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SIRT7 regulates the TGF-β1-induced proliferation and migration of mouse airway smooth muscle cells by modulating the expression of TGF-β receptor I

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ABSTRACT

Accumulating evidence shows that sirtuin 7 (SIRT7), a key mediator of many cellular activities, plays an important role in the pathogenesis of various diseases; however, little is known about the role of SIRT7 in asthma, which is characterized by airway remodeling. This study investigated the potential role of SIRT7 in regulating the proliferation and migration of airway smooth muscle (ASM) cells, which are critical events during airway remodeling in asthmatic conditions. The results demonstrated that SIRT7 expression was significantly upregulated in ASM cells treated with transforming growth factor-beta 1 (TGF-β1). Knockdown of SIRT7 inhibited the proliferation, promoted the apoptosis, and suppressed the migration of TGF-β1-treated ASM cells, while overexpression of SIRT7 had the opposite effect. Moreover, knockdown of SIRT7 inhibited protein expression of the TGF-β receptor I (TβRI), whilst overexpression of SIRT7 promoted the expression of TβRI. Importantly, knockdown of TβRI partially reversed the stimulatory effect of SIRT7 overexpression on the TGF-β1-induced proliferation and migration of ASM cells. Taken together, these results demonstrate that SIRT7 is involved in regulating TGF-β1-induced ASM cell proliferation and migration by regulating the expression of TβRI, thus indicating an important role of SIRT7 during airway remodeling in asthma.

1. Introduction

Asthma is characterized by airway remodeling that severely limits airflow in the lungs [1]. It is a complex respiratory disease with an increasing incidence worldwide; however, there is currently no effective cure. Contractile agonists, growth factors, and inflammatory mediators contribute to the hyperproliferation, excessive mass, and enhanced cell migration of airway smooth muscle (ASM) cells in the airway wall, which leads to airway remodeling during the pathogenesis of asthma [2,3]. Therefore ASM cells were suggested as potential targets for the treatment of asthma [4]. It is essential to identify novel targets that are involved in regulating airway remodeling, as these will help to develop effective therapies targeting ASM cells for the treatment of asthma.

Sirtuins are a family of highly-conserved NAD^+-dependent protein deacetylases that play a pivotal role in a number of cellular and metabolic processes [5–7]. Sirtuin 7 (SIRT7) is a recently identified sirtuin that has received particular attention [8]. The SIRT7 gene is located on chromosome 17 (17q25.3) and encodes a 400 amino acid protein [9]. SIRT7 expression is abundant in metabolically-active cells and is enriched in the nucleoli where it facilitates the transcription of rDNA [10,11]. SIRT7 is also involved in regulating a variety of biological activities including metabolism, cellular stress, and chromatin remodeling [12–15], and SIRT7 dysregulation is implicated in numerous diseases including cancers, cardiovascular disease, and neurological disease [16–21]. However, whether SIRT7 regulates airway remodeling in asthma remains unknown.

Transforming growth factor-β1 (TGF-β1) is a pluripotent cytokine

Abbreviations: ASM, airway smooth muscle; CCK-8, Cell Counting Kit-8; FBS, fetal bovine serum; RT-qPCR, real-time quantitative polymerase chain reaction; SIRT7, sirtuin 7; TβRI, TGF-β receptor I; TGF-β1, transforming growth factor-beta 1

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that acts as a potent regulator of connective tissue synthesis and cell proliferation [22]. Upon binding with TGF-β receptors, such as TGF-β receptor I (TβRI), TGF-β1 initiates a series of signal transduction events mediated by downstream Smad proteins [23]. TGF-β1 is upregulated in asthma and contributes to airway remodeling by regulating the production of extracellular matrix and the proliferation of ASM cells [24–26]. TGF-β1-induced ASM cell proliferation and migration was previously used as an in vitro model to investigate airway remodeling [27,28].

Accumulating evidence shows that SIRT7 is a multifunctional protein that regulates numerous cellular processes and is implicated in various diseases [29]; however, the involvement of SIRT7 in the pathogenesis of asthma has not been established. Therefore this study was designed to investigate the potential role of SIRT7 in regulating TGF-β1-induced ASM cell proliferation and migration in vitro based on the hypothesis that SIRT7 participates in airway remodeling. SIRT7 expression was significantly upregulated in ASM cells treated with TGF-β1. Knockdown of SIRT7 inhibited the proliferation, promoted the apoptosis, and suppressed the migration of ASM cells treated with TGF-β1, whilst overexpression of SIRT7 had the opposite effect. Notably, SIRT7 knockdown inhibited the protein expression of TβRI, whilst SIRT7 overexpression promoted the expression of TβRI. Importantly, TβRI knockdown partially reversed the effect of SIRT7 overexpression on TGF-β1-induced ASM cells. Taken together, these results demonstrate that SIRT7 is involved in regulating TGF-β1-induced ASM cell proliferation and migration by regulating the expression of TβRI, thus indicating an important role for SIRT7 in asthma airway remodeling. This suggests that SIRT7 may serve as a potential therapeutic target for the treatment of asthma.

2. Materials and methods

2.1. Cell culture

Primary ASM cells were isolated from the tracheae of C57BL/6 mice (Experimental Animal Center of Xi'an Jiaotong University, Xi'an, China) according to a previously reported method [30]. In brief, mice were euthanized and the tracheae were excised and washed. Afterward, the cells were enzymatically dissociated using 0.2% collagenase type IV and 0.05% elastase (Sigma, St. Louis, MO, USA) at 37 °C for 30 min. After centrifugation at 500 × g for 6 min, cell pellets were resuspended in Dulbecco's Modified Eagle's Medium–Ham's F-12 Medium (1:1) (Gibco, Rockville, MD, USA) supplemented with 0.2% NaHCO3, 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin mix. Cells were cultured in humidified air containing 5% CO_2 at 37 °C. The culture media was refreshed every 3 days, and cells were used for subsequent experiments when they reached passages 2–4. Animal experiments were approved by the Institutional Animal Care and Use Committee, The Second Affiliated Hospital, Xi'an Jiaotong University.

2.2. RNA extraction and real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted using Trizol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) in accordance with the manufacturer's protocol. Total RNA was reverse transcribed using M-MLV Reverse Transcriptase (Takara, Dalian, China), and real-time PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) on an ABI 7500 Real-Time PCR System. GAPDH was used as the internal control for the normalization of gene expression. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

2.3. Western blot analysis

Proteins were extracted from cultured cells using RIPA lysis buffer. Proteins were subsequently separated by electrophoresis using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis before being transferred to a PVDF membrane. Membranes were incubated with 5% skim milk for 1 h at 37 °C before they were incubated with primary antibodies against SIRT7, TβRI, TGF-β1, and GAPDH (Abcam, Cambridge, MA, USA) at 4 °C overnight. The next day, membranes were probed using horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Targeted protein signals were detected using an enhanced chemiluminescence kit (Pierce, Rockford, IL, USA), and band intensities were measured using Image-Pro Plus 6.0 software.

2.4. Cell transfection

The siRNAs targeting SIRT7 and TβRI and negative control siRNAs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and transfected into cells as per the manufacturer's instructions. The full-length cDNA of SIRT7 was subcloned into the pcDNA3.1 vector to generate the pcDNA3.1-SIRT7 expression vector. The empty pcDNA3.1 vector was used as a control. Vectors were transfected into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.5. Cell proliferation assay

Cell proliferation was detected using the Cell Counting Kit-8 assay (CCK-8; Beyotime Biotechnology, Haimen, China) according to the manufacturer's instructions. Briefly, cells were seeded into 96-well plates and cultured overnight. After the indicated treatment, cells were treated with a CCK-8 solution at 10 μL/well and cultured for 2 h at 37 °C. The optical density (OD) values were subsequently measured at 450 nm using a microplate reader (Bio-Rad, Sunnyvale, CA, USA).

2.6. Cell apoptosis assay

Cell apoptosis was determined using a commercial kit to measure caspase-3 activity (Beyotime Biotechnology). Briefly, cells were lysed using lysis buffer after the indicated treatments. Approximately 50 μL of supernatant, 40 μL of reaction buffer solution, and 10 μL of Ac-DEVD-pNA (2 mM) were mixed together and incubated for 2 h at 37 °C. The OD values were measured at 405 nm.

2.7. Cell migration assay

Cell migration was detected using a transwell chamber and 24-well plates. Briefly, cells were transfected with SIRT7 siRNA or the pcDNA3.1-SIRT7 vector for 48 h before being resuspended in 200 μL of serum-free media in the upper chamber. Meanwhile, 500 μL of media supplemented with 10% FBS was added to the lower chamber. Cells were cultured for 24 h at 37 °C. Residual cells on the upper surface of the filter were discarded using cotton swabs, whilst migrated cells on the lower surface of the filter were fixed with 4% formaldehyde and stained with 0.1% crystal violet. The number of stained cells was counted under an optical microscope.

2.8. Data analysis

Quantitative data are expressed as mean ± standard deviation (SD). SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. Differences were compared using the Student's *t*-test or one-way analysis of variance. Differences were regarded as statistically significant at $P < 0.05$.

3. Results

3.1. SIRT7 expression is increased in ASM cells following TGF-β1 treatment

To investigate the potential role of SIRT7 in asthma, the expression