Knockdown of LSD1 alleviates the IL-1 β -induced chondrocyte apoptosis, inflammation and ECM degradation via TRIM32-mediated autophagy

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Abstract

Osteoarthritis (OA) is a common joint disease with characteristics of chronic inflammation and articular cartilage degeneration. It has been proved that LSD1 was up-regulated in OA cartilage tissues, but its role and regulatory mechanism in OA are unclear. Herein, interleukin 1 beta (IL-1 β)-treated human chondrocytes was performed as a cell model of OA. Then, LSD1 expression was found that up-regulated in OA cartilage tissues and IL-1 β -induced chondrocytes. Knockdown of LSD1 increased cell viability, while decreased apoptosis rate and inflammatory cytokines secretion levels in IL-1 β -induced chondrocytes. In addition, knockdown of LSD1 reduced the expression of catabolic proteins (MMP-13 and ADAMTS-5) and enhanced the expression of anabolic proteins (Collagen II and Aggrecan) in chondrocytes after IL-1 β stimulation. Moreover, overexpression of TRIM32 repressed chondrocyte viability, while promoted IL-1 β -induced chondrocyte apoptosis, inflammation and ECM degradation. The expression of LSD1 and TRIM32 in OA cartilage was positively correlated, and knockdown of LSD1 down-regulated TRIM32 expression of chondrocytes. Our data further indicated that LSD1 regulated autophagy of chondrocytes through modulating TRIM32. Overexpression of TRIM32 reduced the effect of LSD1 knockdown on IL-1 β -induced chondrocytes, while activating autophagy by Rapamycin further reversed this reduction. Therefore, our study shows that knockdown of LSD1 inhibited IL-1 β -induced chondrocyte apoptosis, inflammation and ECM degradation via TRIM32-mediated autophagy.

Introduction

Osteoarthritis (OA) is a common degenerative joint disease with a significant increase in incidence with age, and is mainly manifested by joint swelling and long-term chronic pain, which seriously affects the quality of life of patients[1, 2]. OA is always characterized by the reduction of chondrocytes and the degradation of cartilage extracellular matrix (ECM) with tissular inflammation[3, 4]. In the onset of OA, abnormal pathological changes in articular cartilage tissue break the balance of anabolism and catabolism in chondrocytes, and ultimately lead to the degradation of ECM[5–7]. Therefore, maintaining chondrocyte viability is one of the key factors to prevent OA.

Lysine specific histone demethylase (LSD1) is a flavin adenine dinucleotide (FAD) dependent monoamine oxidase[8, 9]. It can specifically catalyze the demethylation of lysine 4 (H3K4me1, H3K4me2) and lysine 9 (H3K9me1, H3K9me2) of histone H3 that are monomethylated and dimethylated, thereby regulating the transcription activity of target genes[10, 11]. In recent years, numerous functional studies have found that LSD1 plays an important role in tumorigenesis, embryonic differentiation, heterochromatin formation, and pluripotent stem cell formation[12–14]. It has been reported that LSD1 expression was up-regulated in OA cartilage tissues, and it can act as an anabolic activity regulator of articular chondrocytes[10, 11]. However, the role and underlying mechanisms of LSD1 in OA have not been clarified.

TRIM32 (Tripartite motif 32) is a transcription factor with E3 ubiquitin linkase activity[15, 16]. Its protein structure contains the characteristic zinc finger domain, B-boxes domain and coiled domain of the TRIM family, as well as characteristic 6 A repetitive NHL domain[17–19]. Studies have found that it participates

in the occurrence and development of a variety of tumors and neurological diseases by regulating cell proliferation, differentiation, development, and apoptosis[19–21]. A recent paper reveals that TRIM32 contributes to the inflammation of rheumatoid arthritis[22]. Whereas, whether TRIM32 plays a role in the pathogenesis of OA remains unknown.

In this study, we examined the expression of LSD1 and TRIM32 in normal and OA cartilage, and investigated the functional role and underlying mechanisms of LSD1 and TRIM32 in IL-1 β -induced OA model, which might provide potential therapeutic targets for OA treatment.

Materials & Methods

tissue samples

The OA cartilage tissues were obtained from 14 patients who underwent arthroplasty for OA between June 2019 and July 2020 at Yantaishan Hospital. The normal cartilage tissues were obtained from 10 patients who suffered from a femoral neck fracture without OA or rheumatic arthritis at the hospital. The study was approved by the institutional ethics committee of Yantaishan Hospital, and written informed consents were obtained from all patients.

Cell culture

Human chondrocytes were obtained from the BeNa Culture Collection (Beijing, China), and were maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco, MA, USA) containing 10% fetal bovine serum (FBS; Gibco, MA, USA) and 1% penicillin/streptomycin (Solarbio, Beijing, China) at 37°C in an atmosphere of 5% CO₂.

Transfection

The small interfering RNA (siRNA) against LSD1 (si-LSD1) and matched control (si-NC), pcDNA3.1-TRIM32 overexpression vector and pcDNA3.1 empty vector were all purchased by Genepharma Co, Ltd (Shanghai, China). After transfected with vectors by using Lipofectamine 2000 Transfection Reagent (Invitrogen, CA, USA) for 48 h, chondrocytes were treated with IL-1 β (Beyotime, Shanghai, China) for 24 h.

Quantitative real-time PCR (qRT-PCR) assay

The total RNAs of cartilage tissues or chondrocytes was extracted using TRIzol (Invitrogen, MA, USA). The total RNA (1 μ g) was reverse transcribed into cDNA using M-MLV Reverse Transcriptase (Invitrogen). qRT-PCR was performed using SYBR® Green (Promega, Madison, WI, USA), with GAPDH used for normalization. The primers used for qRT-PCR are: LSD1 (forward) 5'-TTCTGGAGGGTATGGAGACG-3' and (reverse) 5'-CCTTCTGGGTCTGTTGTGGT-3'; TRIM32 (forward) 5'-CCCGGACAGTTAACGTGGAA-3' and (reverse) 5'-CGGGCTCATGTCCATCTCTC-3'; GAPDH (forward) 5'-GAAGGTGAAGGTCTGGAGTCA-3' and (reverse) 5'-TTGAGGTCAATGAAGGGGTC-3'.

Western blot analysis

The total protein of cartilage tissues or chondrocytes was extracted by RIPA lysis buffer (Beyotime, Haimen, China), and the protein concentration was determined by BCA assay (Beyotime, Haimen, China). Protein samples were transferred to PVDF membrane by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Then the membranes were incubated with primary antibodies against LSD1 (1:1000; ab129195; Abcam); TRIM32 (1:1000; ab131223; Abcam); MMP-13 (1:800; ab39012; Abcam); ADAMTS-5 (1:800; ab41037; Abcam); Collagen II (1: 1000; ab34712; Abcam); Aggrecan (1: 1000; ab3778; Abcam); p-mTOR (1:1000; ab137133; Abcam); mTOR (1:1000; ab2732; Abcam); p62 (1:1000; ab56416; Abcam); LC3 α /LC3 β (1:1000; ab48394; Abcam); beclin1 (1:1000; ab62557; Abcam) or GAPDH (1:2000; ab8245; Abcam) at 4°C overnight. The membrane was then incubated with a secondary antibody named horseradish peroxidase-labeled rabbit anti-rat IgG (1:2000; ab6721; Abcam) at room temperature for 2 hours. Finally, the blots were visualized using the electrochemiluminescence kit (Thermo Fisher Scientific, MA, USA) and Image Lab 3.0 software (Bio-Rad; CA; USA).

Cell viability assay

Cell viability was detected by cell-counting kit-8 assay (CCK-8; Dojindo Laboratory, Kumamoto, Japan). Briefly, Chondrocytes (5×10^3 cells/well) were cultured in a 96-well plate and treated with a medium supplemented with 10% CCK-8 solution at 37°C for 4 hours. Then, the absorbance of each well was assessed by a microplate reader (BIOTEK, Winooski, Vermont, USA) at 450 nm.

Apoptosis assay

The total number of apoptotic chondrocytes was quantified by flow cytometry with fluorescein isothiocyanate (FITC)-labeled Annexin V and propidium iodide (PI) (Enzo Life Sciences Inc., Farmingdale, NY) according to manufacturer's protocol.

Enzyme-linked-immunosorbent-assay (ELISA)

The cell culture supernatant was collected and the secretion levels of inflammatory cytokines, including IL-8, IL-6 and TNF- α in chondrocytes were detected using ELISA kits according to the manufacturer's instructions.

Statistical analysis

All experiments were repeated three times, and the data was analyzed by SPSS 20.0 statistical software. Data are means $\pm$ standard deviation (SD). The correlation was analyzed by Pearson' correlation test. Statistical significance was assessed using student's *t*-test and one-way ANOVA, and $P < 0.05$ was considered to be statistically significantly relevant.

Results

1 LSD1 was overexpressed in OA cartilage tissues and IL-1 β -induced chondrocytes

Quantitative real-time (qRT)-PCR and Western blot assay were performed to examine the expression of LSD1 in OA (n = 14) and normal cartilage tissues (n = 6). As shown in Fig. 1A and 1B, the expression of LSD1 was up-regulated in OA cartilage tissues. Besides, a cell model of OA was established by IL-1 β -induced human chondrocytes. After IL-1 β stimulation, the LSD1 expression of chondrocytes increased in a dose-dependent manner (Fig. 1C and 1D).

2 Knockdown of LSD1 inhibited IL-1 β -induced apoptosis, inflammation and ECM degradation

To determine the role of LSD1 in OA, LSD1 siRNA was transfected into IL-1 β -induced chondrocytes. The results of transfection efficiency indicated that LSD1 siRNA significantly decreased LSD1 expression in IL-1 β -induced chondrocytes (Fig. 2A and 2B). Afterwards, IL-1 β inhibited cell viability of chondrocytes while LSD1 siRNA attenuated this inhibition (Fig. 2C). IL-1 β promoted apoptosis rate, and production of IL-8, IL-6 and TNF- α , while LSD1 siRNA blocked this promotion (Fig. 2D and 2E). In addition, LSD1 siRNA reduced the expression of catabolic markers (MMP-13 and ADAMTS-5) and enhanced the expression of anabolic markers (Collagen II and Aggrecan) in chondrocytes after IL-1 β stimulation (Fig. 2F-2H).

3 Overexpression of TRIM32 enhanced IL-1 β -induced apoptosis, inflammation and ECM degradation

As shown in Fig. 3A, TRIM32 was highly expressed in OA cartilage tissues, compared with normal control group. Then, the expression of TRIM32 was elevated by its overexpression vectors (pcDNA-TRIM32) in IL-1 β -induced chondrocytes (Fig. 3B and 3C). The data of functional experiments further revealed that pcDNA-TRIM32 repressed cell viability while facilitated apoptosis, inflammatory response, and ECM degradation in IL-1 β -induced chondrocytes.

4 Down-regulated LSD1 induced autophagy through modulating TRIM32

Interestingly, LSD1 and TRIM32 expression were positively correlated in OA cartilage tissue, and TRIM32 expression of IL-1 β -induced chondrocytes was down-regulated after LSD1 knockdown, which suggested that LSD1 could modulate TRIM32 expression (Fig. 4A-4C). Recent studies have reported that TRIM32 is a key activator of mammalian target of rapamycin (mTOR), which participates in the progression of OA via regulating autophagy[23–25]. Here, we explored whether LSD1 and TRIM32 regulates mTOR-mediated OA autophagy. After IL-1 β treatment, LSD1 siRNA reduced the expression of p-mTOR/mTOR and p62, and pcDNA-TRIM32 retarded this reduction (Fig. 4D and 4E). Moreover, LSD1 siRNA increased the expression of LC3 β /LC3 α and beclin1, and pcDNA-TRIM32 alleviated this increase (Fig. 4D and 4F). These findings indicated that knockdown of LSD1 induced OA autophagy via modulating TRIM32.

5 Knockdown of LSD1 activated TRIM32-mediated autophagy to suppress IL-1 β -induced apoptosis, inflammation and ECM degradation

To investigate the role of TRIM32-mediated autophagy in LSD1-regulated chondrocytes, a mTOR inhibitor Rapamycin was used to induce autophagy. After knockdown of LSD1, pcDNA-TRIM32 inhibited cell viability, and promoted apoptosis, inflammation and ECM degradation in IL-1 β -induced chondrocytes (Fig. 5). Furthermore, Rapamycin attenuated the effect of pcDNA-TRIM32 on LSD1-knockdown chondrocytes, which suggested that LSD1 regulated IL-1 β -induced chondrocytes by activating TRIM32-mediated autophagy.

Discussion

OA is a clinically common chronic degenerative disease, which mainly manifests as joint pain, swelling and/or restricted movement, and is the main cause of decreased mobility and dysfunction in the elderly[26, 27]. Damage and loss of articular cartilage is the key to the pathological process in OA[28, 29]. Inflammatory cytokines such as IL-1 β can cause a series of inflammatory cascade reaction in chondrocytes, and ultimately leads to the destruction of chondrocytes and degradation of EMC[30, 31]. Therefore, IL-1 β -stimulated chondrocytes was used to establish a cell model of OA in this study. It has been reported that LSD1 expression is increased in OA cartilage compared with normal cartilage[11], and our data confirmed this. Besides, our data also found that LSD1 expression was up-regulated in IL-1 β -induced OA model *in vitro*.

Autophagy plays an important role in the homeostasis maintenance of chondrocytes for clearing dysfunctional organelles and macromolecules[32, 33]. With the degeneration of cartilage, autophagy is inhibited and induces the homeostasis imbalance of chondrocytes, eventually leading to cell death[34, 35]. Consistent with previous studies[36, 37], we found that autophagy was down-regulated in IL-1 β -induced chondrocytes. mTOR, a serine/threonine protein kinase, is a key repressor of autophagy, which is modulated by multiple upstream signaling pathways to regulate autophagy level[24, 38]. It has been found that parathyroid hormone (PTH)-(1–34) and Astragaloside IV ameliorate chondrocyte apoptosis via increasing autophagy by repressing mTOR[7, 39]; Xue et al. and Kong et al. have reported that inhibition of mTOR attenuates inflammation of chondrocytes in rats with OA[40, 41]. What is more, numerous experimental data indicate that activating mTOR-mediated autophagy can inhibit the ECM degradation in IL-1 β -induced chondrocytes[39, 40, 42]. These evidences suggest that mTOR-mediated autophagy plays an important role in the progression of OA.

LSD1 is the first confirmed histone demethylase that can specifically remove the methyl modification of H3K9me1/2 and restore it to the unmethylated state, thereby affecting the transcriptional activation of target genes[8, 12]. Previous studies have shown that LSD1 regulates the level of autophagy to programme oocyte death and reduce cancer cell viability via modulating p62 expression [43, 44].

Furthermore, Wei et al. found that LSD1 mediates autophagy to regulate ovarian cancer progression through the mTOR signaling pathway[45]. Shi et al. reported that LSD1 down-regulates autophagy of myoblast cells via the activity the mTOR signaling pathway[46]. A recent study has revealed that knockdown of LSD1 attenuates ox-LDL-induced inflammation of RAW264.7 cells by promoting mTOR-mediated autophagy[47]. In this study, we found that knockdown of LSD1 suppressed IL-1 β -induced chondrocyte apoptosis, inflammation and ECM degradation by activating mTOR-mediated autophagy.

TRIM32 has been reported that be overexpressed in fibroblast-like synoviocytes (FLS) of OA patients[22], and the present study indicated that TRIM32 expression was up-regulated in OA cartilage tissues and IL-1 β -induced chondrocytes. It is noted that LSD1 modulates TRIM37 expression in luminal breast cancer cells[12], our study showed that knockdown of LSD1 down-regulated TRIM32 expression in IL-1 β -induced chondrocytes. Recently, papers have found that TRIM32 contributes to autophagy in muscle cells through the regulation of p62 activity[48]. Moreover, a *in vivo* experimental data shows that TRIM32 deficiency mice increases autophagy level by reducing mTOR activity[23]. In this study, we found that LSD1 knockdown activated mTOR-mediated autophagy by modulating TRIM32, and that may be the molecular mechanism of LSD1 knockdown on chondrocyte apoptosis, inflammation and ECM degradation.

In conclusion, our study found that LSD1 and TRIM32 were overexpressed in OA cartilage tissues and IL-1 β -induced chondrocytes, and their expression showed a strong positive correlation. Knockdown of LSD1 enhanced chondrocyte viability, and repressed apoptosis, inflammation and ECM degradation via activating mTOR-mediated autophagy by regulating TRIM32. Therefore, our study may provide potential therapeutic targets for OA therapy.

Declarations

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Ethics approval and consent to participate

The study was approved by the institutional ethics committee of Yantaishan Hospital, and written informed consents were obtained from all patients.

Consent for publication

Not applicable

Conflicts of Interest

The authors declare no conflict of interest.

Availability of data and material

All datasets for this study are included in the manuscript/supplementary files.

Acknowledgements

Not applicable

Author Contributions

WQX and XFL designed the experiments and wrote the paper; WQX, XFL, WQQ, XW, HS and WLL carried out the experiments; YHC performed the statistical analysis.

References

1. Woodell-May, J. E., and S. D. Sommerfeld. 2020. Role of Inflammation and the Immune System in the Progression of Osteoarthritis. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society* 38: 253–257.
2. Zhong, G., H. Long, S. Ma, Y. Shunhan, J. Li, and J. Yao. 2019. miRNA-335-5p relieves chondrocyte inflammation by activating autophagy in osteoarthritis. *Life Sciences* 226: 164–172.
3. Jia, Y., C. Pang, K. Zhao, J. Jiang, and Y. Qian. 2019. Garcinol Suppresses IL-1 β -Induced Chondrocyte Inflammation and Osteoarthritis via Inhibition of the NF- κ B Signaling Pathway. *Inflammation* 42: 1754–1766.
4. Zhang, L., S. Ma, H. Su, and J. Cheng. 2018. Isoliquiritigenin Inhibits IL-1 β -Induced Production of Matrix Metalloproteinase in Articular Chondrocytes. *Molecular Therapy-Methods & Clinical Development* 9: 153–159.
5. Chen, J., Y. T. Gu, J. J. Xie, C. C. Wu, J. Xuan, W. J. Guo, Y. Z. Yan, L. Chen, Y. S. Wu, and X. L. Zhang. 2018. Gastrodin reduces IL-1 β -induced apoptosis, inflammation, and matrix catabolism in osteoarthritis chondrocytes and attenuates rat cartilage degeneration in vivo. *Biomedicine & Pharmacotherapy* 97: 642–651.
6. Chern, C. M., H. Zhou, Y. H. Wang, C. L. Chang, W. F. Chiou, W. T. Chang, C. H. Yao, K. T. Liou, and Y. C. Shen. 2020. Osthole ameliorates cartilage degradation by downregulation of NF- κ B and HIF-2 α pathways in an osteoarthritis murine model. *Eur J Pharmacol* 867: 172799.
7. Li, Y., Y. Wu, K. Jiang, W. Han, J. Zhang, L. Xie, Y. Liu, J. Xiao, and X. Wang, Mangiferin Prevents TBHP-Induced Apoptosis and ECM Degradation in Mouse Osteoarthritic Chondrocytes via Restoring Autophagy and Ameliorates Murine Osteoarthritis, *Oxidative medicine and cellular longevity* 2019 (2019) 8783197.
8. Yang, G. J., P. M. Lei, S. Y. Wong, D. L. Ma, and C. H. Leung, Pharmacological Inhibition of LSD1 for Cancer Treatment, *Molecules (Basel, Switzerland)* 23 (2018).

9. Karakaidos, P., J. Verigos, and A. Magklara, LSD1/KDM1A, a Gate-Keeper of Cancer Stemness and a Promising Therapeutic Target, *Cancers* 11. 2019.
10. Durand, A. L., A. Dufour, E. Aubert-Foucher, C. Oger-Desfeux, M. Pasdeloup, S. Lustig, E. Servien, G. Vaz, E. Perrier-Groult, F. Mallein-Gerin, and J. E. Lafont, The Lysine Specific Demethylase-1 Negatively Regulates the COL9A1 Gene in Human Articular Chondrocytes, *Int J Mol Sci* 21 (2020).
11. El Mansouri, F. E., S. S. Nebbaki, M. Kapoor, H. Afif, J. Martel-Pelletier, J. P. Pelletier, M. Benderdour, and H. Fahmi. 2014. Lysine-specific demethylase 1-mediated demethylation of histone H3 lysine 9 contributes to interleukin 1 β -induced microsomal prostaglandin E synthase 1 expression in human osteoarthritic chondrocytes. *Arthritis Res Ther* 16: R113.
12. Hu, X., D. Xiang, Y. Xie, L. Tao, Y. Zhang, Y. Jin, L. Pinello, Y. Wan, G. C. Yuan, and Z. Li. 2019. LSD1 suppresses invasion, migration and metastasis of luminal breast cancer cells via activation of GATA3 and repression of TRIM37 expression. *Oncogene* 38: 7017–7034.
13. Cao, K., C. K. Collings, M. A. Morgan, S. A. Marshall, E. J. Rendleman, P. A. Ozark, E. R. Smith, and A. Shilatifard. 2018. An Mll4/COMPASS-Lsd1 epigenetic axis governs enhancer function and pluripotency transition in embryonic stem cells. *Science advances* 4: eaap8747.
14. Storck, W. K., V. T. Bicocca, M. R. Rountree, S. Honda, T. Ormsby, and E. U. Selker. 2020. LSD1 prevents aberrant heterochromatin formation in *Neurospora crassa*. *Nucleic Acids Res* 48: 10199–10210.
15. Di Rienzo, M., M. Piacentini, and G. M. Fimia. 2019. A TRIM32-AMBRA1-ULK1 complex initiates the autophagy response in atrophic muscle cells. *Autophagy* 15: 1674–1676.
16. Wang, M., W. Luo, Y. Zhang, R. Yang, X. Li, Y. Guo, C. Zhang, R. Yang, and W. Q. Gao. 2020. Trim32 suppresses cerebellar development and tumorigenesis by degrading Gli1/sonic hedgehog signaling. *Cell death and differentiation* 27: 1286–1299.
17. Luo, Q., X. Wu, Y. Nan, W. Chang, P. Zhao, Y. Zhang, D. Su, and Z. Liu. 2020. TRIM32/USP11 Balances ARID1A Stability and the Oncogenic/Tumor-Suppressive Status of Squamous Cell Carcinoma. *Cell Rep* 30: 98–111.e115.
18. Zhao, T. T., F. Jin, J. G. Li, Y. Y. Xu, H. T. Dong, Q. Liu, P. Xing, G. L. Zhu, H. Xu, S. C. Yin, and Z. F. Miao. 2018. TRIM32 promotes proliferation and confers chemoresistance to breast cancer cells through activation of the NF- κ B pathway. *J Cancer* 9: 1349–1356.
19. Wei, L., J. S. Zhang, S. F. Ji, H. Xu, Z. H. Zhao, L. Zhang, L. Pang, J. F. Zhang, P. B. Yang, and H. Ma. 2019. Knockdown of TRIM32 Protects Hippocampal Neurons from Oxygen-Glucose Deprivation-Induced Injury. *Neurochem Res* 44: 2182–2189.
20. Du, Y., W. Zhang, B. Du, S. Zang, X. Wang, X. Mao, and Z. Hu. 2018. TRIM32 overexpression improves chemoresistance through regulation of mitochondrial function in non-small-cell lung cancers. *Onco Targets Ther* 11: 7841–7852.
21. Prajapati, P., D. Gohel, A. Shinde, M. Roy, K. Singh, and R. Singh. 2020. TRIM32 regulates mitochondrial mediated ROS levels and sensitizes the oxidative stress induced cell death. *Cell Signal* 76: 109777.

22. Liang, T., M. Song, K. Xu, C. Guo, H. Xu, H. Zhang, and L. Xu. 2020. TRIM32 promotes inflammatory responses in rheumatoid arthritis fibroblast-like synoviocytes. *Scand J Immunol* 91: e12876.
23. Zhu, J. W., M. M. Zou, Y. F. Li, W. J. Chen, J. C. Liu, H. Chen, L. P. Fang, Y. Zhang, Z. T. Wang, J. B. Chen, W. Huang, S. Li, W. Q. Jia, Q. Q. Wang, X. C. Zhen, C. F. Liu, S. Li, Z. C. Xiao, G. Q. Xu, J. C. Schwamborn, M. Schachner, Q. H. Ma, and R. X. Xu, Absence of TRIM32 Leads to Reduced GABAergic Interneuron Generation and Autism-like Behaviors in Mice via Suppressing mTOR Signaling, *Cerebral cortex* (New York, N.Y. 1991. 30 (2020) 3240–3258.
24. Yang, H., Y. Wen, M. Zhang, Q. Liu, H. Zhang, J. Zhang, L. Lu, T. Ye, X. Bai, G. Xiao, and M. Wang. 2020. MTORC1 coordinates the autophagy and apoptosis signaling in articular chondrocytes in osteoarthritic temporomandibular joint. *Autophagy* 16: 271–288.
25. Wu, J., L. Kuang, C. Chen, J. Yang, W. N. Zeng, T. Li, H. Chen, S. Huang, Z. Fu, J. Li, R. Liu, Z. Ni, L. Chen, and L. Yang. 2019. miR-100-5p-abundant exosomes derived from infrapatellar fat pad MSCs protect articular cartilage and ameliorate gait abnormalities via inhibition of mTOR in osteoarthritis. *Biomaterials* 206: 87–100.
26. Luo, X., J. Wang, X. Wei, S. Wang, and A. Wang. 2020. Knockdown of lncRNA MFI2-AS1 inhibits lipopolysaccharide-induced osteoarthritis progression by miR-130a-3p/TCF4. *Life Sci* 240: 117019.
27. Zhao, X., H. Li, and L. Wang. 2019. MicroRNA-107 regulates autophagy and apoptosis of osteoarthritis chondrocytes by targeting TRAF3. *International Immunopharmacology* 71: 181–187.
28. Sining, Kang, Jun, Zhang, Ye, and Yuan. 2018. Abietic acid attenuates IL-1 β -induced inflammation in human osteoarthritis chondrocytes. *International Immunopharmacology* 64: 110–115.
29. Rao, Z., S. Wang, and J. Wang. 2018. Protective effects of psoralidin on IL-1 β -induced chondrocyte apoptosis. *Molecular Medicine Reports* 17: 3418–3424.
30. Shijin, L., X. Xungang, and C. Minghua. 2015. Matrine inhibits IL-1 β -induced expression of matrix metalloproteinases by suppressing the activation of MAPK and NF- κ B in human chondrocytes in vitro. *International journal of clinical and experimental pathology* 8: 4764–4772.
31. Piao, S., W. Du, Y. Wei, Y. Yang, X. Feng, and L. Bai. 2020. Protectin DX attenuates IL-1 β -induced inflammation via the AMPK/NF- κ B pathway in chondrocytes and ameliorates osteoarthritis progression in a rat model. *Int Immunopharmacol* 78: 106043.
32. Wang, S., Z. Deng, Y. Ma, J. Jin, F. Qi, S. Li, C. Liu, F. J. Lyu, and Q. Zheng. 2020. The Role of Autophagy and Mitophagy in Bone Metabolic Disorders. *International journal of biological sciences* 16: 2675–2691.
33. Ma, W. D., Z. Weng, J. Zhang, and S. Zhang. 2020. Y, BNIP3 decreases the LPS-induced inflammation and apoptosis of chondrocytes by promoting the development of autophagy. *J Orthop Surg Res* 15: 284.
34. Zhao, Y., Y. Zuo, H. Huo, Y. Xiao, X. Yang, and D. Xin. 2014. Dexamethasone reduces ATDC5 chondrocyte cell viability by inducing autophagy. *Molecular Medicine Reports* 9: 923–927.
35. Castrogiovanni, P., S. Ravalli, and G. Musumeci. 2020. Apoptosis and Autophagy in the Pathogenesis of Osteoarthritis. *Journal of investigative surgery: the official journal of the Academy of Surgical*

36. Xu, Zhao, Xiaowen Yun, Li, Junnan Lin, Wang, Xuejun, and Juntian. 2018. Ozone induces autophagy in rat chondrocytes stimulated with IL-1 β through the AMPK/mTOR signaling pathway. *Journal of Pain Research* 11: 3003–3017.
37. Chen Cai, S. M., Bo Yan, Wen Liu, Xiao Yang, Liuxun Li, and Ting Wang. 2019. Anmin Jin MiR-27a promotes the autophagy and apoptosis of IL-1 β treated articular chondrocytes in osteoarthritis through PI3K/AKT/mTOR signaling. *Aging (Albany NY)* 11: 6371–6384.
38. Zhang, Y., F. Vasheghani, Y. Li, M. Blati, K. Simeone, H. Fahmi, B. Lussier, P. J. Roughley, D. Lagares, and J. Pelletier. 2015. Cartilage-specific deletion of mTOR upregulates autophagy and protects mice from osteoarthritis. *Annals of the Rheumatic Diseases* 74: 1432–1440.
39. Liu, J., Q. Meng, H. Jing, and S. Zhou. 2017. Astragaloside IV protects against apoptosis in human degenerative chondrocytes through autophagy activation. *Mol Med Rep* 16: 3269–3275.
40. Kong, C., C. Wang, Y. Shi, L. Yan, J. Xu, and W. Qi, Active vitamin D activates chondrocyte autophagy to reduce osteoarthritis via mediating the AMPK-mTOR signaling pathway, *Biochemistry and cell biology = Biochimie et biologie cellulaire* 98. 2020. 434–442.
41. Xue, J. F., Z. M. Shi, J. Zou, and X. L. Li. 2017. Inhibition of PI3K/AKT/mTOR signaling pathway promotes autophagy of articular chondrocytes and attenuates inflammatory response in rats with osteoarthritis. *Biomed Pharmacother* 89: 1252–1261.
42. Wang, F., J. Liu, X. Chen, X. Zheng, N. Qu, B. Zhang, and C. Xia. 2019. IL-1 β receptor antagonist (IL-1Ra) combined with autophagy inducer (TAT-Beclin1) is an effective alternative for attenuating extracellular matrix degradation in rat and human osteoarthritis chondrocytes. *Arthritis Res Ther* 21: 171.
43. Chao, A., C. Y. Lin, A. N. Chao, C. L. Tsai, M. Y. Chen, L. Y. Lee, T. C. Chang, T. H. Wang, C. H. Lai, and H. S. Wang. 2017. Lysine-specific demethylase 1 (LSD1) destabilizes p62 and inhibits autophagy in gynecologic malignancies. *Oncotarget* 8: 74434–74450.
44. He, M., T. Zhang, Z. Zhu, S. Qin, H. Wang, L. Zhao, X. Zhang, J. Hu, J. Wen, H. Cai, Q. Xin, Q. Guo, L. Lin, B. Zhou, H. Zhang, G. Xia, and C. Wang. 2020. LSD1 contributes to programmed oocyte death by regulating the transcription of autophagy adaptor SQSTM1/p62. *Aging Cell* 19: e13102.
45. Wei, Y., T. Han, R. Wang, J. Wei, K. Peng, Q. Lin, and G. Shao. 2018. LSD1 negatively regulates autophagy through the mTOR signaling pathway in ovarian cancer cells. *Oncol Rep* 40: 425–433.
46. Shi, Y. X., Y. J. He, Y. Zhou, H. K. Li, D. Yang, R. Y. Li, Z. L. Deng, and Y. F. Gao. 2020. LSD1 negatively regulates autophagy in myoblast cells by driving PTEN degradation. *Biochem Biophys Res Commun* 522: 924–930.
47. Zhuo, X., Y. Wu, Y. Yang, L. Gao, X. Qiao, and T. Chen. 2019. Knockdown of LSD1 meliorates Ox-LDL-stimulated NLRP3 activation and inflammation by promoting autophagy via SESN2-mediated PI3K/Akt/mTOR signaling pathway. *Life Sci* 233: 116696.
48. Overå, K. S., J. Garcia-Garcia, Z. Bhujabal, A. Jain, A. Øvervatn, K. B. Larsen, V. Deretic, T. Johansen, T. Lamark, and E. Sjøttem, TRIM32, but not its muscular dystrophy-associated mutant, positively

Figures

Figure 1

LSD1 was overexpressed in OA cartilage tissues and IL-1 β -induced chondrocytes (A) qRT-PCR analysis of LSD1 relative expression in normal cartilage samples (n = 6) and OA cartilage samples (n = 14), with GAPDH used for normalization. *P<0.05 vs normal group. (B) Western blot analysis of LSD1 relative protein expression in normal cartilage samples (n = 3) and OA cartilage samples (n = 3), with GAPDH used for normalization. *P<0.05 vs normal group. (C, D) The relative mRNA and protein expressions of LSD1 in chondrocytes treated with escalating IL-1 β concentration for 24 h.

Figure 2

Knockdown of LSD1 inhibited IL-1 β -induced apoptosis, inflammation and ECM degradation (A, B) After chondrocytes transfected with LSD1 siRNA (si-LSD1) and its negative control vector (si-NC), the relative expression of LSD1 in IL-1 β -induced chondrocytes was detected by qRT-PCR and Western blot. * $P < 0.05$ vs normal group. (C) The cell viability was measured by CCK-8 kit. (D) The apoptosis rate was assessed by Annexin V-FITC/PI apoptosis detection kit. (E) The secretion levels of inflammatory cytokines, including IL-8, IL-6 and TNF- α , were examined by ELISA assay. (F) The relative protein expression of catabolic markers (MMP-13 and ADAMTS-5) and anabolic markers (Collagen II and Aggrecan). * $P < 0.05$.

Figure 3

Overexpression of TRIM32 enhanced IL-1 β -induced apoptosis, inflammation and ECM degradation (A) The relative mRNA expression of TRIM32 in normal cartilage samples (n = 6) and OA cartilage samples (n = 14). *P<0.05 vs normal group. (B, C) The relative expression of TRIM32 in IL-1 β -induced chondrocytes was determined after transfected with TRIM32 overexpression vector (pcDNA-TRIM32) and its negative control vector (pcDNA3.1). (C) The cell viability, (D) apoptosis rate, (E) inflammatory cytokines levels, and (F) the relative protein expression of ECM-related genes was detected after transfection. *P<0.05.

Figure 4

Down-regulated LSD1 induced autophagy through modulating TRIM32 (A) The correlation between LSD1 and TRIM32 mRNA expression in OA cartilage tissues were analysed by Pearson's correlation analysis ($r = 0.7789$, $P < 0.05$). (B, C) The relative expression of TRIM32 was measured after chondrocytes transfected with si-LSD1 and si-NC. * $P < 0.05$. (D) Chondrocytes were transfected with pcDNA-TRIM32 or/and si-LSD1 before IL-1 β stimulation. The expression of autophagy related proteins, including p-mTOR, mTOR, p62, LC3I, LC3II and beclin1 were detected. * $P < 0.05$ vs control group, # $P < 0.05$ vs IL-1 β group, & $P < 0.05$ vs IL-1 β +si-LSD1 group, & $P < 0.05$ vs IL-1 β +pcDNA-TRIM32 group.

Figure 5

Knockdown of LSD1 activated TRIM32-mediated autophagy to suppress IL-1 β -induced apoptosis, inflammation and ECM degradation Chondrocytes were treated with pcDNA-TRIM32 or/and si-LSD1 or/and Rapamycin before IL-1 β stimulation. (A) The cell viability, (D) apoptosis rate, (E) inflammatory cytokines levels, and (F) the relative protein expression of ECM-related genes was detected after

transfection. *P<0.05 vs control group, #P<0.05 vs IL-1 β group, &P<0.05 vs IL-1 β +si-LSD1 group, &P<0.05 vs IL-1 β +pcDNA-TRIM32 group.