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Effects of SIRT1/Akt pathway on chronic inflammatory response and lung function in patients with asthma

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Abstract. – OBJECTIVE: Asthma is the most common chronic airway inflammatory disease. Sirtuin 1 (SIRT1) exerts a crucial effect on regulating chronic inflammatory responses. Therefore, this study aims to explore the effect of SIRT1 on the pathogenesis of asthma.

MATERIALS AND METHODS: Serum level of SIRT1 in asthma patients and healthy controls was detected by Western blot. Correlation between SIRT1 level and pulmonary function in asthma patients was analyzed. Subsequently, asthma model in mouse was established. Primary airway epithelial cells were extracted from asthma mice and control mice to detect SIRT1 level. Furthermore, relative levels of Akt and interleukin 6 (IL-6) were detected in 16HBE cells. Regulatory effects of Akt on SIRT1 in 16HBE cells were determined as well.

RESULTS: SIRT1 was highly expressed in serum of asthma patients, which was negatively correlated with FEV1/FVC (r=-0.27, **p<0.01). Both mRNA and protein levels of SIRT1 were downregulated in primary airway epithelial cells extracted from asthma mice compared with those from controls. SIRT1 knockdown in 16HBE cells upregulated IL-6 expression, which was reversed by Akt inhibitors.

CONCLUSIONS: SIRT1 regulates IL-6 level via Akt pathway, thereafter affecting pulmonary function in asthma patients.

Key Words:
Asthma, SIRT1, Akt pathway.

Introduction

Asthma is the most common airway chronic inflammatory disease, with about 235 million asthma patients globally. In China[1] more than 20 million people are affected by asthma, with the incidence rate of 1.24%. Injury and abscission of airway epithelial cells are the major pathological features of asthma, but its specific occurrence and development mechanisms remain unclear. Studies have shown that the proportion of eosinophils in the peripheral blood and airway mucosa of asthma patients is relatively high. The majority of cytokines are abundantly produced, such as interleukin 6 (IL-6), IL-2 and IL-17[2]. As antigen-presenting cells, dendritic cells are capable of initiating immune responses and modulating the ratio of TH1/Th2 cells and Th17 cells, and they are also closely related to the pathogenesis of asthma[3]. Sirtuin 1 (SIRT1) is a member of the human sirtuins family and it is a silent information regulator[4-6]. As a NAD^+-dependent protein deacetylase, SIRT1 is mainly involved in cell growth, gene repair, gene transcription and cell senescence. SIRT1 is closely related to many diseases, including cardiovascular disease, osteoarthropathy, diabetes mellitus, lung cancer, gastric cancer, rheumatoid arthritis, asthma and COPD (chronic obstructive pulmonary disease)[7]. Recent studies[8] have found that SIRT1 can be applied as an anti-inflammatory factor. SIRT1 expression is downregulated in lung tissues of asthma patients. On the contrary, serum level of SIRT1 is upregulated in asthma patients, which is negatively correlated to pulmonary function. It is suggested that elevated serum level of SIRT1 may be used as a diagnostic marker for asthma, whereas decreased level of SIRT1 in lung tissues could be used as a therapeutic basis for asthma[9]. It has been found in other studies that SIRT1 expression in the lung tissue of OVA-induced asthma mouse is inhibited, thus upregulating levels of IL-6, IL-4 and other cytokines[9-11]. Highly expressed IL-6 can serve as an indicator for worse FEV1 (forced expiratory volume in one second)

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and FVC (forced vital capacity)[12,13]. At present, the specific mechanism of SIRT1 in the pathogenesis of asthma is rarely reported. The role of immune factors in the pathogenesis of asthma has gradually been well recognized. Studies[14-17] have shown that IL-6, IL-10, and tumor necrosis factor-α (TNF-α) are greatly involved in asthma development. IL-6 is a glycoprotein produced by airway macrophages and bronchial epithelial cells, whose level has been identified to be closely related to symptoms and pulmonary function of asthma patients[17]. IL-6 is highly expressed in acute phase of asthma compared with that in the remission phase. Serum level of IL-6 declines with infection control. Hence, IL-6 is regarded as an indicator to distinguish between acute and remission phase of bronchial asthma.

Materials and Methods

Experimental Animal

Male BALA/c mice in SPF (specific-pathogen-free) level weighing 18-20 g were selected in the experiment. The experimental procedures were in line with International Animal Welfare Standards. Asthma mouse model was established by ovalbumin (OVA) induction. Mice in control group received intraperitoneal injection of isodose normal saline. This study was approved by the Animal Ethics Committee of The Second People's Hospital of Liaocheng Animal Center.

Cell Culture

Human bronchial epithelial cell line 16HBE was cultured in RPMI-1640 (Roswell Park Memorial Institute-1640, Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS (fetal bovine serum) (Gibco, Rockville, MD, USA). 16HBE cells were maintained in a 5% CO_2 incubator at 37°C. Primary airway epithelial cells were extracted from asthma mice and control mice, and cultured in the same condition of 16HBE cells.

Small Interfering RNA

Corresponding small interfering RNAs (siRNA) were constructed by GenePharma (Shanghai, China). The sequences of siRNAs were as follows: Akt siRNA: 5'-GACGGGCACATTAAGATCA-3', siRNA NC: 5'-TTCTCCGAACGTGTCATGT-3'; SIRT1 siRNA: 5'-TCGAACAATTCTTAAAGAT-3'; siRNA NC: 5'-TTCTCCGAACGTGTCACGT-3'.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA in cells and tissues was extracted using TRIzol method (Invitrogen, Carlsbad, CA, USA) according to the instructions of PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). RNA concentration was detected using spectrometer, followed by qRT-PCR based on the instructions of SYBR Premix Ex Taq™ (TaKaRa, Otsu, Shiga, Japan). The relative gene expression was calculated using $2^{-\Delta Ct}$ method. Primer sequences used in this study were as follows: SIRT1, F: 5'-CTAGGACTTCTCCATACTC-3', R: 5'-CTGATAGGGTGTACGAAGGA-3'; IL-6, F: 5'-GAACTGTAAGCATCTTCGACTG-3', R: 5'-AGTGTTGCGTGCTGAGTG-3'; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Western Blot

Cells and tissues were lysed by radioimmunoprecipitation assay (RIPA) (Beyotime, Shanghai, China) to extract the total protein, followed by gel electrophoresis. Protein samples were separated and incubated with primary antibody (Cell Signaling Technology, Danvers, MA, USA) overnight at 4°C. Subsequently, membranes were incubated with corresponding secondary antibodies. Finally, an image of the protein band was captured by the Tanon detection system using enhanced chemiluminescence (ECL) reagent (Thermo Fisher, Waltham, MA, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 statistical software (IBM, Armonk, NY, USA) was used for data analysis and GraphPad Prism5.0 (La Jolla, CA, USA) was introduced for figure editing. Data were expressed as mean ± standard deviation ($\bar{x}\pm s$). Measurement data and classification data were compared using the *t*-test and chi-square test, respectively. $p<0.05$ considered the difference was statistically significant.

Results

Serum Level of SIRT1 was Upregulated in Asthma Patients and Negatively Correlated With Pulmonary Function

Serum level of SIRT1 in asthma patients was detected by Western blotting. The results man-

ifested that the serum level of SIRT1 in asthma mice was higher than that of control mice (Figure 1A and 1B). Correlation analyses showed that SIRT1 expression was negatively correlated with FEV1/FVC (r=-0.27, $^{**}p<0.01$, Figure 1C). The above results indicated that serum level of SIRT1 was negatively related to the severity of asthma.

SIRT1 was Inhibited in Airway Epithelial Cells of Asthma Mouse

Primary airway epithelial cells were extracted from asthma mice and control mice. Both mRNA and protein levels of SIRT1 expression were downregulated in primary airway epithelial cells of asthma mice compared with those of control mice (Figure 2A and 2B). To verify the regulatory effect of SIRT1 on IL-6 level, siRNA SIRT1 and negative control were first constructed. It was found that transfection of siRNA SIRT1 in 16HBE cells remarkably upregulated IL-6 level (Figure 2C and 2D).

IL-6 was Regulated by Akt Pathway

To explore whether IL-6 level could be regulated by Akt pathway, 16HBE cells were treated with Akt activator SC-79. Both mRNA and protein levels of IL-6 were elevated after SC-79 treatment in 16HBE cells (Figure 3A and 3B). On the contrary, Akt knockdown inhibited IL-6 expression at mRNA and protein levels (Figure 3C and 3D).

SIRT1 Regulated IL-6 Expression via Akt Pathway

To verify the regulatory effect of SIRT1 on IL-6 and Akt pathway, 16HBE cells were co-transfected with Akt siRNA and SIRT1 siRNA. The upregulated IL-6 level induced by SIRT1 knockdown was greatly reversed by Akt inhibition (Figure 4A and 4B). It was concluded that SIRT1 regulated IL-6 expression in 16HBE cells *via* Akt pathway.

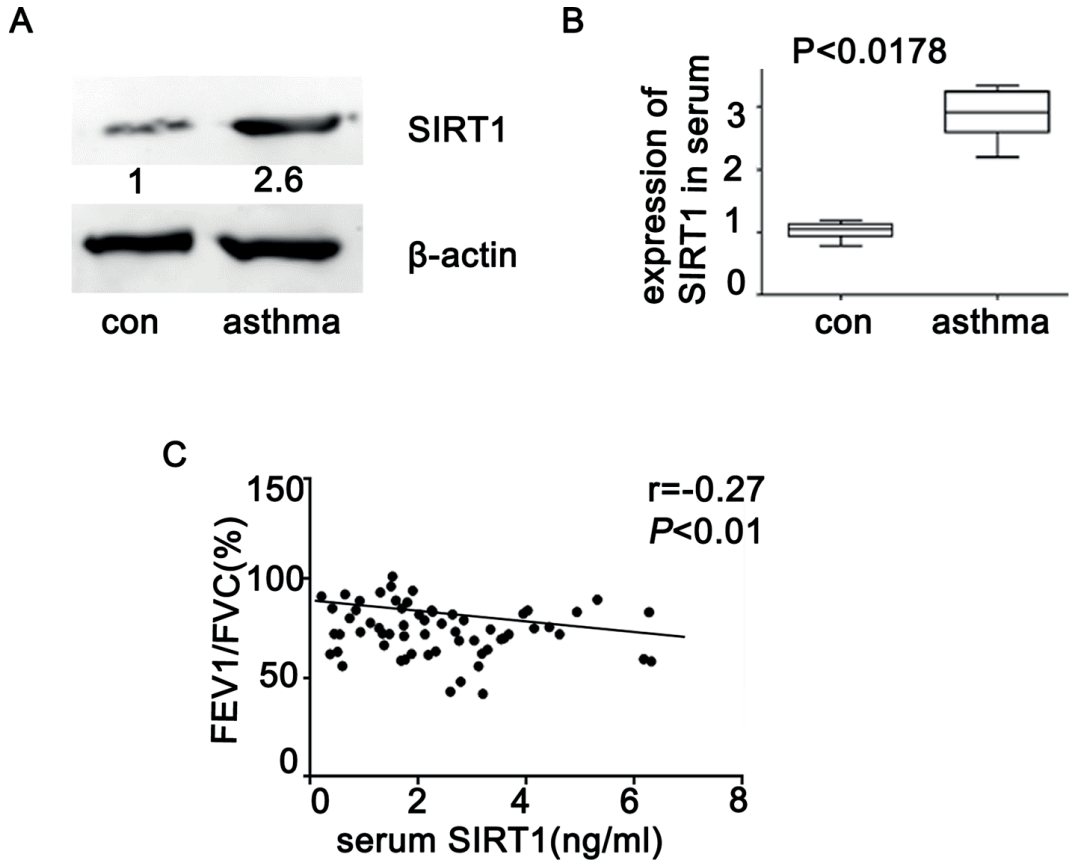

Figure 1. Serum level of SIRT1 was upregulated in asthma patients and negatively correlated to pulmonary function. ***A,*** SIRT1 expression in serum sample of asthma patients. ***B,*** SIRT1 expression in peripheral blood sample of asthma patients. ***C,*** SIRT1 expression was negatively correlated to FEV1/FVC (r=-0.27, $^{**}p<0.01$).

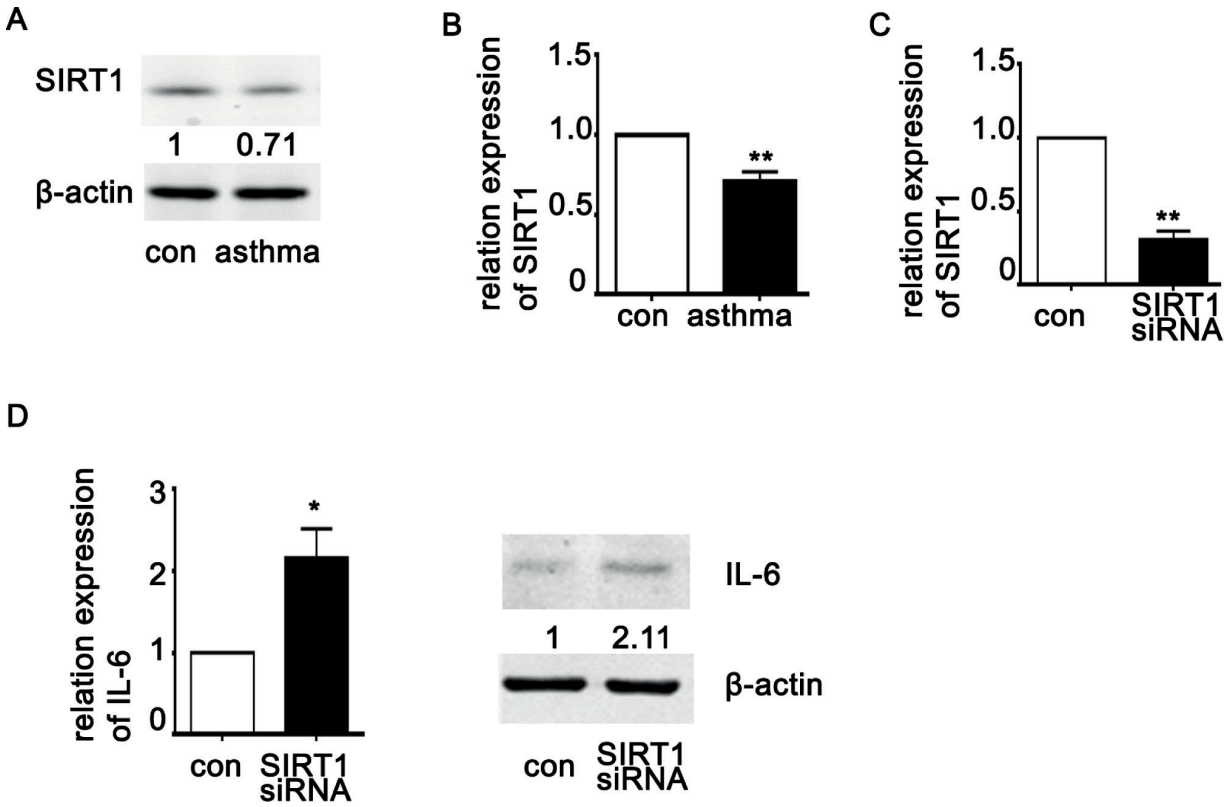

Figure 2. SIRT1 was inhibited in airway epithelial cells of asthma mouse. ***A-B,*** Both mRNA and protein levels of SIRT1 expression were downregulated in primary airway epithelial cells of asthma mice compared with those of control mice. ***C-D,*** SIRT1 knockdown in 16HBE cells remarkably upregulated IL-6 level.

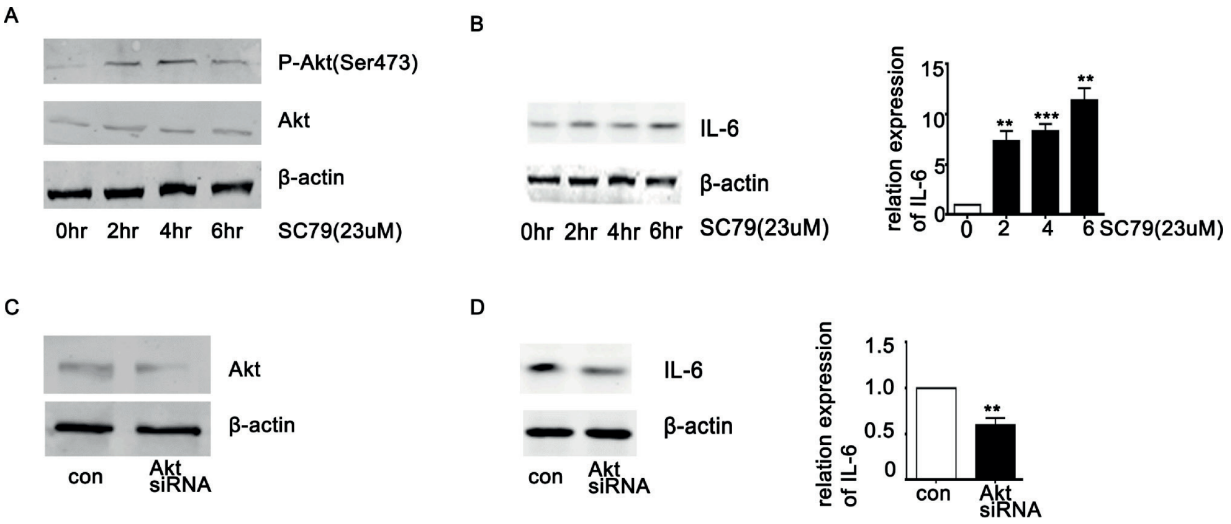

Figure 3. IL-6 was regulated by Akt pathway. ***A-B,*** Both mRNA and protein levels of IL-6 were elevated after SC-79 treatment in 16HBE cells. ***C-D,*** Akt knockdown inhibited IL-6 expression at mRNA and protein levels.

Discussion

Asthma-induced chronic airway inflammation is regulated by various cytokines. SIRT1, as an intracellular regulator of inflammation, is rarely studied in asthma development. Studies have shown that SIRT1 can inhibit inflammatory responses by regulating NF-κB and P38 MAPK pathways[16]. SIRT1 serves as an intracellular information molecule to participate in the development of various diseases. It has been found that SIRT1 is closely related to chronic airway inflammatory diseases and has an inhibitory effect on chronic inflammation[18-22]. This study verified that knockdown of SIRT1 upregulated IL-6 expression in 16HBE cells. We speculated that SIRT1 may exert a specific role in asthma-induced chronic inflammation.

IL-6 is a multifunctional cytokine produced by airway macrophages and bronchial epithelial cells. IL-6 expression is regulated by the transcription factor NF-kB and Akt pathway[23,24]. Studies have shown that expression levels of inflammatory cytokines are closely related to the symptoms and pulmonary function of asthma mice[15]. IL-6 is highly expressed in the acute phase of asthma compared with that in the remission phase, indicating that IL-6 level is related to susceptibility of lung infections. Since serum level of IL-6 decreases with infection control, it can be used as an indicator to distinguish between acute and remission of bronchial asthma[15,17]. IL-10 is also a major immunosuppressive cytokine, which is largely produced by Th2 cells, macrophages, and $CD8^+$ T cells[17]. IL-10 inhibits asthma development by directly inhibiting airway inflammation through multiple regulations[15-17]. IL-6 and IL-10 may be sensitive indicators for assessing the disease condition of bronchial asthma. In this study, Akt inhibitor was found to inhibit SIRT1-induced IL-6 expression in airway epithelial cells. Although advance progress has been made in the treatment of asthma, its etiology and pathogenesis have not been fully elucidated. Current treatment methods can only relieve and stabilize asthma symptoms. The pathogenesis of asthma may involve immunity, nerve system, endocrine, genetic genes, etc. Among them, the immune mechanism is mostly studied in regulating asthma development. It is well known that $CD4^+$Th2 immune response and relative cytokines are involved in asthma. Therefore, Th2 inhibitor is applied as a potential therapeutic target for asthma[3]. This study demonstrated that serum level of SIRT1 was remarkably elevated in asthma patients. SIRT1 expression was negatively correlated with FEV1 and disease severity, but positively associated with IL-17 level and neutrophil count in asthma patients[25], indicating that SIRT1 may be involved in the regulation of neutrophils asthma[26,27].

Conclusions

SIRT1 is highly expressed in serum of asthma patients but lowly expressed in airway epithelial cells. SIRT1 regulates IL-6 level *via* Akt pathway, so as to affect pulmonary function in asthma patients.

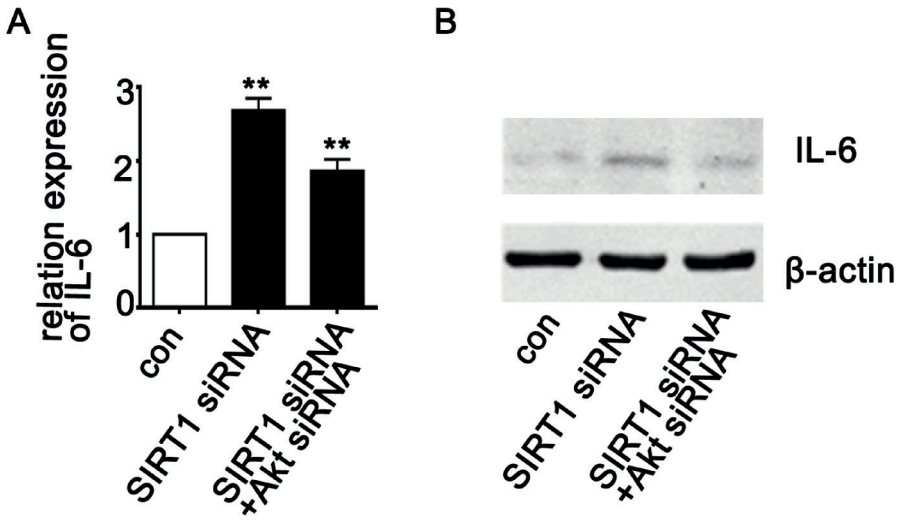

Figure 4. SIRT1 regulated IL-6 expression *via* Akt pathway. ***A-B,*** The upregulated IL-6 level induced by SIRT1 knockdown was greatly reversed by Akt inhibition.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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