LncRNA SAL-RNA1 Knockdown Suppressed Pink1-Mediated Mitophagy Protects Against CS-Induced Alveolar Cells Senescence Through Regulation of Sirt1

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Abstract

Background

LncRNAs pays an important roles in the regulation of alveolar cells in cigarette smoke (CS) induced chronic obstructive pulmonary disease (COPD). However, the role of lncRNA SAL-RNA1 in promoting mitophagy and reduces senescence of alveolar cells in COPD is unknow.

Methods

This study is aims to elucidate the underlying mechanism of LncRNA SAL-RNA1 and phosphatase and tensin homolog (PTEN)-induced putative protein kinase 1 (PINK1) expression were upregulated in alveolar of emphysema mice and CS treated alveolar epithelial cells. LncRNA SAL-RNA1 knockdown suppressed mitophagy and induced senescence of alveolar cells while overexpressing lncRNA SAL-RNA1 promoted mitophagy and reduced the senescence of alveolar cells. In addition, lncRNA SAL-RNA1 interacted with Sirt1 and regulated the upgradation of Sirt1.

Results

We further found lncRNA SAL-RNA1 regulated Pink1 expression through Sirt1. Mechanistically, lncRNA SAL-RNA1 regulated PinK1-mediated mitophagy and senescence of alveolar cells through regulation Sirt1.

Conclusion

Finally, overexpression lncRNA SAL-RNA1 promote mitophagy and relieved emphysema.

Introduction

Cell senescence is one of the most important feature of emphysema, which is impaired cell regeneration, induces the loss of alveolar structure and airspace enlargement[1]. Although therapeutic interventions have shown to postpone the progression of emphysema, the morbidity and mortality of emphysema remain high and new treatment measures are need[2]. epithelial cells (EC) are the multifunctional secretory cells, which play important roles in maintaining alveolar homeostasis and gas exchange and the destruction of the alveolar structure in pathological states will lead to emphysema[3]. Many studies also show that excessive senescence of alveolar cells contributes to the progression of emphysema[4]. Recent studies also show that autophagy dysregulation is closely related to the senescence of alveolar cells[5]. However, the molecular mechanisms is still unknow whether control mitophagy could alleviate the senescence of lung structure.

Recent advances have shown that long non-coding RNAs(lncRNAs) play important roles in the regulation of AEC senescence in emphysema[6]. LncRNA SAL-RNA1(*XLOC_023166*) was found to lower abundance in senescence cells and delay senescence[7, 8]. In addition, LncRNA SAL-RNA1 knockdown enhanced the appearance of phenotypic traits of senescence, including an enlarged airspace, positive β-galactosidase activity, and lower sirt1 levels[9, 10]. These evidence reveals that lncRNA SAL-RNA1 play direct regulatory roles in this important cellular process[11, 12]. However, whether the aberrant expression of lncRNA SAL-RNA1 occurs during the senescence of AEC in emphysema is still unclear.

In the present study, we investigate the role of lncRNA SAL-RAL in the mitophagy and senscence of AEC and determine lncRNA SAL-RNA1 regulates CS-induced senscence of AEC through regulating protein expression of Pink in vivo and in vitro.

Meterials And Methods

Cell culture Normal airways were obtained from 1st through 4st order bronchi from pneumonectomy and lobectomy specimens for primary lung cancer. Informed consent was obtained from all surgical participants as part of an approved ongoing research protocol by the ethics committee of Zhejiang University school of Medicine. Human bronchial epithelial cells (HBEC) were isolated with protease treatment and characterized as previously described[13]. HBEC were serially passaged and used for experiments until passage 3. The majority of experiments were performed with HBEC from non-COPD patients. HBEC were cultured in DMEM (Beyotime, MA0212) with 10% fetal calf serum (Beyotime,C0227) and penicillin-streptomycin (Beyotime, C0222).

Antibodies and reagents Antibodies used were rabbit anti-p21(Abcam,ab109520), rabbit anti-PINK1(Abcam,ab216144), rabbit anti-MAP1LC3B/LC3B (Abcam, ab247327),rabbit anti-MAP1LC3B/LC3B(Novus, 600–1384),rabbit anti-TOMM20 (Abcam,ab186735), Mouse anti-ACTB/β-actin(Abcam, ab8226), The following reagents were used: bafilomycin A1 (BafA1; Sigma-Aldrich, B1793), MG-132 (Enzo Life Sciences, BML-P102), CM-H2DCFDA (Life Technologies, C6827), Hoechst 33258 (Sigma-Aldrich, B2883), MitoSOX Red (Molecular Probes/Life Technologies, M36008).

Small interfering and transfection pcDNA-SAL-RNA1 were constructed by inserting SAL-RNA1 cDNA. pcDNA was used as a negative control. For SAL-RNA1 konckdown, small interfering RNA targeting SAL-RNA1(siRNA-SAL-RNA1)were synthesized by Ribobio Co.,Ltd.(Guangzhou, China).siRNA was used as a negative control. Transfection experiments were conducted using Lipofectamine 2000 reagent (Invitrogen).

Preparation of cigarette smoke extract (CSE) Cigarette smoke extract (CSE) was prepared as previously described with minor modifications[14]. The research reference cigarettes 3R4F were used for experiments (Louisville, KY, USA). Forty milliliters of cigarette smoke were drawn into a syringe and slowly bubbled into sterile serum-free cell culture media in 15-ml BD falcon tubes. One cigarette was used for the

preparation of 10 mL of solution. CSE solution was filtered (0.22 μm; Merck Millipore, SLGS033SS) to remove insoluble particles and was designated as 100% CSE solution.

qRT-PCR Total RNAs were extracted from glomeruli and podocytes using Trizol reagent (Beyotime Biotechnology) according to the manufacturer's instructions. RNAs were transcribed into cDNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA). PCR mixtures (25 μl) containing 2.5 μM of primer pairs, cDNA template, and SYBR Green Real-Time PCR Master Mixes (AppliedBiosystems) were subjected to PCR amplification using QuantStudio 5 Real-Time PCR System (Applied Biosystems). The relative SAL-RNA1 and SIRT1 expressions were calculated using the $2^{-\Delta\Delta CT}$ method. GAPDH was used as an internal reference.

Measurement of ROS production HBEC were grown on 8-well culture slides. HBEC transfected with pcDNA,pcDNA SAL-RNA1 were treated with CSE at 48 h post transfection or SRT1720 administration. HBEC were transfected with control siRNA, SAL-RNA1 siRNA, and were treated with CSE at 48 h post-transfection or selisistat. HBEC were fixed after 24-h treatment with CSE. Mitochondrial ROS production was analyzed by MitoSOX Red staining according to the manufacturer's instructions, which was evaluated by fluorescence microscopy (Olympus, Tokyo, Japan and Keyence, BZ-X700).

RNA pull-down Pierce Magnetic RNA-Protein Pull-Down Kit (Thermo Scientific) was used to determine the interaction between SAL-RNA1 and SIRT1. HBEC were transfected with biotinylated SAL-RNA1 and negative control (NC). The lysate was incubated with streptavidin magnetic beads. Then, the SAL-RNA1-SIRT1 complex bound to the beads were eluted and detected by western blot analysis.

RNA immunoprecipitation (RIP) RIP assay was performed to confirm the interaction between SAL-RNA1 and SIRT1. Briefly, HBEC were lysed with RIP lysis buffer.The lysate was incubated with an anti-SIRT1 rabbit polyclonal antibody at 4°C overnight with Magnetic Beads.protein A/G. The beads were washed with cold RIP wash buffer for six times. Then, beads were resuspended in 150 μl Proteinase K buffer and incubated at 55°C for 30 min. RNA was extracted and purified. qRT-PCR was then conducted with the purified RNA. Total RNAs (input controls) and isotype controls (normal mouse IgG) were simultaneously determined.

Ubiquitination assay To examine whether SNHG17 affects Mst1 ubiquitination, HA-Ub, FLAG-SIRT1, siRNA-control, siRNA-SAL-RNA1 were transfected into HBEC using transfection reagent for 12 h. After transfecting, HBEC were treated with MG132 (10 μM) for 4 h and then collected and lysed. Western blot analysis of cell lysates was used to confirm Mst1 protein expression. Then, cell lysates were immunoprecipitated with an anti-FLAG antibody at 4°C followed by immunoblotting with an anti-HA antibody.

SA–β-gal activity assay. SA–β-gal activity was quantitatively measured by the rate of conversion of 4-methylumbelliferyl-β-d-galactopyranoside (MUG) to the fluorescent hydrolysis product 4-methylumbelliferone (4-MU) at pH 6.0, as described previously. Briefly, lung tissues were homogenized in the lysis buffer (5 mM CHAPS, 40 mM citric acid, 40 mM sodium phosphate, 0.5 mM benzamidine, and

0.25 mM PMSF, pH 6.0), and kept on ice for 1 hour. The lysates were centrifuged for 5 minutes at 12,000*g*, and the supernatant was mixed with 2× reaction buffer (40 mM citric acid, 40 mM sodium phosphate, 300 mM NaCl, 10 mM β-mercapto-ethanol, and 4 mM MgCl2 [pH 6.0] with 1.7 mM MUG), which was placed into a 37°C water bath for 3 hours. Finally, 50 µl of the reaction mix was added to 500 µl of 400 mM sodium carbonate stop solution (pH 11.0), which was read at 150 µl/well in a 96-well plate using a SpectrumMax M5 plate reader (Molecular Devices) with excitation at 360 nm, emission at 465 nm, 40 µs integration, and gain held constant at 46. Normalized SA–β-gal activity was expressed as observed fluorescence (rate of conversion of 4-methylumbelliferyl-β-d-galactopyranoside to 4-MU) divided by milligram total protein in the assay.

Mouse model and CS exposure protocol C57BL/6J (CLEA Japan INC, Tokyo, Japan) and B6.129-SAL-RNA1$^{-/-}$ (Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences) mice were purchased, and were maintained in the animal facility at the Zhejiang University School of Medicine. All experimental procedures were approved by the Zhejiang University School of Medicine Animal Care Committee. 6 to 8-week-old mice were exposed using a whole-body exposure system (SCIREQ 'InExpose') within a barrier facility. Mice were exposed at a total suspended particulates of 200 mg/m^3 using 3R4F cigarettes for 5 days a week. After 6 months, immediately following sacrifice, the right lung was inflated with 10% buffered formalin at a pressure of 20 cm H_2O. Fixed lungs were embedded in paraffin and cut into 4-µm sections for tissue staining. The sections stained with hemaoxylin & eosin (H-E) and Picro-Sirius Red Stain (PSR) according to conventional protocols for histopathological evaluation. Immunohistochemistry was performed on the paraffin-embedded lung tissues as previously described with minor modifications[15]. The left lungs were removed and homogenized for protein evaluation or the frozen sections stained with SA-β- gal staining and immunofluorescence staining. Tracheotomy was performed to insert a tracheal tube for collecting bronchoalveolar lavage fluid (BALF) sample with PBS (Wako, 041-20211; total 3 ml). The cell numbers in BALF were counted using a hemocytometer. Differential cell counts in BALF were analyzed on 300 cells stained with Diff-Quick (Sysmex, 16920).

Immunofluorescence staining HBEC were grown on 8-well culture slides. HBEC transfected with pcDNA SAL-RNA1 were treated with CSE at 48 h post-transfection or selisistat. HBEC were transfected with control siRNA, SAL-RNA1 siRNA and were treated with CSE at 48 h post-transfection or SRT1720 administration. HBEC were treated with BafA1 6 h before collection. HBEC were fixed with 100% methanol (Wako, 137–01823). After blocking with 1% BSA (Sigma-Aldrich, A2153) for 60 min, the primary and secondary antibodies were applied according to the manufacturers' instructions. Confocal laser scanning microscopy analysis of BEAS-2B cells was performed using mouse anti-TOMM20 and rabbit anti-MAP1LC3B (Cell Signaling Technology, 3868), and evaluated by fluorescence microscopy (Carl Zeiss LSM880, Tokyo, Japan). Quantitative measure of immunofluorescence staining to evaluate mitophagic activity was analyzed by dividing the yellow intensity of mitophagy areas by the red intensity of mitochondrial areas. Fluorescence microscopy analysis of phospho-histone H2AFX and Hoechst 33258 staining were performed in lung frozen sections and HBEC, and evaluated by fluorescence microscopy (Olympus, BX60, Tokyo, Japan and Keyence, BZ-X700, Tokyo, Japan).

Lung morphometry. Mouse lungs (which had not been lavaged) were inflated with 1% low-melt agarose at a pressure of 25 cm H_2O, then fixed with 4% neutral buffered PFA[16]. Fixed lung was dehydrated, embedded in paraffin, and sectioned into 4-μm sections using a rotary microtome (MICROM International GmbH). H&E staining was performed on the lung midsagittal sections to determine Lm of airspace using MetaMorph software (Molecular Devices)[17]. Ten randomly selected ×100 fields per slide were photographed in a blinded manner, and the images were manually thresholded. The airway and vascular structures were eliminated from the analysis.

Measurement of lung mechanics. Lung mechanical properties, including lung compliance and R_L, were determined as described previously[18]. Briefly, the mouse was weighed, deeply anesthetized by i.p. injection of pentobarbital (90 mg/kg BW) and pancuronium (0.5 mg/kg BW), and tracheostomized. The trachea was cannulated, and the cannula was connected to a computer-controlled small animal ventilator (FlexiVent; SCIREQ). Estimated lung compliance, R_L, and Rn at 3 cmH2O positive end expiratory pressure were obtained by fitting a model to each impedance spectrum. The calibration procedure removed the impedance of the equipment and tracheal tube within this system.

Electron microscopy Electron microscopy was performed as previously described[19]. Pieces from mouse lung were fixed with 2% glutaraldehyde, 0.1 M phosphate buffer (pH 7.4) and after 48 h of incubation were dehydrated with a graded series of ethanol. Fixed lung pieces were then embedded in epoxy resin (Epok812; Oken, 02–1001). Ultrathin sections were stained with uranyl acetate and lead citrate and observed with the Hitachi H-7500 transmission electron microscope (Hitachi, Tokyo, Japan). For quantitative evaluation of mitochondria in airway epithelial cells, 10 airway epithelial cells imaged at 10,000X magnification were selected for each sample and mitochondria were counted.

Statistics Data are shown as the average (± SEM) taken from at least 3 independent experiments. Comparisons between 2 different groups were determined by Student t test for parametric data or Mann-Whitney test for nonparametric data. One-way analysis of variance was used for multiple comparisons and Tukey or Bonferroni post-hoc tests used to test for statistical significance. Significance was defined as $P < 0.05$. Statistical software used was Prism v.5 (GraphPad Software, Inc., San Diego, CA).

Results

SAL-RNA1 deficiency aggravates pulmonary emphysema.

Our recent observation of the positive correlation between SAL-RNA1 and the pulmonary function test indicates that SAL-RNA1 RNA levels can be implicated in the mechanism of COPD progression[19]. To determine the role of SAL-RNA1 in the development of pulmonary emphysema, we exposed to *SAL-RNA1* KO mice, as well as their WT littermates, to CS. Lung SAL-RNA1 level was decreased in SAL-RNA1$^{-/-}$ mice, compare with their WT littermates(Fig. 1A). Lung parenchymal emphysema in response to CS exposure were examined in both wild-type and SAL-RNA1 KO mice. CS exposure for 6 months induced a modest airspace enlargement in wild-type mice, whereas SAL-RNA1 KO mice started to exhibit airspace

enlargement after just 4 months of CS exposure, which was augmented at 6 month(Fig. 1,B). Deficiency of SAL-RNA1 significantly augmented 6 month CS induced increase in alveolar mean linear intercept (Lm)(Fig. 1,C). Lung compliance was further augmented in SAL-RNA1 KO mice, compared with WT mice exposed to CS for 6 months(Fig. 1,D). SAL-RNA1 deficiency decreased total lung resisitance(R_L), although no significant change in R_L in WT mice was observed after 6 months of CS exposure.(Fig. 1,E).

Accelerate cellular senescence and accumulation of damaged mitochondriain SAL-RNA1 KO mice in response to CS exposure

Accelerate cellular senescence has been widely implicated in COPD pathogenesis. Our recent finding suggest that LncRNA-mediated SIRT1/FOXO3a signaling pathways regulate type Ⅱalveolar epithelial cell senescence in patients with chronic obstructive pulmonary disease[19]. Therefore, it is possible that the protective effect of SAL-RNA1 in emphysema is associated with its ability to regulate cellular senescence. As expected, CS exposure significantly the levels of SA-βgal activity in lungs of SIRT1$^{-/-}$ mice versus wild type mice(Fig. 2,A).To confirm airway epithelial cell senescence, immunohistochemical staining of SIRT1(anti-aging protein) and p21(senescence-associated cyclin dependent kinase inhibitors) were performed. Both airway epithelial cells and alveolar epithelial cells in CS exposed SAL-RNA1 KO mice demonstrated clear positive staining in p21,which was markedly reduced in SIRT1 expression(Fig. 2,B and C).We and others have previously shown that accumulation of fragmented and structurally distorted mitochondria in the airway epithelial cells of COPD/emphysema[20].

To elucidate the accumulation of damaged mitochondria reflecting insufficient mitophagy conferred by SAL-RNA1 deficiency, electron microscopy evaluation was performed. CS treatment significantly increased structural alteration of mitochondria, which was markedly enhanced by SAL-RNA1 deficiency. No obvious damaged mitochondria was observed in airway epithelial cells of wild type mice nor SAL-RNA1$^{-/-}$ mice without CS exposure(Fig. 2,D). TOMM20 protein levels in lung homogenates were significantly increased in lungs from CS exposed SAL-RNA1 KO mice showing that the increased of total mitochondrial counts in airway epithelial(Fig. 2,E). PINK1 protein levels were apparently increased in lungs from CS exposed SAL-RNA1 KO mice, indicating that PINK1 levels may also reflect accumulation of damaged mitochondria(Fig. 2,F).

LncRNA SAL-RNA1 regulated the degradation of Sirt1

We conducted RNA pull-down and RIP assays to confirm the interaction between SAL-RNA1 and Sirt1. The result of RNA pull-down assay showed that Sirt1 protein was detected in the SAL-RNA1 pull-down complex(Fig. 3a),and the result of the RIP assay showed that SAL-RNA1 was accumulated in Sirt1 precipitate(Fig. 3b), indicating SAL-RNA1 interacted with Sirt1. Besides, siRNA-SAL-RNA1 decreased the Sirt1 protein level, and pcRNA-SAL-RNA1 increased the Sirt1 protein level(Fig. 3c), indicating Sirt1 protein level was positively regulated by SAL-RNA1. Whereas the mRNA level of Sirt1 in MAEC did not significantly change after the transfection of siRNA-SAL-RNA1(Fig. 3d), which indicating that SAL-RNA1 may affect the ubiquitination of Sirt1. Later MAEC were treated with CHX, and we found CHX promoted

the degradation of Sirt1 protein in MAEC transfected with siRNA-SAL-RNA1(Fig. 3e). Moreover, siRNA-SAL-RNA1 promoted the ubiquitination of SIRT1(Fig. 3f).

LncRNA SAL-RNA1 regulated pink1 expression through Sirt1

To gain a better understanding of mechanisms for enhanced PINK1 protein levels in COPD with concomitant SAL-RNA1 overexpression through SIRT1, PINK1 protein levels were examined in the setting of both overexpression and deficiency in HBEC, respectively. SAL-RNA1 overexpression significantly ebhanced the protein level of PINK1 in HBEC, which were significantly recovered by selisistat, a sirt1 inhibitors, without affecting SAL-RNA1 expression in response to CSE (Fig. 4a and b).siRNA-SAL-RNA1 significantly inhibitor the protein level of pink1, and SRT1720(Sirt1 activator) abolished the inhibitor effect of siRNA-SAL-RNA1 on pink1 expression in the present of CSE exposure(Fig. 4d), without affecting SAL-RNA1 expression(Fig. 4c).These finding suggesting that lncRNA SAL-RNA1 promoted pink1 expression through regulating Sirt1.

LncRNA SAL-RNA1 regulated mitophagy and senscence of AEC through Sirt1

CS exposed SAL-RNA1 KO mice demonstrated high PINK1 protein levels with concomitant accumulation of damaged mitochondria, suggesting the pivotal role of SAL-RNA1 in regulating mitophagy in COPD pathogenesis. To investigate whether lncRNA SAL-RNA1 regulated mitophagy and senscence of HBEC through Sirt1, combinations of overexpression and knockdown experiments were performed. Mitophagy was examined by means of colocalization analysis of confocal laser scanning microscopy images of TOMM20 stained mitochondria and MAP1LC3B dots in HBEC.CSE induced mitophagy shown by colocalization of TOMM20 and MAP1LC3B, which was further enhanced by SIRT1 overexpression, the effect of which was no alterations by SAL-RNA1 overexpression(Fig. 5a). Consistent with our previous findings[21], CSE induced mitophagy was apparently inhibited by siRNA-SAL-RNA1 and SIRT1 deficiency, respectively. Conversely, SAL-RNA1 overexpression failed to reserve SIRT1 deficiency mediated reduction of CSE induced mitophagy(Fig. 5b).

In line with mitophagy status, increased mitochondrial ROS(MitoSOX Red)and mitochondrial mass(TOMM20), and accelerated cellular senescence(p21 staining) by CS exposure,were enhanced by SAL-RNA1 knockdown and SIRT1 deficiency in HBEC,respectively(Fig. 5(c-e)). CS induced accumulation of mitochondrial ROS and mitochondrial mass, as well as accelerated cellular senescence enhanced by SAL-RNA1 knockdown ,were clearly attenuated by SRT1720.SAL-RNA1 overexpression failed to attenuated those alternations in the setting of selisistat(Fig. 5(c-e)).

Discussion

Recent studies reported that mitochondrial dysfunction is one of the causes COPD, and accumulations of damaged mitochondria are found in lung tissues[22, 23]. Mitophagy plays a protective role in COPD for it

select removal of damaged or dysfunctional mitochondria. The function and regulation of PINK1-mediated on the reactive oxygen species inflammasome remain unknown in lung disease, including COPD. In this study, we showed that Pink1-mediated mitophagy was induced in HBEC in COPD, both in vivo and in vitro. Then, we demonstrated the promotes roles of lncRNA SAL-RNA1 induced Pink1-mediated mitophagy in COPD by using SAL-RNA1 knockout mice, siRNA, and pcRNA[24]. Furthermore, we elucidate that a deficiency of Pink1-mediated mitophagy increased mitochondrial ROS production, airspace was enlarged, contributing to AECs senscence in COPD[25, 26]. However, the molecular mechanisms that regulated Pink1-parkin pathway activation in mitophagy induced senscence of HBEC are not fully discovered.

Sirt1, a family of NAD-dependent deacetylases, has been reported that it may participate in aging and age-related pathologies[27]. Recent papers demonstrated that there be a connection between Sirt1 activity and the induction of autophagy[28]. Sirt1 knockdown decreased the amount of alveoli and suppressed HBEC senscence-related signaling pathway SIRT1/FOXO3,thereby aggravating the injury of COPD[29]. In addition, Sirt1 knockdown reduces the expression of Parkin, decrease Parkin to the mitochondria, and attenuating the mitophagy of HBEC treated with CS[30]. In this study, the Sirt1 protein level was decreased in lungs of COPD mice and CS treated HBEC. Silencing Sirt1 reduces protein level of Parkin, whereas the activation of Sirt1 promotes mitophagy and suppressed CS induced cellular senscence, which were consistent with previous reported.

Many research has demonstrated that lncRNAs can regulate the stability of protein though binding to these protein[12, 31].In this study, Representative histology and pathological showed that the injury is substantially severity in lungs of SAL-RNA1$^{-/-}$ mices exposed to CS than of normal mices exposed to CS. We and other previously shown that the level of Sirt1 is decreased in the lung of patients with COPD[32]. We found lncRNA SAL-RNA1 could bind to Sirt1, and positively regulate the Sirt1 protein level. In our study, we demonstrated that SAL-RNA1 could increase the interaction of Sirt1 and Pink1.The ubiquitination and ubiquitindependent proteolysis of Sirt1 were promote by siRNA-SAL-RNA1, indicating lncRNA SAL-RNA1 reduce the ubiquitination and degradation of Sirt1. We results illustrates that lncRNA SAL-RNA1 provide a higher degree of complexity to the control of pathogenesis of COPD, which may promotes the autophagy signaling and gave rise to the airspace enlarged and the FEV20 declined.

Mitochondria provide an essential source of energy to drive cellular processes and are particularly importantly in COPD progression[33]. Cumulative evidence demonstrates the importance of mitochondrial quality control in lung function during autophagy and aging[34]. It is increasingly recognized that mitophagy is critical for attenuating mitochondrial impairment, especially the damaged mitochondrial clearance[35]. Pink1 mediated autophagy signaling promoted the damaged mitochondrial clearance and contributed to detoxified[36]. Overexpression of SAL-RNA1 upregulated expression levels of autophagy receptors, such as LC3,in HBEC. Overexpression of SAL-RAL1 showed a reduction in ROS accumulation, while inhibite of Sirt1,Pink1,or LC3 abolished this protective effect, indicating that SAL-RNA1 regulated ROS pathology via Sirt1-Pink1 activated mitophagy[37, 38].

COPD represents the leading cause of death across the world. It is invaluable to understand the function of lncRNA SAL-RNA1 in regulation mitophagy which protects lung from airspace enlarged and the FEV20 declined.

Abbreviations

CS: cigarette smoke; COPD:chronic obstructive pulmonary disease; PINK1:phosphatase and tensin homolog (PTEN)-induced putative protein kinase 1 ; EC:epithelial cells ; lncRNAs:long non-coding RNAs; HBEC:Human bronchial epithelial cells ;CSE:Cigarette smoke extract ; NC:negative control

Declarations

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Author's contributions

Hui Jiang and **Yaqing Li** carried out the experiment studies, participated in the sequence alignment and drafted the manuscript. **Hui Jiang** and **Yaona Jiang** carried out the histopathological and immunohistochemical examination. **Dong Yuan** participated in the animal study. **Hui Jiang** participated in the design of the study and performed the statistical analysis. **Yaqing Li** conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The software and all relevant raw data are freely available to scientists.

Ethics approval and consent to participate

The ethics approval was obtained from Ethics Committee of Zhejiang Provincial People's Hospital, People's Hospital of Hangzhou Medical College.

Consent for publication

Not applicable

Human and animal rights

We obey to the rules of Ethic Committee of Animal Rights in the present study.

Competing interests

The authors declare that there are no conflicts of interest.

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Figures

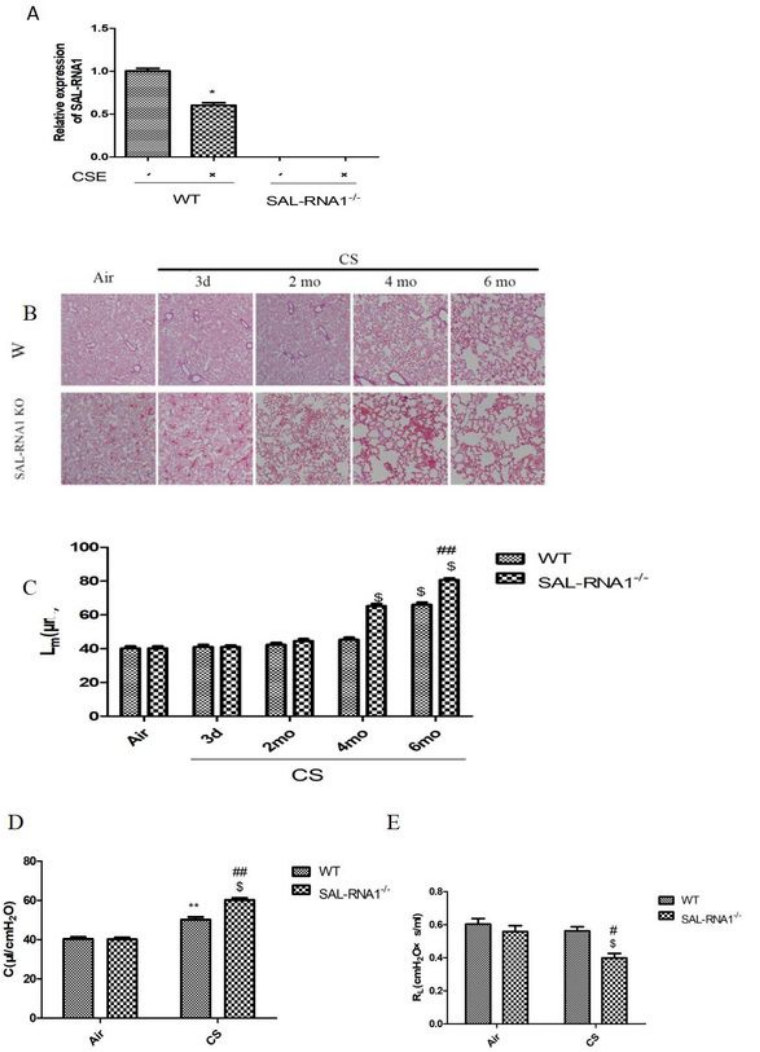

Figure 1.SAL-RNA1 deficiency augmented airspace enlarge and increased lung compliance in emphysematous mice.A LncRNA SAL-RNA1 expression in lung was detected using qRT-PCR.B and C:SAL-RNA1 KO mice susceptible to developing airspace in response to 6 months of CS exposure. D and E: Lung compliance was increased in SAL-RNA KO mice compare with WT littermates in response to 6 months of CS exposure. H & E-stained images are representative of experiments from 2 separate mice. Original magnification, × 100.Scale bars:100um.n=3-4 per group.*p＜0.05,**p＜0.01,$p<0.01versus air;#p<0.05,##p＜0.01versus WT.

Figure 1

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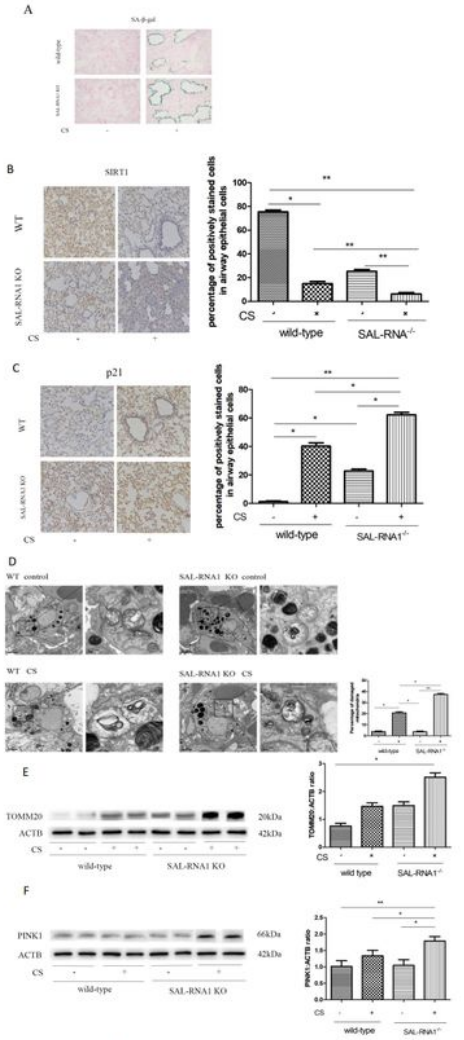

Figure 2. Accelerated cellular senescence,alteration of mitochondrial structure,mitochondrial mass in SAL-RNA1$^{-/-}$ mice after 6 months cigarette smoke exposure. A:CS exposure increased lung SA-β-gal activity, whereas SA-β-gal activity was increased by SAL-RNA1 deficiency when exposed to CS for 6 months. B:Immunohistochemical staining of SIRT1 in common-air and CS-exposed mouse lungs.Bar:100 μm.The lower panel is the percentage(average ± SEM) of positively stained away epithelial cells.*p<0.05,**p<0.001,by ANOVA and Tukey post-hoc test.C:Immunohistochemical staining of p21 in control-air and CS-exposed mouse lungs. Bar: 100 μm. The lower panel is the percentage (average ± SEM) of positively stained airway epithelial cells. *P < 0.05, **P < 0.001, by ANOVA and Tukey post-hoc test.D:Electron microscopy detection of mitochondria in airway epithelial cells with cilia of control-air- and CS-exposed mouse lungs. Bar: 2 μm. Shown in upper panel is percentage of damaged mitochondria (average ± SEM) taken from 10 image fields (10,000 X) for each sample (n = 4 in each group). Shown in the lower panel is average (±SEM) of mitochondrial counts taken from 10 image fields (10,000 X) for each sample (n = 4 in each group). *P < 0.05, **P < 0.001, by ANOVA and Bonferroni post-hoc test.E:WB using anti-TOMM20 and anti-ACTB antibodies, of lung homogenates from control-air- and CS-exposed mice for 6 months. The lower panel is the average (±SEM) taken from densitometric analysis of WB. Treatment groups were composed of control-air-exposed wild-type mice (n = 10), CS-exposed wild-type mice (n = 9), control-air-exposed SAL-RNA1 KO mice (n = 8), CS-exposed SAL-RNA1 KO mice (n = 9) *P < 0.05, by ANOVA and Tukey post-hoc test. F: WB using anti-PINK1 and anti-ACTB antibodies, of lung homogenates from control-air- and CS-exposed mice for 6 months. The lower panel is the average (±SEM) taken from densitometric analysis of WB. *P < 0.05, by ANOVA and Tukey post-hoc test.

Figure 2

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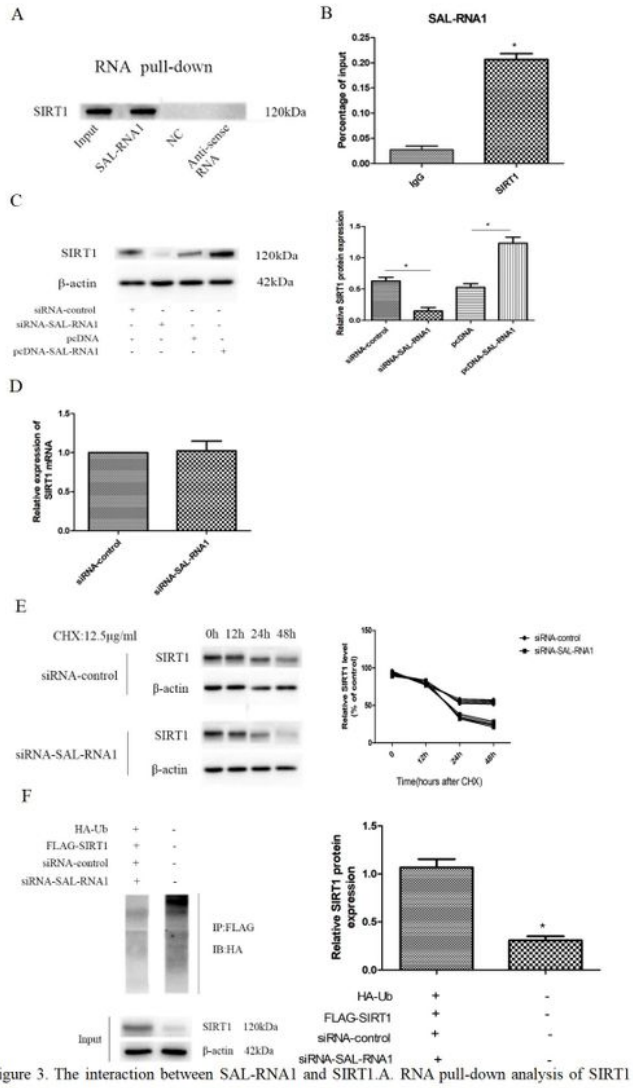

Figure 3. The interaction between SAL-RNA1 and SIRT1.A. RNA pull-down analysis of SIRT1 expression in the SAL-RNA1 pull-down complex.NC,negative control of the SAL-RNA1 group. B. RIP analysis of SAL-RNA1 expression in SIRT1 precipitate. IgG was used as a negative control. *p<0.05vs IgG. C.SIRT1 protein level in HBEC transfected siRNA-control, siRNA-SAL-RNA1,pcDNA, pcDNA-SAL-RNA1.D Relative mRNA levels of SIRT1 in HBEC which were transfected with siRNA-control or siRNA-SAL-RNA1.E. HBEC were transfected with siRNA-control or siRNA-SAL-RNA1,then treated with CHX for 0h,12h,24h and 48h. SIRT1 protein level in HBEC at different time points was detected using western blot. F. HBEC were transfected with HA-Ub, FLAG-SIRT1,siRNA-control,siRNA-SAL-RNA1 for 12h, followed by 10 μ m MG123 treatment for 4 hour. Lysates of HBEC confirmed for SIRT1 expression(bottom) and subjected to immunoprecipitation using an anti-FLAG antibody, followed by immunoblotting with an anti-HA antibody(top).Input,lysates of HBEC.

Figure 3

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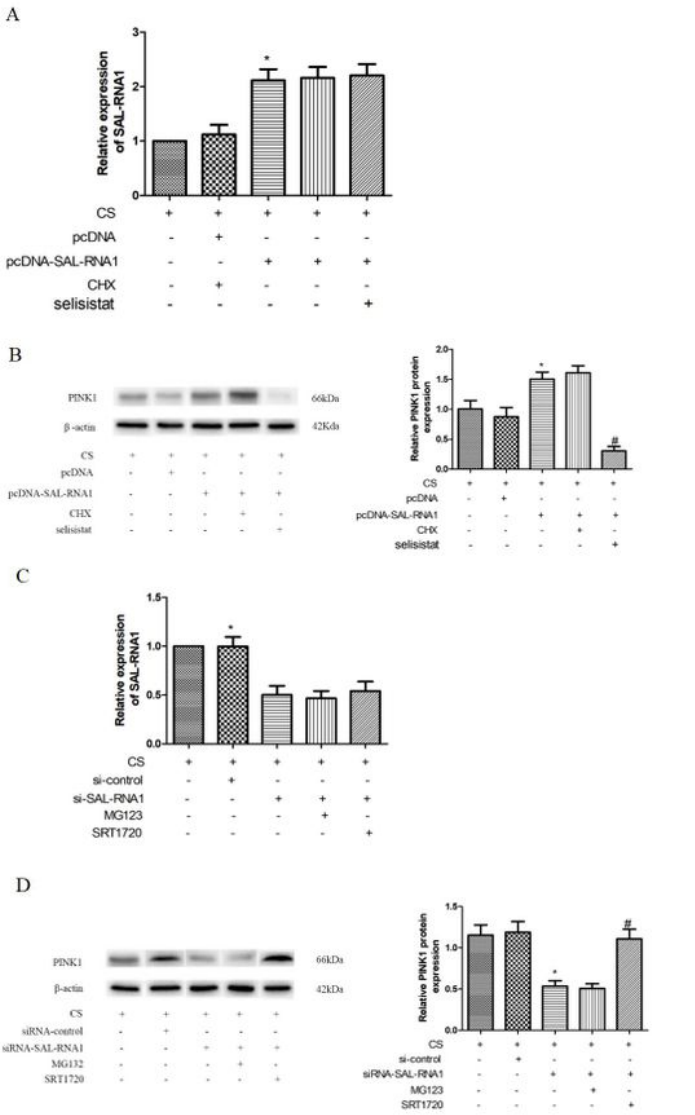

Figure 4. SAL-RNA1 regulated Parkin expression through SIRT1. HBEC were divided into CS,CS+pcDNA,CS+pcDNA-SAL-RNA1,CS+pcDNA-SAL-RNA1+CHX and CS+pcDNA-SAL-RNA1 +selisistat groups.A. The expression level of SAL-RNA1 in HBEC was measured by qRT-PCR.B. The protein level of PINK1 in HBEC were detected using western blot. HBEC divided into CS,CS+siRNA-control,CS+siRNA-SAL-RNA1,CS+siRNA-SAL-RNA1+MG123,CS +siRNA-SAL-RNA1+SRT1720 groups.C The expression level of SAL-RNA1 in HBEC was measured by qRT-PCR. D. The protein level of PINK1 in HBEC ere detected using western blot. *p<0.05vs CS+pcDNA or CS+siRNA-control; #p<0.05vs CS+pcDNA SAL-RNA1+CHX or CS+siRNA-SAL-RNA1+MG123.

Figure 4

See image above for figure legend.

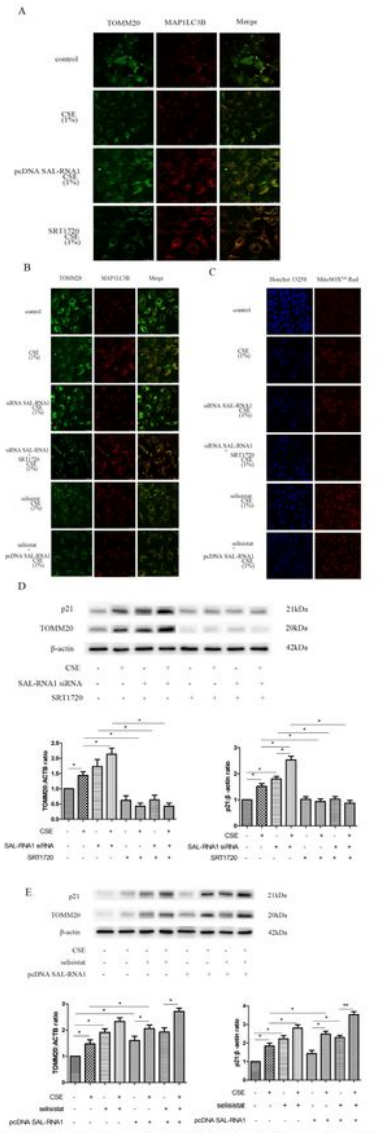

Figure 5.Dominant role of SIRT1 in the regulation of CSE-induced mitophagy.A. Colocalization analysis of confocal laser scanning microscopy images of TOMM20 staining and MAP1LC3B staining. HBEC were transfected with pcDNA SAL-RNA1, treated with SRT1720.CSE(1%) treatment was started 48h post-transfection or SRT1720 administration.Baf(A1(20nM) treatment was started 6h before fixation and HBEC were fixed after 24 hour treatment with CSE. BAR:100μm. Shown percentage in merged image was calculated by dividing yellow intensity of mitophagy area by red intensity of mitochondrial area.B.Colocalization analysis of TOMM20 staining and MAP1LC3B staining. HBEC were transfected with the indicated combination of siRNA and SRT1720 administration,or transfected with the indicated combination of pcDNA and selisistat, respectively. Bar: 100 μm. Shown percentage in merged image was calculated by dividing yellow intensity of mitophagy area by red intensity of mitochondrial area.C.Photographs of Hoechst 33258 and MitoSOX Red fluorescence staining. HBEC were transfected with the indicated combination of siRNA and SRT1720 administration,or transfected with the indicated combination of pcDNA and selisistat, respectively. CSE (1%) treatment was started 48 h post-transfection or SRT1720 administration. HBEC were fixed after 24-h treatment with CSE. Bar: 100 μm.D-E. WB using anti-p21, anti-TOMM20, and ACTB antibodies. HBEC were transfected with the indicated combination of siRNA and SRT1720 administration,or transfected with the indicated combination of pcDNA and selisistat, respectively. CSE (1%) treatment was started 48 h post-transfection or SRT1720 administration,and protein samples were collected after 48-h treatment. Shown is a representative experiment of 4 showing similar results. The middle panel is the average (±SEM) relative increase in p21 normalized to ACTB and the right panel is the average (± SEM) relative increase in TOMM20 normalized to ACTB, which are taken from densitometric analysis of WB from 4 independent experiments. *P < 0.05, by paired Student t test.

Figure 5

See image above for figure legend.