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Long non-coding RNA activated by transforming growth factor beta alleviates lipopolysaccharide-induced inflammatory injury via regulating microRNA-223 in ATDC5 cells

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ABSTRACT

Osteoarthritis (OA) is a conversant joint disease, which seriously threatens the health of the elderly, and even leads to disability. Long non-coding RNA-activated by transforming growth factor beta (lncRNA-ATB) has been reported in diverse cancers. However, the functions of lncRNA-ATB in OA remain uninvestigated. The current study aimed to explore the impacts of lncRNA-ATB on lipopolysaccharide (LPS)-induced inflammatory injury in ATDC5 cells and to uncover the underlying mechanism. LPS-induced ATDC5 cell injury model was constructed, and the effects of lncRNA-ATB on LPS-injured cells were explored via analyzing cell viability, apoptosis, iNOS, COX-2, and inflammatory cytokines (IL-6 and TNF-α). Subsequently, the relationship between lncRNA-ATB and microRNA (miR)-223 was detected, and whether miR-223 was involved in modulating LPS-induced cells injury in ATDC5 cells was investigated. Finally, MyD88/NF-κB and p38MAPK pathways were assessed to explore the underlying mechanism. Results showed that LPS repressed cell viability, induced apoptosis, and promoted iNOS, COX-2, IL-6 and TNF-α expression. Additionally, we observed that lncRNA-ATB expression was down-regulated in LPS-injured cells, and lncRNA-ATB overexpression significantly alleviated LPS-induced inflammatory injury in ATDC5 cells. Interesting results revealed that miR-223 expression was down-regulated by lncRNA-ATB and miR-223 overexpression declined the protective effect of lncRNA-ATB on LPS-injured ATDC5 cells. Further, the signaling pathway experiments showed that lncRNA-ATB inhibited MyD88/NF-κB and p38MAPK pathways by down-regulating miR-223 in LPS-injured cells. These data demonstrated that lncRNA-ATB protected ATDC5 cells against LPS-induced inflammatory injury by repressing MyD88/NF-κB and p38MAPK pathways, which was mediated by down-regulation of miR-223.

1. Introduction

Osteoarthritis (OA) is a chronic joint disease which is characterized by degeneration, destruction and hyperosteogeny of articular cartilage [1]. It has become a principal cause of disability in the elderly [2]. Initially, there is no obvious symptom of OA, but along with the disease progresses, several symptoms are appeared, including joint pain, transient stiffness, swelling and limitation of range of joint motion [3–5]. Currently, joint replacement surgery is usually implemented to recover the joint function in the advance stage of OA [6]. Evidence from 2014 OA Research Society International (OARSI) guidelines for the non-surgical management of OA demonstrated that biomechanical interventions, corticosteroid injection, exercise, self-management and education, power training, and weight management were appropriate treatment for all individuals with OA [7]. However, it is still lack of effective diagnosis and treatment methods to control the progress of OA in the early stage. In consideration of the limitations for the treatment of OA, the novel and effective strategies are urgently needed.

Long non-coding RNAs (lncRNAs) are a class of non-protein-coding RNAs with longer than 200 nucleotides in length, which play vital roles in mediating gene expression as well as affecting various biological processes [8,9]. Increasing researches corroborated that numerous lncRNAs are intimately associated with the pathogenesis of OA [10,11]. Approximately 150–4000 kinds of lncRNAs were reported in OA articular cartilage cells, which exert indispensable functions in the articular cartilage matrix synthesis, neovascularization, autophagy and apoptosis of cartilage cells [12–14]. LncRNA activated by transforming growth factor beta (lncRNA-ATB) is a novel cancer-associated lncRNA, and its abnormal expression has been discovered in multiple cancers, such as breast cancer [15], colon cancer [16] and pancreatic cancer

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[17]. Accumulating evidences discovered that dysregulation of lncRNA-ATB could promote cell growth, migration and invasion in the distinguished type cancer cells [18,19]. However, the functions of lncRNA-ATB in OA are still not well documented and needed to be investigated.

Herein, the study attempted to investigate the anti-inflammatory effect of lncRNA-ATB on chondrocyte ATDC5 cells. Lipopolysaccharide (LPS) was used to construct an ATDC5 cell injury model mimicking OA *in vitro*. Subsequently, the effect of lncRNA-ATB on LPS-induced inflammatory injury in ATDC5 cells was explored. Additionally, the effect of microRNA (miR)-223 on lncRNA-ATB functions in LPS-injured ATDC5 cells was studied. The signaling pathways of MyD88/NF-κB and p38MAPK were investigated to reveal the potential molecular mechanisms. These findings might provide a promising therapeutic option for the treatment of OA.

2. Materials and methods

2.1. Cell culture and treatment

The ATDC5 murine chondrocyte cells (RIKEN Cell Bank, Tsukuba, Japan) were cultured in Dulbecco's modified Eagle's medium/Ham's F12 (DMEM/F12, Life Technologies, Carlsbad, CA, USA) with 5% fetal bovine serum (FBS, Life Technologies) in a humidified incubator containing 5% CO_2 at 37 °C. After the 5 to 10 passages, cells were used in the present study and were maintained in growth medium in a 75 cm^2 flask. The culture medium was replaced every 3 days until confluence was achieved about 80–90%. All these cells were treated by the different concentrations (0, 2.5, 5, 7.5 and 10 μg/ml) of LPS for 12 h.

2.2. Cell counting kit-8 (CCK-8) assay

ATDC5 cells were seeded at an initial density of 5×10^3 cells/well in 96-well plates and incubated under the conventional culture conditions. After stimulation with LPS (0, 2.5, 5, 7.5 and 10 μg/ml) for 12 h, 10 μl CCK-8 solution (Dojindo Molecular Technologies, Gaithersburg, MD) was added to the culture plate, and the plate was cultured in a CO_2 incubator for another 1 h at 37 °C. The optical density was measured at 450 nm (OD_{450}) using a Microplate Reader (Bio-Rad, Hercules, CA, USA).

2.3. Apoptosis assay

The percentage of apoptotic cells was detected by using a common apoptosis kit (Annexin V-FITC/PI apoptosis detection kit, BD Biosciences, Franklin Lakes, NJ, USA) followed by flow cytometry. After administrations, the treated cells were washed with phosphate buffered saline (PBS, Gibco, Carlsbad, CA, USA) and stained in 5 μl Annexin V-FITC and 5 μl PI at room temperature in the dark for 15 min. Afterward, cell apoptosis was evaluated by FACS (Beckman Coulter, Fullerton, CA, USA), and the data were analyzed by using FlowJo software (Treestar, Ashland, OR, USA).

2.4. Enzyme-linked immunosorbent assay (ELISA)

ATDC5 cells cultured in 24-well plates were pre-treated with 5 μg/ml LPS for 12 h at 37 °C. Subsequently, the culture supernatant was collected and the concentrations of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were measured by corresponding ELISA kit (R& D Systems, Abingdon, UK) according to the protocols supplied by the manufacturer.

2.5. Cell transfection

The full-length of lncRNA-ATB was constructed in pcDNA3.1 and named pc-lncRNA-ATB. The empty pcDNA3.1 plasmid from Invitrogen (San Diego, CA, USA) was used as a control. Additionally, miR-223 mimic, miR-223 inhibitor and the corresponding controls (mimic NC and inhibitor NC) were synthesized by Life Technologies Corporation (Carlsbad, CA, USA). All these expression plasmids were transfected into ATDC5 cells and analyzed by Lipofectamine 3000 reagent (Life Technologies Corporation). As the highest transfection efficiency was presented at 48 h, we collected these transfected cells at 72 h post-transfection for the subsequent experiments.

2.6. Real-time quantitative PCR (RT-qPCR)

Total RNA was extracted from the stimulated cells by using Trizol reagent (Life Technologies Corporation). One Step SYBR® PrimeScript®PLUS RT-RNA PCR Kit (TaKaRa Biotechnology, Dalian, China) was used for analyzing the expression level of lncRNA-ATB. For measurement of miR-223, the extracted RNA was reversed by TaqMan® MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Massachusetts, USA), then, the TaqMan MicroRNA Assay supplemented with the TaqMan Universal Master Mix II (Applied Biosystems, Foster City, CA, USA) was used for detecting the expression level of miR-223. For the quantitative analysis of mRNAs of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), IL-6 and TNF-α, MultiscribeTM Reverse transcription Kit (Applied Biosystems) supplemented with random hexamers or oligo (dT) and Fast START Universal SYBR Green Master (ROX) (Roche, Heiman, Germany) was used. These data were analyzed by using the classic $2^{-\Delta\Delta CT}$ method, β-actin and U6 were used for normalizing the expression levels in each sample [20].

2.7. Western blot assay

ATDC5 cells after LPS stimulation were treated with radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Applygen Technologies Inc., Beijing, China). Samples with equivalent proteins were separated with 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene defluoride (PVDF) membranes. Subsequently, these membranes were blocked in 5% skim milk and incubated with primary antibodies of pro-Caspase-3 (ab32150), cleaved Caspase-3 (ab13585), pro-Caspase-7 (ab32067), pro-Caspase-9 (ab135544), cleaved Caspase-9 (ab2324), iNOS (ab15323), COX-2, (ab15191), IL-6 (ab7737), TNF-α (ab6671), myeloid differentiation factor 88 (MyD88, ab133739), t-p65 (ab16502), phosphorylated (p)-p65 (ab86299), t-IκBα (ab178846), p-IκBα (ab133462), t-p38MAPK (ab32142), p-p38MAPK (ab47363) and β-actin (ab6276) (all from Abcam, Cambridge, UK) and cleaved-Caspase-7 (#8438, Cell Signaling Technology, Beverly, MA, USA) at 4 °C overnight. After this, the membranes were washed with TBST and probed with the secondary antibody of horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (ab205718, 1:2000, Abcam) for 1 h at room temperature. Then, 200 μl Immobilon Western Chemiluminescent HRP Substrate (Millipore, MA, USA) were supplemented to cover the membrane surface. The signals were then captured and the intensity of the bands was quantified by using Image Lab™ Software (Bio-Rad).

2.8. Statistical analysis

All experiments were repeated at least three times. All results in the present study were presented as the mean ± standard deviation (SD). Statistical analyses were performed using Graphpad 6.0 statistical software (GraphPad Software Inc., La Jolla, USA). The *P*-values were calculated using a one-way analysis of variance (ANOVA) following with Bonferroni or student's *t*-test. A *P*-value of < 0.05 was considered to indicate a statistically significant result.

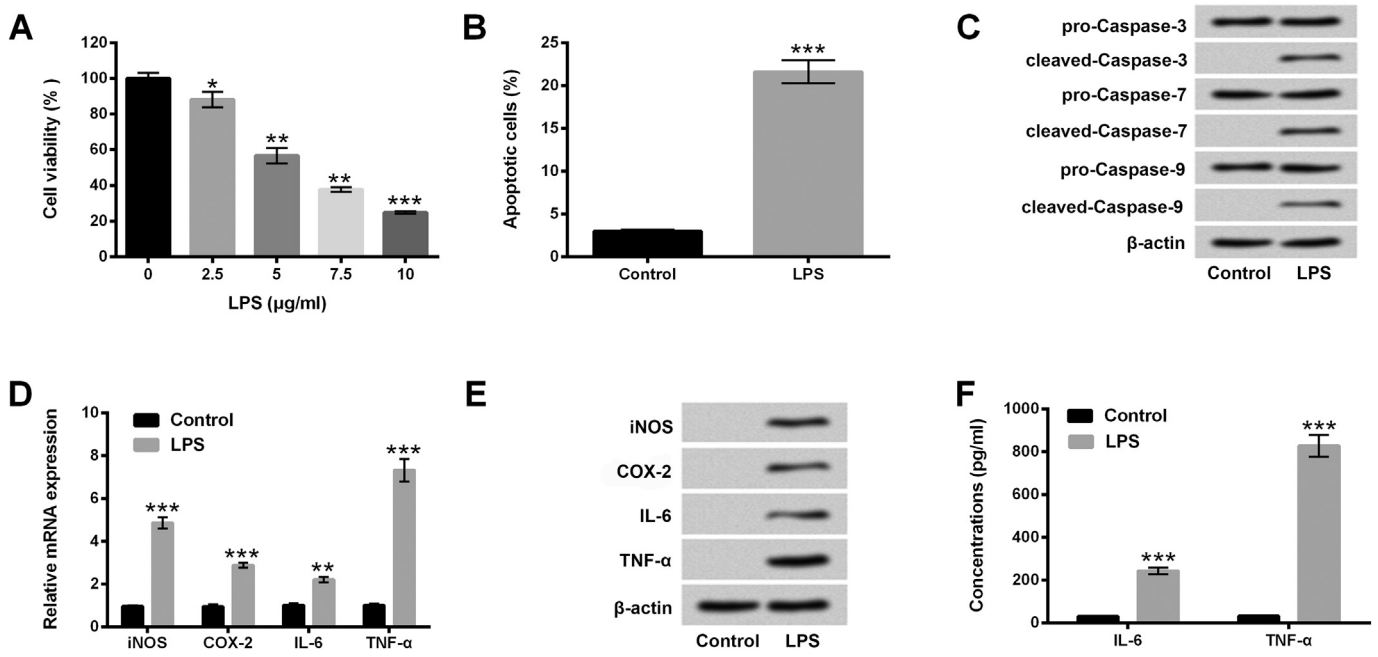

Fig. 1. LPS-induced inflammatory injury in ATDC5 cells.
The different concentrations of LPS were utilized to stimulate ATDC5 cells. (A) Cell viability was tested by CCK-8 assay after stimulation with 5 μg/ml LPS. (B) Cell apoptosis and (C) cleaved-Caspase-3, cleaved-Caspase-7 and cleaved-Caspase-9 expression were detected by flow cytometry and western blot, respectively. (D) The mRNA and (E) protein levels of iNOS, COX-2, IL-6 and TNF-α were assessed by RT-qPCR and western blot, respectively. (F) The concentrations of IL-6 and TNF-α were determined by ELISA. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

3. Results

3.1. LPS-induced inflammatory injury in ATDC5 cells

A LPS-induced inflammatory injury model *in vitro* has been widely used to mimic various inflammatory diseases. In this study, we used the different concentrations (0, 2.5, 5, 7.5 and 10 μg/ml) of LPS to stimulate ATDC5 cells, simultaneously observed the change of viability in ATDC5 cells. The results in Fig. 1A showed that cell viability of ATDC5 cells was significantly decreased by LPS at the different concentrations of 2.5 ($P < 0.05$), 5 ($P < 0.01$), 7.5 ($P < 0.01$) and 10 μg/ml ($P < 0.001$), indicating that LPS decreased ATDC5 cell viability in a dose dependent manner. Subsequently, the concentration of LPS at 5 μg/ml was selected as an optimum concentration for the following experiments. The percentage of apoptotic cells was obviously induced after treatment with 5 μg/ml LPS compared with control group ($P < 0.001$, Fig. 1B). Meanwhile, western blot assay revealed that LPS promoted the protein levels of cleaved-Caspase-3, cleaved-Caspase-7 and cleaved-Caspase-9 (Fig. 1C). Additionally, we found that LPS administration increased the mRNA and protein levels of iNOS, Cox-2, IL-6 and TNF-α in ATDC5 cells ($P < 0.01$ or $P < 0.001$, Fig. 1D and E). ELISA analytical results revealed that the concentrations of IL-6 and TNF-α were increased by LPS administration in ATDC5 cells ($P < 0.001$, Fig. 1F). All these data certified that LPS could induce ATDC5 cell inflammatory injury, implying that a cell injury model *in vitro* was successfully constructed.

3.2. LPS affected the expression level of lncRNA-ATB in ATDC5 cells

LncRNA-ATB has been reported to play an oncogenic role in various types of malignancies. However, whether lncRNA-ATB participates in regulating LPS-induced ATDC5 cell inflammatory injury remains uninvestigated. In the next experiment, the expression level of lncRNA-ATB was examined by RT-qPCR assay after stimulation with 2.5 and 5 μg/ml LPS. As shown in Fig. 2A, the expression level of lncRNA-ATB was significantly reduced by LPS treatment as compared with control group ($P < 0.05$ or $P < 0.001$). In addition, the expression plasmids of pc-lncRNA-ATB and the empty pcDNA3.1 were transfected into ATDC5 cells. We observed that the expression level of lncRNA-ATB was significantly up-regulated in pc-lncRNA-ATB-transfected cells compared with that in pcDNA3.1-transfected cells ($P < 0.01$, Fig. 2B). These data suggested that LPS down-regulated lncRNA-ATB expression in ATDC5 cells, and lncRNA-ATB might be involved in mediating the inflammatory injury in LPS-injured ATDC5 cells.

3.3. LncRNA-ATB weakened LPS-induced inflammatory injury in ATDC5 cells

To confirm above results, the impacts of lncRNA-ATB on cell viability, apoptosis and inflammatory response in ATDC5 cells were assessed. The results showed that lncRNA-ATB overexpression strikingly promoted cell viability, repressed apoptosis and declined cleaved-Caspase-3, cleaved-Caspase-7 and cleaved-Caspase-9 expression in LPS-injured ATDC5 cells ($P < 0.05$, $P < 0.01$ or $P < 0.001$, Fig. 3A–C). More interestingly, we observed that lncRNA-ATB overexpression decreased the mRNA and protein levels of iNOS, COX-2, IL-6 and TNF-α in LPS-injured ATDC5 cells ($P < 0.05$ or $P < 0.001$, Fig. 3D and E). Further, the promoting effects of LPS on the concentrations of IL-6 and TNF-α were notably restrained by lncRNA-ATB overexpression ($P < 0.05$ or $P < 0.001$, Fig. 3F). All these data demonstrated that lncRNA-ATB could alleviate LPS-induced inflammatory injury in ATDC5 cells.

3.4. LncRNA-ATB down-regulated miR-223 expression in ATDC5 cells

MiR-223 seems to be a positive regulator in inflammatory response. Nevertheless, whether miR-223 participates in lncRNA-ATB functions to affect LPS-induced inflammatory injury in ATDC5 cells is still unclear. We subsequently utilized RT-qPCR to investigate the relevance between lncRNA-ATB and miR-223 in ATDC5 cells. The results showed that lncRNA-ATB overexpression prominently impeded the expression level of miR-223 in ATDC5 cells compared with the corresponding

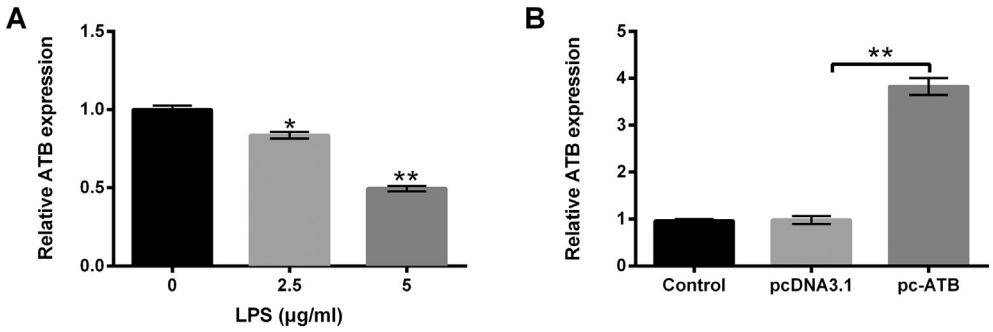

Fig. 2. LPS regulated lncRNA-ATB expression in ATDC5 cells.
(A) The disparate concentrations (0, 2.5 and 5 μg/ml) of LPS were utilized to stimulate ATDC5 cells, lncRNA-ATB expression in LPS-treated ATDC5 cells was detected by RT-qPCR assay. (B) The expression plasmids of pc-lncRNA-ATB and pcDNA3.1 were transfected into ATDC5 cells, and the transfection efficiency of these plasmids was examined by RT-qPCR. *, $P < 0.05$, **, $P < 0.01$.

control ($P < 0.05$, Fig. 4A). Additionally, miR-223 mimic was transfected into ATDC5 cells to regulate miR-223 expression in ATDC5 cells. As shown in Fig. 4B, miR-223 expression was significantly up-regulated in miR-223 mimic-transfected cells compared with NC group ($P < 0.001$). The data indicated that the transfection efficiency of miR-223 mimic was well, and could use to accelerate miR-223 expression in ATDC5 cells in the subsequent studies. Additionally, we observed that miR-223 expression was up-regulated in LPS-treated cells at the concentrations of 2.5 ($P < 0.05$) and 5 μg/ml ($P < 0.001$, Fig. 5A). More importantly, miR-223 overexpression distinctly decreased cell viability and induced apoptosis in LPS-treated cells ($P < 0.01$ or $P < 0.001$, Fig. 5B and C). Protein levels of cleaved-Caspase-3, cleaved-Caspase-7 and cleaved-Caspase-9 were all facilitated by miR-223 overexpression in LPS-treated cells (Fig. 5D). Additionally, the protein and mRNA levels of iNOS, COX-2, IL-6 and TNF-α in LPS-treated cells were all increased by miR-223 overexpression ($P < 0.01$ or $P < 0.001$, Fig. 5E and F). Further, the concentrations of IL-6 and TNF-α were also augmented by miR-223 overexpression in LPS-treated cells ($P < 0.01$, Fig. 5F). MiR-223 inhibitor showed the opposite results in Fig. 5B–F ($P < 0.05$, $P < 0.01$ or $P < 0.001$). These data testified that miR-223 was associated with LPS-induced inflammatory injury in ATDC5 cells.

3.5. LncRNA-ATB relieved LPS-induced inflammatory injury in ATDC5 cells by repression of miR-223

After transfection with miR-223 mimic, the impacts of miR-223 on LPS-induced inflammatory injury were investigated. Results testified that overexpression of miR-223 significantly reversed the promoting effect of lncRNA-ATB overexpression on cell viability ($P < 0.05$, Fig. 6A). The apoptotic cells and cleaved-Caspase-3, cleaved-Caspase-7 and cleaved-Caspase-9 expression were remarkably enhanced by miR-223 overexpression in ATDC5 cells after stimulation with LPS and overexpression of lncRNA-ATB ($P < 0.05$, Fig. 6B and C). Simultaneously, overexpression of miR-223 increased the mRNA and protein levels of iNOS, COX-2, IL-6 and TNF-α in ATDC5 cells after treatment with LPS and overexpression of lncRNA-ATB ($P < 0.001$, Fig. 6D and E). Besides, we found that miR-223 overexpression reversed the inhibitory effect of lncRNA-ATB overexpression on the concentrations of IL-6 and TNF-α in LPS-injured ATDC5 cells ($P < 0.05$ or $P < 0.001$, Fig. 6F). All results from above indicated that miR-223 repressed the

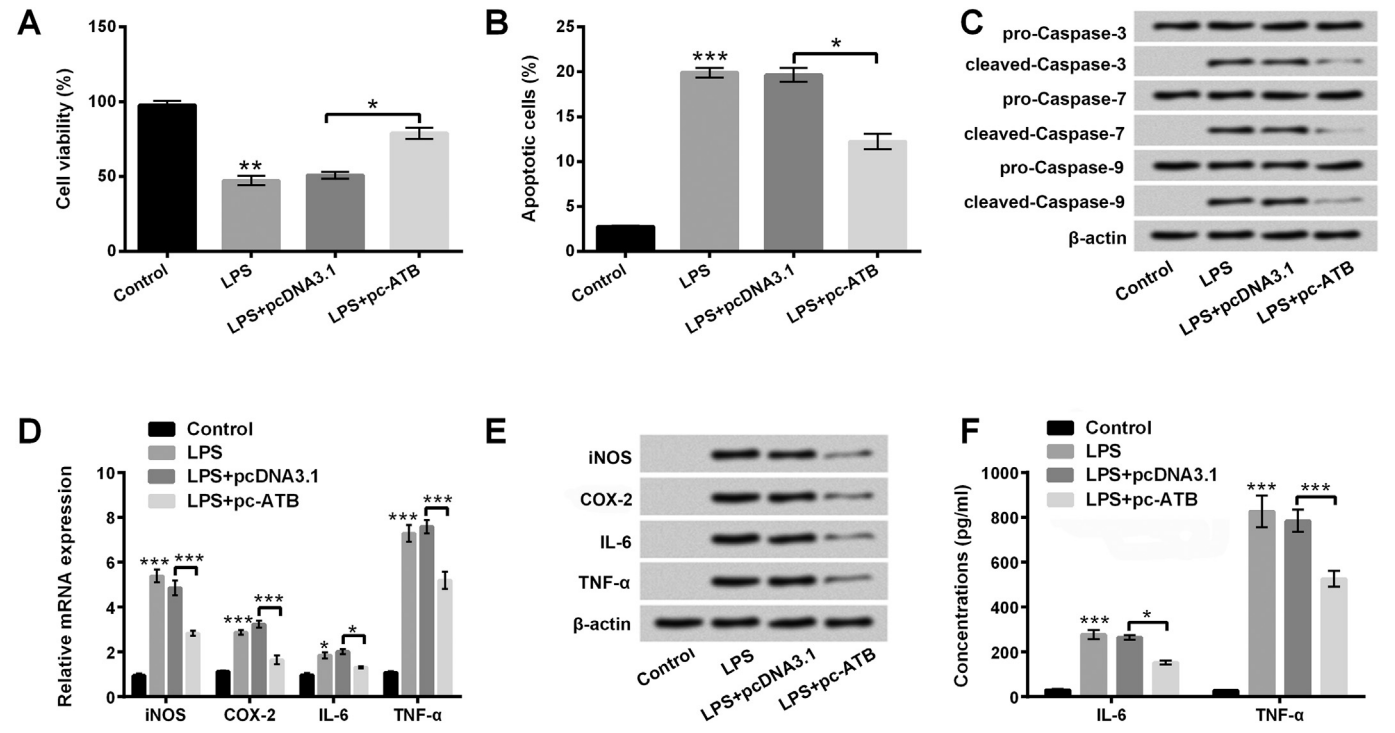

Fig. 3. LncRNA-ATB mitigated LPS-induced inflammatory injury in ATDC5 cells.
After administration with 5 μg/ml LPS, ATDC5 cells were transfected with pc-lncRNA-ATB and pcDNA3.1. (A) Cell viability, (B) cell apoptosis, and (C) the apoptosis-related proteins were detected by CCK-8, flow cytometry and western blot, respectively. (D) The mRNA and (E) the protein levels of iNOS, COX-2, IL-6 and TNF-α were analyzed by RT-qPCR and western blot, respectively. (F) The concentrations of IL-6 and TNF-α were examined by ELISA. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

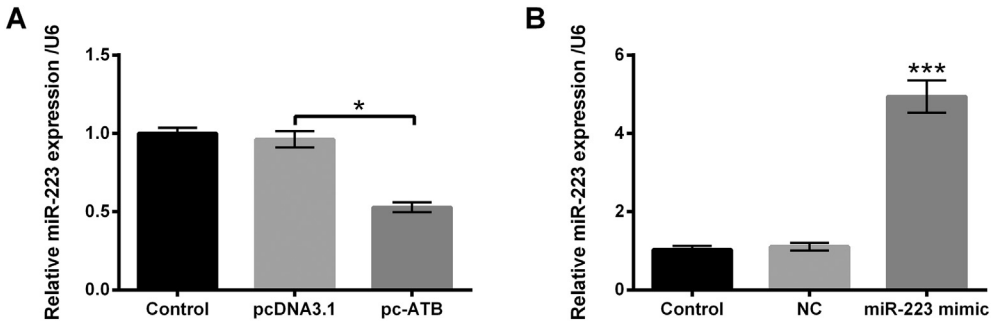

Fig. 4. LncRNA-ATB repressed miR-223 expression in ATDC5 cells.
(A) After transfection with pc-lncRNA-ATB and pcDNA3.1, miR-223 expression was examined by using RT-qPCR. (B) ATDC5 cells were transfected with miR-223 mimic and NC, and miR-223 expression was also assessed after transfection by RT-qPCR assay. *, $P < 0.05$, ***, $P < 0.001$.

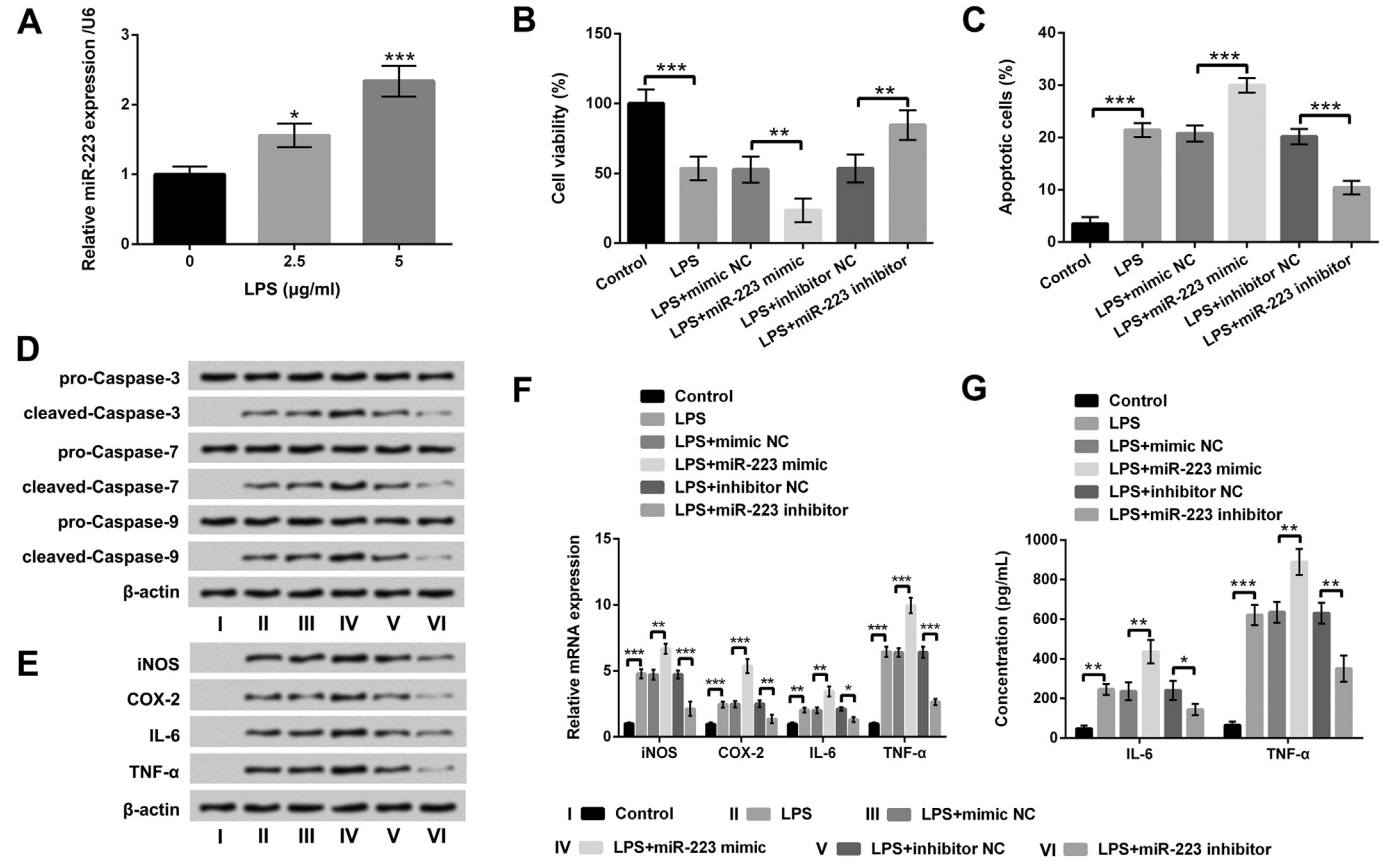

Fig. 5. MiR-223 regulated LPS-induced inflammatory injury in ATDC5 cells.
(A) After stimulation with 2.5 and 5 μg/ml LPS, miR-223 expression was determined by RT-qPCR. After administration with 5 μg/ml LPS, ATDC5 cells were transfected with miR-223 mimic or miR-223 inhibitor. (B) Cell viability, (C) cell apoptosis, and (D) cleaved-Caspase-3, cleaved-Caspase-7 and cleaved-Caspase-9 expression were detected by CCK-8, flow cytometry and western blot, respectively. (E) The protein and (E) the mRNA levels of iNOS, COX-2, IL-6 and TNF-α were analyzed by western blot and RT-qPCR assay, respectively. (F) The concentrations of IL-6 and TNF-α were examined by ELISA. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

protective effect of lncRNA-ATB on LPS-injured ATDC5 cells.

3.6. LncRNA-ATB inhibited MyD88/NF-κB and p38MAPK pathways via regulation of miR-223

Finally, we explored the influences of lncRNA-ATB in MyD88/NF-κB and p38MAPK pathways to disclose the potential molecular mechanisms. The results displayed that MyD88, p-p65, p-IκBα, and p-p38MAPK protein levels were all up-regulated in LPS-treated ATDC5 cells ($P < 0.05$ or $P < 0.001$, Fig. 7A–D). Whereas, the activated effects of LPS on MyD88/NF-κB and p38MAPK pathways were inhibited by lncRNA-ATB overexpression ($P < 0.05$ or $P < 0.001$, Fig. 7A–D). The regulatory effect of miR-223 overexpression on these signaling pathways was contrary to lncRNA-ATB overexpression in LPS-injured ATDC5 cells ($P < 0.05$ or $P < 0.001$, Fig. 7A–D). These data indicated that lncRNA-ATB overexpression hindered MyD88/NF-κB and p38MAPK pathways through regulation of miR-223 expression.

4. Discussion

We utilized LPS to treat ATDC5 cells to construct an inflammatory injury model *in vitro*. Then, we discovered that lncRNA-ATB expression was significantly restrained in LPS-treated ATDC5 cells. Overexpression of lncRNA-ATB observably mitigated LPS-induced inflammatory injury in ATDC5 cells. Additionally, we found that lncRNA-ATB down-regulated miR-223 expression in LPS-stimulated ATDC5 cells, simultaneously miR-223 overexpression reversed the protective effect of lncRNA-ATB on LPS-injured ATDC5 cells. Finally, the results displayed

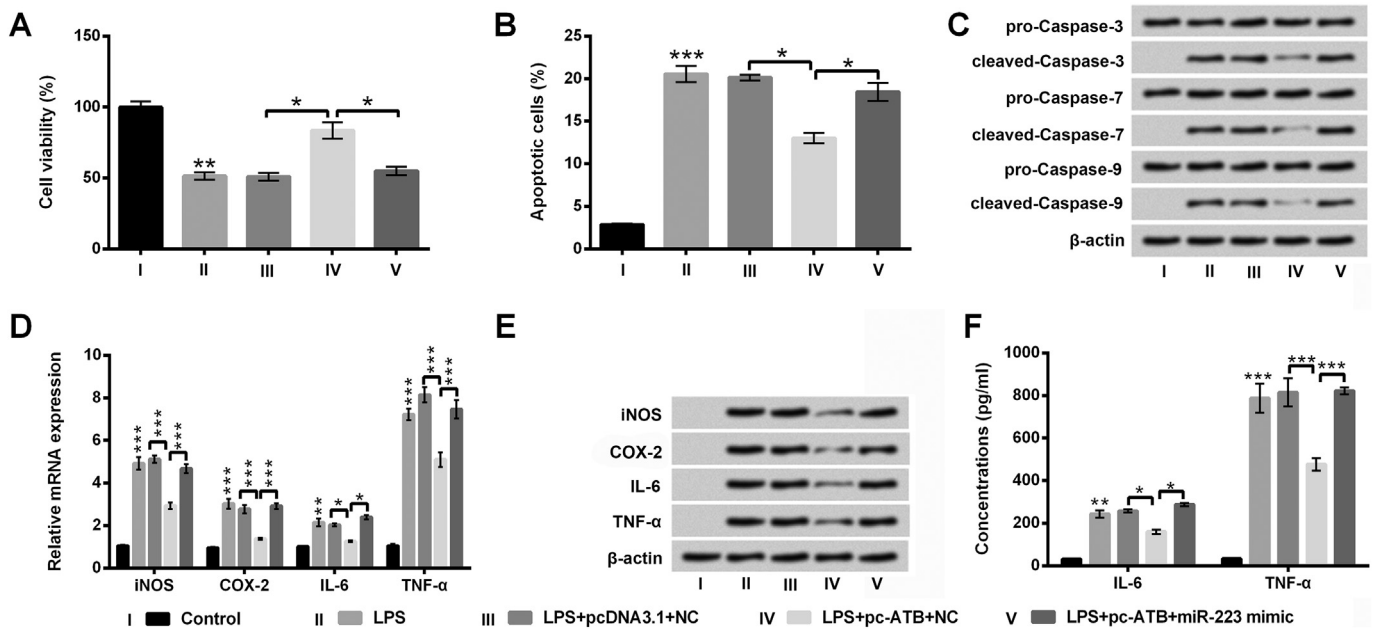

Fig. 6. LncRNA-ATB protected ATDC5 cells against LPS-induced inflammatory injury by repression of miR-223.
After administration with 5 μg/ml LPS, ATDC5 cells were transfected with pc-lncRNA-ATB or miR-223 mimic. (A) Cell viability, (B) cell apoptosis, and (C) cleaved-Caspase-3, cleaved-Caspase-7 and cleaved-Caspase-9 expression were detected by CCK-8, flow cytometry and western blot, respectively. (D) The mRNA and (E) iNOS, COX-2, IL-6 and TNF-α protein levels were analyzed by RT-qPCR and western blot, respectively. (F) The concentrations of IL-6 and TNF-α were examined by ELISA. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

that lncRNA-ATB impeded MyD88/NF-κB and p38MAPK signaling pathways through down-regulation of miR-223 in LPS-injured cells.

Inflammation is increasingly recognized as a crucial driving factor for OA cartilage pathology [21]. Various kind of inflammatory factors play a key role in the development of OA, and are closely related to the degradation of cartilage [22,23]. Increasing studies have corroborated that LPS could affect cell proliferation and apoptosis, as well as induce the secretions of inflammatory factors in several inflammatory diseases, including OA [24,25]. An interesting study exhibited that LPS could increase iNOS expression in osteoarthritic synoviocytes [26].

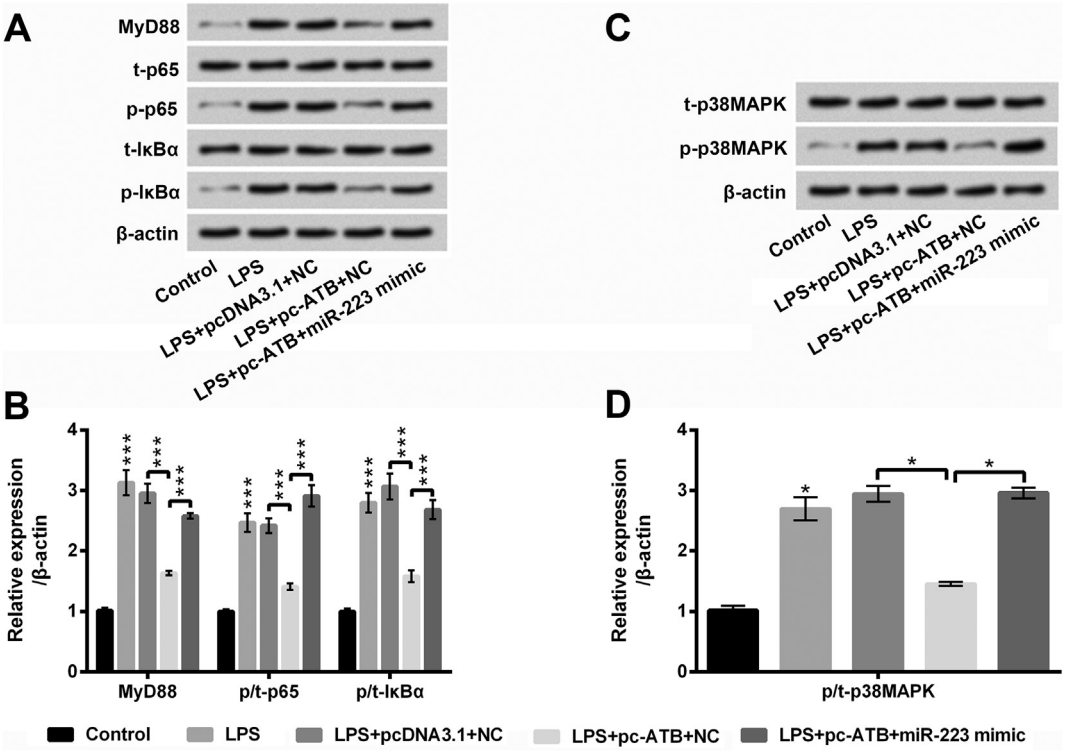

Fig. 7. LncRNA-ATB inhibited MyD88/NF-κB and p38MAPK pathways via regulation of miR-223.
After administration with 5 μg/ml LPS, ATDC5 cells were transfected with pc-lncRNA-ATB or miR-223 mimic. (A) MyD88, p/t-p65 and p/t-IκBα, as well as (B) p/t-p38MAPK protein levels were examined by western blot assay in ATDC5 cells. *, $P < 0.05$, ***, $P < 0.001$.

Additionally, Chen et al. found that IL-1β, IL-6 and COX-2 presented at high expression levels in patients with OA [27]. Recently, LPS has been widely utilized to construct the inflammatory injury model in OA cartilage [28]. Thus, we assessed cell viability, apoptosis, and secretions of iNOS, COX-2, IL-6 and TNF-α after treatment with LPS. We found that LPS significantly repressed cell viability, facilitated apoptosis, and induced iNOS, COX-2, IL-6 and TNF-α productions, indicating that the inflammatory injury model was successfully constructed in ATDC5 cells.

Recent study affirmed that lncRNAs could improve inflammatory injury in OA chondrocytes [29]. As Li et al. uncovered that lncRNA growth arrest-specific transcript 5 (GAS5) mitigated LPS-induced inflammatory injury and apoptosis via regulating Kruppel-like factor 2 (KLF2) expression in ATDC5 cells [30]. Evidence from Sun et al. authenticated that lncRNA RP11-445H22.4 inhibition attenuated LPS-induced injury through mediating miR-301a expression in ATDC5 cells [31]. It is reported that lncRNA-ATB is an indispensable cancer-related lncRNA, which acts as an oncogene in diverse cancers [32,33]. However, the impact of lncRNA-ATB on LPS-induced inflammatory injury in ATDC5 cells remains indistinct. In our research, results revealed that lncRNA-ATB expression was declined by LPS in ATDC5 cells. Importantly, we found that lncRNA-ATB overexpression significantly promoted cell viability, inhibited apoptosis, as well as repressed inflammatory factors expression in LPS-injured cells. These data indicated that lncRNA-ATB could ameliorate LPS-induced inflammatory injury in ATDC5 cells.

Recently, the involvements of miRNAs in the cartilage metabolism and in the development and pathogenesis of OA have been widely investigated [34,35]. Several miRNAs, such as miR-9, miR-27, miR-34a, miR-140, miR-146a, miR-558 and miR-602 have been proven to be aberrantly expressed in OA [34]. Furthermore, Wu et al. certified that miR-1246 aggravated LPS-induced inflammatory injury in ATDC5 cells via regulating HNF4γ expression [36]. Zhao et al. stated that suppression of miR-203 weakened LPS-induced injury via targeting MCL-1 in C28/I2 chondrocytes [37]. MiR-223 is a novel miRNA, increased miR-223 has been discovered in the patients with rheumatoid arthritis patients, and also confirmed to be associated with osteoclastogenesis and bone erosion [38,39]. Kim et al. testified that miR-223 exerted the protective effect against cartilage destruction [40]. Recent studies corroborated that EPB41L3, FBXW7 and ITGA3/ITGB1 were direct target genes of miR-223 in the different diseases [41–43]. Wang et al. demonstrated that miR-223 could repress LPS-induced inflammatory response by directly targeting Irak1 in the nucleus pulposus cells [44]. Additionally, Chen et al. discovered that miR-223 aggrandized the production of pro-inflammatory cytokines IL-6 and IL-1β in macrophages through directly targeting signal transducer and activator of transcription 3 (STAT3) [45]. However, the impact of miR-223 on LPS-induced inflammatory injury in ADTC5 cells is still unclear. In our study, we tried to explore the effect of lncRNA-ATB on miR-223 expression at the level of transcription by using RT-qPCR. We found that the expression of miR-223 was restrained by lncRNA-ATB. Hirata et al. reported that MALAT1 knockdown significantly increased miR-205 expression in renal cancer cells, however after overexpression of miR-205, the expression of MALAT1 was significantly decreased, indicating the reciprocal effects between MALAT1 and miRNA-205 [46]. Based on this study, we suspected that after overexpression of miR-223, the expression level of lncRNA-ATB might be down-regulated in ATDC5 cells. Increasing evidence demonstrated that lncRNA could interact with the miRNA as a competing endogenous RNA (ceRNA) to participate in the regulation of the expression of target genes, thereby exerting the central effects on the progression of different diseases [46]. In the present study, we observed that miR-223 overexpression reversed the effect of lncRNA-ATB on LPS-injured cells. LncRNA-ATB functioned by down-regulation of miR-223, indicating that miR-223 played an important role in LPS-induced inflammatory injury in ATDC5 cells. If there is complementary sequence between lncRNA-ATB and miR-223, we suspected that lncRNA-ATB could sponge miR-223 to affect LPS-induced injury in ADTC5 cells. Further studies are still needed to explore these speculations in the future.

MyD88 is a key adapter protein of Toll-like receptor 4 (TLR4) signaling pathway, which can initiate the transduction of downstream inflammatory factors [47]. NF-κB is considered to be a vital regulator in all stages of inflammation, which has been proven to regulate the secretion of pro-inflammatory cytokines (IL-1β, IL-6, IL-17 and TNF-α) that participate in the pathogenesis of OA [48]. p38 is the most important member of the MAPK family controlling inflammatory response, and suppression of p38MAPK signaling pathway can decrease OA chondrocyte apoptosis and decline the expression of pro-inflammatory cytokines [49]. Recent study confirmed that the inflammatory response regulated by lncRNA might be through mediation of the relevant inflammatory pathways [50]. Therefore, in the current study, we explored the impact of lncRNA-ATB on these signaling pathways to uncover the underlying mechanism. The results emerged that lncRNA-ATB inhibited MyD88/NF-κB and p38MAPK pathway activations through regulation of miR-223 in LPS-injured ATDC5 cells.

Taken together, the study stated that lncRNA-ATB ameliorated LPS-induced inflammatory injury in ATDC5 cells by blocking MyD88/NF-κB and p38MAPK pathways via repression of miR-223. These evidences point out that lncRNA-ATB exerted the anti-inflammatory effect on OA, which may be a diagnostic, therapeutic and prognostic biomarker for OA. There are some limitations in the current research: lack of *in vivo* experiment; whether there is an interaction between lncRNA-ATB and miR-223 remains unclear. Additionally, it is unclear whether the expression level of lncRNA-ATB is down-regulated in ATDC5 cells after overexpression of miR-223. Further studies are still needed to solve these limitations in the future.

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Competing interests

The authors declare that they have no competing interests.

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