Revista Argentina de Clínica Psicológica
2020, Vol. XXIX, N°3, 1118-1127
DOI: 10.24205/03276716.2020.941

LncRNA SNHG1 improves chondrocyte apoptosis and inflammation microenvironment by down-regulating miR-195-5p

Baiqi Liu*, Donghui Xu, Xiujuan Ding, Dexin Che

Abstract

Background: Osteoarthritis (OA) is a chronic progressive bone disease, which still lacks effective treatment strategies. Many evidences show that long non-coding RNA (LncRNA) is closely related to the progression of OA. However, the effect of small nucleolar RNA host gene 1 (SNHG1) on OA apoptosis and inflammatory microenvironment have not been described in detail.

Methods: Firstly, the expression levels of SNHG1 and miR-195-5p in cartilage tissue and normal cartilage tissue of OA patients and rats were detected. Secondly, the effects of SNHG1 and miR-195-5p on apoptosis, inflammation and proliferation of OA chondrocytes were studied through a series of cell function experiments. Next, the relationship between SNHG1 and miR-195-5p was studied. Finally, SNHG1 promoter and miR-195-5p inhibitor were injected to explore the in vivo effects on OA rat models.

Results: We found that the SNHG1 expression was down-regulated and the miR-195-5p expression was up-regulated in OA tissue. In addition, increasing SNHG1 or knocking down miR-195-5p expression can reduce apoptosis rate, improve inflammatory microenvironment of OA chondrocytes, and restore cell proliferation. Besides, we clarified that SNHG1 could act as a molecular sponge for miR-195-5p. Finally, we found that SNHG1 promoter and miR-195-5p inhibitor could reduce apoptosis and inflammatory reaction in OA rat models.

Conclusion: Adjusting the SNHG1-miR-195-5p axis, increasing SNHG1 or knocking down miR-195-5p is beneficial to improving the apoptosis and inflammatory microenvironment of chondrocytes and provides new insights for the mechanism of OA progression.

Keywords: osteoarthritis, SNHG1, miR-195-5p, apoptosis, inflammatory microenvironment

Introduction

Osteoarthritis (OA) is a common joint disease. Joint function damage and pain are typical manifestations. Severe cases can make people disabled and bring great negative impact on the normal life of patients (Pan and Jones, 2018; Qin et al, 2017). The blasting fuse of OA involves joint injury, obesity, aging and other factors. The pathological mechanism is related to inflammatory microenvironment and apoptosis created by excessive release of inflammatory factors in chondrocytes (Mathiessen and Conaghan, 2017; Sun et al., 2017). At present, we can't cure OA but can only relieve symptoms or prevent diseases. However, exploring genes with high correlation of pathological regulation in the expression profile of OA-related genes are helpful for us to find molecular therapeutic approaches to curb OA (Bartels et al., 2016; Fan et al., 2018). Hence, based on the pathological mechanism of OA, this study will explore the potential molecular targets of the course of OA, hoping to provide new ideas for its treatment, which is also quite significant to improve the quality of life of patients.

[a]Department of Orthopeadic Surgery, The First Hospital of Qiqihar, Qiqihar, Heilongjiang Province, China.
[b]Department of Orthopeadic Surgery, Qiqihar Hospital Affiliated to Southern Medical University,Qiqihar, Heilongjiang Province, China.
**Corresponding Author:Baiqi Liu*
Email: lbqdxj@163.com

Long non-coding RNA-microRNA (LncRNA-miRNA) is a highly involved molecular regulatory network in organism physiology and pathology. LncRNA is often used as an upstream dominant gene, serving as a molecular sponge for miRNA negative regulation, and participating in the progression of OA and other diseases (Huang, 2018; Li et al., 2017). Li et al. (Li et al., 2017) reported that LncRNA PVT1-miR-488-3p molecular regulatory network could mediate apoptosis of chondrocytes, thus playing a role in pathological process of OA. Pan et al. (Pan et al., 2018) studied that LncRNA MALAT1-miR-19b molecular regulatory axis could regulate inflammatory injury of animal OA in vitro model. However, the main molecule in this study was the small nucleolar RNA host gene 1 (SNHG1)-miR-195-5p, and we found that there were potential targeting sites in starbase. Among them, SNHG1 participated in the carcinogenic mechanism of various cancer types of cells, and also participated in the SNHG1-miR-577-WNT2B axial network in osteosarcoma to regulate tumor progression (Tian et al., 2018; Jiang et al., 2018). However, miR-195-5p has been proved to play a regulatory part in the molecular mechanism of osteosarcoma regulated by LncRNA SNHG12 or XIST in many studies (Yang et al., 2018; Yang et al., 2018). At the moment, there are few pathological mechanisms of both in OA, but we found that the joint research of SNHG1 and miR-195-5p has been reported in hepatocellular carcinoma and colorectal cancer, and it has been proved that there is a targeted regulatory relationship (Huang et al., 2019; Bai et al., 2019).

We suspect that SNHG1-miR-195-5p regulatory network mediates chondrocyte apoptosis and inflammatory microenvironment.

Materials and methods

Collection of OA tissue samples

The samples were taken from 52 OA patients, including 30 males and 22 females with an average age of 58.9±5.3 years, and 52 non-OA patients, including 28 males and 24 females with an average age of 59.3±4.8 years. The collection time was from October 2017 to October 2019. We excluded all patients with malignant tumors, other orthopedic diseases or inflammation, or those in pregnancy and lactation, or those with severe physical dysfunction. This experiment has been approved by the Hospital Ethics Committee and all patients have provided an informed consent form.

Cell culture

Human chondrocytes C-28/I2 (Beina Chuanglian Biotechnology Institute, Beijing, China, BNCC339995 C-28/I2) were purchased and cultured in DMEM medium containing 10%PBS (Kanglang Biotechnology Co., Ltd., Shanghai, China, KL-P0032) at 37℃, 5%CO_2.

OA cell models

The models were established as described above (Shu et al., 2019), and the cells were placed in an environment of 5 μg/ml lipopolysaccharide (LPS) (Hengdu Biotechnology Co., Ltd., Shanghai, China, L2880) and intervened 5 h at 37℃. Cells without LPS intervention were used as controls.

Cell transfection

Chondrocytes were transfected with Lipofectamine™ 2000 kit (Woosen Biotechnology Co. Ltd, Hangzhou, China, 11668019), and the operation procedures were strictly in accordance with the kit instructions. Among them, the main transfectants include SNHG1 over-expression plasmid (SNHG1), empty vector plasmid (pcDNA3.0), miR-195-5p inhibitor (inhibitor), miR-195-5p mimetic and miR negative control (miR-NC). Twenty-four hours after transfection, the transfection efficiency was verified by RT-qPCR.

Animal models

SD rats (female, weight 225±25 g) (Fuerbo Biotechnology Co., Ltd., Shanghai, China) were purchased, and they were fed based on feeding standard, and animal experiments have been approved by the Animal Protection Committee of our hospital. The OA rat models were established by the improved Hulth method (Wang et al., 2016). Under general anesthesia, the left knee joint was opened, the anterior cruciate ligament was transected, and the medial meniscus was excised. After the operation, the medial-lateral stress test and the anterior drawer test were used to verify the success of the operation. In contrast, the rats in the control group underwent sham operation and only the articular cavity was opened without any intervention. All rats were forced to move after the operation. SNHG1 and inhibitor (60 mg/kg body

weight for 3 consecutive days) were injected into their joints in the model group 3 weeks after the operation. Normal saline was injected into the simple model group and the control group. Thus, the rats were divided into control group (n=10), model group (n=10), SNHG1 intervention group based on model group (SNHG1, n=10), and inhibitor intervention group based on model group (inhibitor, n=10).

Two weeks after the last injection, rats were euthanized and cartilage tissue was collected.

Real-time quantitative PCR

The total RNA was extracted from tissues and cells via Trizol reagent (Simgen Biochemical Reagent Development Co., Ltd., Hangzhou, China, 5301100), and it was reverse transcribed with reverse transcription kit (Jiehui Bogao Biotechnology Co., Ltd., Beijing, China, K1622), and then the synthesized complementary DNA was amplified. The primer design was handed over to Hasense Biological Medicine Technology co., Ltd., wuxi, China. MRNA employed β-Actin as internal reference, miRNA employed U6 as internal reference, and the relative expression was analyzed by $2^{-\Delta\Delta ct}$.

Apoptosis assay

Transfected chondrocytes were collected and washed with PBS (Huamaike Biotechnology Co., Ltd., Beijing, China, 331452). We added 500 μL of binding buffer containing FITC (2 μL) (Jinsui Biotechnology Co., Ltd., Shanghai, China, J31405) and PI (5 μL) (Chreagen Biotechnology Co., Ltd., Beijing, China, 13624) in a dark environment, and the apoptosis rate was measured 1 h later by flow cytometry (AMG0002051, Beijing, China).

Enzyme-linked immunosorbent assay (ELISA)

ELISA kits (Hengfei Biotechnology Co., Ltd., Shanghai, China, CSB-E08055r-1, CSB-E04640r-1, CSB-E11987r-1) were used to measure IL-1β, IL-6, TNF-α in human chondrocytes and rat cartilage tissue. The operation instructions were strictly followed. At last, the absorbance was measured at 450 nm with a practical microplate reader (Image Trading Co., Ltd., Beijing, China, 21261000).

Cell proliferation test

MTT kit (Baiolebo Technology Co., Ltd., Beijing, China, SY0502-YQV) was used for detection. Cells were first inoculated into 96-well plates (density: 5×10^4 cells/well), 20 μL of MTT solution was added to each well at different incubation times, and incubated 4 h at 37℃. Then, 150 μL of DMSO was mixed into each well one by one, shaken for 10 min, and finally absorbance value was measured at 450 mm wavelength by enzyme reader.

Western blot analysis

Proteins in cells or tissues were isolated by RIPA buffer (Xinhua Lvyuan Technology Co., Ltd., Beijing, China, SS0656), ionized by 10% SDS-PAGE (Enjing Biotechnology Co., Ltd., Nanjing, China, E1WP304) and then transferred to PVDF membrane (Chreagen Biotechnology Co., Ltd., Beijing, China, ISEQ00011), and sealed 1 h with sealing solution (Hengfei Biotechnology Co., Ltd., Shanghai, China, PW0046). Next, the primary antibody, including IL-1β, IL-6, TNF-α, Caspase-3, Bax, Bcl-2, β-Actin, was incubated with the membrane at 4℃ all night long. The antibodies were all purchased from Beijing Baiaolaibo Technology Co., Ltd. After that, the sample was hybridized with HRP-coupled secondary antibody (Bio-Rad Laboratories, Shanghai, China, 170-6515) at 37℃. Finally, protein bands were analyzed by chemiluminescence kits (Dingguo Changsheng Biotechnology Co., Ltd, Beijing, China, ECL-0013).

Bioinformatics analysis

The SNHG1-miR-195-5p relationship was predicted by online prediction tools starBase (http: //starbase.sysu.edu.cn/), lncRNABase (http: //starbase.sysu.edu.cn/mirLncRNA.php) and RegRNA2.0 (http: //regrna2.mbc.nctu.edu.tw/detection.html).

Dual-luciferase report assay

Complementary DNA fragments of wild-type (Wt) and mutant (Mut) SNHG1 and miR-195-5p fragments were subcloned into downstream of the luciferase gene in the luciferase report vector. The SNHG1 fragment was co-transfected with miR-195-5p mimetic (miR-195-5p) or miR-NC; after 48 h, luciferase activity in the cell lysate was continuously measured using a dual-luciferase report kit

(Solarbio Technology Co., Ltd., Beijing, China, D0010).

RNA immunoprecipitation

Determination was carried out by EZMagna RIP kit (Guyan Industrial Co., Ltd., Shanghai, China, GOY-E5944). Cells were lysed, incubated 1 h with protein A magnetic beads and primary antibody, then immunoprecipitated 8 h at 4℃. Finally, the expression levels of SNHG1 and miR-195-5p were detected after RNA purification.

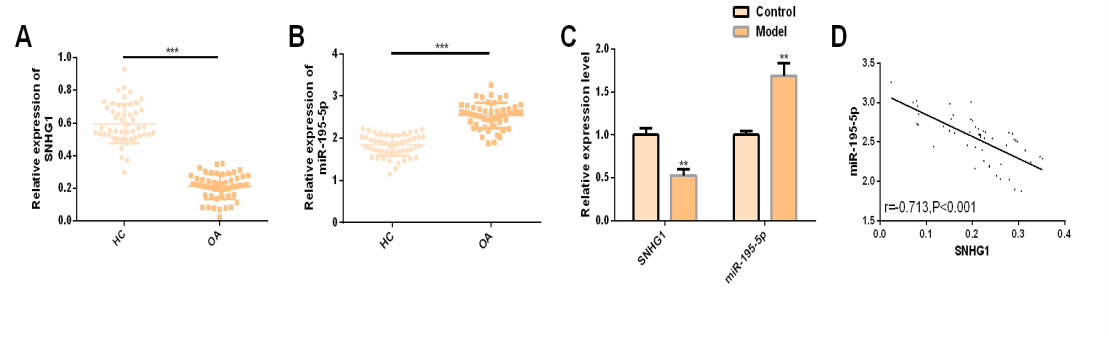

Figure 1: **expression levels of SNHG1 and miR-195-5p**

A-B: A lower level of SNHG1 and a higher level of miR-195-5p are present in cartilage tissue of OA patients.
C: Cartilage tissue in rat OA models also has low expression of SNHG1 and high expression of miR-195-5p.
D: There is a negative correlation between SNHG1 and miR-195-5p in cartilage tissue of OA patients (r = -0.713, P < 0.001).
Note: compared with Control or between two groups, $^{**}P < 0.01$, $^{***}P < 0.001$.

RNA pull-down experiment

Biotinylated miR-195-5p-Wt, miR-195-5p-Mut and Bio-NC (negative control) were used respectively and then transfected into chondrocytes. After 48 h, the cell lysate was incubated with M-280 Streptomyces magnetic beads to detect the SNHG1 level in the RNA complex bound to the beads.

Statistical methods

Statistical analysis and picture drawing were carried out with GraphPad 6 software package. The data were expressed as mean±standard deviation. All experiments were carried out independently for at least 3 times. Independent-samples t test, one-way analysis of variance (ANOVA), LSD-t test, repeated measures ANOVA and Bonferroni test were applied to analyze the data difference, and P < 0.05 showed that the difference had statistical significance. The correlation between SNHG1 and miR-195-5p was tested by Pearson correlation coefficient.

Results

SNHG1 is down-regulated while miR-195-5p is up-regulated in OA

SNHG1 was down-regulated in cartilage tissue of OA patients, while miR-195-5p was up-regulated, both of which had similar manifestations in rat OA models ($P < 0.001$). Correlation analysis revealed that there was a remarkable negative correlation between them (r = -0.713, $P < 0.001$). (Figure 1)

Over-expression of SNHG1 can reduce the high apoptosis rate of chondrocytes induced by LPS and improve inflammatory microenvironment

We transfected the over-expression plasmid into chondrocytes to realize the over-expression of SNHG1. Cell analysis manifested that LPS-induced apoptosis of chondrocytes, expression of inflammatory factors IL-1β, IL-6, TNF-α and protein levels increased obviously, and cell proliferation was remarkably inhibited. However, after SNHG1 was over-expressed, the expression of chondrocytes was markedly improved, but there was still a marked difference compared with the

control group. The above analysis results had statistical significance ($P < 0.05$). (Figure 2)

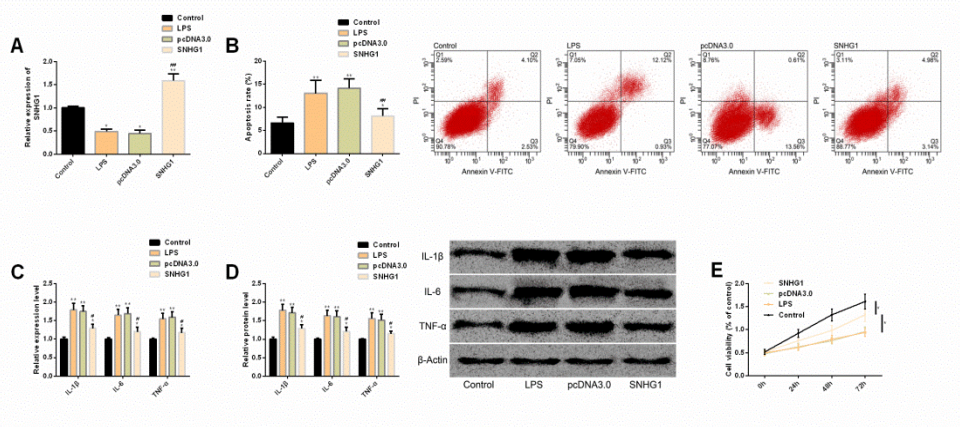

Figure 2: **effect of SNHG1 on LPS-induced chondrocytes**

A: SNHG1 transfection efficiency
B: LPS-induced apoptosis rate of chondrocytes increases markedly, while SNHG1 can inhibit apoptosis level, and its flow cytometry is shown.
C-D: Inflammatory factor expression and protein level in chondrocytes induced by LPS increase obviously, while SNHG1 can improve this inflammatory state, and its protein map is shown.
E: LPS-induced proliferation of chondrocytes is inhibited, while SNHG1 can relieve this inhibition.
Note: compared with Control, $^{*}P < 0.05$, $^{**}P < 0.01$; compared with LPS, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

Knocking down miR-195-5p can reduce the high apoptosis rate of chondrocytes induced by LPS and improve inflammatory microenvironment

Similarly, we knocked down miR-195-5p expression by transfecting miR-195-5p inhibitory sequence into chondrocytes. The results of cell function tests manifested that chondrocytes showed higher apoptosis rate, higher levels of inflammatory factors (including transcription and protein levels) and lower cell proliferation level under LPS intervention. However, after knocking down miR-195-5p expression, the above results were close to the control group, but there is still a certain distance. The above results had statistical significance ($P < 0.05$). (Figure 3)

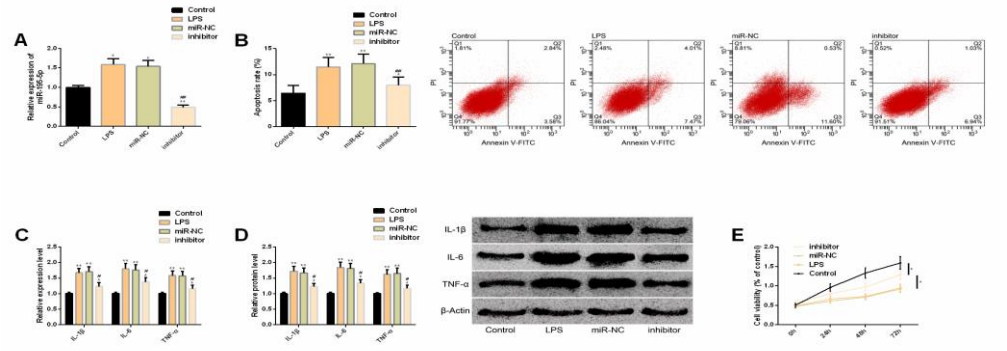

Figure 3: **effect of miR-195-5p on LPS-induced chondrocytes**

A: transfection efficiency of miR-195-5p
B: effect of miR-195-5p on chondrocyte apoptosis rate and its flow cytometry
C-D: effect of miR-195-5p on transcription and protein level of inflammatory factors in chondrocytes and its protein map
E: effect of miR-195-5p on chondrocyte proliferation
Note: compared with Control, $^{*}P < 0.05$, $^{**}P < 0.01$; compared with LPS, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

miR-195-5p is the direct target of SNHG1

Through bioinformatics analysis, we found that miR-195-5p and SNHG1 had potential targeting sites. In the dual-luciferase report analysis, the miR-195-5p mimetic only significantly reduced SNHG1-Wt (instead of SNHG1-Mut). In RNA immunoprecipitation analysis, SNHG1 was highly recruited in miR-195-5p complex containing Ago2. In the pull-down experiment, SNHG1 was only pulled down by biotin-labeled miR-195-5p-WT. Besides, we also found that low levels of miR-195-5p exist in chondrocytes transfected with SNHG1 over-expression sequence. The above results had statistical significance ($P < 0.05$). (Figure 4)

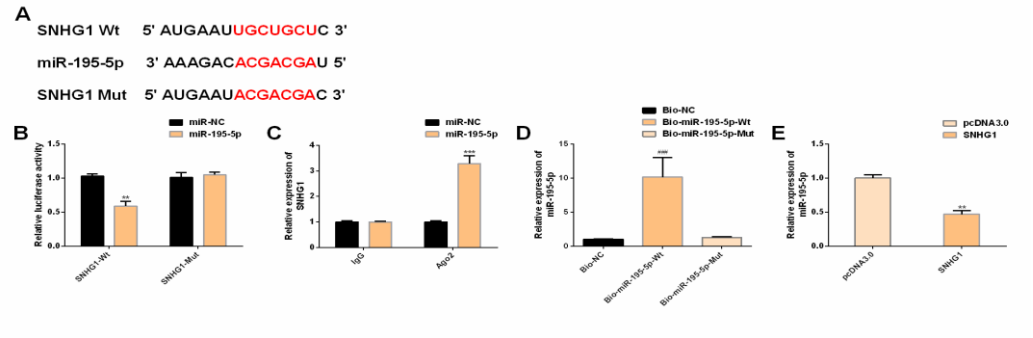

Figure 4: **expression levels of SNHG1 and miR-195-5p**

A: potential binding site of miR-195-5p-SNHG1
B: dual-luciferase report
C: RNA immunoprecipitation experiment
D: RNA pull-down experiment
E: miR-195-5p-SNHG1 relationship
Note: compared with miR-NC/pcDNA3.0, $^{**}P < 0.01$, $^{***}P < 0.001$; compared with LPS, $^{\#\#\#}P < 0.001$.

SNHG1-miR-195-5p regulatory axis can improve the level of apoptosis-related factors and inflammatory microenvironment in OA rats in vivo

The OA rat model was injected with SNHG1 promoter and miR-195-5p inhibitor for treatment. The results showed that the transcription and protein levels of apoptosis factors Caspase-3 and Bax/Bcl-2 were dramatically lower than that of the model group, but still remarkably higher than that of the control group. Besides, inflammatory factors IL-1β, IL-6 and TNF-α had similar results. The above results had statistical significance ($P < 0.05$). (Figure 5)

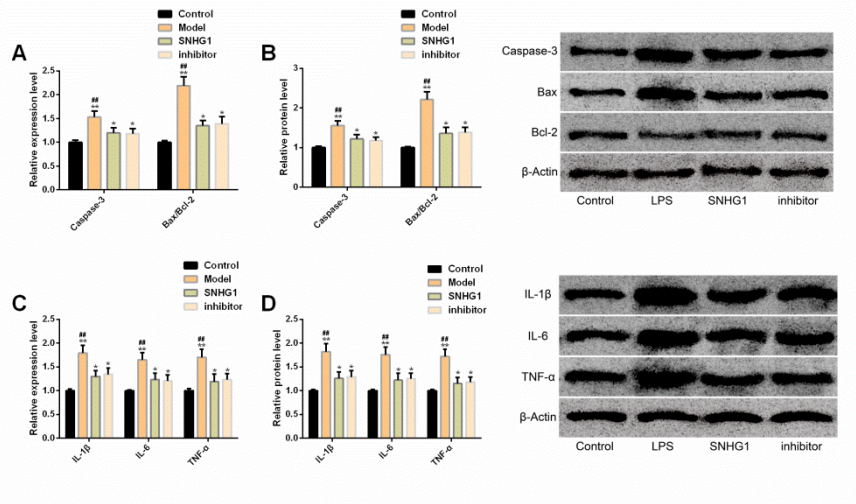

Figure 5**: effect of SNHG1-miR-195-5p regulatory axis on OA rats**

A-B: effect of SNHG1-miR-195-5p regulatory axis on transcription and protein levels of apoptosis related factors in OA rats and its protein map

C-D: effect of SNHG1-miR-195-5p regulatory axis on inflammatory factor transcription and protein level in OA rats and its protein map

Note: compared with Control, $^{*}P < 0.05$, $^{**}P < 0.01$; compared with Model, $^{\#\#}P < 0.01$.

Discussion

OA has a worldwide influence, and women are at greater risk of disease. Moreover, due to limited activities, the disease can also lead to psychological depression and bring negative effects on people's physical and mental health to varying degrees (Vina and Kwoh, 2018; Vitaloni et al., 2019). Research shows that LncRNA-miRNA integrated network can regulate the progression of OA. Dynamic monitoring and human intervention may be beneficial to the reversal of OA (Chen et al., 2019). Lei et al. (Lei et al., 2019) pointed out that enhancing SNHG1 expression could alleviate metabolic dysfunction and inflammatory state of OA by targeting inhibition of miR-16-5p and mediating p38 MAPK-NF-κB signaling pathway, suggesting that SNHG1 could alleviate its effect in the progression of OA and might become a new therapeutic target. Shu et al. (Shu et al., 2019) reported that miR-195-5p had an abnormally high level in OA; knocking down its expression could reduce the apoptosis level and inflammatory degree of chondrocytes by targeting REGγ, suggesting that developing miR-195-5p inhibitor was helpful to curb OA progression. In this study, we mainly focus on SNHG1 and miR-195-5p to explore the molecular mechanism of OA in vivo and in vitro, hoping to provide new insights for treatment, which is quite significant to improve the physical and mental health of patients.

During the progression of OA, chondrocyte apoptosis is generally induced by excessive mechanical stress, which will also lead to cartilage tissue destruction, resulting in body cartilage disability and limited movement (Xu et al., 2019). The low proliferation level of chondrocytes is also one of the cellular events of early OA, which may be tied to the metabolic dysfunction of chondrocytes (Boehme and Rolauffs, 2018). Inflammatory microenvironment of OA patients is often accompanied by excessive activation of inflammatory factors, which is also the fuse of cell dysfunction (Wang et al., 2017). Studies have shown that the anti-inflammatory properties of OA drugs are often reflected in the secretion inhibition of inflammatory factors such as IL-1β, IL-6, TNF-α (Yang et al., 2019). In our study, SNHG1 was down-regulated and miR-195-5p was up-regulated in OA tissue, both of which had abnormal imbalance, indicating that both might mediate the pathological process of OA. In vivo study, we increased the SNHG1 expression by transfecting the expression plasmid into chondrocytes. Cell function analysis found that high level of SNHG1 could significantly

reduce the apoptosis rate, inhibit the secretion of inflammatory factors IL-1β, IL-6, TNF-α, etc., thus improving the inflammatory microenvironment of chondrocytes, and could also restore the proliferation level of cells. The above results showed that developing SNHG1 promoter was conducive to improve OA condition by maintaining the molecular microenvironment of chondrocytes, including regulating apoptosis, proliferation and inflammatory state. Similarly, we found that down-regulation of miR-195-5p had similar effect on chondrocytes as upregulation of SNHG1, which suggested that human intervention on both expressions was helpful to improve OA. Afterwards, we explored the mechanism of action of the two and found that there was a binding site between SNHG1 and miR-195-5p. The miR-195-5p mimetic could significantly reduce SNHG1-Wt (instead of SNHG1-Mut), the miR-195-5p complex containing Ago2 could recruit SNHG1, and only biotin-labeled miR-195-5p-Wt was pulled down by SNHG1. What's more, over-expressing SNHG1 could knock down miR-195-5p level. Based on the above results, SNHG1, as an endogenous RNA, could act as a molecular sponge of miR-195-5p to realize negative regulation of its expression, and there was an exact targeting relationship between the two. Our data showed that SNHG1 could target miR-195-5p to mediate the development of OA chondrocytes, and mainly played a regulatory role in chondrocyte apoptosis, proliferation and inflammatory microenvironment.

We also conducted in vivo studies to verify the effects of SNHG1 promoter and miR-195-5p inhibitor in OA mice. The results showed that higher levels of Caspase-3, Bax/Bcl-2 (transcription and protein level) and higher levels of inflammatory factors (IL-1β, IL-6, TNF-α) appeared in the cartilage tissue of rats in the model group, suggesting that those rats had shown obvious OA progression such as cartilage apoptosis and inflammatory microenvironment. However, when we intervened the model group rats with SNHG1 promoter or miR-195-5p inhibitor, the above results were significantly improved, suggesting that SNHG1 promoter or miR-195-5p inhibitor could significantly inhibit apoptosis factors and inflammatory factors in the model group rats at transcription and protein levels, which was beneficial to prevent cartilage apoptosis and repair inflammatory microenvironment. Hu et al. (Hu et al., 2018) studied that the effects of apoptosis factors in OA rats were confirmed. Down-regulating pro-apoptotic factors Caspase-3, Bax and up-regulating anti-apoptotic factor Bcl-2 had important effects on inhibiting chondrocyte apoptosis, and thus playing a protective role, which was also consistent with the results of this study.

Although this study has been completed, there is still room for improvement. First of all, we can supplement and explore the potential downstream targets of miR-195-5p and study the mechanism of SNHG1 on them. Secondly, we can also increase the research on signal transduction pathways, find the possible inflammatory pathways mediated by SNHG1 and miR-195-5p, and explore their potential association with OA pain. Furthermore, we can increase the diagnostic value of SNHG1 combined with miR-195-5p for early OA and further explore its potential clinical value.

To summarize, SNHG1-miR-195-5p regulatory network mediates chondrocyte apoptosis and inflammatory microenvironment. Developing SNHG1 promoter or miR-195-5p inhibitor may be beneficial to OA treatment.

References

[1] Bai J, Xu J, Zhao J. (2019). lncRNA SNHG1 cooperated with miR-497/miR-195-5p to modify epithelial–mesenchymal transition underlying colorectal cancer exacerbation. *Journal of cellular physiology*, 235(2): 1453-1468. DOI:10.1002/jcp.29065

[2] Bartels E M, Juhl C B, Christensen R. (2016). Aquatic exercise for the treatment of knee and hip osteoarthritis. Cochrane *Database of Systematic Reviews*, (3). DOI: 10.1002/14651858.CD005523.pub2

[3] Boehme K A, Rolauffs B. (2018). Onset and progression of human osteoarthritis—Can growth factors, inflammatory cytokines, or differential miRNA expression concomitantly induce proliferation, ECM degradation, and inflammation in articular cartilage? *International journal of molecular sciences*, 19(8): 2282. DOI: https://DOI.org/10.3390/ijms19082282

[4] Chen Y, Lin Y, Bai Y. (2019). A Long Noncoding RNA (lncRNA)-Associated Competing Endogenous RNA (ceRNA) network identifies

eight lncrna biomarkers in patients with osteoarthritis of the knee. *Medical science monitor: international medical journal of experimental and clinical research*, 25: 2058.

[5] Fan Q, Liu Z, Shen C. (2018). Microarray study of gene expression profile to identify new candidate genes involved in the molecular mechanism of leptin-induced knee joint osteoarthritis in rat. *Hereditas*, 155(1): 4. DOI:https:// DOI.org/10.1186/s41065-017-0039-z

[6] Hu P F, Chen W P, Bao J P. (2018). Paeoniflorin inhibits IL-1β-induced chondrocyte apoptosis by regulating the Bax/Bcl-2/caspase-3 signaling pathway. *Molecular medicine reports*, 17(4): 6194-6200. DOI: 10.3892/mmr.2018.8631

[7] Huang D, Wei Y, Zhu J. (2019). Long non-coding RNA SNHG1 functions as a competitive endogenous RNA to regulate PDCD4 expression by sponging miR-195-5p in hepatocellular carcinoma. *Gene*, 714: 143994. DOI:https:// DOI.org/10.1016/j.gene.2019.143994

[8] Huang Y. (2018). The novel regulatory role of lnc RNA-mi RNA-mRNA axis in cardiovascular diseases. *Journal of cellular and molecular medicine*, 22(12): 5768-5775. DOI:https:// DOI.org/10.1111/jcmm.13866

[9] Jiang Z, Jiang C, Fang J. (2018). Up-regulated lnc-SNHG1 contributes to osteosarcoma progression through sequestration of miR-577 and activation of WNT2B/Wnt/β-catenin pathway. *Biochemical and biophysical research communications*, 495(1): 238-245. DOI: 10.1016/j.bbrc.2017.11.012

[10] Lei J, Fu Y, Zhuang Y. (2019). LncRNA SNHG1 alleviates IL-1β-induced osteoarthritis by inhibiting miR-16-5p-mediated p38 MAPK and NF-κB signaling pathways. *Bioscience reports*, 39(9):123-132. DOI:10.1042/BSR20191523

[11] Li Y F, Li S H, Liu Y. (2017). Long noncoding RNA CIR promotes chondrocyte extracellular matrix degradation in osteoarthritis by acting as a sponge for Mir-27b. *Cellular Physiology and Biochemistry*, 43(2): 602-610. DOI:10.1159/000480532

[12] Li Y, Li S, Luo Y. (2017). LncRNA PVT1 Regulates Chondrocyte Apoptosis in Osteoarthritis by Acting as a Sponge for miR-488-3p. *DNA and cell biology*, 36(7): 571. DOI:10.1089/dna.2017.3678

[13] Mathiessen A, Conaghan P G. (2017). Synovitis in osteoarthritis: current understanding with therapeutic implications. *Arthritis research & therapy*, 19(1): 18. DOI:10.1186/s13075-017-1229-9

[14] Pan F, Jones G (2018). Clinical perspective on pain and pain phenotypes in osteoarthritis. *Current rheumatology reports*, 20(12): 79. DOI: 10.1007/s11926-018-0796-3

[15] Pan L, Liu D, Zhao L. (2018). Long noncoding RNA MALAT1 alleviates lipopolysaccharide-induced inflammatory injury by upregulating microRNA-19b in murine chondrogenic ATDC5 cells. *Journal of cellular biochemistry*, 119(12): 10165. DOI: https:// DOI.org/10.1002/jcb.27357

[16] Qin J, Barbour K E , Murphy L B (2017). Lifetime risk of symptomatic hand osteoarthritis: the Johnston County osteoarthritis project. *Arthritis & Rheumatology*, 69(6): 1204-1212. DOI: https:// DOI.org/10.1002/art.40097

[17] Shu Y, Long J, Guo W. (2019). MicroRNA-195-5p inhibitor prevents the development of osteoarthritis by targeting REGγ. *Molecular Medicine Reports*, 19(6): 4561. DOI:10.3892/mmr.2019.10124

[18] Sun J, Wei X, Lu Y. (2017). Glutaredoxin 1 (GRX1) inhibits oxidative stress and apoptosis of chondrocytes by regulating CREB/HO-1 in osteoarthritis. *Molecular immunology*, 90: 211. DOI:https:// DOI.org/10.1016/j.molimm.2017.08.006

[19] Tian T, Qiu R, Qiu X. (2018). SNHG1 promotes cell proliferation by acting as a sponge of miR-145 in colorectal cancer. *Oncotarget*, 9(2): 2128. DOI:10.18632/oncotarget.23255

[20] Vina E R, Kwoh C K. (2018). Epidemiology of osteoarthritis: literature update. *Current opinion in rheumatology*, 30(2): 160. DOI:10.1097/BOR.0000000000000479

[21] Vitaloni M, Botto-van Bemden A, Contreras R M S. (2019). Global management of patients with knee osteoarthritis begins with quality of life assessment: a systematic review. *BMC musculoskeletal disorders*, 20(1): 493. DOI:10.1186/s12891-019-2895-3

[22] Wang Q, Wang W, Zhang F. (2017). NEAT1/miR-181c regulates osteopontin (OPN)-mediated synoviocyte proliferation in osteoarthritis. *Journal of cellular biochemistry*, 118(11): 3775-3784. DOI: https:// DOI.org/10.1002/jcb.26025

[23] Xu B, Xing R, Huang Z. (2019). Excessive mechanical stress induces chondrocyte apoptosis through TRPV4 in an anterior cruciate ligament-transected rat osteoarthritis model. *Life sciences*, 228: 158-166. DOI: 10.1016/j.lfs.2019.05.003

[24] Yang C, Wu K, Wang S. (2018). Long non-coding RNA XIST promotes osteosarcoma progression by targeting YAP via miR-195-5p. *Journal of cellular biochemistry*, 119(7): 5646-5656. DOI: https:// DOI.org/10.1002/jcb.26743

[25] Yang H, Huang J, Mao Y. (2019). Vitexin alleviates interleukin-1β-induced inflammatory responses in chondrocytes from osteoarthritis patients: Involvement of HIF-1α pathway. *Scandinavian journal of immunology*, 90(2): e12773. DOI:10.1111/sji.12773