Research Paper

Bamboo Leaf Flavone Regulates microRNA-127-5p to Hinder Interleukin-1 Beta-induced Chondrocyte Damage

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Prior studies have reported that bamboo leaf flavone possess biological and pharmacological activities in mammals. Yet, the effect and possible mechanism of bamboo leaf flavone on interleukin-1 beta-induced chondrocyte damage is still unclear. Methods interleukin-1 beta was used to induce human chondrocytes to establish a cell injury model, followed by exposure to different doses of bamboo leaf flavone. Apoptosis rate was analyzed using flow cytometry. Superoxide dismutase and lactate dehydrogenase activities were assessed using a kit. Interleukin-6 and interleukin-10 levels were determined using enzyme-linked immunosorbent assay. Quantitative reverse transcription-polymerase chain reaction analyzed microRNA-127-5p content. MicroRNA-NC and microRNA-127-5p mimics were transfected into chondrocytes, followed by treatment with interleukin-1 beta for 24 h. Cleaved caspase-3 and cleaved caspase-9 protein expression were monitored using Western blot. After bamboo leaf flavone treatment, apoptosis rate, cleaved caspase-3 and cleaved caspase-9 protein levels, lactate dehydrogenase activity, and interleukin-6 level were decreased, while microRNA-127-5p expression, superoxide dismutase activity, and interleukin-10 level were increased in dose-dependent ways. Upregulated microRNA-127-5p hindered apoptosis rate, cleaved caspase-3 and cleaved caspase-9 protein levels, the activity of lactate dehydrogenase, and the level of interleukin-6, and increased superoxide dismutase activity and interleukin-10 level. MicroRNA-127-5p knockdown could antagonize the effects of bamboo leaf flavone on interleukin-1 beta-mediated chondrocyte apoptosis, oxidative stress and inflammation. Bamboo leaf flavone exposure could inhibit cell apoptosis, oxidative stress and inflammation by up-regulating microRNA-127-5p expression, thereby reducing interleukin-1 beta-induced chondrocyte damage.

Key words: Interleukin-1 beta, human chondrocytes, bamboo leaf flavone, microRNA-127-5p, apoptosis, oxidative stress

As a most prevalent chronic joint disease all over the world, Osteoarthritis (OA) can be responsible for chronic pain, stiffness, and even disability, which is becoming a major public health problem[1,2]. Currently, chronic inflammation, metabolic disorders and matrix degradation of articular cartilage have been considered the primarily characteristics of OA[3,4]. Emerging evidence have indicated that the suppression of chondrocyte apoptosis, oxidative stress, and inflammatory response is beneficial in blocking OA progression[5-7]. However, the pathogenesis of OA has not yet been elucidated. Recently, numerous studies have presented that active components extracted from natural plants have anti-inflammatory and anti-oxidant properties that might hinder OA progression[8,9]. Belong to the phenolic fraction of bamboo leaves, Bamboo Leaf Flavone (BLF) has anti-oxidative stress effects and reduce hippocampal neuronal damage *via* repressing the inflammatory response in the hippocampus of the brain[10,11]. However, studies on the correlation between BLF and OA have not been reported. Considerable laboratory research has shown that microRNAs (miRNAs), a single-stranded non-coding Ribonuclic Acid (RNA) molecule, can modulate diverse biological processes *via* hampering the degradation of target genes[12]. Previous reports have described that miR-127-5p might diminish Interleukin-1 Beta

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(IL-1β)-triggered chondrocyte inflammation and apoptosis[13,14]. Nevertheless, whether miR-127-5p could be a potential target of BLF for OA treatment has not been elucidated. Herein, we focused on whether BLF/miR-127-5p might control IL-1β-challenged chondrocyte injury.

MATERIALS AND METHODS

Reagents:

BLF and human chondrocytes were respectively provided by RisingPointBio (Wuhan, China) and MingzhouBio (Ningbo, China). Gibco (Grand Island, New York, United States of America (USA)) offered Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), and trypsin. Invitrogen (Carlsbad, California, USA) offered Lipofectamine™ 2000. RiboBio (Guangzhou, China) provided miR-127-5p mimics and anti-miR-127-5p. Besides, Trizol reagent, Reverse transcription and fluorescent quantitative Polymerase Chain Reaction (PCR) reagents were purchase from Thermo Fisher (Grand Island, New York, USA). Cell apoptosis detection kit was acquired from Solarbio (Beijing, China). JianchengBio (Nanjing, China) offered Superoxide Dismutase (SOD) and Lactate Dehydrogenase (LDH) detection kits. Mlbio (Shanghai, China) provided IL-6 and IL-10 detection kits. Amyjet (Wuhan, China) and Abcam (Cambridge, United Kingdom) respectively offered Rabbit anti-human cleaved caspase-3, cleaved caspase-9 antibody and secondary antibodies.

Method:

Cell treatment and transfection: Referring to the previous description[15], 10 ng/ml IL-1β was applied to stimulate chondrocytes in DMEM medium for 24 h, marked as IL-1β group. Synchronously, control group was normal cultured chondrocytes. In 6-well plates, chondrocytes were exposed to 0 μg/ml, 100 μg/ml, and 200 μg/ml BLF[16] in IL-1β-triggered DMEM medium, generating IL-1β+BLF-L/M/H group. Based on Lipofectamine method, we overexpressed miR-127-5p by transfecting miR-NC or miR-127-5p mimics into chondrocytes, which were incubated with 10 ng/ml IL-1β-induced DMEM medium, namely IL-1β+miR-NC/miR-127-5p group. In addition, anti-miR-NC or anti-miR-127-5p transfected chondrocytes were exposed to 200 μg/ml BLF and IL-1β in DMEM medium termed as IL-1β+BLF+anti-miR-NC/anti-miR-127-5p group.

Flow cytometry: Cell apoptosis rate was detected in this experiment. In short, collected chondrocytes were subjected to 0.25 % trypsin digestion and 3000 r/min centrifugation for 6 min. After discarding the supernatant, 5 μl Annexin V-Fluorescein Isothiocyante (FITC) and 5 μl Propidium Iodide (PI) were added into cells, which then were analyzed using FACS Calibur flow cytometer.

Assessment of SOD and LDH activates: After being collected chondrocyte culture supernatant from each group, LDH activity was monitored by the kit. In parallel, Radio-Immunoprecipitation Assay (RIPA) lysate was added into chondrocytes, which were centrifuged. Then, supernatant was aspirated and submitted to detect the activity of SOD using the kit.

Enzyme-Linked Immunosorbent Assay (ELISA): In general, the culture medium of chondrocytes was collected. Then, the secretions of IL-6 and IL-10 were examined using ELISA kits.

Quantitative Reverse transcription (qRT)-PCR: After adding 1 ml of Trizol reagent, total chondrocyte RNA were generated, which then were transcribed to complementary Deoxyribonucleic Acid (cDNA) according to the reverse transcription kit. Subsequently, qRT-PCR amplification reaction system; 2.5 μl 10×PCR buffer, 2.5 μl Magnesium sulfate ($MgSO_4$), 2.5 μl Deoxynucleoside Triphosphate (dNTP), 0.5 μl forward primers, 0.5 μl reverse primers, 2 μl cDNA, 25 μl RNase-free double distilled Water (ddH_2O). Procedure includes 95° pre-denaturation for 2 min, 95° denaturation for 60 s, 60° annealing 30 s, annealing at 60° for 60 s, extension at 72° for 30 s, a total of 36 cycles. Finally, U6 acted as the internal reference for miR-127-5p, and data were analyzed with $2^{-\Delta\Delta Ct}$ method.

Western blot:

In brief, collected chondrocytes were mixed with 500 μl RIPA lysate for total cellular protein. Samples were taken for Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) electrophoresis. Then, separated protein gel was shifted to Polyvinylidene Difluoride (PVDF) membrane, which was added with cleaved caspase-3 (1:1000), cleaved caspase-9 (1:1000), Glyceraldehyde 3-Phosphate Dehydrogenase

(GAPDH) (1:2000). After being added with secondary antibody (1:3000) incubated at 37° for 1 h, the results were analyzed using ImageJ software.

Statistical analysis:

The data in this research was analyzed using Statistical Package for the Social Sciences (SPSS) 21.0 and expressed as ($\bar{x}$±s). Difference were recognized as significant when $p<0.05$. Comparison was analyzed based on Student's t-test or one-way Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

Referring to data exhibited in fig. 1 and Table 1, chondrocyte apoptosis rate, cleaved caspase-3 and cleaved caspase-9 protein levels, and LDH activity were improved after IL-1β treatment, but SOD activity was reduced. Meanwhile, BLF exposure might repress apoptosis rate, cleaved caspase-3 and cleaved caspase-9 protein levels, and LDH activity in IL-1β induced chondrocytes with increasing BLF doses, but promote SOD level.

As shown in Table 2, IL-6 secretion were apparently enhanced in chondrocyte after IL-1β treatment, but IL-10 level was decreased. Beyond that, BLF might relieve IL-6 content in IL-1β treated chondrocytes in concentration-dependent ways, and boost IL-10 level.

Results from Table 3 showed that miR-127-5p level was obviously reduced by IL-1β treatment. Furthermore, BLF might significantly overturn the repression of IL-1β on miR-127-5p expression in chondrocyte in a dose-dependent manner.

As displayed in fig. 2 and Table 4, overexpression of miR-127-5p might clearly decline cleaved caspase-3 and cleaved caspase-9 protein level, elevate SOD activity and IL-10 level, and curb LDH activity and IL-6 content in chondrocytes after IL-1β stimulation.

As presented in fig. 3 and Table 5, miR-127-5p deficiency might partly reverse BLF-mediated apoptosis and cleaved caspase-3 and cleaved caspase-9 protein levels inhibition, SOD activity promotion, and LDH activity repression in chondrocytes after IL-1β treatment. Downregulation of miR-127-5p might effectively abrogate BLF-triggered IL-6 content repression and IL-10 level promotion in IL-1β-treated chondrocytes (Table 6).

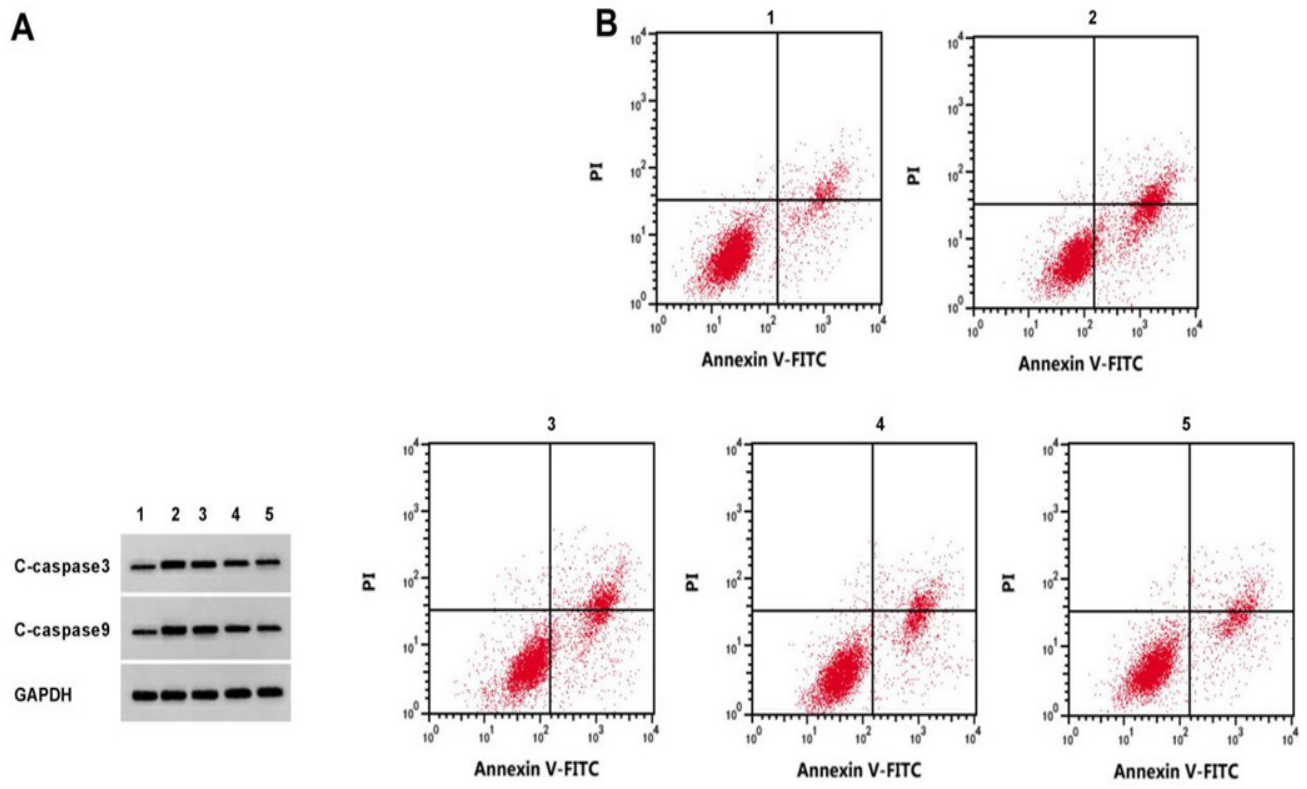

Fig. 1: BLF treatment repressed IL-1β caused chondrocyte apoptosis, (1): Control; (2): IL-1β; (3): IL-1β+BLF-L; (4): IL-1β+ BLF-M and (5): IL-1β+ BLF-H

TABLE 1: BLF RELIEVED IL-1β EVOKED INJURY (x̄±s, n=9)

Group	Apoptosis rate %	Cleaved caspase-3	Cleaved caspase-9	SOD/U·l^{-1}	LDH/U·l^{-1}
Control	6.80±0.51	0.18±0.02	0.25±0.03	309.24±26.74	157.80±15.62
IL-1ß	22.40±1.54*	0.75±0.06*	0.87±0.07*	105.55±12.20*	600.66±27.90*
IL-1ß+BLF-L	19.95±1.06$^{\#}$	0.61±0.04$^{\#}$	0.72±0.06$^{\#}$	149.96±12.64$^{\#}$	522.32±19.02$^{\#}$
IL-1ß+BLF-M	15.28±0.88$^{\#\&}$	0.44±0.03$^{\#\&}$	0.53±0.04$^{\#\&}$	227.20±8.70$^{\#\&}$	401.04±21.94$^{\#\&}$
IL-1ß+BLF-H	11.31±0.67$^{\#\&@}$	0.24±0.02$^{\#\&@}$	0.32±0.02$^{\#\&@}$	261.23±21.62$^{\#\&@}$	281.63±23.76$^{\#\&@}$
F	361.745	379.109	270.276	195.894	590.879
p	0.000	0.000	0.000	0.000	0.000

Note: *p<0.05, $^{\#}$p<0.05, $^{\&}$p<0.05, and $^{@}$p<0.05 *vs.* control, IL-1ß, IL-1ß+BLF-L, and IL-1ß+BLF-M

TABLE 2: BLF HINDERED IL-1β INDUCED INFLAMMATORY FACTORS IN CHONDROCYTES (x̄±s, n=9)

Group	IL-6/pg·ml-1	IL-10/pg·ml-1
Control	46.18±6.39	479.38±33.22
IL-1ß	226.27±15.53*	125.13±13.52*
IL-1ß+BLF-L	191.04±20.27$^{\#}$	200.19±10.98$^{\#}$
IL-1ß+BLF-M	146.28±11.73$^{\#\&}$	289.53±20.29$^{\#\&}$
IL-1ß+BLF-H	79.84±6.13$^{\#\&@}$	382.31±18.40$^{\#\&@}$
F	294.388	414.591
p	0.000	0.000

Note: *p<0.05, $^{\#}$p<0.05, $^{\&}$p<0.05, and $^{@}$p<0.05 *vs.* control, IL-1ß, IL-1ß+BLF-L, and IL-1ß+BLF-M

TABLE 3: INFLUENCES OF BLF ON miR-127-5p LEVEL (x̄±s, n=9)

Group	miR-127-5p
Control	1.00±0.00
IL-1ß	0.16±0.02*
IL-1ß+BLF-L	0.32±0.03$^{\#}$
IL-1ß+BLF-M	0.57±0.04$^{\#\&}$
IL-1ß+BLF-H	0.77±0.05$^{\#\&@}$
F	948.408
p	0.000

Note: *p<0.05, $^{\#}$p<0.05, $^{\&}$p<0.05, and $^{@}$p<0.05 in comparison with control, IL-1ß, IL-1ß+BLF-L, and IL-1ß+BLF-M

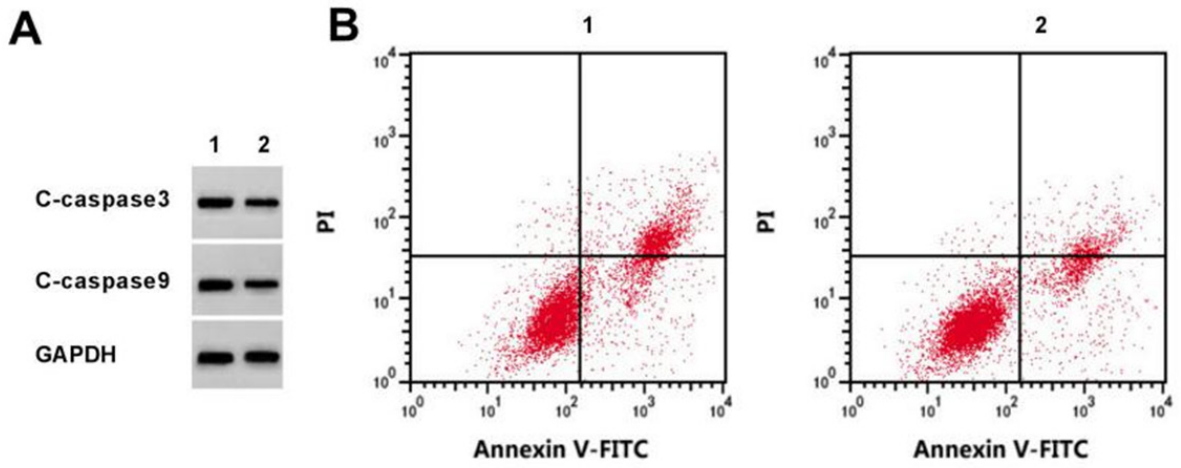

Fig. 2: miR-127-5p affected chondrocyte apoptosis, (1): IL-1β+miR-NC and (2): IL-1β+miR-127-5p

TABLE 4: EFFECTS OF miR-127-5p ON APOPTOSIS (x̄±s, n=9)

Group	miR-127-5p	Apoptosis rate %	Cleaved caspase-3	Cleaved caspase-9	U·l^{-1}		pg·ml^{-1}	
					SOD	LDH	IL-6	IL-10
IL-1β+miR-NC	1.00±0.00	22.61±1.68	0.76±0.07	0.84±0.06	106.18±11.49	603.71±49.31	232.84±19.34	127.75±12.63
IL-1β+miR-127-5p	3.67±0.11*	12.88±0.76*	0.35±0.03*	0.36±0.04*	242.71±11.99*	333.46±19.62*	116.19±9.38*	326.69±25.10*
t	72.818	15.831	16.151	19.969	24.664	15.277	16.281	37.936
p	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: *p<0.05 *vs.* IL-1β+miR-NC

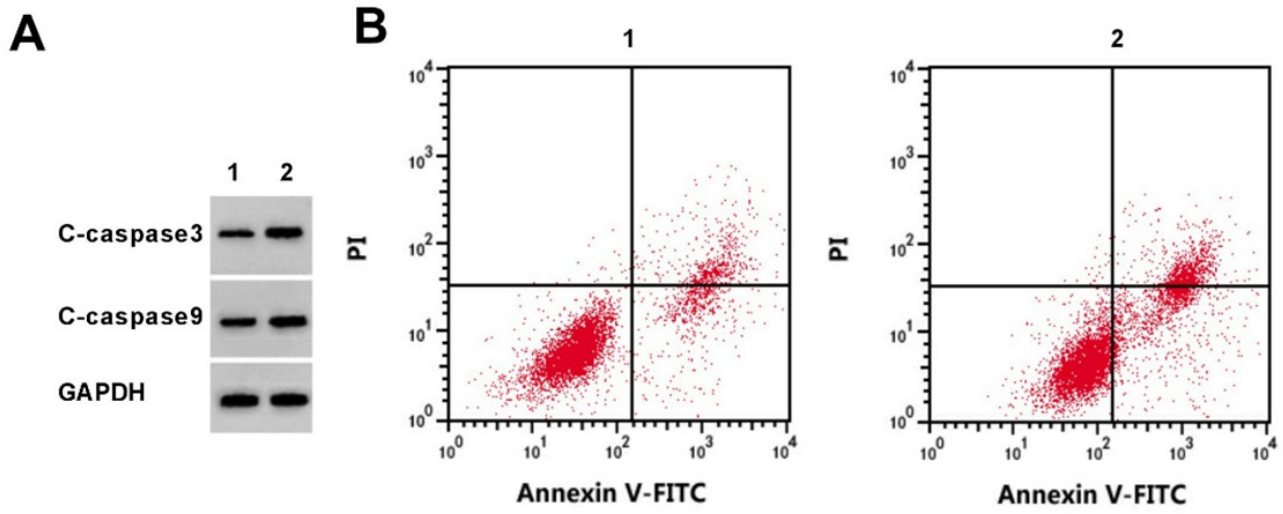

Fig. 3: Effect of interfering miR-127-5p on IL-1β-induced apoptosis of chondrocytes after BLF treatment, (1): IL-1β+ BLF+anti-miR-NC and (2): IL-1β+ BLF+anti-miR-127-5p

TABLE 5: BLF/miR-127-5p REGULATED CHONDROCYTE DAMAGE (x̄±s, n=9)

Group	miR-127-5p	Apoptosis rate %	Cleaved caspase-3	Cleaved caspase-9	U·l^{-1}	
					SOD	LDH
IL-1β+BLF+anti-miR-NC	1.00±0.00	11.27±0.84	0.22±0.02	0.31±0.03	265.59±21.51	280.59±30.49
IL-1β+BLF+anti-miR-127-5p	0.30±0.04*	21.22±1.29*	0.68±0.05*	0.75±0.05*	126.32±9.39*	547.16±39.09*
t	52.500	19.391	25.626	22.638	17.802	16.131
p	0.000	0.000	0.000	0.000	0.000	0.000

Note: *p<0.05, relative to IL-1β+ BLF+anti-miR-NC

TABLE 6: EFFECTS OF BLF AND ANTI-miR-127-5p ON IL-1β INDUCED INFLAMMATORY FACTORS IN CHONDROCYTES (x̄±s, n=9)

Group	pg·ml^{-1}	
	IL-6	IL-10
IL-1β+BLF+anti-miR-NC	80.53±7.44	377.59±30.59
IL-1β+BLF+anti-miR-127-5p	207.82±22.31*	159.88±13.62*
t	16.237	19.505
p	0.000	0.000

Note: *p<0.05, relative to IL-1β+ BLF+anti-miR-NC

Substantial laboratory work has revealed that chondrocyte apoptosis and oxidative stress are closely associated with OA progression and development. Furthermore, chondrocyte inflammatory response might induce apoptosis and thus causes chondrocyte damage. IL-1β, a well-known inflammatory cytokine, might contribute to the inflammatory response of chondrocytes, thereby mediating chondrocyte apoptosis[17]. Moreover, numerous miRNAs might participate in the process of OA development *via* modulating target gene expression[18]. Nevertheless, whether miRNAs can be used as a target for OA treatment with herbal medicines remains unknown.

With anti-inflammatory and anti-oxidative stress effects, BLF might attenuate rat inflammation model and hepatic ischemia-reperfusion injury[19,20]. But the influence of BLF on IL-1β-induced chondrocyte damage is not yet known. Herein, our data displayed that IL-1β might apparently improve chondrocyte apoptosis and cleaved caspase-3 and cleaved caspase-9 protein levels, consistent with the former report[21]. Furthermore, we found that BLF might relieve IL-1β-induced chondrocyte apoptosis and cleaved caspase-3 and cleaved caspase-9 in concentration-dependent manners, implying the repression of BLF on IL-1β triggered chondrocyte apoptosis. In agreement with previous studies[22,23], our work presented that IL-1β treatment might decrease SOD activity and IL-10 content, and enhance LDH activity and IL-6 level. However, these influences were partially overturned by BLF exposure in a dose-dependent ways. These findings implied that BLF treatment might effectively weaken chondrocyte injury evoked by IL-1β.

miR-127-5p overexpression was demonstrated to abate OA progression *via* repressing chondrocyte apoptosis and inflammation[24,25]. Herein, BLF treatment might gradually ameliorate IL-1β mediated miR-127-5p content inhibition in chondrocytes, indicating BLF/miR-127-5p take part in regulating IL-1β mediated chondrocytes. Interfering miR-127-5p might partly recede the repression of BLF on IL-1β caused chondrocyte apoptosis, oxidative stress, and inflammation. The above observation validated that BLF treatment might constrain IL-1β evoked chondrocyte damage *via* upregulating miR-127-5p.

In summary, BLF might partly relieve IL-1β-induced chondrocyte apoptosis, oxidative stress, and inflammation through targeting miR-127-5p, providing a new direction for OA treatment. However, the specific mechanism of action needs to be further explored.

Conflict of interests:

The authors declared no conflict of interests.

REFERENCES

1. Jiang Y. Osteoarthritis year in review 2021: Biology. Osteoarthr Cartil 2022;30(2):207-15.
2. Zhang Y, Jordan JM. Epidemiology of osteoarthritis. Clin Geriatr Med 2010;26(3):355-69.
3. Molnar V, Matisic V, Kodvanj I, Bjelica R, Jelec Z, Hudetz D, *et al.* Cytokines and chemokines involved in osteoarthritis pathogenesis. Int J Mol Sci 2021;22(17):9208.
4. Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. Lancet 2019;27;393(10182):1745-59.
5. Archer CW, Francis-West P. The chondrocyte. Int J Biochem Cell Biol 2003;35(4):401-4.
6. Wang J, Luo X, Cai S, Sun J, Wang S, Wei X. Blocking HOTAIR protects human chondrocytes against IL-1β-induced cell apoptosis, ECM degradation, inflammatory response and oxidative stress *via* regulating miR-222-3p/ADAM10 axis. Int Immunopharmacol 2021;98:107903.
7. Li W, Wang Y, Tang Y, Lu H, Qi Y, Li G, *et al.* Quercetin alleviates osteoarthritis progression in rats by suppressing inflammation and apoptosis *via* inhibition of IRAK1/NLRP3 signaling. J Inflamm Res 2021;14:3393-403.
8. Wang S, Ding P, Xia X, Chen X, Mi D, Sheng S, *et al.* Bugan Rongjin decoction alleviates inflammation and oxidative stress to treat the postmenopausal knee osteoarthritis through Wnt signaling pathway. Biomed Eng Online 2021;20(1):103-13.
9. Hu J, Zhou J, Wu J, Chen Q, Du W, Fu F, *et al.* Loganin ameliorates cartilage degeneration and osteoarthritis development in an osteoarthritis mouse model through inhibition of NF-κB activity and pyroptosis in chondrocytes. J Ethnopharmacol 2020;247:112261.
10. Cheng Y, Wan S, Yao L, Lin D, Wu T, Chen Y, *et al.* Bamboo leaf: A review of traditional medicinal property, phytochemistry, pharmacology, and purification technology. J Ethnopharmacol 2023;306:116166.
11. Zhou XY, Ying CJ, Wang SS. Bamboo leaf flavonoids reduce cognitive dysfunction in diabetic rats by inhibiting inflammatory response in hippocampus. Chin Med Innov 2016;13(35):21-3.
12. Ali Syeda Z, Langden SS, Munkhzul C, Lee M, Song SJ. Regulatory mechanism of microRNA expression in cancer. Int J Mol Sci 2020;21(5):1723.
13. Zhang Y, Zhao P, Li S, Mu X, Wang H. circSCAPER knockdown attenuates IL-1β-induced chondrocyte injury by miR-127-5p/TLR4 axis in osteoarthritis. Autoimmunity 2022;55(8):577-86.
14. Liu H, Zhao H, Huang Y, Lei M. circ_0002715 promotes the development of osteoarthritis through regulating LXN by sponging miR-127-5p. J Orthop Surg Res 2023;18(1):230.
15. Guo XZ, Gao BL, Guo W. Effect of lncRNA FGD5-AS1 on IL-1β-induced apoptosis of articular chondrocytes by targeting miR-103a-3p. Chin J Osteop 2020;26(6):832-7.

16. Wang DD. Protective effects of three plant extracts on H_2O_2 induced oxidative damage in PC12 cells. Shandong Normal Univ 2016:1-102.
17. Zhang Y, Zeng Y. Curcumin reduces inflammation in knee osteoarthritis rats through blocking TLR4/MyD88/NF-κB signal pathway. Drug Dev Res 2019;80(3):353-9.
18. Liu H, Yan L, Li X, Li D, Wang G, Shen NN, *et al.* microRNA expression in osteoarthritis: A meta-analysis. Clin Exp Med 2023;23(7):3737-49.
19. Yang M, Zhang S, Wang XY. Anti-inflammatory components of bamboo leaf flavonoids and their pharmacodynamics in different mouse models of inflammation. Hubei J Tradit Chin Med 2017;39(3):1-5.
20. Xia QF, Xu YB, Liu Q. Effect and mechanism of bamboo leaf flavonoids on hepatic ischemia-reperfusion injury in rats. Int J Chin Mater Med 2016;38(11):1005-8.
21. Wang BW, Jiang Y, Yao ZL. Aucubin protects chondrocytes against IL-1 beta-induced apoptosis *in vitro* and inhibits osteoarthritis in mice model. Drug Des Devel Ther 2019;13(1):3529-38.
22. Wang XB, Zuo RT. Effects of cucurbiturin B on IL-1β-induced chondrocyte injury and extracellular matrix metabolic disorder. Tianjin Med 2019;47(12):15-9.
23. Liu WJ, Li QB, Cai L. Muscone reduces interleukin-1β-induced chondrocyte injury by regulating the expression of microrNA-223. Chin J Lab Surg 2021;38(9):1744-7.
24. Zhou ZB, Huang GX, Fu Q, Han B, Lu JJ, Chen AM, *et al.* circRNA. 33186 contributes to the pathogenesis of osteoarthritis by sponging miR-127-5p. Mol Ther 2019;27(3):531-41.
25. Liu C, Cheng P, Liang J, Zhao X, Du W. Circular RNA circ_0128846 promotes the progression of osteoarthritis by regulating miR-127-5p/NAMPT axis. J Orthop Surg Res 2021;16(1):307.