, TNF-α, and IL-10 in the cultured medium was measured by ELISA. *p < 0.05 compared with control group; #p < 0.05 compared with IL-1β + sh-NC group; $p < 0.05 compared with IL-1β + vector group. Columns are presented as the mean ± sem (n ≥ 3).

of *ZEB2* knockdown on the IL-1β-induced inflammatory response. As shown in Figure 7(A)–(C), the suppressed protein expression levels of iNOS and COX-2, and the decreased content of proinflammatory cytokines (IL-6 and TNF-α) induced by *ZEB2* knockdown, were reversed by LiCl treatment. Moreover, the upregulation of the IL-10 level in IL-1β-treated cells transfected with sh-ZEB2 plasmids was decreased after LiCl treatment (Figure 7D).

Discussion

The present study investigated the effects and functional mechanism of *ZEB2* in IL-1β-induced injury of chondrocytes. We found that the expression levels of *ZEB2* were increased in the cartilage tissues of OA patients and IL-1β-treated chondrocytes. These findings imply that *ZEB2* plays important roles in OA development. As enzymes secreted by chondrocytes that can degrade

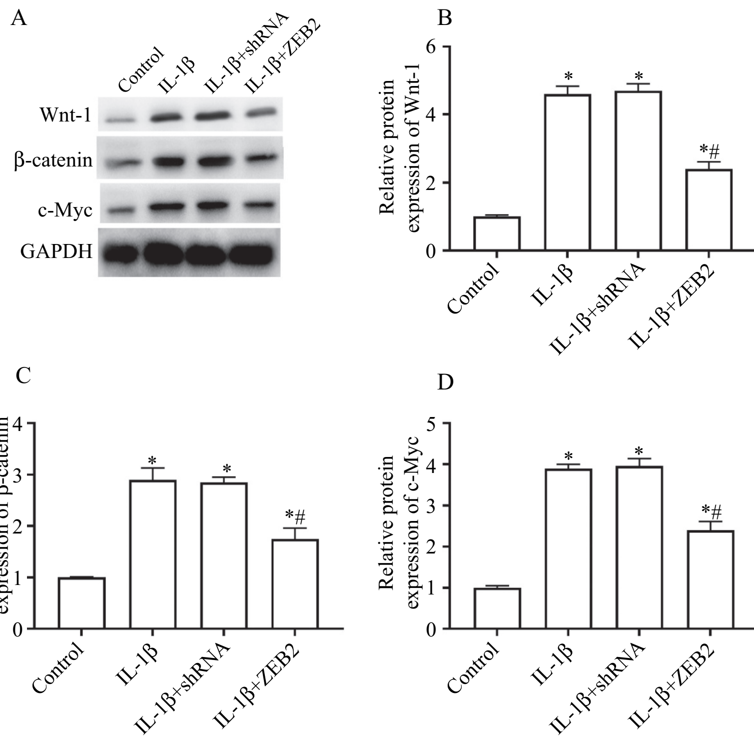

Figure 5. Effect of ZEB2 inhibition on interleukin-1β (IL-1β)-induced Wnt/β-catenin activation in chondrocytes. Cells were transfected with ZEB2 knockdown or overexpression plasmid, and then cultured in IL-1β (10 ng/mL) for 24 h. (A–D) Protein expression of Wnt1, β-catenin, and c-Myc in chondrocytes was assessed by Western blot. *p < 0.05 compared with control group; #p < 0.05 compared with IL-1β + sh-NC group. Columns are presented as the mean ± sem.

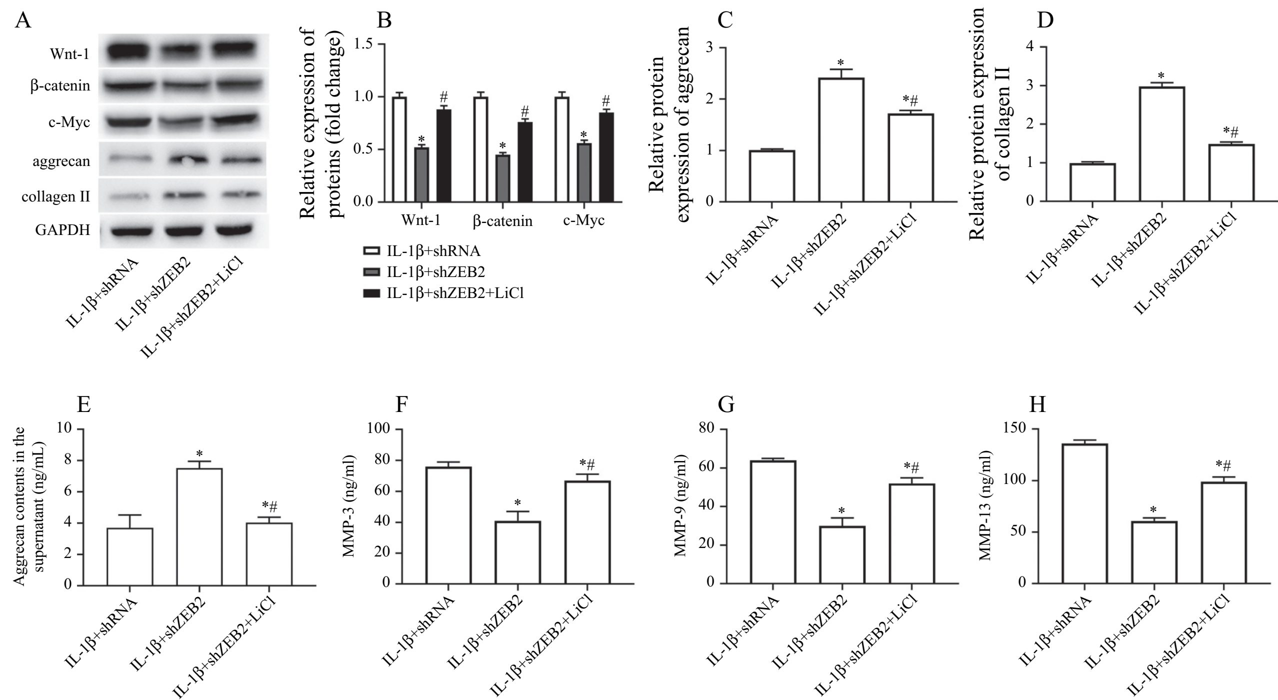

Figure 6. Wnt agonist reversed the effect of ZEB2 knockdown on cartilage degradation. Human chondrocytes transfected with sh-ZEB2 or sh-NC were treated with 10 mM LiCl for 24 h followed by interleukin-1β (IL-1β) (10 ng/mL). (A) Protein levels of Wnt1, β-catenin, c-Myc, aggrecan, and collagen II were assessed by performing Western blot in IL-1β-treated cells. (B) Expression of Wnt1, β-catenin, and c-Myc. (C, D) Protein expression of aggrecan and collagen II in chondrocytes. (E–H) The content of aggrecan and matrix metalloproteinases (MMPs) in supernatants was examined by enzyme-linked immunosorbent assay. *p < 0.05 compared with IL-1β + sh-NC group; #p < 0.05 compared with IL-1β + sh-ZEB2 group. Columns are presented as the mean ± sem (n ≥ 3).

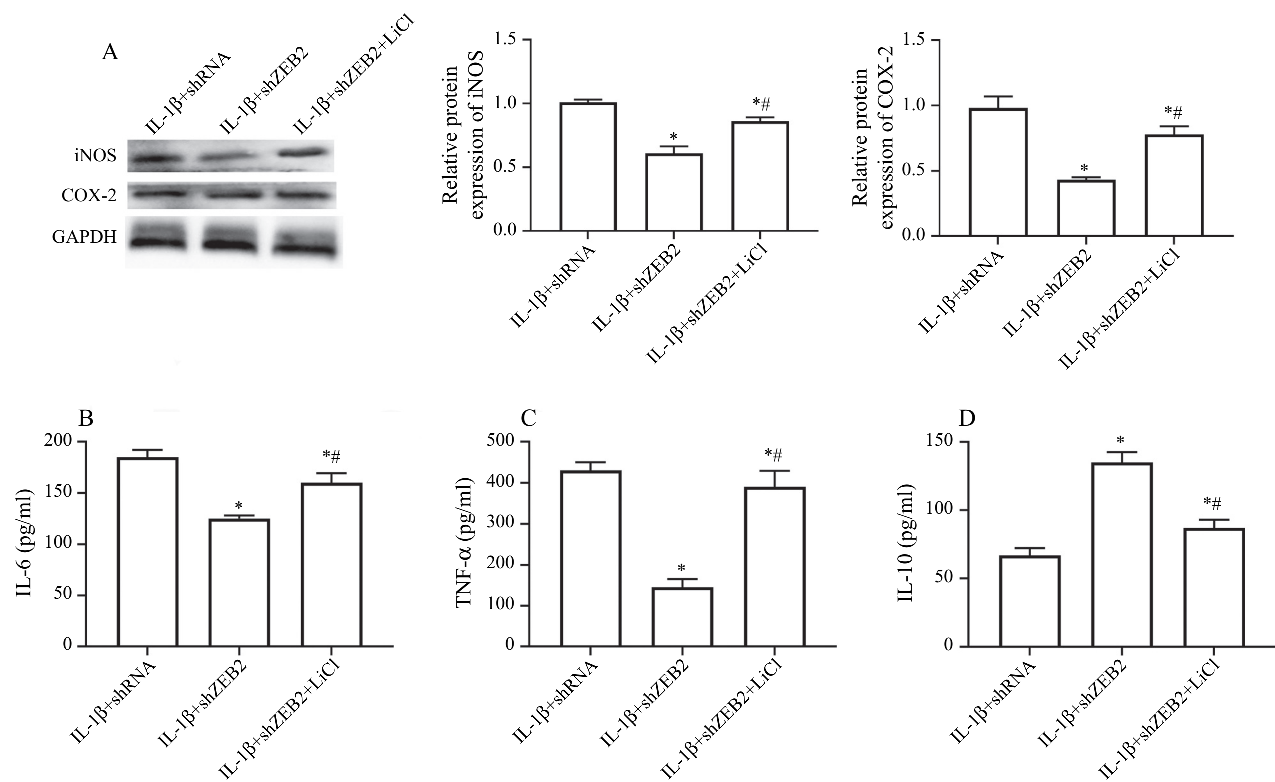

Figure 7. Wnt agonist reversed the effect of ZEB2 knockdown on the inflammatory response. Human chondrocytes transfected with sh-ZEB2 or sh-NC were treated with 10 mM LiCl for 24 h followed by interleukin-1β (IL-1β) (10 ng/mL). (A) Protein expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) was assessed by Western blot. (B)–(D) The content of IL-6, tumour necrosis factor-α (TNF-α), and IL-10 in the cultured medium was measured by enzyme-linked immunosorbent assay. *$p < 0.05$ compared with IL-1β + sh-NC group; #$p < 0.05$ compared with IL-1β + sh-ZEB2 group. Columns are presented as the mean ± sem ($n \geq 3$).

cartilage matrix components, the increased secretion of MMPs will aggravate cartilage matrix damage during the process of OA (26). Yao et al indicated that the upregulation of *ZEB2* played an osteosarcoma-suppressing role, and *ZEB2* knockdown inhibited the protein levels of MMP-2 and MMP-9 in MG-63 cells (20, 27). Moreover, *ZEB2* overexpression increased the expression of the MMP family in endotheliocytes (28). Our data showed that *ZEB2* knockdown decreased the cartilage degradation by reducing the content of MMPs. Thus, these findings suggest that *ZEB2* silencing may have a protective effect on OA by inhibiting cartilage degradation.

Previous studies have shown that inflammation plays a significant role in OA by inducing the release of multiple inflammatory cytokines and mediators (29). Inflammatory factor IL-1β interferes with the normal function of chondrocytes by worsening the microenvironment of chondrocytes, causing joint cartilage degeneration, and accelerating the pathological progress of OA (30, 31). Several studies have indicated that suppressing the inflammatory response of chondrocytes is conducive to controlling and suppressing the progression of OA, and this is becoming a key link in the treatment of OA (12, 32, 33). The aggravated inflammatory response and accumulated local inflammatory cytokines facilitate the degradation of cartilage during OA pathogenesis (34). Katsura et al reported that *ZEB1* and/or *ZEB2* knockdown led to the depression of inflammatory cytokines, such as IL-6 and IL-8, connected with poor prognosis in breast cancer patients (19). In rats with chronic constriction injury, *ZEB2* has been shown to be remarkably upregulated in a time-dependent manner, and the loss of *ZEB2* depressed the neuroinflammation process via the inhibition of *COX-2, IL-1*β, and *IL-6* in rat microglial cells (35). In addition, *ZEB2* was notably upregulated in the lungs of mice in a chronic obstructive pulmonary disease model, and overexpression of *ZEB2* promoted cellular senescence and inflammatory responses (18). However, the expression of *ZEB2* was reported to be significantly downregulated in response to lipopolysaccharide, and overexpression of *ZEB2* resulted in a decrease in TNF-α and IL-6 secretion in HK-2 cells (36). As a member of the same family as *ZEB2, ZEB1* was found to be upregulated in knee OA articular cartilage tissues and contributed to OA progression, as well as cartilage senescence (37, 38). Until now, the direct evidence demonstrating the regulatory role of *ZEB2* in OA pathogenesis has been weak. From previous studies, we learned that *ZEB2* is a key mediator gene in various

inflammatory diseases, including OA, in which the core pathogenic reaction of the body is an inflammatory response. In addition, it was known that the main immune cell environment of chondrocytes is macrophages, and regulating immune infiltration has also been indicated as a crucial way to address OA problems (39). Kim et al demonstrated that *ZEB2* had a positive correlation with macrophage infiltration and could be a meaningful diagnostic target for ovarian cancer (40). In atherosclerosis, a cardiac disease with an inflammatory pathological progress similar to OA, the downregulation of *ZEB2* was considered to have the effect of inhibiting the myocardial inflammatory reaction and alleviating pathological progression (41). Thus, we inferred that *ZEB2* may play a role in OA pathogenesis. As a conclusion, our study found that the inhibition of *ZEB2* decreased the IL-1β-induced inflammatory response. These findings suggest that *ZEB2* may play a bidirectional role in inflammation.

The Wnt/β-catenin signalling pathway was involved in various cellular events, such as cell proliferation, differentiation, and migration, as well as cartilage homoeostasis and joint remodelling (42, 43). As demonstrated in previous studies, the canonical pathway of Wnt/β-catenin signalling is the stimulation of Wnt in the subcellular location and the intracellular activation of β-catenin (44). According to current research in humans, Wnt/β-catenin signalling has been indicated as a susceptibility factor during OA progression. Overactivation of Wnt/β-catenin signalling has been proven to be a crucial trigger for OA degeneration (45). The inhibition of the Wnt/β-catenin pathway has a protective role in the inflammatory response and cartilage degradation in OA (46), whereas β-catenin upregulation in degenerative cartilage indicates that a diminished capacity to limit Wnt signalling results in cartilage loss. Chen et al pointed out that salinomycin could serve as a therapeutic agent during OA pathogenesis, by depressing Wnt/β-catenin signalling (47). Another study reported that the decreased expression of *ZEB2* inhibited the expression of *β-catenin, c-Myc*, and *cyclinD1* (48). In addition, Weng et al indicated that *ZEB2* directly targeted at *smad7* resulted in the reduced expression of *β-catenin* in oligodendrocyte precursor cells (49). In our study, we found that the upregulation of *Wnt1, β-catenin*, and *c-Myc* induced by IL-1β was significantly inhibited by *ZEB2* knockdown, and the inhibition of the inflammatory response and cartilage degradation by *ZEB2* silencing was significantly reversed by a Wnt agonist. These results demonstrate that the protective effects of *ZEB2* inhibition in chondrocytes are mediated, at least in part, by its inhibiting the activation of the Wnt/β-catenin pathway.

Conclusion

ZEB2 expression was significantly upregulated in the cartilage tissues of OA patients and IL-1β-stimulated chondrocytes. Furthermore, *ZEB2* silencing inhibited IL-1β-induced cartilage degradation and the inflammatory response through the Wnt/β-catenin pathway. These results show that *ZEB2* inhibition had a protective effect on the damaged OA chondrocytes, effectively regulated OA, and promoted the repair of articular cartilage. The findings suggest that *ZEB2* may be a promising therapeutic target for OA.

However, this was a primary exploration on the specific effect of *ZEB2* alteration in OA progression. This study may be limited by its basic research on an in vitro aspect. With regard to the regulatory mechanism, the way in which *ZEB2* mediates the immune phenomena surrounding the cartilage and then impacts the development of OA is another issue for further research. The search for specific inhibitory drugs of *ZEB2* is also limited based on the current evidence. We infer that crisper-cas9 gene editing technology could be used to construct specific biomacromolecules carrying *ZEB2* inhibition for targeted therapy, and we will investigate this in our future research.

Authors' contributions

Feng Wang contributed to the study conception and design. Material preparation and data collection were performed by Yongzhi Li and Zhiping Sun. Data analysis was performed and software was run by Wenxiong Li and Zhen Xiao. The manuscript was written and revised by Zhibin Li. All authors commented on previous versions of the manuscript, and read and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

The data and materials used to support the findings of this study are available from the corresponding author upon reasonable request.

Ethical approval

The study was carried out in accordance with the Declaration of Helsinki and approved by the ethics committee of the Affiliated Hospital of Shaanxi University of Chinese Medicine (number SZFYIEC-PJ-2022[90]). Informed consent was obtained from all participants.

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