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Antagonism of cysteinyl leukotriene receptor 1 (cysLTR1) by montelukast suppresses cell senescence of chondrocytes

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ABSTRACT

Aging is closely associated with osteoarthritis (OA). Although its underlying mechanisms remain unknown, cellular senescence in chondrocytes has become an important therapeutic target for the treatment of OA. Cysteinyl leukotriene receptors (cysLTRs) mediate the pathobiological function of cysteinyl leukotrienes (cysLTs). However, the roles of cysLTRs in the pathogenesis of OA have not been reported before. In the current study, we found that cysLTR1 but not cysLTR2 is expressed in human primary chondrocytes. In addition, stimulation with tumor necrosis factor α (TNF-α) resulted in a significant increase in the expression of cysLTR1. Interestingly, montelukast, a specific cysLTR1 antagonist, attenuated TNF-α-induced up-regulation of the activity of senescence-associated β-galactosidase (SA-β-Gal). In addition, TNF-α led to cell cycle arrest at the G0/G1 phase, which was prevented by treatment with montelukast. Notably, montelukast reduced expression of the senescence markers p53, p21 and PAI-1. In addition, montelukast ameliorated TNF-α-induced K382 acetylation of p53 by promoting the expression of SIRT1. Silencing of SIRT1 using SIRT1 siRNA broke the inhibitory effects of montelukast on K382 acetylation of p53. Importantly, silencing of cysLTR1 reversed the reduction of SIRT1 expression as well as the K382 acetylation of p53. Our findings strongly implicate that cysLTR1 has the capacity to regulate cellular senescence in chondrocytes. It is suggested that montelukast may be a potential therapeutic agent for chondro-protective therapy.

1. Introduction

Osteoarthritis (OA) is the most common degenerative joint disease, affecting millions of people worldwide [1]. Aging is one of the most important risk factors of OA. Chondrocytes, the only type of cell present within articular cartilage, play a casual role in maintaining the metabolism balance of the cartilage matrix [2]. Interestingly, previous studies have shown that cellular senescence plays an important role in the pathological progression of OA. Chondrocytes from OA patients display numerous senescence-associated phenotypes, as well as increased activity of SA-β-Gal, telomere attrition, and increased expression of senescent-associated genes [3]. It has been found that a low degree of inflammation resulting from the release of pro-inflammatory factors is involved in the pathogenesis of OA [4]. The pro-inflammatory cytokine tumor necrosis factor α (TNF-α) induces an imbalance between anabolic and degradative mechanisms, which may cause extrinsic stress-induced senescence in chondrocytes [5,6].

Cysteinyl leukotrienes (cysLTs) are a group of highly bioactive lipids synthesized from arachidonic acid. Previous studies have shown that cysLTs play a crucial role in both physiological and pathological conditions [7]. CysLTs, including leukotriene C4 (LTC4), LTD4, and LTE4, are proinflammatory bioactive lipids that have a pathobiological function in asthma [8]. CysLTs exhibit several biological activities through two specific G protein-coupled receptor (GPCR) subtypes named cysLTR1 and cysLTR2 [9]. Montelukast (MK), a potent and specific cysLTR1 antagonist, is widely used in patients with chronic asthma [10]. Montelukast can modulate airway remodeling in asthma by exerting anti-inflammatory and anti-oxidant actions [11]. In addition to chronic asthma, the pharmacological effects of montelukast have been studied in other diseases. For example, montelukast reversed the upregulation of cysLTR1 and NF-kB p65 and activated caspase-3 expression induced by Aβ1–42 in Alzheimer's disease [12]. Montelukast could also attenuate infarct volume, brain atrophy, neuron loss, and behavioral dysfunction after focal cerebral ischemia in rodents [13]. However, the pharmacological roles of montelukast in the pathogenesis of OA have not been reported before. Therefore, in the current investigation, we evaluated the effects of montelukast on *in vitro* cell senescence of chondrocytes.

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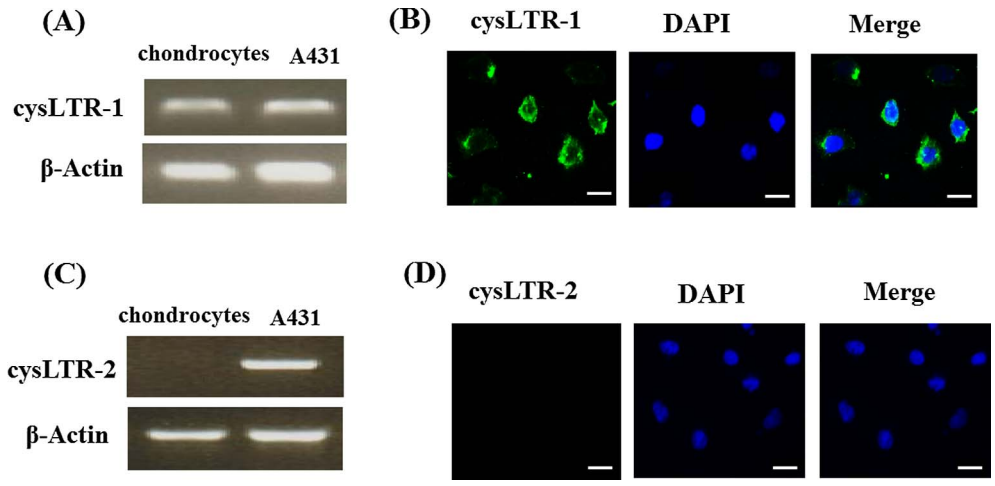

Fig. 1. Cysteinyl leukotriene receptor 1 (cysLTR1) but not cysLTR2 is expressed in human primary chondrocytes. Human A431 cells were used as a positive control. (A) Expression of cysLTR1 at the mRNA level was determined by RT-PCR analysis; (B) expression of cysLTR1 at the protein level was determined by cysLTR1 immunofluorescence staining experiments; (C) expression of cysLTR2 at the mRNA level was determined by RT-PCR analysis; (D) expression of cysLTR2 at the protein levels was determined by cysLTR2 immunofluorescence staining experiments. Scale bars, 50 μM. Experiments were repeated for 3 times.

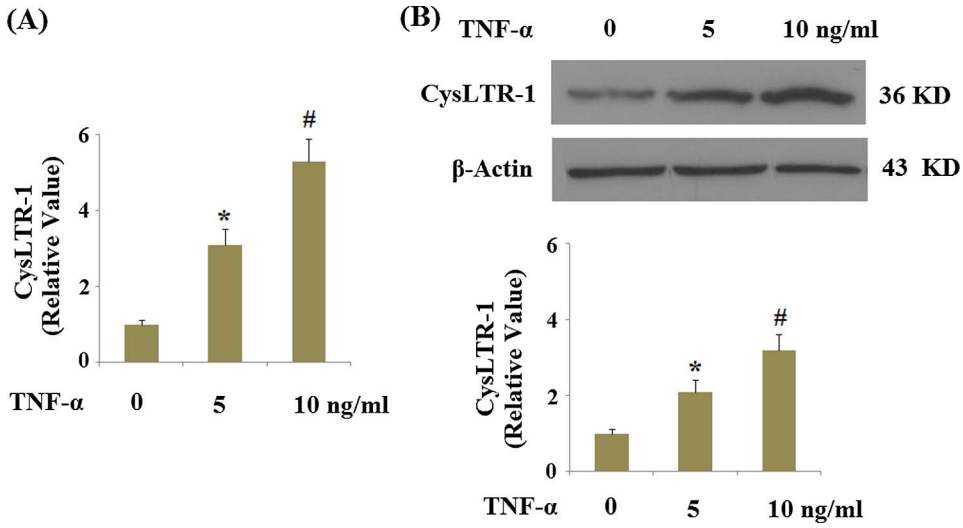

Fig. 2. TNF-α increased expression of cysLTR1 in human primary chondrocytes. Cells were treated with TNF-α at concentrations of 5 and 10 ng/ml for 24 h. (A) Expression of cysLTR1 at the mRNA level was determined by the RT-PCR analysis; (B) expression of cysLTR1 at the protein level was determined by the western blot analysis (*, $P < .0001$ vs. vehicle group; #, $P < .0001$ vs. 5 ng/ml TNF-α treatment group, $n = 5–6$).

2. Materials and methods

2.1. Chondrocyte isolation and culture

Healthy human cartilage was obtained from 18 adults undergoing hip replacement due to femoral neck or distal femoral tumor. Briefly, cartilage slices were collected and minced into small pieces. Samples were treated with 0.5 mg/ml trypsin for 20 min at 37 °C, followed by incubation overnight with 2 mg/ml clostridial collagenase at 37 °C. After centrifugation at 500*g*, chondrocytes were suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Montelukast (#Y0001434, Sigma-Aldrich, USA) was dissolved in 0.1% dimethyl sulfoxide (DMSO). Control cultures received carrier solvent (0.1% DMSO), unless otherwise specified.

2.2. Real-time polymerase chain reaction (PCR)

After appropriate stimulation, total intracellular RNA was isolated from chondrocytes using Trizol reagent (Roche Applied Science, USA). The concentration and quality of each total RNA sample were examined using a NanoDrop ND1000 spectrophotometer (Thermo Fisher Scientific, USA). An equal amount of RNA (1 μg) was then used to synthesize cDNA via reverse transcription PCR (RT-PCR) using the iScript RT-PCR kit (Bio-Rad, USA). Real-time PCR was carried out on a 7500 Real-Time PCR System (Applied Biosystems, USA) using SYBR Green PCR master mix (Bio-Rad, USA). The reference gene β-actin was used as an internal reference. Gene expression is presented as $2^{-\Delta\Delta Ct}$ with respect to non-stimulated cells. The following primers were used in this study: CysLTR1, forward 5′-ATG TTC ACA AAG GCA AGT GG-3′ and reverse 5′-TGC ATC CTA AGG ACA GAG TCA-3′; CysLT2R, forward 5′-ACC CCT TCC AGA TGC TCC A-3′ and reverse 5′-CGT GCT TTG AAA TTC TCT CCA-3′; β-actin, forward 5′-AAC CCT AAG GCC AACCGT GAA-3′ and reverse 5′-TCA TGA GGT AGT CTG TCA GGT C-3′.

2.3. Western blot analysis

Human primary chondrocytes were washed 3 times in ice-cold PBS and lysed with cell lysis buffer (Cell Signaling Technologies, USA) containing cocktail inhibitor (1 mg/ml) (Sigma-Aldrich, USA) and 1 mM phenylmethylsulphonyl fluoride (PMSF, Sigma-Aldrich, USA). An equal amount of protein sample (20 μg) was resolved on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon P, USA). After blocking with 5% non-fat dried milk for 2 h at room temperature (RT), the blots were sequentially probed with primary antibodies for 12 h at 4 °C and peroxidase-conjugated secondary antibodies for 2 h at RT. Blots were developed using an enhanced chemiluminescence (ECL) kit (GE Healthcare, USA) [14].