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LncRNA SNHG1 Alleviates the IL-1β-Induced Chondrocytes Inflammatory Injury *via* Targeting miR-143-3p/KLF2 Axis

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Abstract

Background: Osteoarthritis (OA), a chronic degenerative joint disease, is short of definitive therapeutic methods until now. More and more studies showed that long non-coding RNAs (lncRNAs) performed essential roles in OA. This study is aimed at investigating the effect of lncRNA SNHG1 on IL-1β-stimulated chondrocytes inflammatory injury in mice.

Materials and Method: As the latest studies indicated, the mouse chondrocytes, stimulated by IL-1β, were used for constructing a model of inflammatory injury. The functions of SNHG1 overexpression or inhibition in regulating apoptosis, inflammation and Extracellular Matrix (ECM) in IL-1β injured chondrocytes were evaluated. Then, bioinformatics methods were used to screen possible targets of SNHG1, and the relationships among SNHG1, miRNA-143-3p, and Kruppel Like Factor 2 (KLF2) were verified by qRT-PCR and luciferase reporter gene assays. Furthermore, the functional mechanism of SNHG1 was explored through miR-143-3p overexpressed or the knockdown of KLF2 as recovery experiments.

Results: SNHG1 was reduced in mouse chondrocytes stimulated by IL-1β. Moreover, overexpression of SNHG1 reduced apoptosis inhibited the inflammatory response and the degradation of ECM. Mechanistically, SNHG1, which functions as a sponger, directly bound to miRNA-143-3p. Additionally, transfection of miRNA-143-3p mimics reversed the inhibition of apoptosis and ECM degradation exerted by SNHG1 overexpression. SNHG1 exerted its function by indirectly regulating KLF2, a downstream target of miRNA-143-3p.

Conclusion: Our study demonstrated that lncRNA SNHG1 protected chondrocytes against IL-1β-stimulated inflammatory injury by targeting miRNA-143-3p/KLF2 axis. It is suggested that SNHG1 might be a potential therapeutic target in OA.

Keywords: MicroRNAs; Osteoarthritis; Long non-coding; SNHG1

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Introduction

Osteoarthritis (OA) belongs to a common degenerative joint illness, which is caused by various factors. Its main features are cartilage degeneration and destruction, and bone hyperplasia. Epidemiological statistics suggested that the incidence of people over 55 years of age is 44% to 70% [1]. However, there are no effective treatments to prevent the occurrence and delay the progression of OA [2,3]. The pathogenic factors of OA generally lie in trauma, age, menopause, and endocrine imbalances. These factors change the biochemical pathway of chondrocytes, leading to increased inflammatory cytokines and the degradation of the Extracellular Matrix (ECM) [4]. It was reported that abnormal gene expression in chondrocytes is related to chondrocytes inflammatory response and ECM synthesis and degradation [5]. Therefore, it is needful to explore the mechanisms affecting chondrocytes inflammation and matrix degradation, which may provide new targets for treating OA.

Long non-coding RNAs (LncRNAs) are a group of RNAs in eukaryotic cells that are longer than 200 nucleotides, and they do not have the function of encoding proteins [6]. Recently, increasing

studies showed that lncRNAs participated in the regulation of gene expression in many diseases, including OA [7-9]. For example, lncRNA HULC, which was highly up-regulated in liver cancer, reduced the inflammatory damage of ATDC5 cells [10]. Tang et al. reported that lncRNA TUG1 promoted the degradation of cartilage extracellular matrix [9]. These studies have proved LncRNAs fulfilled significant functions in OA.

MicroRNAs (miRNAs), generally 18 to 22 nucleotides long, are small non-coding RNAs molecules that could mediate the transcription and degradation of mRNA via their complementary 3′-Untranslated Region (3′-UTR) [11]. As some reports went, miRNAs took part in inflammation-related diseases like OA and promoted or inhibited OA development [12-14]. According to the latest researches, it was demonstrated that lncRNAs performed their functions by the interaction with miRNAs or mRNAs [15-17]. SNHG1 helped cell proliferation and tumorigenesis by the negative regulation of miR-137 in colorectal cancer [18]. Cao et al. revealed that SNHG1 regulated neuroinflammation by sponging the miR-7/NLRP3 axis [19]. However, the function of SNHG1 in OA has not been illustrated. Therefore, it's needful to explore SNHG1's role in OA further.

Our study was aimed at determining SNHG1's potential mechanisms in OA chondrocytes. Moreover, we used bioinformatics software (Starbase) and various experimental methods to reveal that SNHG1 positively regulated KLF2 through sponging miR-143-3p, and mediated the apoptosis, the inflammatory response and the degradation of ECM in OA chondrocytes.

Materials and Methods

Primary chondrocyte isolation and culture

The Ethics Committee of the Third Affiliated Hospital of Nanchang University agreed with our research. The Animal Center of Nanchang University provided male BALB/c mice (1 week old). Mouse primary chondrocytes were isolated like the previous study reported [20]. The obtained chondrocytes were cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM; Gibco, USA) with 10% fetal bovine serum (FBS, Gibco, USA) and 100 units/ml penicillin/streptomycin (Solarbio, China) at 37°C with 5% CO2. The chondrocytes from passages 2 to 3 were only used in the following experiments to avoid changes in phenotype.

The chondrocytes at 60% to 70% confluency were induced with different concentrations of IL-1β (0, 1, 5, 10 ng/ml, Sangon Biotech, China). Moreover, we determined that the appropriate concentration of IL-1β was 10n g/ml for subsequent experiments.

MTT assay

The cell viability of the treated chondrocytes was detected by Cell Proliferation Reagent Kit (MTT, Solarbio, China) and referring to the manufacturer's instructions. Briefly, 96-well plates were used for culturing cells (4×10^4). After various treatments in the control group and the inflammation group (3 repeated holes in each group), MTT solution (20 μL) was put to each well. After 4 h, adding DMSO to dissolve them. Finally, cell viability was tested by the microplate spectrometer (Bio-Rad, USA).

Apoptosis assay

The apoptotic rate of the treated chondrocytes was evaluated by flow cytometry (BD, USA) and Annexin V-FITC/PI Apoptosis Detection Kit (Keygen, China) with the guidance of the supplier's protocol. In short, the chondrocytes were first washed by using Phosphate-Buffered Saline (PBS). Next, the cells were resuspended in 500 ul binding buffer, and then incubated with 5 μL Annexin V-FITC and 5 μL PI in the dark at room temperature for 15 min. Finally, apoptosis was evaluated by the flow cytometry and analyzed by the FlowJo software. In general, the experimental grouping is the same as the MTT assay.

Quantitative reverse transcription PCR (qRT-PCR)

TRIzol reagent (Invitrogen, USA) helped to extract the total RNA of chondrocytes. Next, the PrimeScript™ RT Reagent kit (Takara, Japan), according to the manufacturer's instructions, was used for reversely transcribing the RNA into cDNA. Moreover, the Real-time PCR system (BioRad) and the SYBR Premix (Takara, Japan) were utilized for performing qRT-PCR. For effectively analyzing all data, our study adopted the $2^{-\Delta\Delta Ct}$ method [21]. As for the related primer sequences, Table 1 showed all the details.

Cell transfection

The overexpression plasmids of SNHG1 were synthesized by GenePharma (China) (pcDNA3.1 vector (pc-DNA) as a negative control). And all of the siRNAs (si-SNHG1, si-KLF2, si-NC), and the miRNAs mimics (miRNA-143-3p mimics, mimics-NC) were purchased from GenePharma. Plasmids or oligonucleotides were respectively transfected into chondrocytes by using Lipofectamine 2000 (Life Technologies, USA) with the protocol of the manufacturer. After 48-h transfection, 10 ng/ml of IL-1β was utilized to induce the cells for 24 h.

Dual-luciferase reporter assay

GenePharma (China) was applied to construct the synthesized 3′-UTR sequences, which were added to the pmirGLO vectors. Similarly, the pMIR-reporter plasmids of the wild-type SNHG1 (SNHG1-Wt), the mutated SNHG1 (SNHG1-Mut), the wild-type KLF2 (KLF2-Wt), and the mutated KLF2 (KLF2-Mut) were constructed as the above. These plasmids and miR-143-3p mimics or the control were transfected into the 293T cells by Lipofectamine 2000. After transfection for 48 h, the relative luciferase activity was detected by the Dual-Luciferase Reporter System (Promega, USA).

Western blot

The chondrocytes were dissolved in 200 ul of the RIPA protein lysis buffer and then centrifuged at 12500 g for 10 min. The supernatant was then collected for further experiments or storage at − 80°C. The protein extracts were isolated by 10% SDS-PAGE and then transferred to PVDF membrane (Millipore, USA). Next, the membrane was blocked by 5% fat-free milk for 1 h. Subsequently, the PVDF membranes were respectively incubated in the specific primary antibodies (Abcam, UK) at 4°C overnight and washed in PBST. Then, the secondary antibody (Proteintech, China) was used to incubate for 1 h. After washed 3 times with PBST, the membrane was developed with the ECL chemiluminescence Kit (Solarbio, China).

Statistical analysis

All data were expressed by mean ± SD. Statistical significance was detected by using SPSS 17.0 software. P-value <0.05 was indicated statistical significance. All results were collected from at least 3 independent experiments.

Results

SNHG1 expression was down-regulated in IL-1β-stimulated chondrocytes injury

We analyzed the data in the GEO database (https://www.ncbi.

Table 1: The sequences of the primers used.

Gene	Sequence
SNHG1-F	CCGCTCGAGCTCATTTTTCCTTGTTCG
SNHG1-R	CGCGGATCCGCAAAGAATTATTTCATC
KLF2-F	ACAGACTGCTATTTATTGGACCTTAG
KLF2-R	CAGAACTGGTGGCAGAGTCATTT
collagenII-F	ACGAAGCGGCTGGCAACCTCA
collagen II-R	CCCTCGGCCCTCATCTCTACATCA
aggrecan-F	CTGTCTTTGTCACCCACACATG
aggrecan-R	GAAGACGACATCACCATCCAG
MMP3-F	AGGGATGATGATGCTGGTATGG
MMP3-R	CCATGTTCTCCAACTGCAAAGG
MMP13-F	CCCTGATGTTTCCCATCTATACC
MMP13-R	TTCATCGCCTGGACCATAAAG
ADAMTS4-F	GAGCTGTGCTATTGTGGAAGA
ADAMTS4-R	CCCTGCCCATTCAAGTTAGT
ADAMTS5-F	CTCGATCCCTAGCTGTCTTTG
ADAMTS5-R	CAGGAGTGGCTTTAGAGTGTAG
TNF-α-F	CCCTCACACTCAGATCATCTTCT
TNF-α-R	GCTACGACGTGGGCTACAG
IL-1β-F	TGCTGATGTACCAGTTGGGG
IL-1β-R	CTCCATGAGCTTTGTACAAG
IL-6-F	CCAGAGTCCTTCAGAGAGATACA
IL-6-R	CCTTCTGTGACTCCAGCTTATC
GAPDH-F	TGGTGAAGCAGGCATCTGAG
GAPDH-R	TGAAGTCGCAGGAGACAACC

F: Forward; R: Reverse

nlm.nih.gov/geo/query/acc.cgi?acc=GSE104793) to explore the clinical significance of lncRNA SNHG1 in OA. Compared with uninjured chondrocytes, the expression of SNHG1 in arthritic chondrocytes was down-regulated (Figure 1A). IL-1β was employed to induce the inflammation injury of chondrocytes [22]. For determining the proper concentration of IL-1β, chondrocytes were processed by different doses of IL-1β, and then we evaluated cell viability. Compared with untreated cells, the 10 ng/mL and 5 ng/mL IL-1β treatment significantly reduced cell viability (Figure 1B). The results showed that the stimulation of IL-1β exerted an evident inhibitory influence on chondrocytes. Therefore, the concentration of IL-1β was 10ng/mL for further experiments. After the treatment with IL-1β, we observed the mRNA expression levels of IL-1β, IL-6 and TNF-α also enhanced obviously (Figure 1C), and the apoptosis rate increased significantly (Figure 1D). The above results confirmed that IL-1β could successfully construct OA injury model in chondrocytes. Finally, qRT-PCR verified that SNHG1 was significantly down-regulated in IL-1β-stimulated chondrocytes (Figure 1E).

The function of SNHG1 in OA chondrocytes

For investigating the regulatory role of SNHG1 in chondrocytes, si-SNHG1 or SNHG1 overexpression vector was transfected into chondrocytes. And we employed qRT-PCR to corroborate the transfection efficiency (Figure 2A). The MTT assays suggested that the overexpression of SNHG1 could improve cell viability (Figure 2B). To further explore the function of SNHG1 in IL-1β-induced chondrocytes, apoptosis and inflammatory cytokines were examined again after transfection. The results revealed that SNHG1 overexpression significantly reduced apoptosis (Figure 2C) and inhibited IL-1β, IL-6 and TNF-α expression levels (Figure 2D). Furthermore, the mRNA and protein levels of major cartilage matrix-degrading enzymes (ADAMTS-4, ADAMTS-5, MMP-3, and MMP13) were increased after the IL-1β treatment, and the overexpression of SNHG1 could inhibit these degradation enzyme expressions (Figures 2E-2H). The mRNA and protein levels of type II collagen and aggrecan, the significant components of cartilage extracellular matrix proteins, were improved after the overexpression of SNHG1

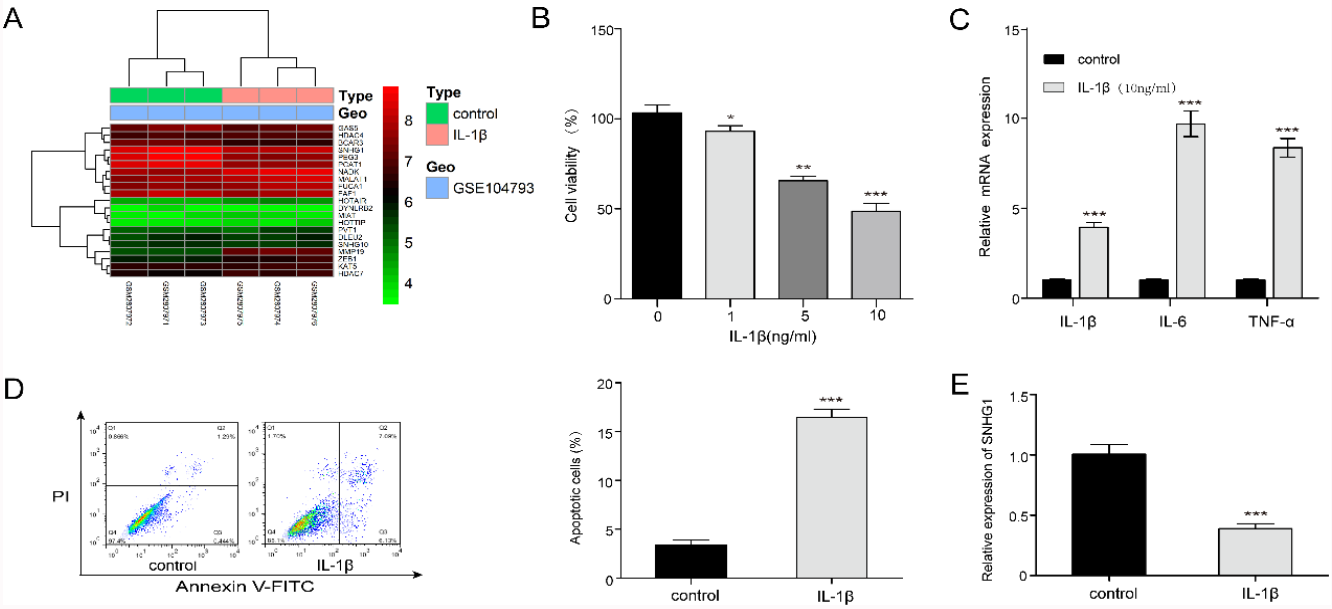

Figure 1: IL-1β induced cell injury in chondrocytes. (A) The data were obtained from the GEO database (GSE104793) to investigate the clinical relevance of SNHG1 in OA. (B) Effects of different concentrations of IL-1β on chondrocyte viability. (C) The expression of inflammatory cytokines after IL-1β stimulation. (D) Flow cytometry was used to confirm apoptosis in IL-1β-induced chondrocytes. (E) qRT-PCR was employed to detect the mRNA expression of SNHG1. The concentration of IL-1β in C-E was 10 ng/mL. *: P<0.05; **: P<0.01; ***: P<0.001.

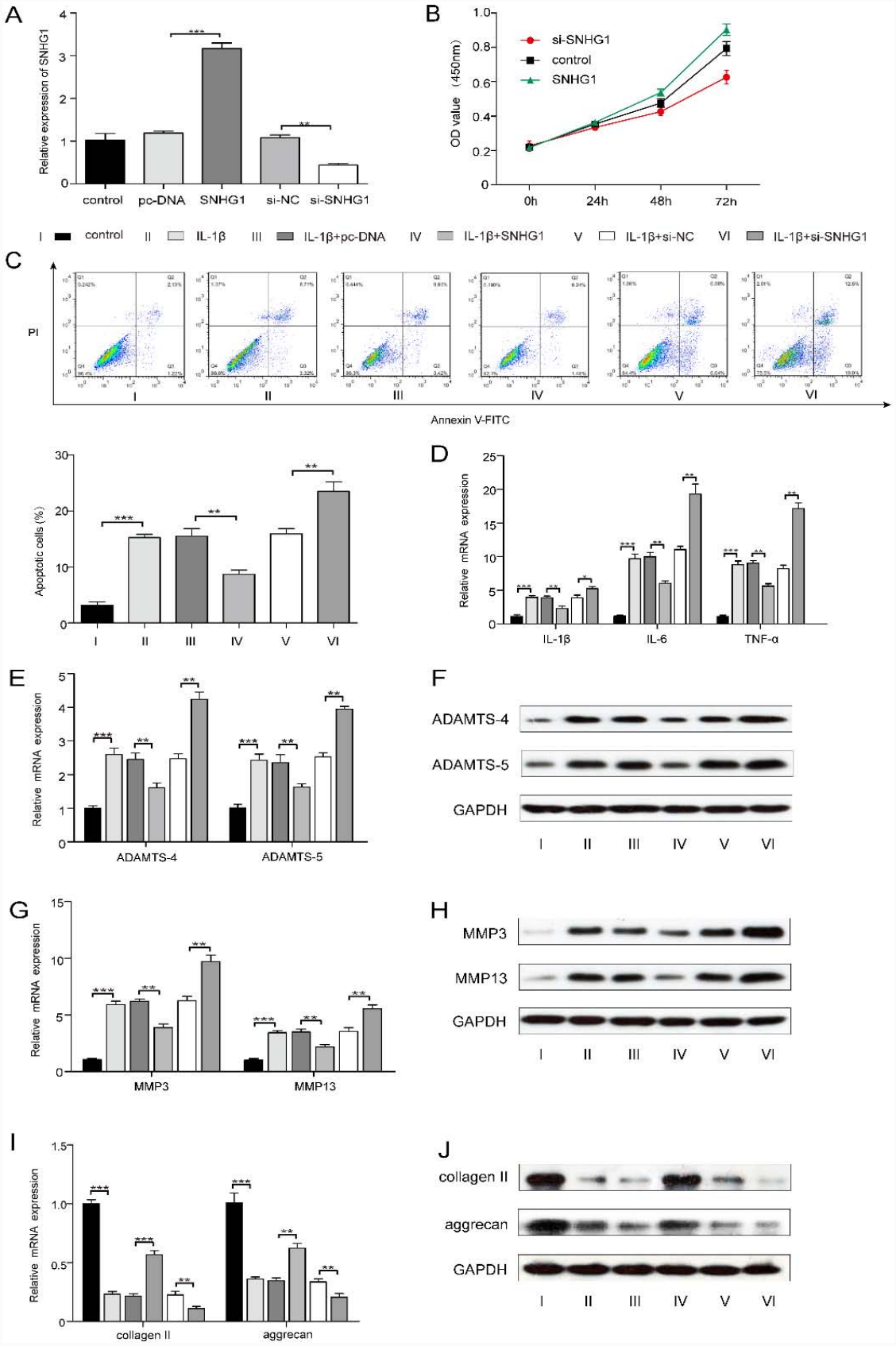

Figure 2: Functional investigation of SNHG1 in chondrocytes. After the transfection with SNHG1 or si-SNHG1, (A) the mRNA expression of SNHG1 and (B) cell viability was detected by qRT-PCR and MTT assay. The transfected chondrocytes were treated with IL-1β, and then (C) the apoptosis was determined by flow cytometry, and (D) the mRNA expression of inflammatory cytokines were analyzed by qRT-PCR. (E-H) the mRNA and protein levels of matrix degradation associated factors were detected by qRT-PCR and western blot. (H) The mRNA and protein levels of type II collagen and aggrecan were examined by qRT-PCR and western blot. *: P<0.05; **: P<0.01; ***: P<0.001.

in IL-1β-induced chondrocytes (Figure 2I, 2J). However, the effect of transfection with si-SNHG1 was contrary to that overexpression of SNHG1 (Figures 2B–2J). In summary, we suggested that SNHG1 regulated the progression of OA by inhibiting inflammatory responses, chondrocyte apoptosis, and the degradation of ECM.

SNHG1 regulated cell viability and ECM degradation *via* sponging miR-143-3p in OA chondrocytes

It's worth noting that the underlying mechanism of SNHG1 in OA remains uncleared. Recent researches revealed SNHG1 could combine directly with miRNA [18,23]. Therefore, we utilized the online bioinformatics software Starbase (http://starbase.sysu.edu.cn/) to search SNHG1's target miRNA. It is predicted that miR-143-3p might be one of the possible targets of SNHG1 (Figure 3A). The relationship between SNHG1 and miR-143-3p was validated by dual-luciferase reporter assays, which verified the surmise that SNHG1 was directly targeted by miR-143-3p in the 293T cells (Figure 3B). And according to the results of qRT-PCR, the expression of SNHG1 reduced after transfecting miR-143-3p mimics (Figure 3C). Functionally, the up-regulation of cell viability, induced by SNHG1, was also restored by miR-143-3p (Figure 3D). Moreover, miR-143-3p inhibited the mRNA and protein expression levels of type II collagen and aggrecan on chondrocytes (Figure 3E, 3F). The above results showed that miR-143-3p might be a downstream target of SNHG1 and affected chondrocytes viability and ECM expression.

SNHG1 indirectly promoted the expression of KLF2 by sponging miR-143-3p

The database of Star base was used to explore the downstream target of miR-143-3p. The result suggested that KLF2 might be a potential target gene of miR-143-3p (Figure 4A). The dual-luciferase reporter assay indicated that miR-143-3p mimics reduced KLF2-Wt luciferase activity, but not KLF2-Mut (Figure 4B). Also, we gained the following findings: miR-143-3p mimics reduced KLF2 expression, SNHG1 could up-regulate KLF2 expression, and miR-143-3p mimics restored the up-regulation of KLF2 by SNHG1 (Figures 4C-4F). The results indicated miR-143-3p directly targets at KLF2. Therefore, we determined that SNHG1 indirectly promoted KLF2 expression through miR-143-3p.

SNHG1 inhibited cell apoptosis, inflammatory response, ECM degradation, and promoted cell viability by regulating KLF2 expression in OA chondrocytes

Finally, the SNHG1 overexpression vector and si-KLF2 were transfected into chondrocytes to explain their interaction further. Functionally, the pro-proliferation, and anti-apoptosis effect,

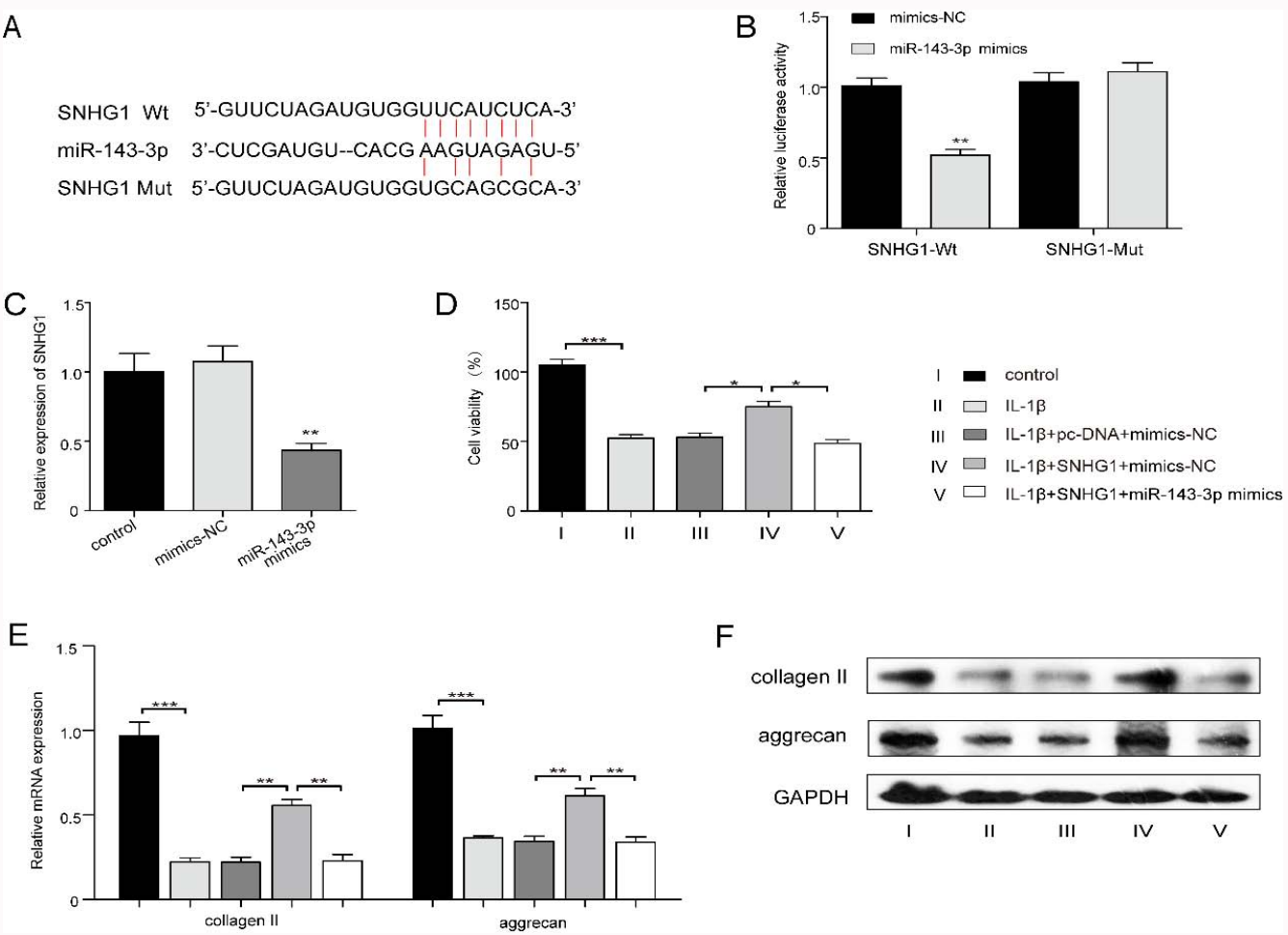

Figure 3: SNHG1 regulated cell viability and ECM degradation via sponging miR-143-3p in IL-1β-induced chondrocytes. (A) The binding site between SNHG1 and miR-143-3p was predicted by the Starbase database. (B) The relevance of SNHG1 and miR-143-3p was determined by dual-luciferase reporter assay in 293T cells. (C) The mRNA expression of SNHG1 in chondrocytes transfected with miR-143-3p mimics or mimics-NC was estimated by qRT-PCR. (D) The cell viability was examined by MTT assay after transfection and IL-1β induction. (E, F) The mRNA and protein levels of type II collagen and aggrecan were detected by RT-PCR and western blot after transfection and IL-1β induction. *: P<0.05; **: P<0.01; ***: P<0.001.

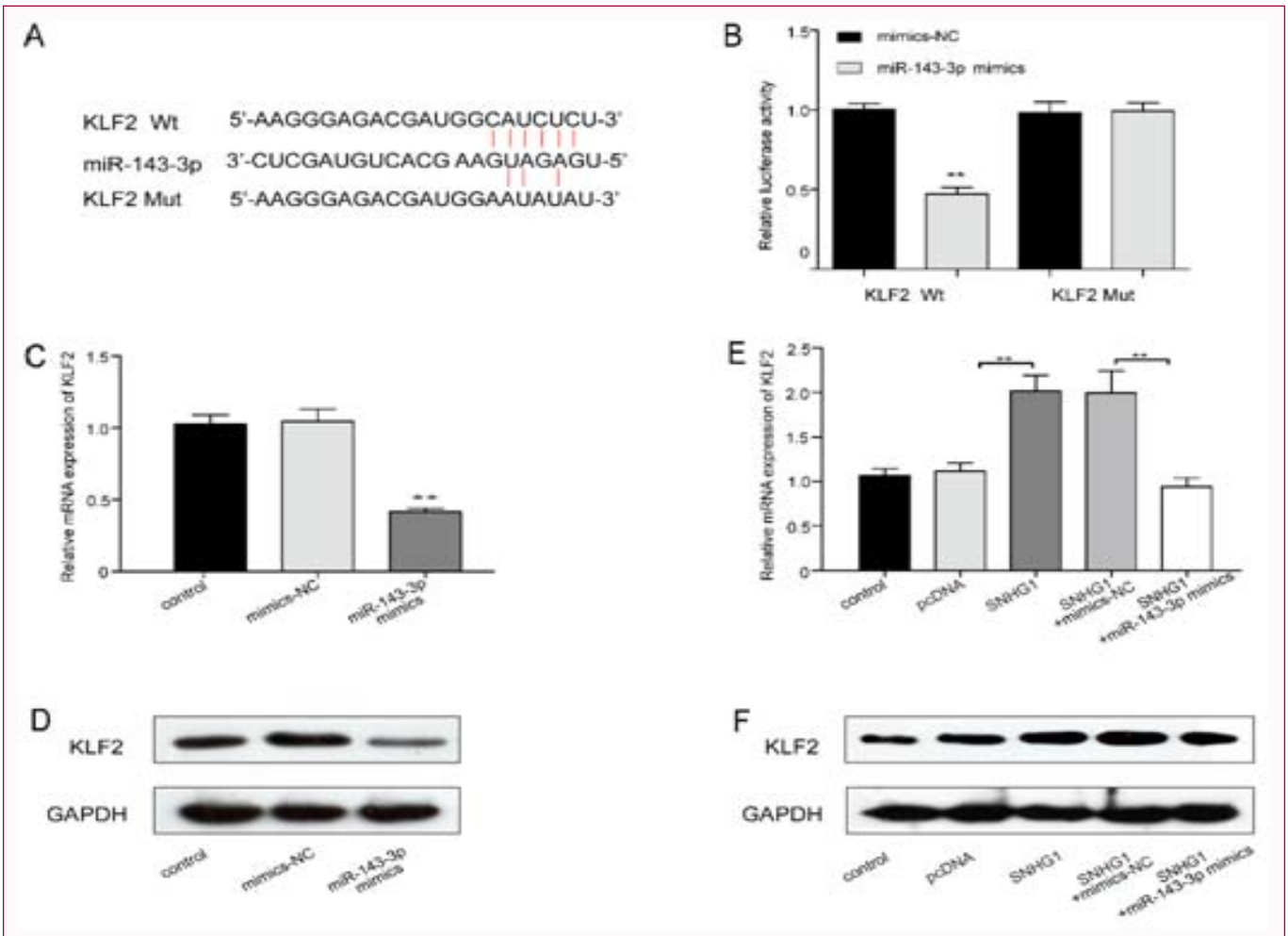

Figure 4: SNHG1 acted as a ceRNA by sponging miR-143-3p and regulated KLF2 expression indirectly. (A) The binding site between miR-143-3p and KLF2 was predicted by the Starbase database. (B) The luciferase activity was detected by dual-luciferase reporter assay. (C, D) The mRNA and protein levels of KLF2 were detected by qRT-PCR and western blot after the transfection with miR-143-3p mimics. (E, F) The mRNA and protein levels of KLF2 were examined by qRT-PCR and western blot after the co-transfection with SNHG1 and miR-143-3p mimics. *: P<0.05; **: P<0.01; ***: P<0.001.

caused by the overexpression of SNHG1, were reversed by the knockdown of KLF2 (Figure 5A, 5B). Also, after the knockdown of KLF2, the expression of inflammatory cytokines, suppressed by the overexpression of SNHG1 in chondrocytes, was partially reversed (Figure 5C). Similarly, the reverse effects of si-KLF2 on the ECM were also identified in chondrocytes with the SNHG1 vector (Figure 5D, 5E). In conclusion, the above data suggested that KLF2 might be involved in the progression of arthritis by affecting the SNHG1/miR-143-3p axis.

Discussion

OA is emerging as a major public health problem. At present, the treatments of OA mainly focus on relieving symptoms. However, the underlying mechanism of its occurrence and development has not been fully elucidated, which would cause most of terminal OA patients need joint arthroplasty at last. Therefore, many studies on OA have focused on the epigenetic regulation of its pathogenesis and potential therapeutic targets, especially non-coding RNA, including lncRNA and miRNA [24].Previous studies have reported that lncRNAs play a pivotal role in the development of bone and cartilage tissue [25], which indicates that lncRNAs may affect the balance between the anabolism and catabolism of articular cartilage, and that lncRNAs may be used for the diagnosis and prognosis of OA. Moreover, studies have shown that lncRNAs, as personalized therapeutic biomarkers, can prevent, stop or even reverse the progress of OA [15,16,26,27]. These lncRNAs may help explain the underlying molecular mechanisms associated with cartilage damage, and may also be beneficial to the development of the new treatments for OA. However, there is no related research on the influence of lncRNA SNHG1 on osteoarthritis. In this study, we found that the expression of lncRNA SNHG1 in inflammatory injured chondrocytes reduced. In addition, after exploring the function of SNHG1 in chondrocytes, we found that the overexpression of SNHG1 mitigated cells' inflammatory damage caused by IL-1β, and that knocking down SNHG1 aggravated cells' inflammatory damage. Further experiments show that miR-143-3p can inhibit the expression of SNHG1, and that the overexpression of miR-143-3p can reverse the protective effect of SNHG1 on the injured chondrocytes caused by IL-1β. Finally, we found that KLF2 is the downstream target gene of miR-143-3p, and that lncRNA SNHG1 indirectly regulates the expression of KLF2 through directly targeting at and combining with miR-143-3p, so as to exert its protective effect on the inflammatory injured chondrocytes.

A large amount of evidence indicate that the development of OA is related to local inflammatory response and the abnormal condition of metabolism [28,29]. Moreover, it is proved that IL-1β, as a pro-inflammatory cytokine, plays a vital role in OA [30]. Many

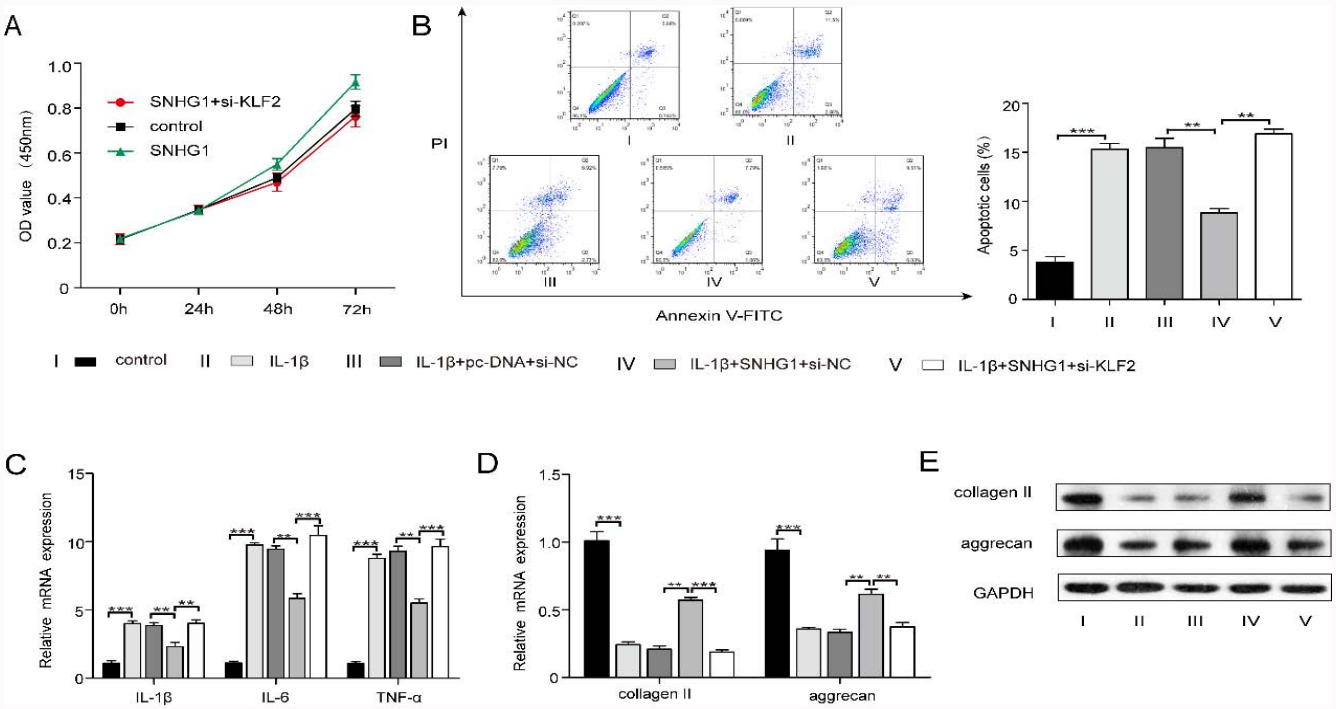

Figure 5: SNHG1 inhibited cell apoptosis, inflammatory response, ECM degradation, and promoted cell viability by regulating KLF2 expression in IL-1β-induced chondrocytes. (A) The cell viability was detected by MTT assay. Transfected chondrocytes were treated with IL-1β, and then (B) the apoptosis was determined by flow cytometry, and (C, ¬D) the mRNA expression of inflammatory cytokines, type II collagen, and aggrecan were analyzed by qRT-PCR. (E) The protein levels of type II collagen and aggrecan were examined by western blot. *: P<0.05; **: P<0.01; ***: P<0.001.

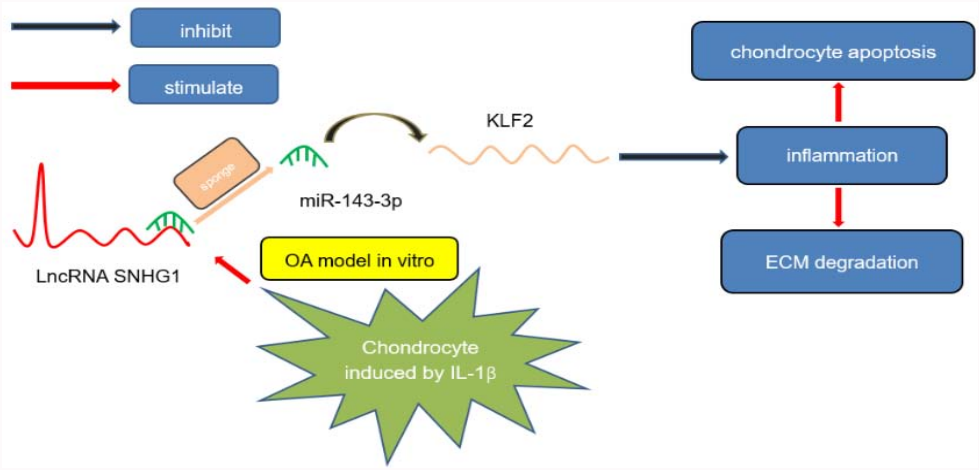

Figure 6: The graphical representation of this manuscript.
In this study, we established the OA model of chondrocytes in mice by IL-1β in order to explore the regulatory role and mechanisms of LncRNA SNHG1 in inflammatory injured chondrocytes. In summary, we identified that SNHG1 was down-regulated in OA chondrocytes and SNHG1 overexpression was proved to alleviate IL-1β-induced inflammatory injury, suggesting it could act as a possible therapeutic target in OA. We further explored and determined that SNHG1 can combine with miR-143-3p to up-regulate the expression of KLF2, thereby reducing the chondrocyte apoptosis, inflammation and degradation of ECM.

studies have shown that IL-1β reduces the synthesis of extracellular matrix components such as collagen II and aggrecan by disrupting the metabolic balance of chondrocytes [31]. Moreover, IL-1β could promote the production of the Main Matrix Degrading Enzymes (MMPs) and (ADAMTS) in chondrocytes [32]. Therefore, inhibiting the expression of IL-1β and IL-1β-induced inflammatory mediators may be a potential therapeutic approach to reduce the progression of OA. In recent years, IL-1β-induced chondrocytes have been widely used to construct inflammatory injury models. For example, Tu et al. used IL-1β to treat rat chondrocytes in order to study the effects of schizandrin and cell viability, inflammatory response and cartilage degradation [33]. Hu et al. found that lncRNA HOTAIR can reduce the IL-1β-induced inflammation of mouse chondrocytes [27]. Therefore, in this study, we chose IL-1β to be an in vitro stimulator in order to construct a model of chondrocyte inflammation. At the same time, we also tested the viability, apoptosis, and inflammatory factor expression of the chondrocytes treated by IL-1β.

After IL-1β treatment of mouse chondrocytes, we found that the expression of lncRNA SNHG1 down-regulated. In order to further clarify the relationship between lncRNA SNHG1 and extracellular

cartilage matrix and inflammatory factors, we used small interfering RNA (si-SNHG1) and overexpressed SNHG1 plasmids to respectively transfect chondrocytes. We found that overexpression of lncRNA SNHG1 can promote chondrocyte proliferation, inhibit the high expression of MMP3, MMP13, ADAMTS4 and ADAMTS5 under the action of IL-1β, and increase the expression of collagen II and aggrecan. In addition, compared with the control group under the intervention of IL-1β, the expression of the inflammatory factors IL-1β, IL-6 and TNF-α obviously reduced, and the apoptosis rate of chondrocytes were also reduced. The transfection of si-SNHG1 reduced the expression of lncRNA SNHG1 in chondrocytes and increased the inflammatory damage induced by IL-1β, which is the opposite of the results obtained by the overexpression of lncRNA SNHG1.These results indicate that lncRNA SNHG1 plays an important role in chondrocytes. Up-regulating the expression of lncRNA SNHG1 can alleviate the degradation of chondrocyte extracellular matrix, inhibit inflammatory reaction and reduce apoptosis.

The ceRNA pathway was widely reported as a classic mechanism of the action of lncRNA [34]. For instance, a study, carried out by Hu et al. indicated that HOTAIR promoted osteoarthritis progression by regulating the expression of miR-17-5p/FUT2/β-catenin axis [27]. LncRNA PVT1 mediated inflammatory injury by targeting the miR-27b-3p/TRAF3 in chondrocytes [35]. Concerning SNHG1, plenty of researches authenticated that SNHG1 could perform its function by antagonizing the miRNAs and up-regulating miRNAs downstream targets' expression. And SNHG1/miRNA 101-3p/ROCK1 axis promoted cell growth, migration and invasion in osteosarcoma [23]. SNHG1 also adjusted PDCD4 expression by interacting miR-195-5p in hepatocellular carcinoma [36]. Many reports indicate that miRNAs have attracted more and more attention due to their important regulatory functions in bone and cartilage [37]. In this regard, we predicted that the potential target of lncRNA SNHG1 is miRNAs through the public database Starbase, and then we selected the most relevant miRNA, namely miR-143-3p. Studies have shown that with the differentiation of Mesenchymal Stem Cells (MSCs) into chondrocytes, the expression of miR-143-3p significantly reduced, and miR-143-3p reduced the level of cartilage marker protein by negatively regulating Bone Morphogenetic Protein 2 (BMPR2) [38]. However, the regulatory role of miR-143-3p in inflammatory injured chondrocytes has not been elucidated. To verify the relationship between lncRNA SNHG1 and miR-143-3p, we indirectly verified that miR-143-3p can directly bind to SNHG1 through the luciferase reporter gene experiment, and that the transfection of miR-143-3p mimic can inhibit the expression of SNHG1 in chondrocytes. In addition, miR-143-3p can also reverse the effects of SNHG1 on the viability, apoptosis and ECM degradation of inflammatory injured chondrocytes.

KLF2, a transcriptional activator, belongs to the zinc finger family. Recently, some researchers reported that KLF2 played a significant regulatory role in various inflammatory diseases. Das et al. suggested that KLF2 regulated K/BxN serum-induced arthritis [39]. Manoharan et al. demonstrated that KLF2 promoted skeletal muscle regeneration by regulating innate immune response [40]. Gao et al. reported that KLF2 can relieve chondrocyte degradation and apoptosis to protect rats from being damaged by experimental OA by inhibiting the oxidation reaction and reducing the expression of MMPs (MMP3, MMP9, and MMP13) [41]. And the suppression of miR-150 can lead to the up-regulation of KLF2, which helps to protect ATDC5 cells from being damaged by IL-1 [42]. Based on the previous studies, we explored the effects of SNHG1 and miR-143-3p on the expression of KLF2. In our study, it was found that KLF2 is a downstream target of miR-143-3p, and that changing SNHG1 and miR-143-3p helps to regulate KLF2 expression. In addition, SNHG1 can inhibit inflammation, apoptosis and ECM degradation by indirectly up-regulating KLF2 expression. However, this effect can be reversed by knocking down KLF2. Our results indicate that LncRNA SNHG1 can regulate IL-1β-induced damage through the miR-143-3p/KLF2 axis.

Conclusion

Collectively, this study demonstrated that LncRNA SNHG1 alleviated the IL-1β-induced chondrocytes inflammatory injury *via* targeting miR-143-3p/KLF2 axis, which may highlight new insights regarding the roles of LncRNAs in OA.

Acknowledgment

Data availability

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

Authors' contributions

LL was the major contributors in implementing experiments and writing the manuscript. FC and JYH performed the data collection and analysis. QCC and SJX conceived and designed the study and critically revised the manuscript for important intellectual content. QL participated in the design of the review and helped to finalize the manuscript. All authors read and approved the final manuscript.

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