Let-7a inhibits proliferation and promotes apoptosis of human asthmatic airway smooth muscle cells

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Received June 30, 2018; Accepted December 13, 2018

DOI: 10.3892/etm.2019.7363

Abstract. The present study aimed to examine the changes of let-7a expression in asthmatic airway smooth muscle cells (ASMCs) and to analyze its effect on the proliferation and apoptosis of ASMCs, as well as the potential mechanism of action. Let-7a expression levels in ASMCs from asthmatic and non-asthmatic subjects were detected using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Furthermore, let-7a mimics were transfected *in vitro* into ASMCs isolated from asthmatic patients, and the effect of let-7a on ASMC proliferation was examined using a Cell Counting Kit-8. In addition, the influence of let-7a on ASMC apoptosis was detected using flow cytometry and a caspase-3/7 activity assay. Target genes of let-7a were predicted using bioinformatics software, and the direct regulatory effect of let-7a on the potential target gene signal transducer and activator of transcription 3 (STAT3) was verified through a dual-luciferase reporter gene assay combined with RT-qPCR and western blot analysis. The results demonstrated that let-7a expression was significantly lower in ASMCs of asthmatic subjects compared with that in ASMCs of normal subjects. Furthermore, upregulation of let-7a expression in asthmatic ASMCs markedly inhibited cell proliferation and promoted cell apoptosis. The results of the dual-luciferase reporter gene assay indicated that let-7a selectively binds with the 3'-untranslated region of the STAT3 mRNA. In addition, let-7a mimics evidently reduced the mRNA and protein expression levels of STAT3 in asthmatic ASMCs. In conclusion, the present study demonstrates that let-7a expression is downregulated in ASMCs from asthmatic patients. Furthermore, let-7a suppresses the proliferation and promotes apoptosis of human asthmatic ASMCs, which may, at least partially, be associated with the downregulation of STAT3 expression.

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Key words: asthma, airway smooth muscle cells, airway remodeling, let-7a, signal transducer and activator of transcription 3

Introduction

Bronchial asthma is a chronic airway inflammatory disease characterized by airway hyperreactivity and reversible flow limitation (1). Epidemiological investigations indicate that ~0.3 billion patients currently suffer from asthma and that 25,000 asthma-associated deaths occur per annum worldwide. Furthermore, the morbidity and mortality of asthma exhibit increasing trends, rendering asthma a major global public health problem (2). Chronic airway inflammation-induced airway remodeling is an important step in the pathogenesis of asthma (3). Airway remodeling mainly involves chronic inflammation-induced massive proliferation of airway smooth muscle cells (ASMCs) and extracellular matrix (ECM) deposition. The proliferation and hypertrophy of ASMCs have a particularly important role in airway remodeling, and are considered as important features thereof (4). In addition, the extent of ASMC proliferation is positively correlated with the severity of asthma (5).

Various factors, including T-helper cell type 1/2 cytokine imbalances, cytokines, matrix metallopeptidase and tissue inhibitor of metallopeptidases, genetic factors, neuromodulation and gene mutation, have important roles in the occurrence and development of asthma (6,7). MicroRNAs (miRNAs/miRs) are a class of highly evolutionarily conserved, small-molecular, non-coding RNAs with a length of 21-23 nt. They interact with the mRNAs of their target genes to reduce their stability and/or inhibit translation, thus negatively regulating target gene expression. miRNAs are involved in a series of important pathophysiological processes, including cell proliferation, differentiation and apoptosis, as well as immune response and tumor formation (8,9). It is estimated that miRNAs, which account for 1-3% of human genes, regulate the expression of >30% of human genes (10). Recently, the role of miRNAs in the genesis and development of asthma has attracted increasing attention (11,12). Furthermore, abnormal miRNA expression may be involved in asthmatic airway remodeling through regulating the proliferation and apoptosis of ASMCs (13-15). Let-7a, a member of the let-7 family, is one of the first identified miRNAs and one of the most abundant miRNAs in lung tissue (16). Let-7a regulates interleukin (IL)-13 secretion, while the latter has a vital role in the asthmatic inflammatory response and airway remodeling (17). Previous studies have indicated that let-7a expression is downregulated in bronchial epithelial

cells and transbronchial lung biopsy tissues obtained during bronchoscopy from asthmatic patients (18,19). Furthermore, silencing of let-7a was demonstrated to significantly alleviate airway inflammation and airway hyperreactivity in asthmatic mice (20,21), suggesting that let-7a is associated with asthma. However, let-7a expression in bronchial SMCs from asthmatic patients as well as its regulatory actions have remained to be fully elucidated. In the present study, changes in let-7a expression levels in ASMCs from asthmatic vs. healthy patients were detected using reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and the effect of let-7a on the proliferation and apoptosis of ASMCs was assessed; furthermore, potential target genes of let-7a were identified.

Materials and methods

Collection of patient samples and primary culture of human ASMCs. ASMCs from asthmatic and non-asthmatic subjects were derived from segmental bronchial biopsy specimens obtained through fiberoptic bronchoscopy at Shengli Oil Field Central Hospital (Dongying, China), including 15 asthmatic and 10 non-asthmatic subjects. None of the subjects had a history of smoking or of respiratory tract infection within the last 3 months. Asthmatic subjects conformed to the diagnostic criteria in the Guidelines for Prevention and Control of Bronchial Asthma in China (22). All subjects had provided written informed consent to participate in the study, and the present study was approved by the Ethics Committee of Shengli Oil Field Central Hospital (Dongying, China). Epithelium, connective tissue, blood vessels and cartilage were removed from the obtained bronchial biopsy specimens (5x5 mm), followed by washing with the ice-cold PBS containing penicillin-streptomycin 3 times. The specimens were then placed in 5 ml tissue culture flasks. Fetal bovine serum (FBS; 1 ml; 10%; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was added, and the smooth muscle tissue block was cut into small pieces and repeatedly minced using iris scissors. The mixture was evenly applied onto the bottom surface of the culture flask with a sterile drinking straw. The culture flask was then inversely placed in a 5% CO_2 incubator at 37˚C. After 2 h, when adherence of the cells was achieved, the flask was turned over and Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS was added from one side. The medium was replaced once every 3-4 days, and after 14 days of culture, hill and valley cell fusion was observed. Following detachment with 0.125% trypsin, the cells were passaged at a ratio of 1:2. Cells at passages 4-8 were used in the experiments. The ASMCs were identified by the typical 'hill and valley' growth pattern and immunocytochemical staining for α-smooth muscle actin (23).

RT-qPCR. Total RNA of was extracted from the cells using TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.), and the purity and content of the extracted RNA were determined using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.) and RNA samples with a A260/A280 ratio between 1.8 and 2.0 were used to synthesize cDNA. Total RNA was reverse transcribed into cDNA using the High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. This reaction was performed at 25˚C for 5 min, 50˚C for 20 min then 75˚C for 5 min. qPCR was performed with SYBR® Premix Ex Taq™ (Takara Bio Inc., Otsu, Japan; cat. no. DRR041A) according to the manufacturer's instructions, using an ABI 7500 real-time fluorescence qPCR machine (Applied Biosystems; Thermo Fisher Scientific, Inc.). U6 and β-actin were used as the internal reference genes for let-7a and signal transducer and activator of transcription (STAT3) detection, respectively. The reaction conditions for PCR were as follows: Initial denaturation at 95˚C for 3 min and 40 cycles of 95˚C for 15 sec and 60˚C for 30 sec. The primers used for the detection of let-7a and STAT3 were as follows: Let-7a forward, 5'-GCGCCTGAGGTAGTAGGTTG-3' and reverse, 5'-CAGTGCAGGGTCCGAGGT-3'; STAT3 forward, 5'-TGCTGGAGGAGAGAATCGT-3' and reverse, 5'-TAGTAGTGAACTGGACGCCG-3'; U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'; β-actin forward, 5'-GGTCATCACCATTGGCAA-3' and reverse, 5'-GAGTTGAAGGTAGTTTCGTGGA-3'. The primers were designed and synthesized by Shanghai Sangon Bioengineering Co., Ltd (Shanghai, China). The relative mRNA expression levels of let-7a and STAT3 were calculated using the $2^{-\Delta\Delta Cq}$ method (24).

Cell transfection. ASMCs from asthmatic subjects in the logarithmic growth phase were inoculated into 6-well plates and divided into a let-7a mimics group and a negative control (NC)-mimics group. Let-7a mimics (5'-UGAGGUAGUAGGUUGUAUAGUU-3'; GenePharma, Shanghai, China) and NC-mimics (5'-UUCUCCGAACGUGUCACGUTT-3'; GenePharma) were transfected into the ASMCs of the corresponding groups using Lipofectamine™ 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) in strict accordance with the manufacturer's protocols. The transfection medium was replaced with normal medium at 6 h after transfection. The transfection efficiency was detected using RT-qPCR.

Cell Counting Kit (CCK)-8 assay. After 24 h of transfection, the cells were collected and seeded into the 96-well plates at a density of 3,000 cells/well. CCK-8 stain (10 μl; Dojindo Molecular Technologies, Inc., Kumamoto, Japan) reaction liquid was added to designated wells every 24 h for 2 h of incubation at 37˚C, and the optical density (OD) value of each well was detected at the wavelength of 450 nm by ELX800 Universal Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA).

Flow cytometry. After 48 h of transfection, the cells were collected and washed with PBS twice, digested with EDTA-free trypsin and collected after centrifugation (111.8 x g, 5 min , 4˚C) to prepare a single-cell suspension ($1x10^6$ cells/ml) for each group individually. Of this cell suspension, 5 ml was filled into a flow tube, followed by addition of 5 μl Annexin V-fluorescein isothiocyanate (BD Biosciences) and 10 μl propidium iodide (BD Biosciences) in succession. The mixtures were incubated for 15 min in the dark, followed by addition of 300 μl binding buffer. The mixtures were then immediately subjected to flow cytometric evaluation (BD FACSCanto II; BD Biosciences, Franklin Lakes, NJ, USA) to determine the apoptotic rate.

Caspase-3/7 activity assay. After 48 h of transfection, the cells were harvested and seeded onto the 96-well plates. Substrates and buffer solution were defrosted at room temperature in strict accordance with the manufacturer's protocol for the Apo-ONE Homogeneous Caspase-3/7 Assay (Promega Corporation, Madison, WI, USA; cat. no. G7790). A total of 100 μl substrate was mixed with 9,900 μl buffer solution to prepare the Apo-ONE Caspase-3/7 reagent. Subsequently, 100 μl of this reaction reagent was added to each well, followed by agitation using a shaker for 30 sec and incubation for 2 h at room temperature in the dark. The fluorescence intensity of each well was then detected using a Tecan Infinite M200 pro plate reader (Tecan Trading AG, Maennedorf, Switzerland), from which the caspase-3/7 activity was determined.

Dual-luciferase reporter gene assay. Target genes of let-7a were predicted using the TargetScan miRNA target gene prediction bioinformatics website (http://www.targetscan.org). The bioinformatics prediction suggested that STAT3 may be a potential target gene for let-7a. The wild-type (WT) and mutant (MUT) reporter gene plasmids were synthesized by GeneCopoeia Co., Ltd. (Guangzhou, China). PCR amplification products of the 3'-untranslated region (3'-UTR) of STAT3 were connected to the *Xba*I enzyme digestion site of the pGL3 plasmid, so as to construct the wild-type pGL3-WT-STAT3 reporter gene plasmid (5'-UGACCUCGGAGUGCGCUA CCUCC-3'). Site-specific mutagenesis was performed on the binding site in the 3'UTR of STAT3 that is complementary to a sequence in let-7a, and the new plasmid obtained after mutagenesis was the pGL3-MUT-STAT3 reporter gene plasmid (5'-UGACCUCGGAGUGCGCAAGCACC-3'). 293 cells (ScienCell Research Laboratories, Inc., San Diego, CA, USA) were divided into 4 groups, namely the STAT3 WT plasmid control group (transfection with pGL3-WT-STAT3 reporter gene plasmid and NC-mimics), STAT3 WT plasmid experimental group (transfection with pGL3-WT-STAT3 reporter gene plasmid and let-7a mimics), STAT3 MUT plasmid control group (transfection with pGL3-MUT-STAT3 reporter gene plasmid and NC-mimics) and STAT3 MUT plasmid experimental group (transfection with pGL3-MUT-STAT3 reporter gene plasmid and let-7a mimics). Cells in each group were split after 24 h of transfection in accordance with the manufacturer's protocol for the dual-luciferase reporter gene system kit (Promega Corp.), and activities of firefly luciferase and Renilla luciferase in each group were detected using a GloMax 20/20 luminometer (Promega Corp.). Renilla luciferase gene was used as the internal reference to verify the transfection efficiency and calculate the relative luciferase activity as follows: Relative luciferase activity = firefly luciferase activity/Renilla luciferase activity.

Western blot analysis. Cells in each group were collected and washed with PBS for 3 times, followed by addition of radioimmunoprecipitation assay lysis buffer (Fermentas; Thermo Fisher Scientific, Inc.) and 1% protease inhibitor (cat. no. 78439; Pierce; Thermo Fisher Scientific, Inc.), and incubation for 20 min on ice. The mixtures were centrifuged (111.8 x g) at 4˚C for 15 min and the supernatant was collected to determine the protein concentration using the bicinchoninic acid method. A total of 20 μg protein per lane was separated by 10% SDS-PAGE, followed by transfer onto the polyvinylidene fluoride membranes (EMD Millipore, Billerica, MA, USA). Subsequent to blocking with 5% skimmed milk at room temperature for 6 h, the membranes were incubated with primary antibodies against STAT3 (cat. no. sc-293151; 1:1,000 dilution) and GAPDH (cat. no. sc-32233; 1:1,000 dilution; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) at 4˚C overnight. The membrane was washed prior to the addition of horseradish peroxidase-conjugated secondary antibody (cat. no. sc-2354; 1:2,000 dilution; Santa Cruz Biotechnology, Inc.) and incubation at 37˚C for 1 h. The membrane was washed and protein bands were visualized using the enhanced chemiluminescence reagent (Western Blotting Detection kit; Applygen Technologies, Inc., Beijing, China) according the manufacturer's protocol. Subsequently, QuantityOne software (version 4.6; Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used for densitometric analysis, and GAPDH was used as the internal reference to determine the relative expression of STAT3.

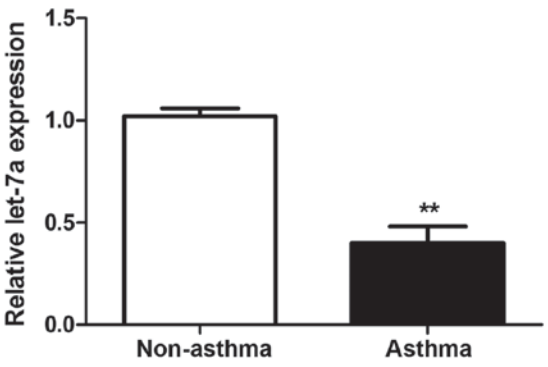

Figure 1. Let-7a expression levels in airway smooth muscle cells from asthmatic subjects were markedly downregulated compared with those from non-asthmatic subjects. **P<0.01 vs. non-asthma group.

Statistical analysis. Data were analyzed by SPSS 19.0 software (IBM Corp., Armonk, NY, USA). Values are expressed as the mean ± standard deviation and comparisons between groups were performed using the Student's t-test. All experiments were repeated at least 3 times. P<0.05 was considered to indicate a statistically significant difference.

Results

Let-7a is downregulated in asthmatic ASMCs. The relative expression levels of let-7a in ASMCs from asthmatic and non-asthmatic subjects were detected using RT-qPCR. As presented in Fig. 1, the relative expression levels of let-7a in ASMCs from asthmatic subjects were significantly decreased compared with those in the ASMCs from non-asthmatic subjects (P<0.01).

Let-7a mimics inhibit the proliferation and promote the apoptosis of asthmatic ASMCs. ASMC proliferation has a vital role in airway remodeling (4). In order to determine the potential effect of let-7a on the proliferation of asthmatic ASMCs, asthmatic ASMCs were transfection with let-7a mimics, and the successful upregulation of let-7a was confirmed by RT-qPCR (P<0.01; Fig. 2A). The results of the CCK-8 assay

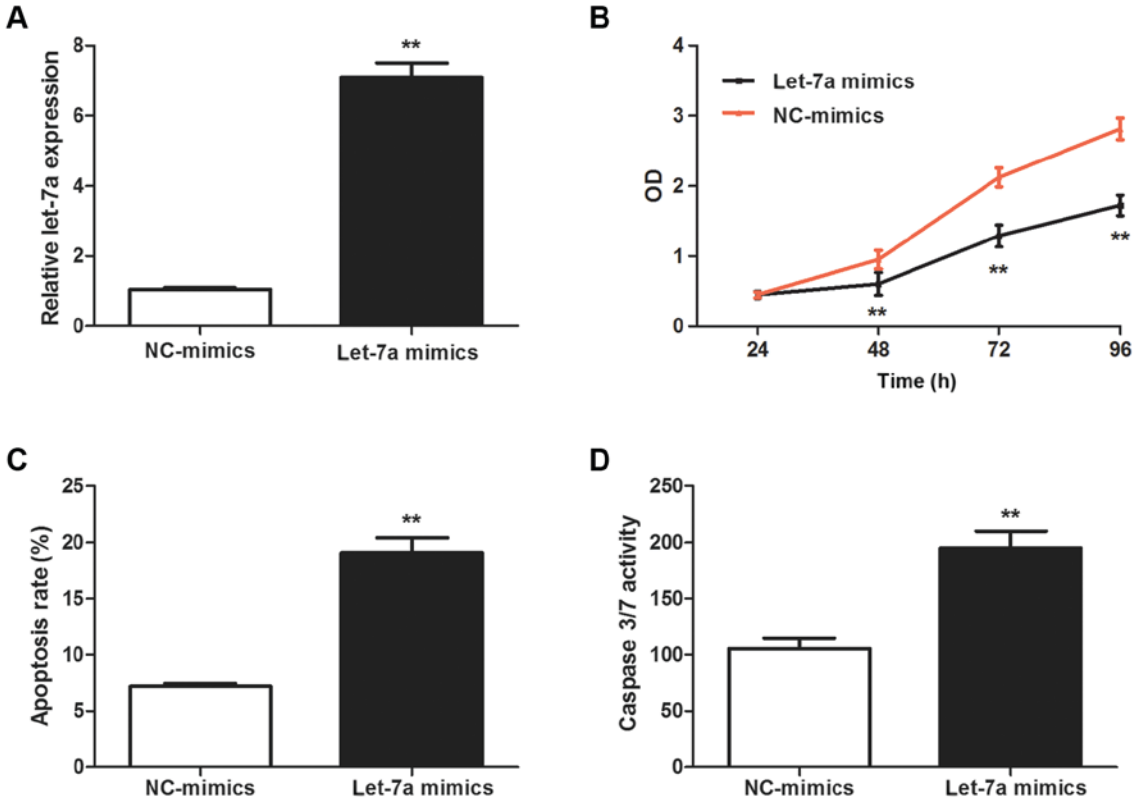

Figure 2. Upregulation of let-7a inhibits the proliferation and promotes apoptosis of asthmatic ASMCs. (A) Compared with the NC-mimics group, let-7a expression levels in the let-7a mimics group were markedly upregulated. (B) A Cell Counting Kit-8 assay indicated that let-7a mimics significantly suppressed the proliferation of asthmatic ASMCs. (C) Flow cytometric analysis indicated that let-7a mimics markedly promoted apoptosis of asthmatic ASMCs. (D) Caspase-3/7 activity analysis indicated that let-7a mimics notably promoted caspase-3/7 activity of asthmatic ASMCs. **P<0.01 vs. NC mimics. OD, optical density; ASMCs, airway smooth muscle cells; NC, negative control.

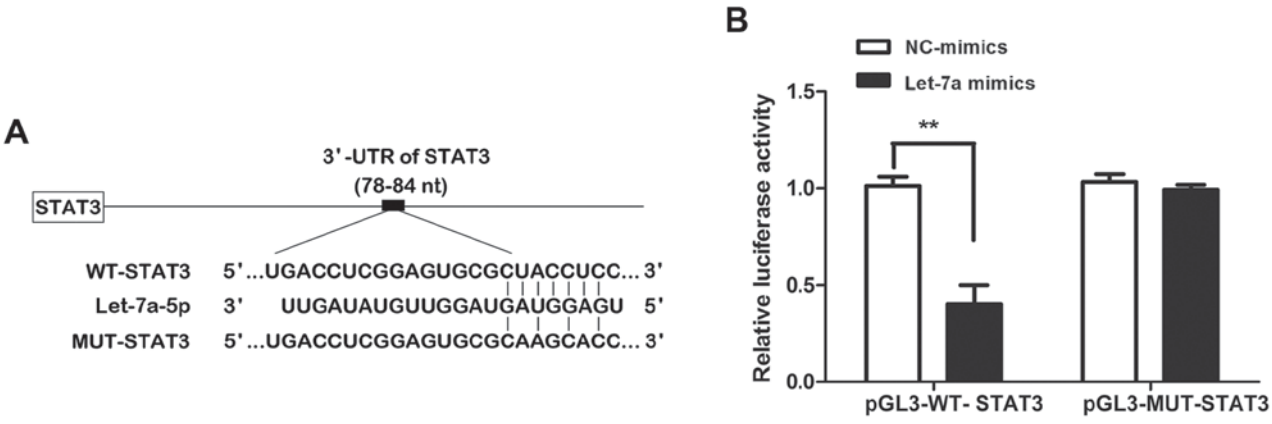

Figure 3. STAT3 was validated as a direct target gene of let-7a. (A) let-7a and STAT3 complementary binding sequences. (B) Let-7a mimics evidently suppressed the luciferase activity of wild-type pGL3-WT-STAT3, while NC mimics had no such effect. **P<0.01 vs. NC mimics. STAT3, signal transducer and activator of transcription; WT, wild-type; MUT, mutated; UTR, untranslated region.

indicated that let-7a mimics significantly suppressed the proliferation of asthmatic ASMCs (P<0.01; Fig. 2B). Flow cytometric analysis indicated that let-7a mimics significantly enhanced the apoptosis of asthmatic ASMCs (P<0.01; Fig. 2C). Furthermore, let-7a mimics significantly increased the activity of caspase-3/7 in asthmatic ASMCs (P<0.01; Fig. 2D).

STAT3 is the direct regulatory target gene of let-7a. To determine the precise molecular biological mechanisms by which let-7a regulates the proliferation and apoptosis of asthmatic ASMCs, potential target genes of let-7a were analyzed and predicted using TargetScan. The analysis suggested that STAT3 is a potential target gene of let-7a due to the presence of theoretical complementary binding sites in the 3'-UTR of STAT3 mRNA with the seed sequence of let-7a (Fig. 3A). To further verify whether STAT3 is a direct target gene of let-7a, a dual-luciferase reporter gene assay was performed to confirm the effect of let-7a on the luciferase activity of the 3'-UTR of

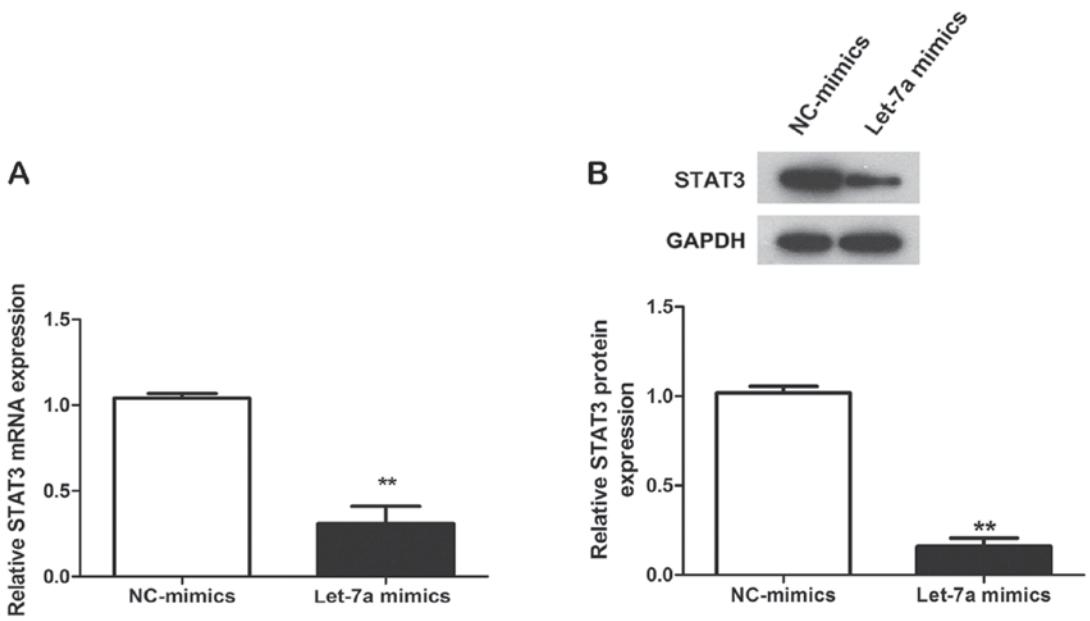

Figure 4. Let-7a mimics inhibited the expression of STAT3 mRNA and protein in asthmatic ASMCs. (A) Reverse transcription-quantitative polymerase chain reaction analysis demonstrated that let-7a mimics significantly reduced the expression levels of STAT3 mRNA in asthmatic ASMCs. (B) Western blot analysis demonstrated that upregulation of let-7a apparently reduced the expression levels of STAT3 protein in asthmatic ASMCs. **P<0.01 vs. NC mimics. STAT3, signal transducer and activator of transcription; ASMCs, airway smooth muscle cells.

STAT3 mRNA. As presented in Fig. 3B, let-7a mimics evidently suppressed the luciferase activity of the pGL3-WT-STAT3 reporter gene vector, while they had no obvious inhibitory effect on the luciferase activity of the pGL3-MUT-STAT3 reporter gene vector (P<0.01). This revealed that let-7a specifically binds with the 3'-UTR of the STAT3 mRNA, and that STAT3 is a direct target gene of let-7a.

Let-7a mimics inhibit the mRNA and protein expression of STAT3 in asthmatic ASMCs. Finally, the mRNA and protein expression of STAT3 in asthmatic ASMCs transfected with let-7a mimics or NC-mimics was detected by RT-qPCR and western blot analysis, respectively. The expression levels of STAT3 mRNA and protein in the let-7a mimics group were significantly lower than those in the NC-mics group (P<0.01; Fig. 4A and B), suggesting that upregulation of let-7a significantly inhibits the expression of STAT3 mRNA and protein in asthmatic ASMCs, further confirming that STAT3 is a target regulated by let-7a.

Discussion

Asthma is a common respiratory tract disease, which is characterized by airway remodeling and airway hyperreactivity. Airway remodeling refers to a series of structural changes in the airway wall under the persistent stimulation of mitogens, which are featured by epithelial damage, gland hyperplasia and hypertrophy, ASMC proliferation, ECM deposition and basilar membrane thickening. Of these, ASMC proliferation is the pathological foundation leading to structural changes in airway tissues (25), which not only aggravate airway stenosis but also increase airway hyperreactivity, thus having a crucial role in the genesis and development of airway remodeling (26). As a result, the prevention of ASMC proliferation to control the genesis of asthmatic airway remodeling has become a hotspot of current asthma research. In the last decade, an increasing number of studies have focused on ASMCs in asthma and have proposed to target them as a therapeutic strategy (27,28).

Certain miRNAs have been verified to participate in the regulation of various biological processes, including cell proliferation, differentiation, apoptosis and tissue development through regulating target gene expression, and to have a key role in the genesis and development of multiple diseases (29,30). Existing data regarding miRNAs and bronchial asthma suggest that miRNAs are involved in almost all pathophysiological processes of bronchial asthma, including allergic inflammation in the airways dominated by eosinophil infiltration, ASMC hyperreactivity and airway remodeling (11). In addition, patients with different severities of asthma may exhibit differences in their miRNA expression profile (31). Furthermore, multiple physiological functions of ASMCs, including contraction, proliferation and apoptosis, are also regulated by miRNAs. Hu *et al* (32) reported that overexpression of miR-10a markedly reduced the proliferation of human ASMCs, while inhibition of miR-10a promoted their proliferation. The underlying mechanisms included the specific inhibition of the phosphatidylinositide-3 kinase catalytic subunit α by miR-10a, which blocked the AKT signaling pathway, as well as cyclins and cyclin-dependent kinases. Furthermore, another study identified that ras homolog family member A (RhoA) expression in ASMCs is negatively regulated by miR-133a, while IL-13 caused an upregulation of RhoA through reducing the miR-133a content in ASMCs. RhoA was identified as the key molecule to induce ASMC dysfunction, which may lead to excessive contraction, abnormal proliferation and apoptotic disorders of ASMCs (33). Therefore, correcting the aberrant expression of critical miRNAs in ASMC may potentially delay airway remodeling and the associated decline in lung function, which may be a promising approach for treating asthma.

Let-7a is a member of the let-7a family, the aberrant expression of which has been identified in multiple diseases, including malignancies, Alzheimer's disease, asthma, allergic rhinitis and allergic dermatitis (34). Solberg *et al* (18) verified through miRNA microarrays combined with RT-qPCR that let-7a is markedly downregulated in bronchial epithelial cells of asthmatic patients. Rijavec *et al* (19) reported that let-7a was distinctly downregulated in transbronchial lung biopsy tissues obtained during bronchoscopy from patients with severe asthma compared with patients with mild asthma and non-asthmatic controls. Levänen *et al* (35) discovered that the levels of let-7a were obviously decreased in the broncho-alveolar lavage fluid of asthmatic patients. However, to the best of our knowledge, the expression of let-7a in asthmatic ASMCs has not been previously reported. The present study indicated that let-7a is notably downregulated in ASMCs from asthmatic subjects. Therefore, it may be speculated that aberrant let-7a expression is associated with the dysfunction of ASMCs in asthmatic patients. Johnson *et al* (36) suggested that Let-7a inhibited pathways controlling cell proliferation, and may also be the major regulator of cell proliferation. Furthermore, Cheng *et al* (37) discovered that upregulation of let-7a expression in bone mesenchymal stem cells suppressed the proliferation of pulmonary artery SMCs through the STAT3/bone morphogenetic protein receptor type 2 signaling pathway, thus delaying the progression of pulmonary hypertension. Furthermore, it has been verified that upregulation of let-7a expression markedly inhibits the proliferation of vascular SMCs (38); in addition, let-7a inhibits the proliferation of multiple types of tumor cell and promotes tumor cell apoptosis (39). In the present study, to determine whether aberrant let-7a expression in asthmatic ASMCs affects their proliferation and apoptosis, ASMCs from asthmatic patients were transfected with let-7a mimics to successfully upregulate let-7a expression. A CCK-8 assay indicated that let-7a mimics markedly suppressed the proliferation of asthmatic ASMCs. Furthermore, flow cytometric analysis and a caspase-3/7 activity assay suggested that let-7a mimics markedly enhanced the apoptosis and caspase-3/7 activity of asthmatic ASMCs, thereby verifying that let-7a affects the remodeling process of asthmatic airway tissues through suppressing the proliferation and promoting the apoptosis of asthmatic ASMCs.

The miRNA target prediction database TargetScan was then used to further explore the downstream targeted regulatory mechanisms by which let-7a regulates the function of asthmatic ASMCs. The bioinformatics prediction suggested that let-7a directly and specifically regulates STAT3, as theoretical complementary binding sites in the 3'-UTR of STAT3 mRNA with the seed sequence of let-7a were identified. Furthermore, let-7a was confirmed in previous studies to inhibit hepatoma cell proliferation through specifically regulating STAT3 gene expression (40), which also enhanced the sensitivity of hepatoma cells to cetuximab through specifically regulating the expression of the STAT3 gene (41). In the present study, the dual-luciferase assay indicated that let-7a specifically binds with the 3'-UTR of STAT3. In addition, RT-qPCR and western blot analysis indicated that upregulation of let-7a evidently reduces the mRNA and protein expression levels of STAT3 in asthmatic ASMCs, further suggesting that STAT3 is a target gene regulated by let-7a. STAT3 is a member of the STAT family, and is an important signal transcription factor in the Janus kinase/STAT signal transduction pathway. It possesses multiple important biological activities, and is involved in processes including cell proliferation, differentiation, survival, angiogenesis and cancer metastasis, through regulating the expression of various genes and cytokines (42). STAT3 has been verified as a key regulatory factor in airway remodeling. Allergic asthma and airway hyperresponsiveness were significantly relieved in asthmatic mice with STAT3 knockout (43). Furthermore, STAT3 inhibitor was reported to significantly inhibit airway remodeling and lung inflammation in asthmatic mice (44). STAT3 was demonstrated to promote ASMC proliferation through inducing the expression of chemokines and growth factors (45). Knockout of STAT3 significantly inhibited the proliferation of SMCs in human airway tissues (46). Platelet-derived growth factor was reported to induce the proliferation of human ASMCs through activation of STAT3 (47). Shi *et al* (48) also confirmed that upregulation of STAT3 expression significantly promoted the proliferation of human ASMCs.

In conclusion, the present study suggested that let-7a is downregulated in ASMCs of asthmatic patients. In addition, let-7a was indicated to inhibit the proliferation and promote the apoptosis of human ASMCs, which may be associated with the downregulation of STAT3 expression. Let-7a has a vital role during asthmatic airway remodeling.

Acknowledgements

Not applicable.

Funding

The present study was supported by grants of the Natural and Science Foundation of Shandong Province (grant no. ZR2014HL003). The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Availability of data and materials

The datasets generated and analyzed during the study are available from the corresponding author on reasonable request.

Authors' contributions

HL designed the experiments. YC, LQ, ZZ, GH and JZ performed the experiments. YC and HL analyzed the data. HL wrote the manuscript.

Ethics approval and consent to participate

All subjects had provided written informed consent to participate in the study, and the present study was approved by the Ethics Committee of Shengli Oil Field Central Hospital (Dongying, China).

Patient consent for publication

Written informed consent was obtained from all patients prior to publication.

Competing interests

The authors declare that they have no competing interests

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