Phgdh serves a protective role in Il-1β induced chondrocyte inflammation and oxidative-stress damage

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Abstract. The primary pathological changes observed in osteoarthritis (OA) involve inflammation and degeneration of chondrocytes. 3-phosphoglycerate dehydrogenase (Phgdh), a rate-limiting enzyme involved in the conversion of 3-phosphoglycerate to serine, serves as a crucial molecular component of cell growth and metabolism. However, its effects on chondrocytes in OA have not been determined. In the present study, a rat model of OA was used to investigate the expression levels of Phgdh *in vivo* and *in vitro*. Additionally, the role of Phgdh in extracellular matrix (ECM) synthesis, inflammation, apoptosis and oxidative stress levels of chondrocytes was detected *in vitro*. Phgdh expression was decreased in OA, and Phgdh overexpression promoted ECM synthesis, decreased levels inflammatory cytokines, such as Il-6, TNF-α, a disintegrin and metalloproteinase with thrombospondin motifs 5 and MMP13, and decreased apoptosis. Furthermore, expression of Phgdh effectively increased expression levels of the cellular antioxidant enzymes catalase and superoxide dismutase 1, and decreased the levels of reactive oxygen species in chondrocytes; and this may have been regulated by a Kelch like ECH associated protein 1/nuclear factor erythroid 2-related factor 2 axis. Taken together, these results suggest that Phgdh may be used to manage the progression of OA.

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Abbreviations: OA, osteoarthritis; Phgdh, 3-phosphoglycerate dehydrogenase; ECM, extracellular matrix; Adamts-5, a disintegrin and metalloproteinase with thrombospondin motifs 5; Cat, catalase; Sod1, superoxide dismutase 1; ROS, reactive oxygen species; Keap1, Kelch like ECH associated protein 1; Nrf2, nuclear factor erythroid 2-related factor 2; Sox9, sex determining region Y-box 9; Acan, aggrecan; Col2a1, collagen type II α 1 chain; Col1a1, collagen type I α 1 chain

Key words: Phgdh, chondrocytes, inflammation, oxidative stress, osteoarthritis

Introduction

Osteoarthritis (OA) is a common joint disease that affects 50% of the population aged >65 years, and 12% of individuals aged >25 years (1). The primary pathological mechanisms of OA are progressive inflammation of chondrocytes, extracellular matrix (ECM) degradation, loss of articular cartilage, proliferation of subchondral bone and formation of osteophytes, which result in the loss of joint function (2,3). The risk factors for OA include aging, history of joint injury, obesity, sex and genetic and anatomical factors (4). However, the exact molecular pathogenesis of OA remains unclear. Considering that articular cartilage has no self-repair capability and its homeostasis is precisely regulated by a series of factors, for example signal transduction (5), cytokines (6) and hormones (7), understanding the homeostatic mechanism of chondrocytes in the process of OA is of significance for the development of effective therapies for OA.

OA was previously considered to be a non-inflammatory joint disease, but more recent studies have suggested that inflammatory mechanisms are involved in the pathological process of OA chondrocytes (8,9). Inflammatory factors, such as IL-1β, IL-6 and TNF-α, in the microenvironment of the joint cavity may decrease the viability of chondrocytes, and increase necrosis and apoptosis (6). Elevated expression levels of MMPs degrade the ECM of chondrocytes, which is accompanied by an active inflammatory response (10). Additionally, a disintegrin and metalloproteinase with thrombospondin motifs (Adamts) family of proteins, which are also known as aggrecanases, exert a proteoglycan/aggrecan (Acan) depletion effect that is associated with cartilage degradation and inflammation during OA (11). The effects of certain genetic molecules on OA have been determined. For example, inhibiting the expression of m6A methyltransferase complex including methyltransferase-like 3 decreases inflammation and apoptosis of OA chondrocytes, which slows progression of OA (12). Overexpression or activation of silent mating type information regulation 2 homolog 1 decreases loss of cartilage by exerting an inhibitory effect on MMP13 (13). In addition, Adamts-5 is inhibited by microRNA-137, and this decreases levels of inflammatory factors and ECM degradation in OA (14). Therefore, investigation of the molecular function of OA chondrocytes would improve understanding of the molecular pathology of OA, and may highlight potential novel therapeutic targets.

3-Phosphoglycerate dehydrogenase (Phgdh) is a key enzyme involved in serine biosynthesis and serves as a rate-limiting enzyme in the conversion from 3-phosphoglycerate to serine. The serine released during this process provides a large amount of energy and metabolites for cell growth and metabolism (15,16). Elevated levels of Phgdh have been observed in several types of cancer, including colon (17), breast (18) and cervical cancer (19), which suggests that overexpression of Phgdh is associated with a poor prognosis in these types of cancer. Moreover, a previous study suggested that Phgdh-deficient mouse embryonic fibroblasts are more vulnerable to oxidative damage, accompanied by an increase in levels of inflammatory factors (16). These findings suggest that upregulation of Phgdh expression may increase cell viability and proliferation, whereas downregulation of Phgdh may result in oxidative damage and the promotion of an inflammatory response. To the best of our knowledge, however, the role of Phgdh in OA chondrocytes has not yet been studied.

In the present study, the expression levels of Phgdh in OA chondrocytes were assessed and its biological effects on chondrocytes were determined. Phgdh levels were assessed using both an *in vitro* and *in vivo* model. Next, the effect of Phgdh on ECM synthesis, inflammation, apoptosis and oxidative stress levels were determined. The present study aimed to improve understanding of the pathogenesis of OA and highlight potentially novel therapeutic targets for the management of OA.

Materials and methods

Affymetrix microarray analysis. In order to determine the levels of Phgdh in the chondrocytes in an OA rat model, the microarray dataset GSE42295 [(Rat230_2) Affymetrix Rat Genome 230 2.0 Array] from Gene Expression Omnibus was used. There were a total of 12 samples, including 3 cases of surgically induced 2 or 8 week rat OA models, and corresponding sham controls, which underwent surgical incision without structural modification. The differentially expressed genes (DEG) were identified between the sham- and OA-2 and 8 week groups using the GEO2R tool (20). Criteria for classification as a DEG were $P<0.05$ and a $|\log2\text{Fold Change (FC)}|>1$.

Establishment of the in vitro OA model, and isolation and culture of chondrocytes. A total of 36 male newborn (weight, 5-6 g) Sprague-Dawley rats, which were all reared at room temperature under 12/12 h day/night cycles, were purchased from the Experimental Animal Centre of the Kunming Medical University (Kunming, China) for chondrocyte extraction. Briefly, after rats were sacrificed by cervical dislocation without anesthesia, articular cartilage was harvested from the knee joints. The cartilage tissue was cut into 1-3 mm^3 pieces followed by digestion with 2 mg/ml collagenase II (Sigma-Aldrich; Merck KGaA) for 3 h at 37˚C. Finally, the digested chondrocytes were suspended in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 1% penicillin/streptomycin (Beijing Solarbio Science & Technology Co., Ltd.) and 10% FBS (Zhejiang Tianhang Biotechnology Co., Ltd.) at 37˚C with 5% CO_2 in a humidified incubator. The chondrocytes adhered to the plate after 2-3 days of culture, at which point the tissue pieces were discarded and the medium replaced. The cells were cultured for three passages for chondrocyte identification and use in subsequent experiments. IL-1β (10 ng/ml; Beijing Solarbio Science & Technology Co., Ltd.) was used to stimulate chondrocytes to establish the *in vitro* OA model as previously described (21). The present study was approved by the Ethics Committee of Qujing First People's Hospital (approval no. 19-025).

Chondrocyte identification and immunofluorescence analysis. Immunofluorescence analysis was performed for chondrocyte identification and Phgdh detection in IL-1β-induced chondrocytes. Collagen type II α 1 chain (Col2a1), a specific marker for chondrocytes, was utilized for chondrocyte identification via immunofluorescence assay. Briefly, third generation chondrocytes and chondrocytes from the Control and IL-1β groups were harvested, washed using PBS and fixed using 4% paraformaldehyde for 30 min at room temperature. After blocking with 5% BSA (Boster Biological Technology) for 30 min at room temperature, primary antibodies against Col2a1 (1:900; cat. no. 28459-1-AP; ProteinTech Group, Inc.) and Phgdh (1:1,000; cat. no. 14719-1-AP; ProteinTech Group, Inc.) were used to incubate the cells at 4˚C overnight. The following day, the primary antibody was removed and the cells were incubated with the FITC-conjugated mouse anti-rabbit IgG (1:5,000; cat. no. BM2012; Boster Biological Technology) for 1 h at room temperature. After staining the cell nuclei with DAPI (1:10,000; Beijing Solarbio Science & Technology Co., Ltd.) for 5 min at room temperature, images were obtained using a fluorescence microscope (magnification, x100; Olympus Corporation).

Cell transfection. The expression plasmid encoding the full-length open reading frame of rat Phgdh with EGFP tags (pIRES2-EGFP-Phgdh) and the corresponding negative control plasmid were synthesized and purchased from Shanghai GenePharma Co., Ltd. For cell transfection, chondrocytes were seeded into six-well plates at a density of $5x10^6$ cells per well and cultured overnight at 37˚C with 5% CO_2 in a humidified incubator. When confluence reached 70-80% density, Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) reagent was used for transfection according to the manufacturer's protocol. The mixture contained plasmids and transfection reagents (1 μg: 3 μl) and cell transfection with the plasmid was performed at a final concentration of 50 nM; cells were incubated with the transfection mixture and Opti-MEM (Gibco; Thermo Fisher Scientific, Inc.) for 6-7 h at 37˚C with 5% CO_2 in a humidified incubator. Subsequently, DMEM supplemented with 10% FBS was used to culture cells for 48 h. The experiments were grouped as follows: Control (chondrocytes without any treatment); IL-1β (chondrocytes treated with IL-1β); IL-1β + pcDNA (chondrocytes treated with IL-1β and transfected with negative control plasmid) and Il-1β + Phgdh (chondrocytes treated with IL-1β and transfected with Phgdh cDNA plasmid).

Cell viability assay. Chondrocytes were seeded into 96-well plates at a density of $8x10^3$ cells per well and cultured for 24, 48 or 72 h. Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.) assay was used to measure cell viability. Briefly, 10 μl CCK-8 solution was added to the wells and cultured for 2 h. The absorbance was then measured at 450 nm using a microplate reader (Thermo Fisher Scientific, Inc.).

Table I. Primer sequences used for reverse transcription-quantitative PCR.

Gene	Forward primer, 5'→3'	Reverse primer, 5'→3'
Phgdh	GAACCCTGCCTAGTCACTGGA	CCTTAGTAGCTGACCGGACG
Sox9	TCCAGCAAGAACAAGCCACA	CGAAGGGTCTCTTCTCGCTC
Acan	GAATGGGAGCCAGCCTACAC	GAGAGGCAGAGGGACTTTCG
Col2a1	ATTGCCTACCTGGACGAAGC	GACAGGCCCTATGTCCACAC
Col1a1	GCTTCACCTACAGCGTCACT	AAGCCGAATTCCTGGTCTGG
Catalase	AGAGGAAACGCCTGTGTGAG	TAGTCAGGGTGGACGTCAGT
Sod1	ATTCACTTCGAGCAGAAGGCA	ATTGCCCAGGTCTCCAACAT
GAPDH	CTATAAATTGAGCCCGCAGC	ACCAAATCCGTTGACTCCG

Phgdh, 3-phosphoglycerate dehydrogenase; Sox9, sex determining region Y-box 9; Col2a1, collagen type II α 1 chain; Sod1, superoxide dismutase 1; Col1a1, collagen type I α 1 chain; Acan, aggrecan.

Safranin O staining. In order to detect the deposition of glycosaminoglycans (GAGs) from chondrocytes, safranin O staining kit (Beijing Solarbio Science & Technology Co., Ltd.) was used to perform the safranin O staining assay according to the manufacturer's protocol. Cells were harvested and fixed using 95% ethanol for 30 min at room temperature, followed by washing three times with PBS. Next, 0.1% safranin O (Beijing Solarbio Science & Technology Co., Ltd.) solution was used to stain the cells for 10-15 min at room temperature. Staining was observed using a light microscope (magnification, x100; Olympus Corporation) and images were captured.

GAGs detection. Treated chondrocytes were incubated with 60 μg/ml proteinase K (Sigma-Aldrich; Merck KGaA) for 10 h at 56˚C and the digested aliquot was used to detect GAGs and DNA content. Dimethylmethylene blue (DMMB) dye binding experiment was used to measure the GAGs, as previously described (22), and the absorbance was measured at 525 nm using a microplate reader (Thermo Fisher Scientific, Inc.). Hoechst 33258 (1 μg/ml; Sigma-Aldrich; Merck KGaA) was used to incubate samples at room temperature for 5 min and measured at 460 nm on a microplate fluorescence reader (FLx800; BioTek Instruments, Inc.) to detect DNA content. After the levels of GAGs and DNA content were measured separately, production of GAGs was expressed as the GAG/DNA ratio.

Reverse transcription-quantitative (RT-q)PCR. RT-qPCR was performed to quantify gene expression levels of Col2a1, Acan, sex determining region Y-box 9 (Sox9), Col1a1, catalase (Cat) and superoxide dismutase 1 (Sod1). The sequences of the primers used are listed in Table I. Total RNA from differently treated cells was extracted using a total RNA isolation kit (Megentec), according to the manufacturer's protocol. A RT kit (Takara Bio, Inc.) was used to reverse transcribe RNA into cDNA, according to the manufacturer's protocols. qPCR was performed with a Fast Start Universal SYBR Green Master Mix (Roche Diagnostics) using a light Cycler 96 system (Roche Diagnostics). The thermocycling conditions were as follows: 10 min at 95˚C; followed by 40 cycles of 95˚C for 10 sec and 60˚C for sec. Relative gene expression levels were normalized to GAPDH and calculated using the $2^{-\Delta\Delta Cq}$ method (23).

ELISA. The medium of chondrocytes following treatment was collected to investigate the levels of pro-inflammatory cytokines (IL-6 and TNF-α) using specific ELISA kits (cat. nos. PR6000B and PRTA00; R&D Systems, Inc.), according to the manufacturer's protocol. The absorbance at 450 nm was measured using a microplate reader (Thermo Fisher Scientific, Inc.).

Apoptosis assay. Apoptosis of chondrocytes following treatment was investigated using an apoptosis kit (Beijing Solarbio Science & Technology Co., Ltd.). Chondrocytes were stained with 10 μl Annexin V-FITC and 10 μl PI solution in the dark for 10 min at room temperature. Next, cells were rinsed, resuspended in PBS and analyzed using a flow cytometer (BD FACSCalibur™; BD Biosciences) and FlowJo software (version 7; FlowJo LLC).

Measurement of production of reactive oxygen species (ROS). The levels of ROS in chondrocytes were determined using a 2'-7'-dichlorodihydrofluorescein-diacetate (DCFH-DA) kit (Beyotime Institute of Biotechnology). Briefly, serum-free medium containing DCFH-DA (10 μM) was used to incubate the cells at 37˚C for 30 min in the dark. Next, the cells were digested, resuspended in PBS and analyzed using a flow cytometer (BD Biosciences) and FlowJo software (version 7).

Western blot assay. Western blot analysis was used to assess the protein expression levels of Phgdh, Sox9, Acan, Col2a1, Col1a1, MMP13, Adamts-5, Bcl2, Bax, cleaved caspase-3, Kelch like ECH associated protein 1 (Keap1) and Nuclear factor erythroid 2-related factor 2 (Nrf2). Total protein from differently treated cells was extracted using RIPA Lysis Buffer (Boster Biological Technology) supplemented with 1 mM phenylmethylsulfonyl fluoride (Boster Biological Technology). The concentrations of protein were detected using a BCA kit (Nanjing Jiancheng Bioengineering Institute). A total of 50 μg protein from each group was loaded on a 10% SDS gel, resolved using SDS-PAGE and transferred to PVDF membranes (Biosharp Life Sciences). Membranes were blocked using 5% non-fat milk for 1 h at room temperature. Subsequently, membranes were incubated with primary antibodies against Phgdh (1:1,000; cat. no. 14719-1-AP; ProteinTech Group, Inc.), Sox9 (1:1,000;

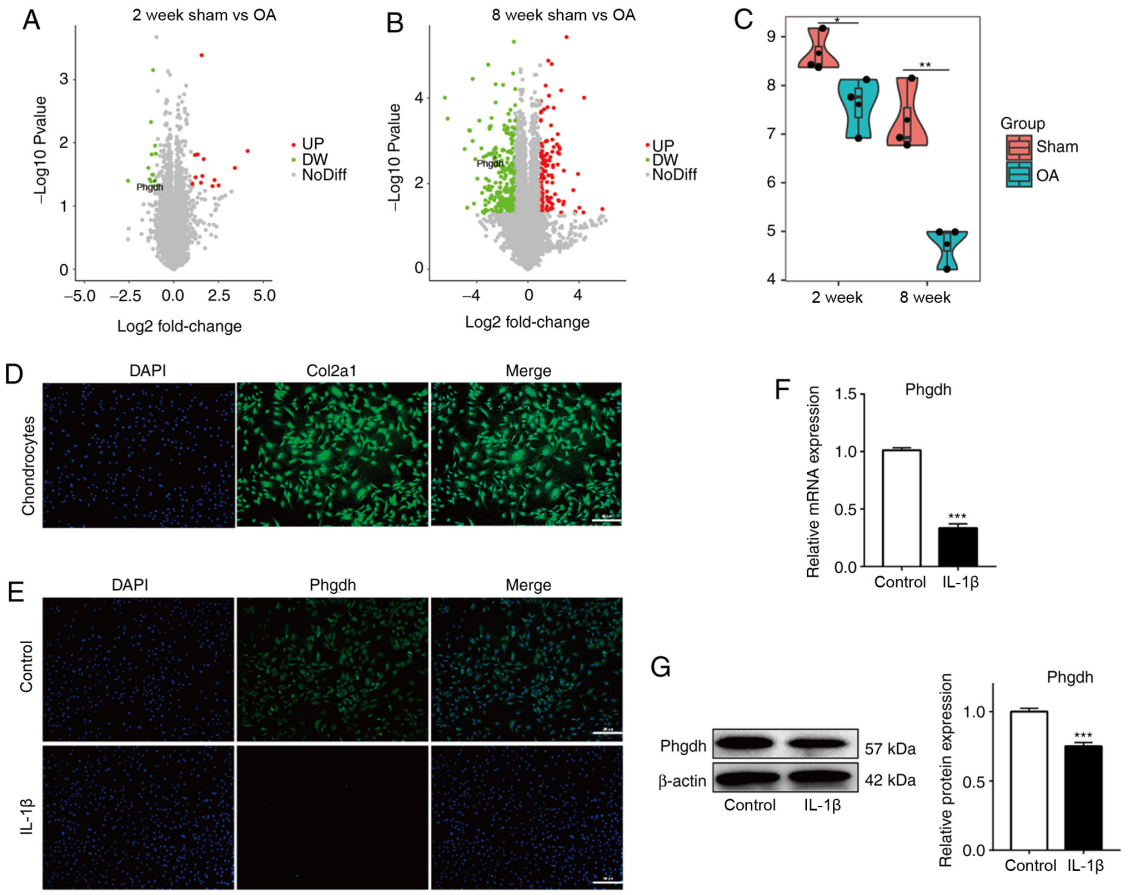

Figure 1. Phgdh expression in an *in vivo* and *in vitro* model of OA. (A) DEGs between the 2 week sham and OA groups. (B) DEGs between the 8 week sham and OA groups. (C) Boxplot of Phgdh expression based on microarray analysis of the different groups. (D) Immunofluorescence assay for detection of Col2a1 in chondrocytes harvested from newborn rats. (E) Immunofluorescence analysis for detection of Phgdh in chondrocytes treated with IL-1β. Scale bar, 200 μm. (F) mRNA and (G) protein expression levels of Phgdh in IL-1β induced chondrocytes. Data are presented as the mean ± SD (n=3). *P<0.05, **P<0.01, ***P<0.001 vs. Control. Phgdh, 3-phosphoglycerate dehydrogenase; OA, osteoarthritis; DEG, differentially expressed gene; Col2a1, collagen type II α 1 chain.

cat. no. 67439-1-Ig; ProteinTech Group, Inc.), Acan (1:1,000; cat. no. 13880-1-AP; ProteinTech Group, Inc.), Col2a1 (1:900; cat. no. 28459-1-AP; ProteinTech Group, Inc.), Col1a1 (1:5,000; cat. no. 67288-1-Ig; ProteinTech Group, Inc.), MMP13 (1:1,000; cat. no. 18165-1-AP; ProteinTech Group, Inc.), Adamts-5 (1:1,000; cat. no. ab41037; Abcam), cleaved caspase-3 (1:1,000; cat. no. 9664S; CST Biological Reagents Co., Ltd.), Bcl2 (1:1,000; cat. no. 3498; CST Biological Reagents Co., Ltd.), Bax (1:1,000; cat. no. 5023; CST Biological Reagents Co., Ltd.), Keap1 (1:1,000; cat. no. 8047; CST Biological Reagents Co., Ltd.), Nrf2 (1:1,000; cat. no. 12721; CST Biological Reagents Co., Ltd.) and β-actin (1:5,000; cat. no. 66009-1-Ig; ProteinTech Group, Inc.). Membranes were incubated with the primary antibodies overnight at 4˚C followed by incubation with HRP-conjugated secondary antibody (1:10,000; cat. no. 7074; CST Biological Reagents Co., Ltd.) for 1 h at room temperature. ECL kit (Beijing Solarbio Science & Technology Co., Ltd.) was used to visualize the protein membranes. Signals were visualized using an Odyssey Infrared Imaging System (LI-COR Biosciences). Densitometry was performed with ImageJ software (version 1.8.0.112; National Institutes of Health).

Statistical analysis. Data were analyzed using SPSS version 22.0 (IBM Corp.) and are presented as the mean ± SD (n=3). All experiments were repeated three times. An unpaired Student's t-test was used to compare differences between two groups and one-way ANOVA followed by Tukey's multiple comparisons post hoc test was used to compare differences between ≥3 groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Phgdh expression is decreased in chondrocytes both in vivo and in an in vitro OA model. First, the expression of Phgdh was investigated in chondrocytes in an *in vivo* model of OA using microarray analysis. In both the 2 and 8 week OA *in vivo* model, Phgdh expression was significantly decreased compared with the corresponding sham group (Fig. 1A and B). Downregulation of Phgdh was greatest in the 8 week OA model (Fig. 1C), suggesting that downregulation of Phgdh may be associated with the severity of OA.

Next, chondrocytes from newborn rats were extracted. Successful harvesting was demonstrated by immunofluorescence analysis to detect expression of Col2a1 (Fig. 1D). IL-1β treatment was used to establish the *in vitro* OA model. Phgdh expression was detected using immunofluorescence staining (Fig. 1E), RT-qPCR (Fig. 1F) and western blotting (Fig. 1G) assays. Phgdh expression in chondrocytes was significantly decreased following IL-1β treatment. These results suggest that Phgdh expression in chondrocytes was decreased in OA.

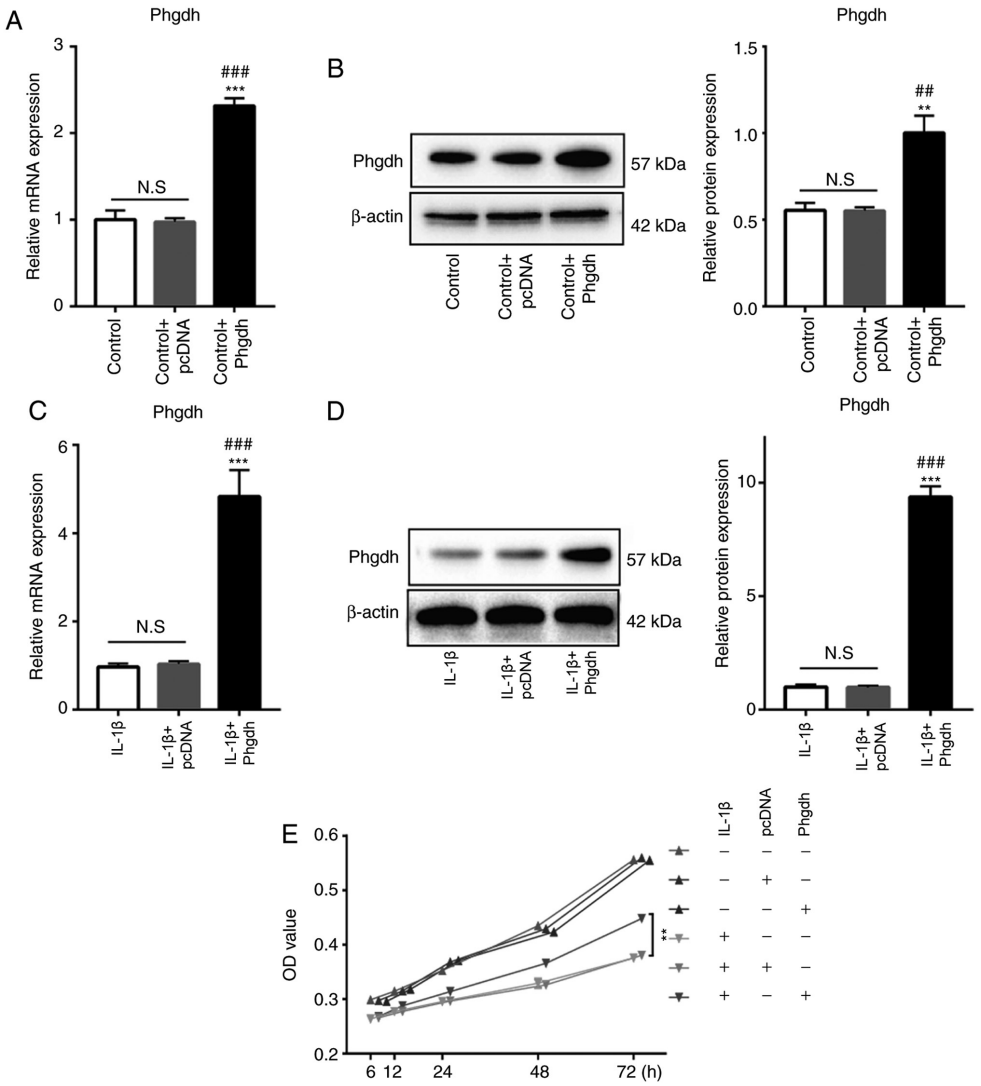

Figure 2. Phgdh increases the viability of chondrocytes treated with IL-1β. (A) mRNA and (B) protein expression levels of Phgdh transfected with Phgdh overexpression vector or negative control plasmid in chondrocytes without IL-1β treatment. (C) mRNA and (D) protein expression levels of Phgdh transfected with a Phgdh overexpression vector or the corresponding negative control plasmid in chondrocytes with IL-1β treatment. (E) Cell viability analysis of chondrocytes following overexpression of Phgdh. Data are presented as the mean ± SD (n=3). **P<0.01, ***P<0.001 vs. Control or IL-1β; $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. Control + pcDNA or IL-1β + pcDNA. Phgdh, 3-phosphoglycerate dehydrogenase; NS, not significant.

Phgdh increases the viability of chondrocytes treated with IL-1β. In order to assess the biological effect of Phgdh on chondrocytes, a Phgdh cDNA overexpression plasmid was used to transfect the chondrocytes. Phgdh mRNA and protein expression levels in chondrocytes in the presence or absence of IL-1β treatment were significantly increased following Phgdh cDNA transfection, whereas the negative control plasmid exhibited no significant effect on Phgdh expression (Fig. 2A-D). CCK-8 assay was used to assess cell viability; the results suggested that Phgdh cDNA and negative control plasmid had no significant effect on the viability of chondrocytes in untreated cells. However, overexpression of Phgdh increased cell viability of chondrocytes treated with IL-1β (Fig. 2E).

Phgdh alleviates IL-1β-induced chondrocyte degeneration. The degeneration of chondrocytes primarily manifests as decreased levels of GAGs and decreased expression of chondrogenic-specific genes, including Col2a1, Sox9 and Acan (24). Safranin-O staining showed that chondrocytes in the IL-1β + Phgdh group exhibited increased GAG staining compared with the IL-1β group (Fig. 3A). Moreover, the DMMB assay also confirmed that GAG levels were increased in the IL-1β + Phgdh group compared with the IL-1β group (Fig. 3B). The expression levels of cartilage-specific genes were consistent with the aforementioned results; Sox9 (Fig. 3C), Acan (Fig. 3D) and Col2a1 (Fig. 3E) expression levels were increased in the IL-1β + Phgdh group compared with the IL-1β group, whereas Col1a1 expression levels were decreased (Fig. 3F). The protein expression levels of Sox9, Acan, Col2a1 and Col1a1 were also assessed. Sox9, Acan, Col2a1 protein expression levels were upregulated in the IL-1β + Phgdh group compared with the IL-1β group, whereas Col1a1 expression levels were decreased (Fig. 3G). There results suggested that overexpression of Phgdh alleviated IL-1β-induced chondrocyte degeneration.

Phgdh decreases IL-1β-induced chondrocyte inflammation and apoptosis. Production of inflammatory factors and expression of matrix-degrading enzymes results in

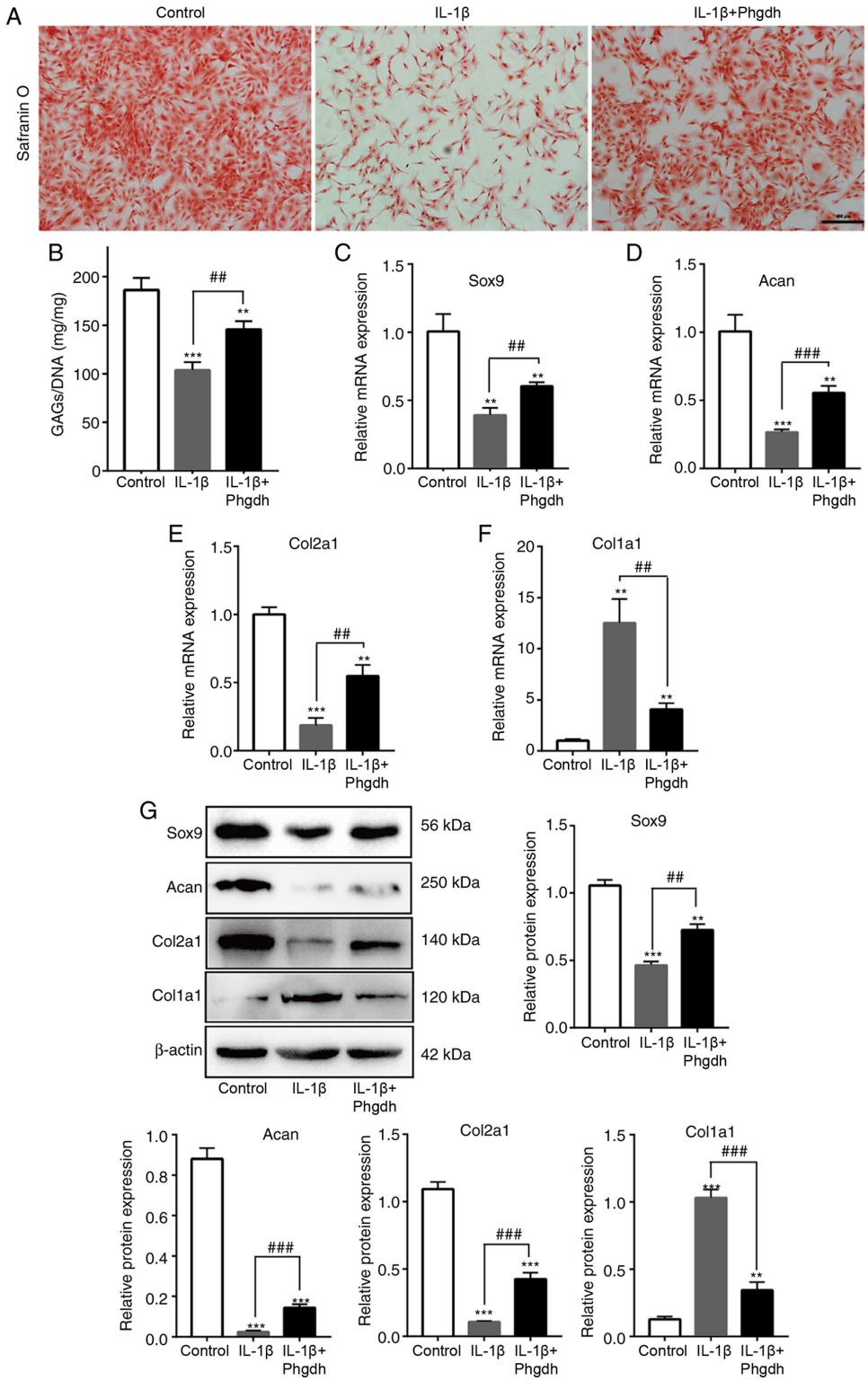

Figure 3. Phgdh alleviates IL-1β-induced chondrocyte degeneration. (A) GAG secretion in the different groups was determined using Safranin O staining. Scale bar, 200 μm. (B) Quantitative analysis of GAGs, normalized to DNA (in mg). mRNA expression levels of (C) Sox9, (D) Acan, (E) Col2a1 and (F) Col1a1. (G) Protein expression levels of Sox9, Acan, Col2a1 and Col1a1. Data are presented as the mean ± SD (n=3). **P<0.01, ***P<0.001 vs. Control; $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. IL-1β. Phgdh, 3-phosphoglycerate dehydrogenase; GAG, glycosaminoglycan; Sox9, sex determining region Y-box 9; Acan, aggrecan; Col2a1, collagen type II α 1 chain; Col1a1, collagen type I α 1 chain.

chondrocyte degeneration (25). The effect of Phgdh on chondrocyte inflammation and apoptosis was investigated. Using ELISA, it was shown that the levels of key inflammatory factors in OA, such as Il-6 and TNF-α, were decreased in the IL-1β + Phgdh group compared with the IL-1β group (Fig. 4A). The protein expression levels of Adamts-5 and MMP13 were also significantly decreased in the Il-1β + Phgdh group (Fig. 4B). Moreover, the apoptotic rate of cells in the IL-1β + Phgdh group was lower than that in the IL-1β group (Fig. 4C). Expression of apoptosis-associated proteins, such as

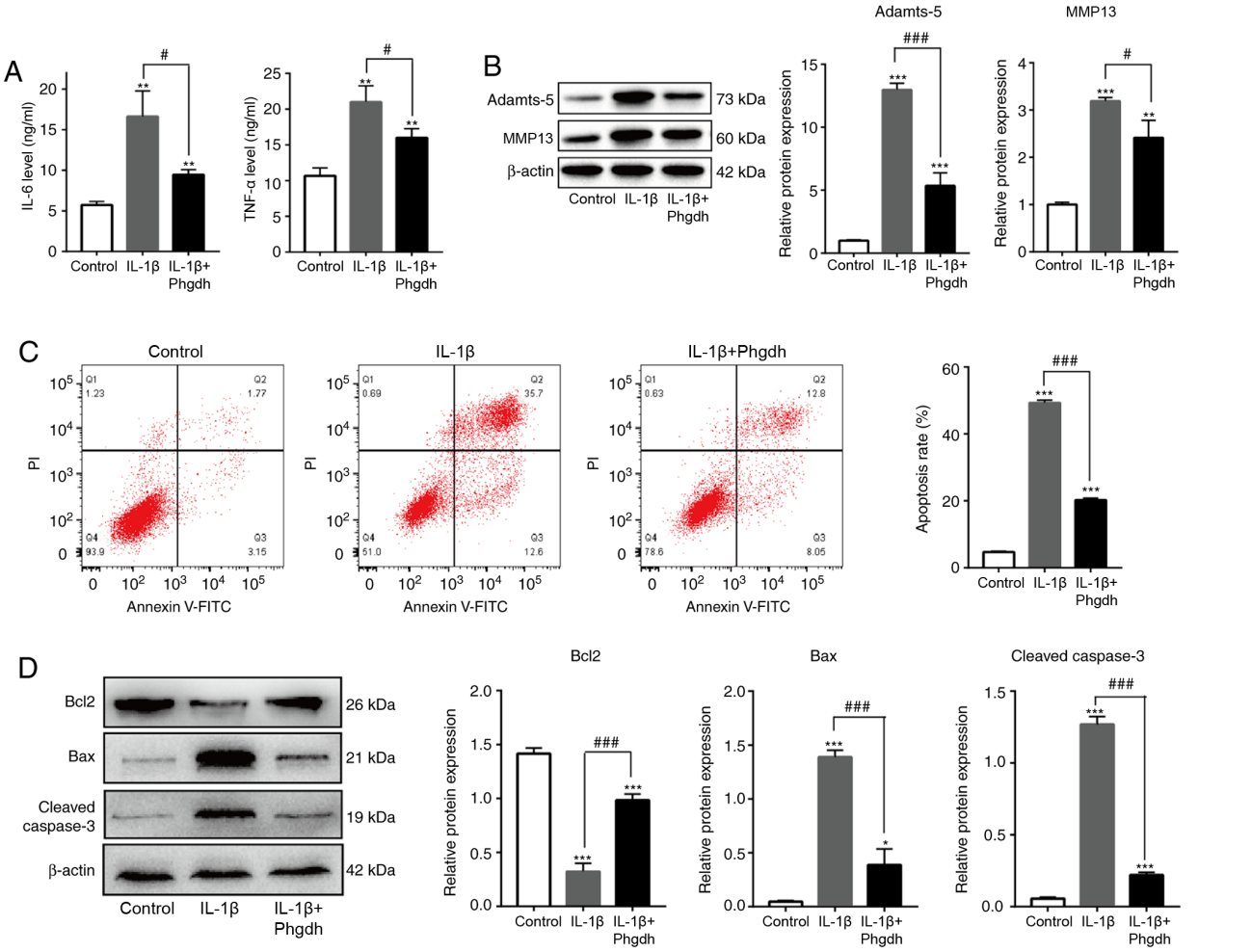

Figure 4. Phgdh decreases IL-1β-induced chondrocyte inflammation and apoptosis. (A) Levels of IL-6 and TNF-α. (B) Protein expression levels of Adamts-5 and MMP13. (C) Apoptotic rate of cells. (D) Expression levels of apoptosis-associated proteins Bcl2, Bax and cleaved caspase-3. Data are presented as the mean ± SD (n=3). *P<0.05, **P<0.01, ***P<0.001 vs. Control; $^{\#}$P<0.05, $^{\#\#\#}$P<0.001 vs. IL-1β. Phgdh, 3-phosphoglycerate dehydrogenase; Adamts-5, a disintegrin and metalloproteinase with thrombospondin motifs 5.

cleaved caspase-3, Bax and Bcl2, were detected. Expression of the pro-apoptotic proteins cleaved caspase-3 and Bax was decreased and that of the anti-apoptotic protein Bcl2 was elevated in the IL-1β + Phgdh group compared with the IL-1β group (Fig. 4D). These results suggested that overexpression of Phgdh alleviated inflammation and apoptosis of chondrocytes treated with IL-1β.

Phgdh decreases oxidative stress damage of chondrocytes treated with IL-1β. Evidence has suggested that an imbalance in oxidative stress is associated with inflammation (26). Studies have also shown that inhibiting oxidative stress levels and improving the antioxidant capacity of cells may decrease inflammation and degeneration of chondrocytes (26,27). In the present study, it was shown that overexpression of Phgdh decreased ROS levels of chondrocytes treated with IL-1β (Fig. 5A). The mRNA expression levels of the anti-oxidant enzymes Cat and Sod1 were measured. The results suggested that Phgdh increased the levels of Cat and Sod1 (Fig. 5B). Key proteins associated with oxidative stress include Nrf2 and Keap1, and their expression was next determined. Overexpression of Phgdh increased expression levels of Nrf2 and decreased those of Keap1 (Fig. 5C). These results suggested that Phgdh may exhibit a regulatory effect on the oxidative stress levels of chondrocytes via the Nrf2/Keap1 axis.

Discussion

OA is the most common disease of joints and is characterized by damaged articular cartilage, which is primarily composed of chondrocytes and cartilage matrix (1). The primary function of chondrocytes is to secrete ECM proteins, such as Acan and Col2a1, to maintain homeostasis of the articular cartilage (28). Alterations to chondrocyte function are accompanied by degeneration of cartilage matrix, which eventually results in the initiation and progression of OA. Protecting or rescuing the function of chondrocytes may thus assist in alleviating the progress of OA (29). Although a number of potential targets and mechanisms of OA chondrocytes have been reported, these studies have not translated into clinically useful therapeutic options due to a lack of sensitivity and specificity (30-33). Therefore, investigating the novel pathogenesis of OA chondrocytes may highlight potential molecules for targeted therapy of OA. In the present study, Phgdh was found to be downregulated in OA chondrocytes and decreased with the progression of OA. Overexpression of Phgdh prevented

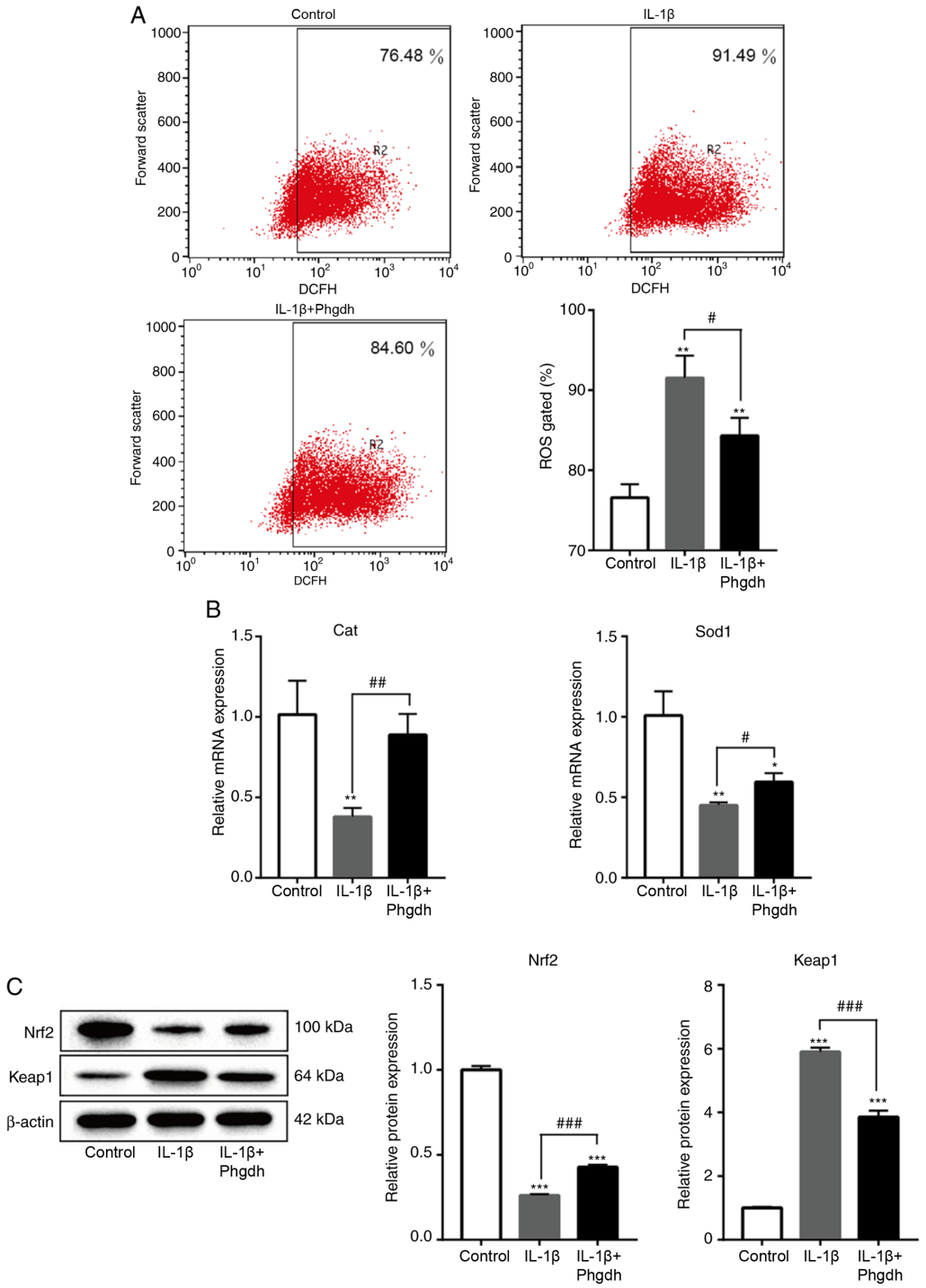

Figure 5. Phgdh decreases oxidative stress damage of chondrocytes treated with IL-1β. (A) Flow cytometry analysis for ROS levels. (B) mRNA expression levels of Cat and Sod1. (C) Protein expression levels of Nrf2 and Keap1 protein. Data are presented as the mean ± SD (n=3). *P<0.05, **P<0.01, ***P<0.001 vs. Control; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. IL-1β. Phgdh, 3-phosphoglycerate dehydrogenase; ROS, reactive oxygen species; Cat, catalase; Sod1, superoxide dismutase; Keap1, Kelch like ECH associated protein 1; Nrf2, nuclear factor erythroid 2-related factor 2.

IL-1β-induced chondrocyte degeneration, inflammation, apoptosis and oxidative damage. Thus, the potential effect of Phgdh on OA chondrocytes was highlighted and the results suggested that Phgdh may be a promising therapeutic target for management of OA.

Phgdh is a key enzyme involved in serine biosynthesis, where it synthesizes serine to provide large amounts of energy and metabolites for cell growth and metabolism (34). In tumors, upregulated expression of Phgdh has been detected in lung, breast, pancreatic and colorectal cancer, where it is positively associated with cell proliferation, migration, invasion and poor prognosis (17,18,35,36). However, downregulation of Phgdh is also associated with certain non-tumor diseases. For example, decreased Phgdh expression, accompanied by low serine levels, is associated with the development of fatty liver disease (37). Moreover, Phgdh deficiency is also a risk factor for the development of Macular Telangiectasia type 2 development, an uncommon bilateral retinal disease (38).

These aforementioned studies suggest that the expression of Phgdh is carefully regulated, and its abnormal expression can result in pathophysiological changes or disease. In the present study, Phgdh expression was shown to be decreased in the chondrocytes of OA and with the progression of OA over time, suggesting that Phgdh was also involved in OA pathogenesis.

Under normal physiological conditions, the activity of synthesis and decomposition of cartilage matrix is in a dynamic balance. Transcription factor Sox9 is a key molecule in maintaining the phenotype of the chondrocyte (39). Sox9 activates a series of downstream signaling molecules to promote the deposition of Col2a1, Acan and GAGs (40). In patients with OA, numerous inflammatory factors, such as IL-1β, IL-6 and TNF-α, inhibit the expression of Sox9 and degrade cartilage ECM by activating a series of proteases, such as MMPs and Adamts (41). Decreasing the level of inflammation and restoring the homeostasis of chondrocytes are key to preventing the progression of OA (42,43). In the present study, in chondrocytes treated with IL-1β, it was observed that inflammatory stimulation decreased the levels of GAGs, and overexpression of Phgdh restored the deposition of GAGs. Furthermore, overexpression of Phgdh offset the inhibitory effect of IL-1β on Sox9, Acan, and Col2a1 expression in chondrocytes and decreased expression levels of the osteogenesis-specific gene Col1a1. In addition, upregulation of Phgdh decreased expression levels of IL-6, TNF-α, MMP13 and Adamts-5 and inhibited apoptosis of chondrocytes treated with IL-1β. These results suggested that Phgdh exerted a protective effect on chondrocyte homeostasis, inflammation and apoptosis.

Oxidative stress is a negative outcome that results from production of free radicals in tissue, and is a key factor for mediating inflammation (44). Oxidative stress damage is primarily the result of increased ROS production and decreased levels of antioxidant enzymes, such as Sod and Cat (45). Oxidative stress is a key factor is associated with the progression of OA. Excessive ROS production activates the inflammatory response via the NF-κB signaling pathway and inhibits synthesis of proteoglycans and ECM in chondrocytes (46). In addition, a lack of Phgdh increases the vulnerability of fibroblasts to oxidative stress damage (16). In the present study, Phgdh exerted a regulatory effect on oxidative stress levels of chondrocytes in OA. Phgdh decreased the levels of ROS and increased expression levels of Cat and Sod1 in chondrocytes treated with IL-1β. Nrf2 is a major antioxidant factor, which normally binds to Keap1 (47). Under oxidative damage, Nrf2 dissociates from Keap1 and translocates to the nucleus to initiate the transcription of antioxidant enzymes (48). It has previously been shown that promoting the expression of Nrf2 decreases the inflammatory response and progression of OA (43). In the present study, overexpression of Phgdh promoted the expression levels of Nrf2 and decreased those of Keap1 in chondrocytes treated with IL-1β, suggesting that Phgdh regulated oxidative stress levels of chondrocytes in OA via Nrf2.

In conclusion, Phgdh was decreased in OA and was associated with OA progression. Moreover, Phgdh was found to be involved in chondrocyte homeostasis: Overexpression of Phgdh decreased inflammation and apoptosis and restored the phenotype of chondrocytes in OA. Additionally, overexpression of Phgdh alleviated oxidative stress damage; this may have involved a Keap1-Nrf2 axis which is involved in the pathological mechanism of OA. Therefore, Phgdh may be a potentially significant target for OA research and treatment.

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Availability of data and materials

The microarray dataset is available in the GSE42295 from Gene Expression Omnibus Database. The data collected and analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

JW and XQ conceptualized and designed the study. HH, KL and HO collected and analyzed data. HH and KL wrote the manuscript. HH, KL, HO, HQ and JW confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Qujing First People's Hospital (approval no. 19-025; Qujing, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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