

Apremilast prevents IL-17-induced cellular senescence in ATDC5 chondrocytes mediated by SIRT1

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Abstract. Osteoarthritis is the most prevalent joint degenerative disease and has been considered a major cause of severe joint pain and physical disability in the elderly. The chondrocyte is the only cell type found in articular cartilage and chondrocyte senescence plays a pivotal role in the pathogenesis of osteoarthritis. Apremilast is an oral PDE4 inhibitor and has been used for the treatment of patients with active psoriatic arthritis. In the present study, the biological function of apremilast was examined in an interleukin (IL)-17-treated chondrocyte model. Expression levels of target genes and proteins were measured using reverse transcription-quantitative PCR, ELISA, and western blotting, respectively. ROS levels in chondrocytes were examined using the fluorescent dye DCFH-DA. Cellular senescence was determined using senescence-associated- β -galactosidase staining. The profile of cell cycle phases was analyzed via flow cytometry. It was revealed that treatment with apremilast reduced the expression of IL-1 β , MCP-1, and the production of ROS. SA- β -gal staining results indicated that the presence of apremilast suppressed IL-17-induced cellular senescence. Furthermore, apremilast prevented IL-17-induced G0/G1 phase cell cycle arrest. In addition, it was demonstrated that apremilast suppressed IL-17-induced expression of p21 and PAI-1. Notably, the silencing of sirtuin 1 (SIRT1) abolished the protective effect of apremilast against IL-17-induced cellular senescence, suggesting that the action of apremilast in chondrocytes is dependent on SIRT1. In conclusion, the present results revealed that apremilast exerted a beneficial effect, thereby protecting chondrocytes from senescence induced by IL-17.

Introduction

Osteoarthritis (OA), is a painful degenerative joint disease that occurs in the elderly across the developed and developing world and has attracted increasing attention in the past decades (1). Although the pathological mechanism of the disease is complicated, aging is considered as the main risk factor in the development of OA (2). The chondrocyte is the only cell type found in articular cartilage. Healthy chondrocytes are responsible for maintaining cartilage homeostasis (3). However, in the condition of OA, chondrocytes produce various pro-inflammatory cytokines and chemokines, which lead to chronic inflammation. Furthermore, high levels of these cytokines result in chondrocyte senescence (4). Cellular senescence plays an important role in OA through G0/G1 phase cell cycle arrest and limiting the proliferative and synthesizing capacities of chondrocytes, resulting in disability of chondrocytes to secrete extracellular matrix components (2). Several signaling pathways have been implicated in the process of cellular senescence. For example, increased expression of plasminogen activator inhibitor-1 (PAI-1) and p21 have been considered as important biomarkers of cellular senescence. Additionally, sirtuin 1 (SIRT1), a nicotinamide dinucleotide (NAD⁺)-dependent deacetylase, plays diverse roles in negatively regulating senescence, aging, and inflammation in different types of cells, including chondrocytes (5). The ATDC5 chondrocyte cell line.

Interleukin (IL)-17 is well known as a member of a multifunctional cytokine family. Research has demonstrated that IL-17 plays an important role in several inflammatory diseases, such as psoriasis, multiple sclerosis (MS), and rheumatoid arthritis (6). Furthermore, studies have revealed the positive relationship between IL-17 concentration and the severity of OA (7,8). The antibody fragment of IL-17 has been used for the treatment of rheumatoid arthritis (9). Although the successful application of IL-17 inhibitors in OA patients has not been reported to date, efforts have been made by both clinical physicians and scientific pharmacologists (10). IL-17 could induce the production of pro-inflammatory cytokines by chondrocytes, thereby triggering a sustained inflammatory response (11). Furthermore, IL-17 enhances the release of matrix metalloproteinases (MMPs), which lead to joint damage and cartilage destruction (12). However, the biological function of IL-17 in chondrocytes remains to be elucidated.

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Apremilast, an oral phosphodiesterase 4 (PDE4) inhibitor, was approved by the US Food and Drug Administration (FDA) in 2014 for the treatment of patients with active psoriatic arthritis and plaque psoriasis (13). Evidence has revealed that apremilast could inhibit PDE4 activity and blocks pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α , IL-1 β , IL-17, and IL-2 in the inflammatory response of psoriasis (14). These results indicate that apremilast may have a protective effect on other inflammatory diseases. Notably, the administration of apremilast revealed efficacy in an atopic dermatitis (AD) mouse model by reducing ear swelling and the expression of monocyte chemoattractant protein-1 (MCP-1), IL-12/IL-23p40, and IL-31 (15). Notably, apremilast has been reported to induce an increase of the anti-inflammatory B-cell subset Breg cells, and a decrease of Th1 cells, IFN γ -producing NKT cells, and IL-17-producing NKT cells (16). However, it is still unknown whether apremilast possesses any efficacy in OA.

Materials and methods

Cell culture and treatment. The ATDC5 chondrocyte cell line was obtained from the Cell Bank of the Cell Engineering Division, RIKEN BioResource Research Center. The cell line was maintained in DMEM/F12 medium (cat. no. 12634010) and supplied with 10% FBS (cat. no. 10100147; both from Gibco; Thermo Fisher Scientific, Inc.) in a 5% CO₂ humidified cell culture incubator at 37°C. For the treatment experiment, the cells were plated in 6-well cell culture plates and treated with IL-17 (10 ng/ml) (R&D Systems, Inc.) in the presence or absence of apremilast (0.5 and 1 μ M) (MedChemExpress) at 37°C for 24 h.

RNA isolation and reverse transcription-quantitative (RT-q) PCR. Total RNA was isolated from ATDC5 cells using TRIzol (cat. no. 15596018; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. The total RNA was treated with DNAase (cat. no. M0303S; New England Biolabs, Inc.) and then quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Inc.). RNA (2 μ g) was reversely transcribed into cDNA using an NGS Reverse Transcription kit (cat. no. A45003; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Gene expression was quantified using the SYBR Green PCR Master Mix (cat. no. 4309155; Thermo Fisher Scientific, Inc.) on an ABI Prism 7000 PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with the following thermocycling conditions: An initial denaturation and enzyme activation at 95°C for 10 min, followed by 35 cycles of 30 sec denaturation at 95°C, an attachment of primers for 1 min at 60°C, and extension at 72°C for 30 sec, and finally one cycle at 72°C for 10 min for final elongation. The following primers were used in the present study: IL-1 β forward, 5'-TGGACCTTCCAGGATGAGGACA-3' and reverse, 5'-GTTTCATCTCGGAGCCTGTAGTG-3'; MCP-1 forward, 5'-CCCAGGAGTGCCTTGATTC-3' and reverse, 5'-CGCCCCATAATTCTGACATC-3'; p21 forward, 5'-GTT CATCTCGGAGCCTGTAGTG-3' and reverse, 5'-AGCTGG CCTTAGAGGTGA-3'); PAI1 forward, 5'-GACACCTC AGCATGTTTCATC-3' and reverse, 5'-AGGGTTGCACTA AACATGTCAG-3'; SIRT1 forward, 5'-TTGTGAAGCTGT

TCGTGGAG-3' and reverse, 5'-GGCGTGGAGGTTTTTCA GTA-3'; and GAPDH (forward, 5'-AAGAGGGATGCTGCC CTTAC-3' and reverse, 5'-CCATTTTGTCTACGGGACGA-3'.

The 2^{- $\Delta\Delta$ C_q} method (17) was used to calculate the relative expression of the target genes, and the expression of GAPDH was used as the internal control.

2,7-Dichlorodihydrofluorescein diacetate (DCFH-DA) staining. Reactive oxygen species (ROS) levels in chondrocytes were measured using the fluorescent dye DCFH-DA (Sigma-Aldrich; Merck KGaA). Briefly, chondrocytes (1x10⁵ cells/ml) were treated with IL-17 or apremilast as aforementioned, then incubated with diluted 5 μ M DCFH-DA in the dark for 60 min at 37°C. Then, the cells were washed with PBS and images were captured with a confocal laser-scanning microscope (Leica TCS SL) (magnification, x10) equipped with an argon laser. The fluorescent quantification was analyzed using the software ImageJ (v2.1.4.7; National Institutes of Health).

Senescence-associated- β -galactosidase (SA- β -gal) staining. Cellular senescence was determined using senescence-associated β -galactosidase (SA- β -gal) staining by a Cellular Senescence Assay kit (product code ab228562; Abcam) according to the instructions from the manufacturer. Briefly, the cells were quickly washed with PBS twice and then fixed with 4% paraformaldehyde (PFA) for 15 min at room temperature followed by incubation with the staining solution overnight at 37°C. The number of SA- β -gal⁺ cells were counted from the total number of cells. The images were analyzed using ImageJ (v2.1.4.7).

Protein isolation and western blotting. Total protein from chondrocytes was lysed using RIPA buffer (cat. no. R0010; Solarbio Life Sciences). The protein concentration was measured using the Pierce BCA protein assay kit (cat. no. 23225; Thermo Fisher Scientific, Inc.). A total of 20 μ g of protein was separated by 8-12% sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE) gel and transferred to polyvinylidene difluoride (PVDF) membranes (EMD Millipore). The blots were then blocked with 5% skim milk diluted in TBST for 1 h at room temperature. The blots were then separately incubated with the following primary antibodies: p21 (1:1,000; cat. no. sc-6246; Santa Cruz Biotechnology, Inc.), PAI-1 (1:2,000; cat. no. 66261-1-Ig; ProteinTech Group, Inc.), SIRT1 (1:1,000; product no. 2310; Cell Signaling Technology, Inc.), β -actin (1:10,000; cat. no. AF7018; Affinity Biosciences) in a cold room (4°C) overnight, and followed by incubation with HRP-conjugated goat anti-mouse IgG (H+L) secondary antibody (1:2,000; cat. no. 31430) or goat anti-rabbit IgG (H+L) secondary antibody (1:2,000; cat. no. G-21234; both from Thermo Fisher Scientific, Inc.) for 1 h at room temperature. Immunoblots were detected with Amersham™ ECL™ Prime reagent (GE Healthcare Life Sciences; Cytiva). The images were captured and analyzed with an EC3 UVP imaging system (Cole-Parmer). The densitometry data were analyzed using the software ImageJ (v2.1.4.7).

ELISA. The mouse MCP-1 ELISA kit (cat. no. SEKM-0108) and mouse IL-1 β ELISA kit (cat. no. SEKM-0002) were purchased from Solarbio Life Sciences. The secretions of

IL-1 β and MCP-1 in chondrocytes were determined according to the manufacturer's instructions. The optical density of the reaction was detected using a 96-well plate reader (BioTek Instruments, Inc.).

Cell cycle analysis. The cell cycle of ATDC5 chondrocytes was assessed with flow cytometry. After IL-17 or apremilast treatment as aforementioned, 1.0×10^6 cells were collected and fixed with 70% ethanol at 4°C overnight. Cells were then permeabilized with 0.1% Triton X-100 in Tween-20, followed by staining using propidium iodide (PI) (50 mg/l) (Sigma-Aldrich, USA) on ice for 5 min. The profile of cell cycle phases was analyzed via flow cytometry on the BD LSR II system (BD Biosciences). The results were analyzed with FlowJo analytical software (version X; Tree Star, Inc.).

SIRT1 siRNA transfection. ATDC5 chondrocytes (2×10^5 cells/well) were plated in 6-well plates. SIRT1-specific or non-specific siRNA (100 nM; Guangzhou RiboBio Co., Ltd.) in serum-free medium was then transfected into cells using Lipofectamine RNAiMax (Invitrogen; Thermo Fisher Scientific, Inc.). The sequence of SIRT1 siRNA was: Forward, 5'-AGAUCAUCAAUUGAAdTdT-3' and reverse, 5'-UUCAAUUGUAUUGAUUCUdTdT-3'. After 4 h of transfection at 37°C, the medium was replaced with normal medium containing 10% FBS and incubation was continued for 48 h.

Statistical analysis. Data are presented as the mean \pm SEM. Statistical analysis was performed using SPSS version 24 software (IBM Corp.), and the difference between groups was assessed using ANOVA followed by Bonferroni's test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Apremilast reduces IL-17-induced expression levels and secretions of pro-inflammatory cytokines. To explore the protective effects of apremilast (Fig. 1A) in chondrocytes, the effects of apremilast on the production of pro-inflammatory cytokines were assessed. Compared with the control group, IL-17 treatment significantly increased the expression levels and secretions of IL-1 β and MCP-1 at both mRNA and protein levels, which were reduced by apremilast in a dose-dependent manner (Fig. 1B and C). This result indicated that apremilast has a potential inhibitory effect on the production of pro-inflammatory cytokines.

Apremilast suppresses IL-17-induced production of ROS. Aberrant ROS production plays a pivotal role in cellular senescence. In the present study, it was examined whether apremilast influenced the generation of ROS. As anticipated, the DCFH-DA staining revealed that IL-17 alone induced ~3.3-fold ROS production, but the presence of 0.5 and 1 μ M concentrations of apremilast decreased IL-17-induced ROS production to only 2.4- and 1.7-fold, respectively (Fig. 2).

Apremilast prevents IL-17-induced cellular senescence. Since apremilast revealed its inhibitory effect on the production of pro-inflammatory cytokines and ROS production induced by

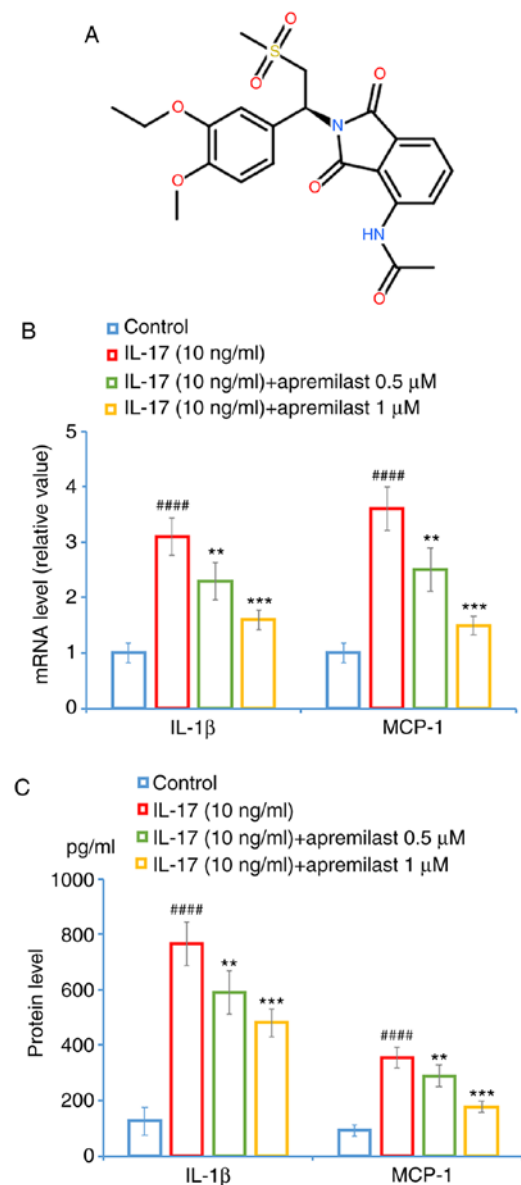


Figure 1. Apremilast prevents IL-17-induced expression and secretions of pro-inflammatory cytokines in mouse ATDC5 chondrocytes. Cells were treated with IL-17 (10 ng/ml) in the presence or absence of apremilast (0.5 and 1 μ M) for 24 h. (A) Molecular structure of apremilast. (B) mRNA levels of IL-1 β and MCP-1. (C) Secretions of IL-1 β and MCP-1. #### $P < 0.0001$ vs. the vehicle control group; ** $P < 0.01$ and *** $P < 0.001$ vs. the IL-17 group. IL, interleukin; MCP-1, monocyte chemoattractant protein-1.

IL-17, it was predicted that apremilast could protect chondrocytes from senescence. As revealed in Fig. 3, IL-17 stimulation induced a 3.7-fold increase of SA- β -gal activity, while 1 μ M apremilast reduced its activity to only 2.1-fold.

Apremilast prevents IL-17-induced cell cycle arrest in the G0/G1 phase. Next, the cell cycle profile was assessed with flow cytometry. Compared to non-treated cells, 7 days of IL-17 stimulation induced a 19% increase in G0/G1 phase cell cycle arrest. In addition, G2/M and S phase cells were reduced from 21.8 and 22 to 17.1 and 7.6%, respectively, by the same IL-17 treatment. However, in the presence of 1 μ M apremilast, the percentage of G0/G1 phase cells was reduced to 64.1%, compared with the 75.3% following exposure to IL-17 only.

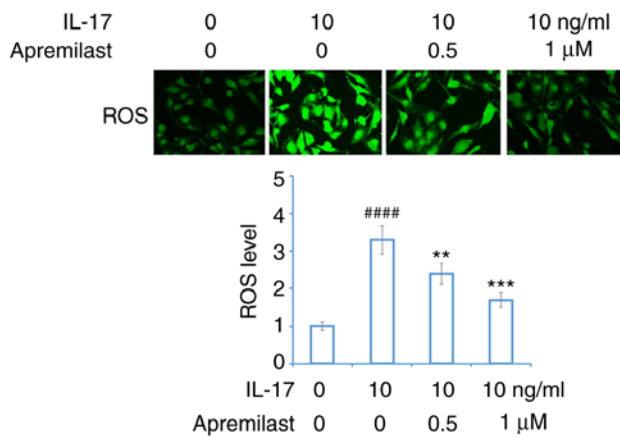


Figure 2. Apremilast prevents IL-17-induced production of ROS in mouse ATDC5 chondrocytes. Cells were treated with IL-17 (10 ng/ml) in the presence or absence of apremilast (0.5 and 1 μ M) for 24 h. Production of ROS was measured using DCFH-DA staining (magnification, $\times 10$). #### $P < 0.0001$ vs. the vehicle control group; ** $P < 0.01$ and *** $P < 0.001$ vs. the IL-17 group. ROS, reactive oxygen species; IL, interleukin; DCFH-DA, 2,7-dichlorodihydrofluorescein diacetate.

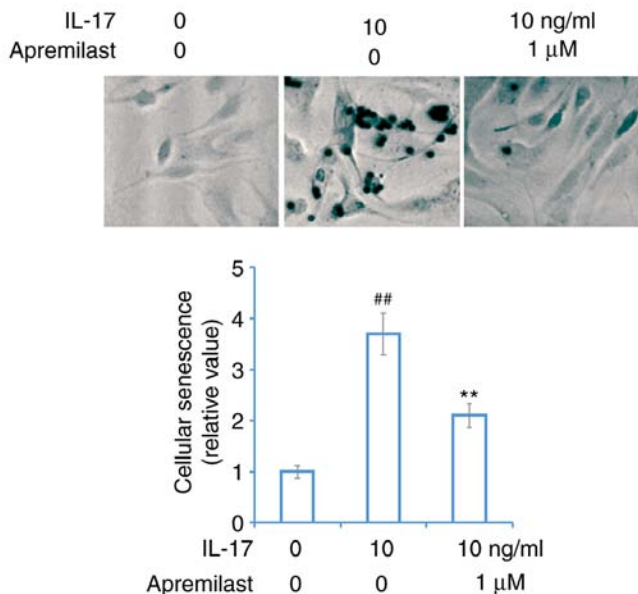


Figure 3. Apremilast prevents IL-17-induced cellular senescence in mouse ATDC5 chondrocytes. Cells were treated with IL-17 (10 ng/ml) in the presence or absence of apremilast (1 μ M) for 7 days. Cellular senescence was measured using SA- β -gal staining. ## $P < 0.01$ vs. the vehicle control group; ** $P < 0.01$ vs. the IL-17 group. IL, interleukin; SA- β -gal, senescence-associated- β -galactosidase.

The percentage of G2/M and S phase cells were rescued to 22.3 and 13.6%, respectively (Fig. 4).

Apremilast inhibits IL-17-induced expression levels of p21 and PAI-1. Thereafter, the effect of apremilast on the expression levels of p21 and PAI-1 were measured. IL-17 stimulation increased the mRNA levels of p21 and PAI-1 to 3.4- and 2.9-fold, respectively, which were reduced to 1.8- and 1.7-fold by 1 μ M apremilast treatment (Fig. 5A), respectively. Furthermore, apremilast reduced the protein levels of p21 and PAI-1 to 1.7- and 1.8-fold, respectively, compared to the

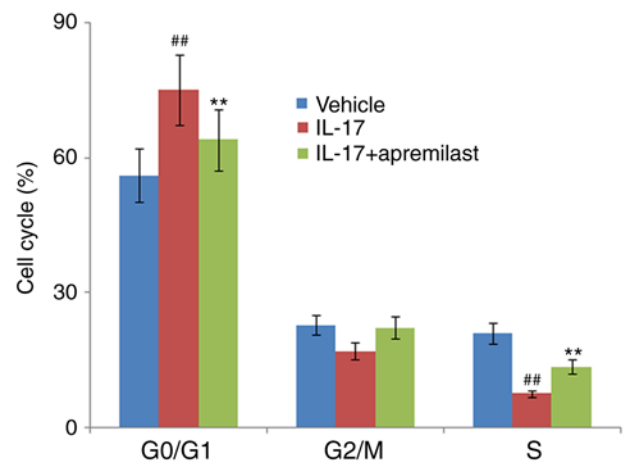


Figure 4. Apremilast prevents IL-17-induced cell cycle arrest in the G0/G1 phase in mouse ATDC5 chondrocytes. Cells were treated with IL-17 (10 ng/ml) in the presence or absence of apremilast (1 μ M) for 7 days. The cell cycle was assayed using flow cytometry. G0/G1 phase, G2/M phase, and S phase of cells were measured. ## $P < 0.01$ vs. the vehicle control group; ** $P < 0.01$ vs. the IL-17 group. IL, interleukin.

2.7- and 2.8-fold increase following exposure to IL-17 only (Fig. 5B).

Inhibitory effects of apremilast in cellular senescence are dependent on SIRT1. SIRT1 is one of the most important anti-aging factors (18). In the present study, the influence of apremilast on SIRT1 expression was also assessed. Compared to the non-treated cells, IL-17 reduced the mRNA level of SIRT1 to 53%, which was recovered to 92%, close to the baseline, in the presence of 1 μ M apremilast (Fig. 6A). It was also confirmed that apremilast rescued the protein level of SIRT1 which had been significantly reduced by IL-17 (Fig. 6B). Moreover, an attempt was made to determine the involvement of SIRT1 in the action of apremilast in chondrocytes. SIRT1 was silenced with SIRT1 siRNA. As revealed in Fig. 7A, in normal SIRT1-expressing cells, apremilast significantly suppressed cellular senescence, however, its effect was almost abolished in SIRT1-silenced cells. The results from western blot analysis in Fig. 7B and C revealed that the inhibitory effect of apremilast on PAI-1 and p21 was abolished by the silencing of SIRT1. These data indicated that the effects of apremilast on chondrocytes depend on the expression of SIRT1.

Discussion

As a common joint degenerative disease, safe and effective therapies against OA are still required. Aside from inflammation, chondrocyte senescence is considered another important event in the pathobiology of OA (19). Cellular senescence refers to irreversible cell growth stagnation after a characteristic number of cell proliferation (20). One of the most important senescence pathways is called stress-induced premature senescence (SIPS), which can be induced by cytokines, DNA damage, oxidative stress, and external stimuli (21). Excessive production of IL-17 has been linked with chondrocyte activation (22). In the present study, apremilast, a PDE4 inhibitor used for the treatment of psoriasis, was investigated as a potential strategy for slowing chondrocyte senescence in the

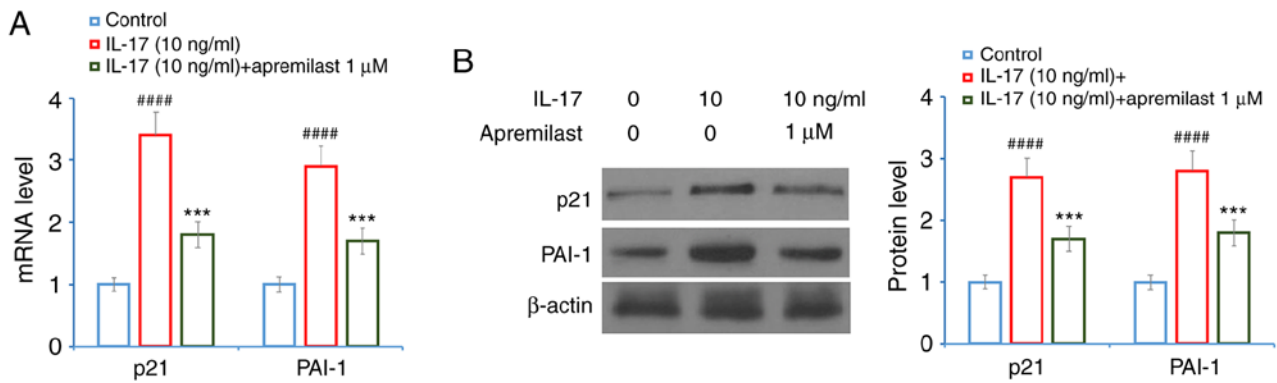


Figure 5. Apremilast prevents IL-17-induced expression of p21 and PAI-1 in mouse ATDC5 chondrocytes. Cells were treated with IL-17 (10 ng/ml) in the presence or absence of apremilast (1 μ M) for 24 h. (A) mRNA levels of p21 and PAI-1. (B) Protein levels of p21 and PAI-1 as measured by western blotting. ####P<0.0001 vs. the vehicle control group; ***P<0.001 vs. the IL-17 group. IL, interleukin; PAI-1, plasminogen activator inhibitor-1.

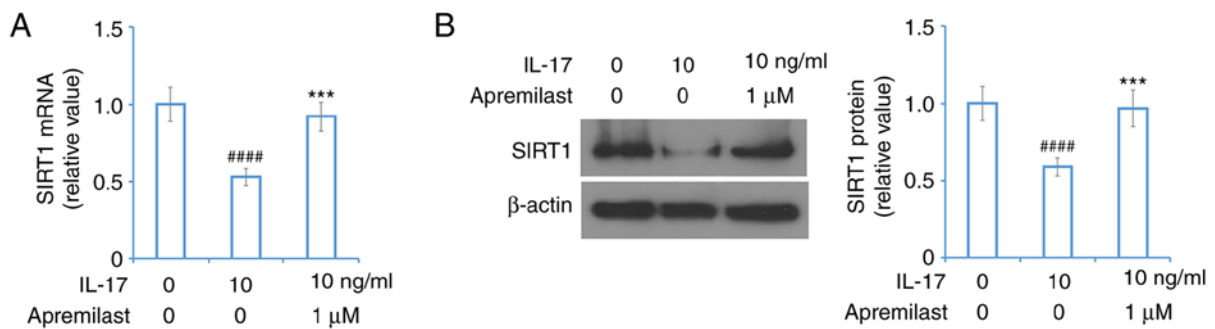


Figure 6. Apremilast prevents IL-17-induced reduction of SIRT1 in mouse ATDC5 chondrocytes. Cells were treated with IL-17 (10 ng/ml) in the presence or absence of a Apremilast (1 μ M) for 24 h. (A) mRNA of SIRT1. (B) Protein level of SIRT1. ####P<0.0001 vs. the vehicle control group; ***P<0.001 vs. the IL-17 group. IL, interleukin; SIRT1, sirtuin 1.

development of OA. First, it was determined that IL-17 treatment significantly increased the production of IL-1 β , MCP-1, and ROS. IL-1 β is one of the most important pro-inflammatory cytokines in various inflammatory diseases including OA, acting as a trigger of the cascades of inflammatory responses. Also, IL-1 β has been demonstrated to have a strong effect on chondrocyte senescence (23). MCP-1 also plays a pivotal role in the development of OA by recruiting immune cells and promoting inflammation (24). Oxidative stress is usually considered as the major inducer of cellular senescence (25). Studies have revealed that increased ROS reduces chondrocyte survival, and this has subsequently been linked to the development of OA (26-28). Further research has demonstrated that ROS could activate several downstream pathways that induce senescence and apoptosis (29).

Furthermore, in this *in vitro* chondrocyte model, our results indicated the ability of apremilast to inhibit chondrocyte senescence, as revealed by its reduction of senescence-associated β -galactosidase (SA- β -gal) activity, which is a cellular marker of senescence (30). Cell cycle arrest is a type of replicative senescence in which cells cease to replicate, and tissue begins to break down. The involvement of expansion and cell cycle arrest in cellular senescence (20). In the present study, it was revealed that IL-17 stimulation elongated quiescent/gap phase (G0/G1), shortened DNA synthesis phase (S phase), and induced senescent phenotype, however, treatment with apremilast reversed the effect of IL-17 on cell cycle arrest.

Mechanistically, it was revealed that apremilast inhibited IL-17-induced expression levels of senescence-related genes. As a downstream gene of p53, the expression of p21 increases in senescent cells and leads to cell cycle arrest by suppressing several cyclin-dependent kinases (31). It has been reported that chondrocyte senescence could be mediated by IL-1 β treatment via the p53/p21/pRb pathway, with the induction of caveolin 1 and activation of p38 mitogen-activated protein kinase (MAPK), resulting in cell senescence and apoptosis (32). Moreover, researchers have revealed that the expression levels of PAI-1 and p21 proteins have a positive correlation with the presence of SA- β -gal (2,33). PAI-1 is a member of the evolutionarily conserved serine protease inhibitor (SERPIN) family and is well known as a marker and mediator of senescence, revealed by its increased expression in senescent cells (34,35). Both p21 and PAI-1 are the direct transcriptional targets of p53 (36). The capacity of apremilast to mitigate the expression of p21 and PAI-1 could account for its ameliorative effect on the G1 phase rescue.

Notably, it was revealed that apremilast rescued the expression of SIRT1. SIRT1 is a master metabolic regulator in different metabolic tissues and is associated with various signaling pathways, including inflammation and senescence (37,38). In cartilage tissues, the expression of SIRT1 proteins is present in the nuclei of chondrocytes. Pro-inflammatory cytokines such as TNF- α could inhibit the expression of SIRT1 (39). In the present study, it was revealed that IL-17 also had a strong inhibitory

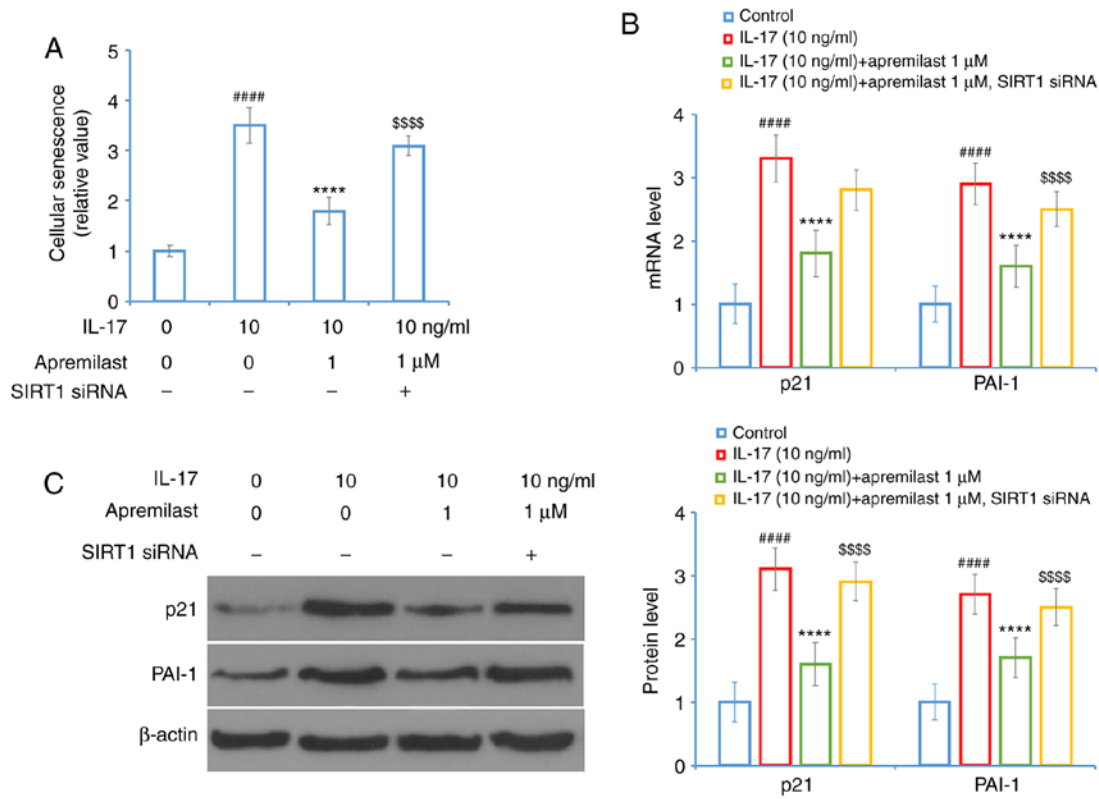


Figure 7. Knockdown of SIRT1 abolishes the protective effects of apremilast against IL-17-induced cellular senescence and the expression levels of p21 and PAI-1. Cells were transfected with SIRT1 siRNA, followed by stimulation with IL-17 (10 ng/ml) in the presence or absence of apremilast (1 μ M) for 24 h. (A) Cellular senescence. (B) mRNA levels of p21 and PAI-1. (C) Protein levels of p21 and PAI-1 as measured by western blotting. ####P<0.0001 vs. the vehicle control group; ****P<0.0001 vs. the IL-17 group; ssssP<0.0001 vs. the IL-17 + apremilast group. SIRT1, sirtuin 1; IL, interleukin; PAI-1, plasminogen activator inhibitor-1.

effect on the expression of SIRT1. It has been demonstrated that the expression of SIRT1 is decreased in degenerated cartilage, resulting in inflammation and chondrocyte senescence (40). However, the function of SIRT1 in OA has not been entirely elucidated. Recently, studies revealed evidence that SIRT1 regulates the expression of extracellular matrix proteins, and silencing of SIRT1 in chondrocytes leads to osteoarthritic gene expression (40,41). Based on this, SIRT1 was considered as a key regulator in the prevention of chondrocyte senescence by apremilast. As anticipated, apremilast rescued the expression of SIRT1 reduced by IL-17. In addition, silencing of SIRT1 abolished the protective effects of apremilast against IL-17-induced cellular senescence. A graphical representation of the underlying mechanism is presented in Fig. 8.

Monoclonal antibodies mediating IL-17 inhibition have been used in the treatment of rheumatoid arthritis (RA), with conflicting results having been reported (9,42). However, IL-17 inhibition appears to be effective in the treatment of psoriasis (43). Notably, a recent study revealed that intra-articular injection of an IL-17-neutralizing antibody reduced joint degeneration in aging OA mice (44). However, IL-17 inhibition has not been trialed in the treatment of OA in patients. Given the heterogeneity of RA and OA, the inhibition of IL-17 may not produce the same outcome in patients. More preclinical data are required for the implementation of the IL-17 inhibition approach in human OA.

Collectively, the present findings revealed that apremilast possessed a protective capacity against IL-17-induced cellular senescence in chondrocytes. Further investigations are

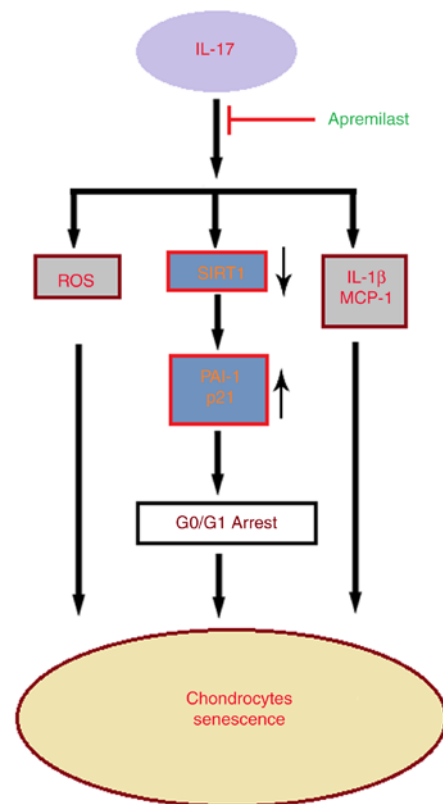


Figure 8. Graphical representation of the underlying mechanism, whereby apremilast prevents IL-17-induced cellular senescence in ATDC5 chondrocytes. IL, interleukin; ROS, reactive oxygen species; SIRT1, sirtuin 1; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1.

required to clarify the mechanisms driving these protective effects of apremilast.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

BW and FL contributed to the conception and design of the study. BW, WS, KB and YL contributed to the acquisition of the data. BW and FL contributed to the analysis of the data. FL contributed to the drafting of the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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