Resveratrol attenuates cigarette smoke extract induced cellular senescence in human airway epithelial cells by regulating the miR-34a/SIRT1/NF-κB pathway

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Abstract

Chronic obstructive pulmonary disease (COPD) is characterized by accelerated lung aging. Smoking is the critical risk factor for COPD. Cellular senescence of airway epithelial cells is the cytological basis of accelerated lung aging in COPD, and the regulation of microRNAs (miRNAs) is the central epigenetic mechanism of cellular senescence. Resveratrol (Res) is a polyphenol with anti-aging properties. This study investigated whether Res attenuates cigarette smoke extract (CSE)-induced cellular senescence in human airway epithelial cells (BEAS-2B) through the miR-34a/SIRT1/nuclear factor-kappaB (NF-κB) pathway. BEAS-2B cells were treated with Res, CSE and transfected with miR-34a-5p mimics. Cellular senescence was evaluated by senescence-associated β-galactosidase (SA-β-gal) staining and expression of senescence-related genes (p16, p21, and p53). The expressions of miR-34a-5p, SIRT1, and NF-κB p65 were examined using quantitative real time polymerase chain reaction and western blotting. The senescence-associated secretory phenotype (SASP) cytokines (IL-1β, IL-6, IL-8, TNF-α) were assessed by enzyme-linked immunosorbent assay. The binding between miR-34a-5p and SIRT1 was confirmed by dual-luciferase reporter assay. The results showed that CSE dose-dependently decreased cell viability and elevated cellular senescence, characterized by increased SA-β-gal staining and senescence-related gene expressions (p16, p21, and p53). Further, CSE dose-dependently increased the expression of miR-34a-5p and SASP cytokines (IL-1β, IL-6, IL-8, TNF-α) in BEAS-2B cells. Pretreatment with Res inhibited CSE-induced cellular senescence and secretion of SASP cytokines (IL-1β, IL-6, IL-8, TNF-α) in a dose-dependent manner. Moreover, Res reversed the CSE-induced down-regulation of SIRT1 and up-regulation of miR-34a-5p and NF-κB p65. SIRT1 is a target of miR-34a-5p. Overexpression of miR-34a-5p via transfection with miR-34a-5p mimic in BEAS-2B cells attenuated the inhibitory effect of Res on cellular senescence, accompanied by reversing the expression of SIRT1 and NF-κB p65. In conclusion, Res attenuated CSE-induced cellular senescence in BEAS-2B cells by regulating the miR-34a/SIRT1/NF-κB pathway, which may provide a new approach for COPD treatment.

Abbreviations: 3'UTR = 3' untranslated region, COPD = chronic obstructive pulmonary disease, CS = cigarette smoke, CSE = cigarette smoke extract, ELISA = enzyme linked immunosorbent assay, miRNA = microRNA, NF-κB = nuclear factor-kappaB, qRT-PCR = quantitative real time polymerase chain reaction, Res = resveratrol, SASP = senescence-associated secretory phenotype, SA-β-gal = senescence-associated β-galactosidase, SIRT = Sirtuin.

Keywords: cellular senescence, cigarette smoke extract, miR-34a, resveratrol, SIRT1

1. Introduction

Chronic obstructive pulmonary disease (COPD) is the leading cause of morbidity and mortality worldwide. COPD is characterized by airway obstruction and chronic inflammation in the lungs, which drive airway remodeling, emphysema and chronic bronchitis. Cigarette smoke (CS) is the most well-established risk factor for COPD. The underlying mechanisms of COPD remain poorly understood, and current treatments primarily focus on relieving symptoms without reducing disease progression and mortality. Therefore, novel insights into the pathogenesis and treatment of COPD are required.

COPD is a disease that results in accelerated aging of the lungs. Cellular senescence is the cytological basis for...
accelerated aging in COPD.[4] Cellular senescence is an irreversible cell cycle arrest condition in which cells are still metabolically active.[5] Senescence of airway epithelial and endothelial cells has been found to accumulate in lungs of COPD patients.[6] Consequently, senescent cells lose regenerative capacity and disturb cellular repair in COPD lungs, leading to emphysema and deteriorating lung function.[7] The expressions of senescence-related biomarkers such as p16, p21, p53, and senescence-associated β-galactosidase (SA-β-gal) can be used to identify senescent cells.[8] Senescent cells also secrete excessive amounts of proinflammatory cytokines, chemokines, growth factors, and proteases, termed the senescence-associated secretory phenotype (SASP), which is a critical difference from terminally differentiated cells.[9] SASP may exert harmful effects on the tissue microenvironment of neighboring cells.[10] In COPD, CS has been identified as a significant inducer of cellular senescence. Cigarette smoke extract (CSE) induces growth arrest and increases the expression of SA-β-gal, p16 and p21 in alveolar epithelial cells.[11] Similar results were reported in which CSE exposure led to enhanced SA-β-gal activity, increased p16 expression and cellular senescence in bronchial epithelial cells.[12]

Sirtuins are conserved NAD+-dependent deacetylases that play vital roles in the promotion of metabolism and longevity.[13] There are 7 sirtuins (SIRT1-7) in mammals, and SIRT1 is a critical factor that inhibits cellular senescence and prolongs organismal lifespan by regulating multiple cellular processes. SIRT1 has been shown to be down-regulated during aging, and up-regulation of SIRT1 can decrease CS-induced cellular senescence.[14] Yao et al found that SIRT1 protects against cellular senescence and inflammation in lungs induced by CS.[15] Nuclear factor-kappaB (NF-κB) is an important transcription factor that is activated in various diseases and has been implicated in SASP and inflammation. SIRT1 has been found to negatively regulate NF-κB signaling.[16] Therefore, activation of SIRT1 inhibits NF-κB activity and reduces inflammation levels, reduces inflammation levels, and subsequently ameliorates vascular endothelial dysfunction with aging.[17]

Micro-RNAs (miRNAs) are a class of small endogenous non-coding RNAs consisting of 18 to 25 nucleotides that negatively regulate target genes.[18] MiRNAs bind the 3′ untranslated region (3′UTR) of target messenger RNAs (mRNAs), leading to direct degradation of mRNA and inhibition of protein translation. Increasing evidence indicates that miRNAs including miR-34a, miR-24, miR-146 play important roles in the aging process by regulating target genes involved in senescence.[19, 20] MiR-34a, a key regulator of SIRT1, is increased in airway epithelial cells from patients with COPD.[21] An antagonist of miR-34a restored SIRT1 expression in airway epithelial cells, reduce markers of cellular senescence, decrease the SASP response, and promote cell proliferation by reversing cell cycle arrest.[22] Collectively, these suggest that miR-34a promotes cellular senescence and that miR-34a antagonists could be employed as anti-aging therapies.

Resveratrol (Res) is a polyphenol found in various plants such as grapes, strawberries, tomatoes, peanuts, cocoa, and sugar cane.[23] Several previous studies demonstrated that Res has many bioactivities, including anti-carcinogenic, anti-inflammatory, cardiovascular -protection, and anti-aging effects.[24] The anti-aging properties of Res have been attributed to several complex mechanisms involving various signaling pathways. Hsu et al reported that Res exerts anti-aging effects by increasing the expression of the anti-aging gene Klotho in the kidney.[25] Ciszar et al demonstrated Res up-regulated the activity of Nrf2 in aged primary vascular smooth muscle cells to decrease secretion of SASP.[26] Furthermore, studies have confirmed that Res administration significantly down-regulates the levels of miR-34a.[27] However, it is still unclear whether Res exerts anti-aging effects by regulating the miR-34a/SIRT1/NF-κB pathway. Therefore, this study aimed to investigate the effect of Res on CSE-induced cellular senescence in airway epithelial cell line (BEAS-2B) and the underlying mechanisms.

2. Methods

2.1. Cell culture and treatment

BEAS-2B cells were purchased from Fu Heng Biology (Shanghai, China) and cultured in Dulbecco’s modified Eagle’s medium (Biological Industries, Beit Haemek, Israel) containing 10% fetal bovine serum (Biological Industries), 1% penicillin/streptomycin at 37°C in an incubator with 5% CO2. BEAS-2B cells were then treated with various concentrations of CSE (2.5%–15%) and/or Res (10, 25 and 50 μM, Sigma-Aldrich, USA) for the indicated time points.

2.2. CSE preparation

CSE was prepared following the methods of Li et al.[27]One cigarette without filter (Lanzhoub Brand; Gansu Tobacco Industry Co., Ltd, China) was aspirated using a vacuum pump. The smoke was drawn into 50 mL of PBS for 2 to 3 minutes. The CSE solution was sterilized through a 0.22-μm filter, and considered as 100% CSE. CSE (100%) was diluted to the desired concentrations with culture medium for each experiment and used within 30 minutes of preparation. The optical densities of CSE solutions prepared in this manner were constant.

2.3. Cell transfection

MiR-34a-5p mimic and scrambled mimic control (miR-NC) were obtained from GenePharma (Shanghai, China). All oligomers were transfected into BEAS-2B cells using Lipopectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s guidelines. Further experiments were conducted 24 hours after transfection. The expression of miR-34a-5p was determined by quantitative real-time polymerase chain reaction (qRT-PCR).

2.4. Cell viability assay

BEAS-2B cells were seeded in 96-well plates at a density of 8 × 103 cells/well. The cells were exposed to 0, 2.5, 5, 7.5, 10, and 15% CSE for 24 hours or 7.5% CSE for 0, 6, 12, 24 and 48 hours. Then, MTT reagent (Sigma) was added and incubated at 37°C for 4 hours in the dark. The supernatants were discarded, and 150 μL DMSO was added to each well. A microplate reader (Synergy H1, Biotek, VT) was used to measure the absorbance at 490 nm. According to this formula, the cell survival rate was calculated as (ODtreatment - ODblank)/ODcontrol - ODblank) × 100%.

2.5. SA-β-gal staining

The SA-β-gal activity was determined using an SA-β-gal staining kit (Beyotime, Shanghai, China). BEAS-2B cells were plated in 24-well plates at a density of 4 × 104 cells/well. After treatment with CSE, Res, and/or transfection, cells were fixed with 4% paraformaldehyde for 15 minutes at room temperature, and then incubated in SA-β-gal staining solution overnight at 37°C. Finally, the cells were observed using a microscope (IX73, Olympus, Tokyo, Japan) to capture images, in which the senescent cells were colored blue.

2.6. qRT-PCR assay

Total RNA was extracted from BEAS-2B cells using RNAiso Plus reagent (Takara, Dalian, China). RNA was reverse transcribed into cDNA using PrimeScript™ RT reagent Kit or Mir-X™ miRNA First-Strand Synthesis Kit (Takara). Primers for mRNA and miRNA were obtained from Accurate Biotechnology (Hunan, China). The primers used are listed in Table 1. qRT-PCR
was performed using the SYBR® Premix Ex Taq™ II kit (Takara) and a Mx3000P Real-Time Thermal Cycler (Agilent, Santa Clara, CA). The relative expression of mRNA or miRNA was calculated using the 2-ΔΔCT method and normalized to GAPDH or U6, respectively.

2.7. Western blot analysis
Total protein was extracted from BEAS-2B cells using RIPA lysis buffer (Solarbio, Beijing, China). Each protein sample (40 μg) was resolved by SDS-PAGE and transferred to a PVDF membrane. Membranes were blocked with 5% skimmed milk for 1 hour and then incubated overnight at 4°C with primary antibodies against SIRT1 (BOSTER, Wuhan, China), NF-κB p65 and β-actin (Signalway Antibody, College Park, MD, USA). After overnight incubation, the membranes were washed with TBST buffer and incubated with goat anti-rabbit IgG (Signalway Antibody) for 2 hours at 1:3000 dilution at room temperature. Finally, blots were quantified using an enhanced chemiluminescence kit and β-actin was used as an internal control.

2.8. Cytokine measurements
The SASP cytokines (IL-1β, IL-6, IL-8, TNF-α) in culture supernatants were measured by enzyme-linked immunosorbent assay (ELISA) (Elabscience Biotechnology Co, Ltd, Wuhan, China) according to the manufacturer’s instructions. Absorbance at 450 nm was measured using a microplate reader (Synergy H1, Biotek, VT).

2.9. Dual-luciferase reporter assay
The binding sites of miR-34a-5p and SIRT1 were predicted using TargetScan. The wild (wt) or mutant (mut) 3’UTR sequence of SIRT1 was inserted into the GP-miR Glo vector (GenePharma). Then, SIRT1-3’UTR-wt vector or SIRT1-3’UTR-mut vector was co-transfected with miR-34a-5p mimic or miR-NC into HEK293T cells using Lipofectamine 2000 reagent (Invitrogen). After 24 hours, the transfected cells were lysed and luciferase activity was measured using a dual-luciferase reporter assay system (Promega, Fitchburg, WI).

2.10. Statistical analysis
All experiments were performed in at least 3 independent assays and are presented as mean ± standard deviation. Statistical analyses were performed using SPSS 21.0 software (SPSS Inc., Chicago, IL). One-way ANOVA was used to compare mean data among multiple groups, and a post hoc LSD test was used for inter-group comparisons. Statistical significance was set at P < .05.

3. Results

3.1. CSE inhibited BEAS-2B cell viability
To evaluate the effect of CSE exposure on cell viability, BEAS-2B cells were exposed to 0, 2.5, 5, 7.5, 10 and 15% CSE for 24 hours or 7.5% CSE for 0, 6, 12, 24 and 48 hours. The MTT assay indicated that the cell viability was decreased in a dose- and time-dependent manner (Fig. 1). When the concentration of CSE reached 10% and 15%, or when cells were exposed to 7.5% CSE for 48 hours, the survival rate of BEAS-2B cells was < 50%. The concentrations of CSE (2.5%–7.5%) were used to treat cells for the following experiments, and experimental times were set at 24 hours as determined by these results.

3.2. CSE exposure induced cellular senescence and increased level of miR-34a in BEAS-2B cells
SA-β-gal is a verified marker of cellular senescence. Senescent cells are stained blue using SA-β-gal staining. To investigate the effect of CSE on cellular senescence, BEAS-2B cells were exposed to various CSE concentrations for 24 hours followed by SA-β-gal staining. As shown in Figure 2A and B, the percentage of SA-β-gal positive cells increased with increasing concentrations of CSE. The senescence-related markers p16, p21, and p53 were measured using qRT-PCR. The results showed that CSE exposure dose-dependently increased the gene expression of p16, p21, and p53 (Fig. 2C-E). Additionally, SASP cytokines including IL-1β, IL-6, IL-8, and TNF-α in cell media were measured by ELISA. Compared to the control group, the levels of these cytokines were elevated after CSE exposure (Fig. 2F–I). These results support the promoting effects of CSE on cellular senescence.

We also evaluated the effect of CSE exposure on miR-34a-5p expression. qRT-PCR assay showed that CSE dose-dependently increased the expression of miR-34a-5p in BEAS-2B cells (Fig. 2J). The results indicate that miR-34a might participate in CSE-induced cellular senescence.

3.3. Res attenuated cellular senescence and regulated miR-34a/SIRT1/NF-κB pathway in CSE-stimulated BEAS-2B cells.
To investigate whether Res alleviated CSE-induced cellular senescence, BEAS-2B cells were pretreated with 10, 25 and 50 μM Res for 6 hours followed by exposure to 7.5% CSE for another 24 hours. As shown in Figure 3A and B, the percentage of SA-β-gal positive cells induced by CSE was reduced by Res pretreatment in a dose-dependent manner. Further, pretreatment with Res dose-dependently attenuated CSE-induced expression of senescence-related markers, including p16, p21, and p53 (Fig. 3C–E). In addition, pretreatment with Res dose-dependently attenuated the levels of IL-1β, IL-6, IL-8, and TNF-α in CSE-treated cells (Fig. 3F–I). These results indicate that Res protected BEAS-2B cells against CSE-induced cellular senescence.

To investigate whether the anti-aging efficacy of Res is associated with the miR-34a/SIRT1/NF-κB pathway, we measured the miR-34a-5p, SIRT1, NF-κB p65 expression using qRT-PCR and western blotting. As shown in Figure 3J, pretreatment with Res decreased the expression of miR-34a-5p in CSE-treated cells. Further, SIRT1 expression was reduced and NF-κB p65 was attenuated in Res-treated cells. These results support the anti-aging effect of Res.
expression was increased after CSE exposure. In contrast, Res pretreatment dose-dependently increased SIRT1 expression and decreased NF-κB p65 expression (Fig. 3K–O). Altogether, these observations suggest that the miR-34a/SIRT1/NF-κB pathway may be implicated in the protective effect of Res on CSE-induced cellular senescence in BEAS-2B cells.

3.4. Overexpression of miR-34a reversed the effects of Res on cellular senescence in CSE-stimulated BEAS-2B cells

A dual-luciferase reporter assay was performed to investigate the target of miR-34a. As shown in Figure 4A and B, miR-34a-5p had sequences complementary to SIRT1. The fluorescence intensity decreased in cells co-transfected with SIRT1-3'UTR-wt vector and miR-34a-5p mimic, however fluorescence intensity showed no significant changes in cells transfected with SIRT1-3'UTR-mut vector. This result implied that SIRT1 is a target of miR-34a.

To determine the function of miR-34a in the inhibitory effect of Res on cellular senescence, we increased its expression by transfecting BEAS-2B cells with miR-34a-5p mimic for 24 hours, then pretreated with 50 μM Res for 6 hours, followed by exposure to 7.5% CSE for another 24 hours. As shown in Figure 4C, the expression of miR-34a-5p was significantly increased in BEAS-2B cells transfected with miR-34a-5p mimic compared in Res + CSE or Res + CSE + miR-NC groups. Furthermore, compared to the Res + CSE group, overexpression of miR-34a-5p enhanced the percentage of SA-β-gal positive cells and elevated the expression of senescence-related markers p16, p21, and p53 (Fig. 4D–H). Additionally, ELISA revealed that overexpression of miR-34a-5p increased the secretion of IL-1β, IL-6, IL-8, and TNF-α (Fig. 4I–L). Thus, overexpression of miR-34a-5p abrogated the inhibitory effect of Res on CSE-induced cellular senescence in BEAS-2B cells. These results confirm that miR-34a is implicated in the inhibitory effect of Res on CSE-induced cellular senescence and secretion of SASP cytokines in BEAS-2B cells.

Next, we investigated whether Res mediates the SIRT1/NF-κB signaling pathway via miR-34a. As shown in Figure 4M–Q, compared to the Res + CSE group, miR-34a-5p overexpression by transfection of BEAS-2B cells with miR-34a-5p mimic decreased the mRNA and protein expression of SIRT1, whereas these effects increased the expression of NF-κB p65. These data indicate that miR-34a overexpression reversed the expression of SIRT1 and NF-κB p65 in BEAS-2B cells treated with Res + CSE, simultaneously abrogating the inhibitory effect of Res on cellular senescence caused by CSE. Collectively, these data suggest that Res attenuates CSE-induced cellular senescence via the miR-34a/SIRT1/NF-κB pathway.

4. Discussion

Airway epithelial cells are the first line of defence against environmental factors and pathogens. Senescence of airway epithelial cells plays an essential role in COPD progression.[24] Cellular senescence impairs the regenerative capacity of stem cells, and senescent cells release SASP cytokines, which contribute to airway inflammation and remodeling in COPD.[25] Cellular senescence is an irreversible process that is typically identified by the increased expression of SA-β-gal and up-regulation of senescence-related markers, including p16, p21, and p53.[21] Senescent cells express β-galactosidase enzyme, which is detectable at a pH of 6 and termed SA-β-gal. Additionally, this enzyme is widely used as a biomarker to identify senescent cells.[22] An important mechanism by which cells maintain the senescent state is to alter expression of senescence-related genes, such as p16, p21, p53, and retinoblastoma protein (pRB).[23] The transcriptional regulators p53 and pRB are the most important factors; p21 works downstream of p53, whereas p16 works upstream of pRB.[24] These components are cyclin-dependent kinase inhibitors that regulate cellular senescence, which can be induced by overexpression of any of these components. In addition, senescent cells secrete multiple inflammatory proteins termed SASP, which cause persistent inflammation and further accelerate senescence.[10] NF-κB-dependent proinflammatory cytokines, including IL-1β, IL-6, IL-8, and TNF-α, are critical components of SASP, and are suggested to be highly conserved and robustly expressed cytokines.[25] Furthermore, a large array of stressors can induce cellular senescence. In vitro and vivo, CS exposure has been found to be sufficient to induce cellular senescence as a stressor. CSE exposure activates senescence-related pathways in human lung fibroblasts, such as p53 and p16-pRB pathways.[26] In the present study, CSE dose-dependently increased the percentage of SA-β-gal stained cells and the expression of p16, p21, and p53 in BEAS-2B cells, accompanied by increased secretion of SASP cytokines including IL-1β, IL-6, IL-8, and TNF-α. Overall, these data indicate that CSE promotes senescence of airway epithelial cells.

Res, a polyphenol found in various plants, has anti-aging properties and prolongs lifespan of several species by targeting multiple cellular processes.[27] Res reduces SASP cytokines in rats induced by CS exposure and suppresses lung aging in mouse models of accelerated aging.[28,29] In vitro, Res inhibits the release of SASP cytokines and the expression of senescence-related markers such as p53, p16, p21 in bone marrow stromal stem cells stimulated by tert-butyl hydroperoxide and promotes osteoblastic differentiation.[30] Similar to these findings, our data showed that Res dose-dependently alleviated CSE-induced cellular senescence.

Figure 1. CSE inhibited BEAS-2B cells viability. BEAS-2B cells were treated with 0%, 2.5%, 5%, 7.5%, 10% and 15% CSE for 24 hours (A) or 7.5% CSE for 0, 6, 12, 24 hours and 48 hours (B). MTT assay was measured to evaluate the cell viability. Data were presented as means ± SD (n = 5). *P < .01, P < .05 versus control. CSE = cigarette smoke extract, MTT = methyl thiazolyl tetrazolium.
increase in the percentage of SA-β-gal positive cells and increased expression of p16, p21, and p53. In addition, Res preconditioning decreased CSE-induced SASP secretion. These results provide direct evidence that Res attenuates CSE-induced senescence in airway epithelial cells. However, the mechanism underlying the anti-aging properties of Res has not been clarified. Therefore, we investigated whether Res attenuates CSE-induced cellular senescence by regulating the miR-34a/SIRT1/NF-κB pathway.

A variety of miRNAs, termed senescence-related miRNAs, can act as senescence inducers. Phillipot et al identified that miR-24 negatively regulates p16 expression and promotes cellular senescence in human chondrocytes. Afanasyeva et al reported that miR-885-5p exerts antitumor effects by interfering with cell cycle progression and cell survival. Additionally, miR-146a has been demonstrated to negatively regulate the expression of TNF-α and IL-8. Meanwhile, miR-34a has been found to act as a senescence promoter that can reduce SIRT1 expression by binding to its 3’UTR and is likely involved in vascular endothelial progenitor cell senescence. Consistent with the above research, dual-luciferase report assays in this study demonstrated that SIRT1 is a direct target of miR-34a. Yamakuchi et al reported that miR-34a inhibits SIRT1 expression and activated p53 to exert antitumor effects. Disayabutr et al showed that miR-34a expression was increased in alveolar epithelial cells of idiopathic pulmonary fibrosis patients and was positively correlated with SA-β-gal expression. NF-κB, a key transcription factor that regulates proinflammatory genes, has been previously found to control SASP release from senescent alveolar epithelial cells. In relation to this, SIRT1 interacts with the RelA/p65 subunit of NF-κB and deacetylates lysine 310, enhancing the expression and activity of NF-κB. Furthermore, SIRT1 knockout or knockdown enhanced NF-κB expression and activity, as well as proinflammatory cytokine release, whereas up-regulation of SIRT1 inhibited NF-κB-mediated inflammatory mediator release. This study showed that CSE dose-dependently
increased miR-34a-5p and NF-κB p65 expression but decreased SIRT1 expression in BEAS-2B cells. Furthermore, Res pretreatment decreased the levels of miR-34a and NF-κB p65 and enhanced SIRT1 expression in CSE-treated BEAS-2B cells. These observations suggest that the miR-34a/SIRT1/NF-κB pathway may be implicated in the protective effect of Res on CSE-induced cellular senescence in BEAS-2B cells.

Following these findings of Res pretreatment, we next investigated whether Res exerted anti-aging effect by inhibiting miR-34a. To confirm this, we transfected BEAS-2B cells with miR-34a-5p mimic to increase miR-34a expression, followed by treatment with Res and CSE. The results showed that miR-34a overexpression abrogated the inhibitory effect of Res on CSE-induced cellular senescence. Simultaneously, the increased expression of SIRT1 and the decreased expression of NF-κB accompanied by decreased secretion of SASP cytokines caused by Res were reversed by miR-34a overexpression. These data indicate that Res
targets miR-34a to regulate the SIRT1/NF-κB pathway and reduce CSE-induced cellular senescence and SASP secretion in BEAS-2B cells.

5. Conclusion

In summary, our results indicate that CSE induces senescence in airway epithelial cells. Additionally, Res attenuates CSE-induced cellular senescence through the miR-34a/SIRT1/NF-κB pathway. These results provide novel research targets and potential treatment strategies for COPD.

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