SIRT1 redresses the imbalance of tissue inhibitor of matrix metalloproteinase-1 and matrix metalloproteinase-9 in the development of mouse emphysema and human COPD

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SIRT1 redresses the imbalance of tissue inhibitor of matrix metalloproteinase-1 and matrix metalloproteinase-9 in the development of mouse emphysema and human COPD. Am J Physiol Lung Cell Mol Physiol 305: L615–L624, 2013. First published September 13, 2013; doi:10.1152/ajplung.00249.2012.—Sirtuin1 (SIRT1), a protein/histone deacetylase, protects against the development of pulmonary emphysema. However, the molecular mechanisms underlying this observation remain elusive. The imbalance of tissue inhibitor of matrix metalloproteinases (TIMPs)/matrix metalloproteinases (MMPs) plays an important role in the pathogenesis of chronic obstructive pulmonary disease (COPD)/emphysema. We hypothesized that SIRT1 protects against emphysema by redressing the imbalance between MMPs and TIMPs. To test this hypothesis, SIRT1-deficient and overexpressing/transgenic mice were exposed to cigarette smoke (CS). The protein level and activity of MMP-9 were increased in lungs of SIRT1-deficient mice exposed to CS compared with wild-type (WT) littermates, and these effects were attenuated by SIRT1 overexpression. SIRT1 deficiency decreased the level of TIMP-1, which was augmented in SIRT1 transgenic mice compared with WT littermates by CS. However, the level of MMP-2, MMP-12, TIMP-2, TIMP-3, or TIMP-4 was not altered by SIRT1 in response to CS exposure. SIRT1 reduction was associated with imbalance of TIMP-1 and MMP-9 in lungs of smokers and COPD patients. Mass spectrometry and immunoprecipitation analyses revealed that TIMP-1 acetylation on specific lysine residues was increased, whereas its interaction with SIRT1 and MMP-9 was reduced in mouse lungs with emphysema, as well as in lungs of smokers and COPD patients. SIRT1 deficiency increased CS-induced TIMP-1 acetylation, and these effects were attenuated by SIRT1 overexpression. These results suggest that SIRT1 protects against COPD/emphysema, in part, via redressing the TIMP-1/MMP-9 imbalance involving TIMP-1 deacetylation. Thus redressing the TIMP-1/MMP-9 imbalance by pharmacological activation of SIRT1 is an attractive approach in the intervention of COPD.

SIRT1; cigarette smoke; MMPs; TIMPs; emphysema; COPD

SIRTUIN1 (SIRT1), a type III histone/protein deacetylase, plays an important role in inflammation, stress resistance, and cellular senescence/aging through the deacetylation of histones, transcription factors, and signaling molecules. It is a redox-sensitive deacetylase that can be posttranslationally modified by oxidants and carbonyl stress, leading to proteasomal degradation (9, 36, 59). This may be one of the reasons for the reduction of SIRT1 level in peripheral lung tissues of smokers and patients with chronic obstructive pulmonary disease (COPD/emphysema (36, 55). Recently, we have shown that SIRT1 protects against pulmonary emphysema in mice (55). However, the mechanisms of SIRT1 in preventing the progression of COPD/emphysema are not fully understood.

The imbalance of tissue inhibitors of metalloproteinases (TIMPs)/matrix metalloproteinases (MMPs) in the lungs has been implicated in the development of COPD/emphysema (4, 7, 17, 29). Genetic overexpression of MMP-9 causes air space enlargement, whereas deficiency of MMP-12 attenuates cigarette smoke (CS)-induced pulmonary emphysema in mice (14, 16). It has been shown that TIMPs are subjected to posttranslational modifications, such as acetylation, oxidation, and nitration, thereby regulating their ability to inhibit MMPs (34, 39, 48, 50, 52). However, it is unknown whether TIMPs undergo these posttranslational modifications, particularly in response to CS leading to upregulation of MMPs, and whether SIRT1 has a regulatory role in redressing the TIMP/MMP imbalance. In light of these findings, we hypothesized that SIRT1 regulates TIMP/MMP imbalance during the development of pulmonary emphysema. To test this hypothesis, SIRT1 heterozygous knockout (SIRT1+/−) and SIRT1 overexpressing/transgenic (SIRT1 Tg), as well as their wild-type (WT) littermates were exposed to CS for 6 mo, and the lung levels and activities of TIMPs and MMPs were measured in these mice. Furthermore, the peripheral lung tissues from smokers and patients with COPD were used to determine the association of SIRT1 reduction with TIMP/MMP imbalance. In addition, we determined the acetylation of TIMP-1, as well as its association with MMP-9 and SIRT1, using the mass spectrometry and immunoprecipitation approaches so as to reveal the potential mechanisms underlying the protection of SIRT1 against pulmonary emphysema.

MATERIALS AND METHODS

Ethics statement. All experiments for animal studies were performed in accordance with the standards established by the United States Animal Welfare Act, as set forth by the National Institutes of Health guidelines. The research protocol for mouse studies was approved by the Institutional Animal Care and Use Committee of the University of Rochester Medical Center.
approved by the University Committee on Animal Research Committee of the University of Rochester. The use of human tissue was approved by the ethics committee of the Helsinki University Central Hospital, Helsinki, Finland. All subjects, including nonsmokers, smokers, and COPD patients, provided informed consent.

Mice and CS exposure. The generation of SIRT1+/− and SIRT1 Tg mice is described previously with their background WT mice being the 129/SvJ and C57Bl/6×129/SvJ strains, respectively (6, 28). SIRT1+/− mice were used in this study, since SIRT1 homozygous knockout mice have low perinatal survival rate (28). Lung SIRT1 protein level was decreased in SIRT1+/− mice, whereas it was increased in SIRT1 Tg mice compared with their WT littermates (55). These mice were housed in the vivarium facility of the University of Rochester with a 12-h light-dark cycle (light on at 6:00 AM). Eight-week-old male mice were used for CS exposure as described previously (54, 55). Briefly, 3R4F cigarettes were used to generate a mixture of sidestream smoke (89%) and mainstream smoke (11%) by a Teague smoking machine (model TE-10, Teague Enterprises, Woodland, CA) at a concentration of ~100 mg/m3 total particulate matter so as to avoid the possible toxicity to mice at a high concentration of long-term CS exposure. Each smoldering cigarette was puffed for 2 s, once every minute for a total of 8 puffs, at a flow rate of 1.05 l/min, to provide a standard puff of 35 cm3. Mice received 5-h exposures per day, 5 days/wk for 6 mo, and were euthanized at 24 h after the last CS exposure.

Human samples. The total 37 lung tissue specimens from life-long nonsmokers, current smokers with normal lung function, and COPD patients were collected by the Department of Medicine and Pathology, Helsinki University Central Hospital (36, 55). The lung samples from three to nine subjects from the above pool in each group of non-smoker, smoker, and COPD were used in the assays. COPD was defined according to the Global Initiative for COPD (GOLD) criteria [forced expiratory volume in 1 s (FEV1) < 80% of predicted, FEV1/forced vital capacity (FVC) < 70% and bronchodilatation effect < 12%]. None of the patients had suffered from acute exacerbation for 2 mo. Tumor-free peripheral lung tissues were immediately stored at −80°C for subsequent immunoblotting and immunoprecipitation assays. The clinical characteristics of the patients used have been described in detail previously (36).

Preparation of whole cell lysate from lung tissues. The preparation of whole cell lysate from lung tissues were described previously (56, 57). Briefly, 100 mg of lung tissue was mechanically homogenized with 0.5 ml of radioimmunoprecipitation assay (RIPA) buffer (50 mmol/l Tris·HCl, 150 mmol/l NaCl, 1 mmol/l EDTA, 0.25% deoxycholate, 1 mmol/l Na3VO4, 1 mmol/l NaF, 1 mg/l leupeptin, 1 mg/l aprotinin, and 1 mmol/l phenylmethylsulfonyl fluoride), and the tissue

Fig. 1. Sirtuin1 (SIRT1) inhibits cigarette smoke (CS)-induced increase of matrix metalloproteinase (MMP)-9 level in mouse lungs. SIRT1 heterozygous knockout (SIRT1+/−), SIRT1 overexpressing/transgenic (SIRT1 Tg), and wild-type (WT) mice were exposed to CS for 6 mo, and the levels of MMPs in the lungs were determined by immunoblotting. CS exposure increased the levels of MMP-9, which were attenuated by SIRT1 overexpression. However, SIRT1 did not exhibit any effect on the level of MMP-2 or MMP-12, although the levels of these enzymes were increased in mouse lungs exposed to CS. Gel pictures shown are representative of at least 3 separate mice. Fold change is indicative of the alteration of MMP-2, MMP-9, and MMP-12 compared with air-exposed WT mice after normalization to GAPDH or β-actin. The reassembly of noncontiguous gel lanes is demarcated by white spaces/boxes. Data are shown as means ± SE (n = 3 to 4 per group). *P < 0.05, **P < 0.01, significant compared with the corresponding air-exposed mice; ***P < 0.001, significant compared with CS-exposed corresponding WT mice.

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homogenates were kept on ice for 45 min to allow total cell lysis. Following centrifugation at 13,000 g in an Eppendorf tube for 5 min, the supernatant was collected as whole cell lysate. The protein levels were measured by use of a BCA kit (Pierce, IL).

**Immunoblot.** Protein samples from lung homogenates were separated on a 7.5–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and separated proteins were electroblotted onto nitrocellulose membranes (Amersham, Arlington Heights, IL). The membranes were blocked for 1 h at room temperature with 5% BSA and then probed with a 1:400–1:1,000 diluted antibodies of anti-MMP-2, anti-MMP-9, anti-MMP-12, anti-TIMP-1, anti-TIMP-2, anti-TIMP-3, and anti-TIMP-4 (Santa Cruz Biotechnology, Santa Cruz, CA), as well as anti-SIRT1 (Cell Signaling, Danvers, MA). The MMP-9 or SIRT1 polyclonal antibodies were used to determine the corresponding proteins. After three washing steps (10 min each), the level of protein was detected using secondary antibody [1:5,000 dilution in 2.5% BSA in PBS containing 0.1% Tween 20 (vol/vol) for 1 h] linked to horseradish peroxidase (Dako, Santa Barbara, CA), and bound complexes were detected by enhanced chemiluminescence (Perkin Elmer, Waltham, MA). Equal loading of the samples was determined by quantification of proteins as well as by reprobing membranes for a housekeeping control (H9252–actin or GAPDH).

**Immunoprecipitation.** Whole lung lysate were used for TIMP-1 immunoprecipitation with a polyclonal antibody against TIMP-1 (1:40 dilution, Santa Cruz Biotechnology), which was added to 200–250 µg of sample proteins in a final volume of 400 µl and incubated for 1 h. Protein-A/G agarose beads (20 µl) (Santa Cruz Biotechnology) were added to each sample and kept overnight at 4°C on a rotating rocker. The beads were washed three times, and then resuspended in 40 µl of RIPA buffer. For immunoblot, the immunoprecipitated TIMP-1 agarose bead suspension was resolved by SDS-PAGE gradient gels. IgG alone and lung tissue lysate (20 µg protein) without TIMP-1 antibody were used as a negative and positive control, respectively. To assess TIMP-1 acetylation, the membranes of immunoprecipitated TIMP-1 were blotted against the anti-acetyl lysine antibody (Cell Signaling, Danvers, MA). The MMP-9 or SIRT1 polyclonal antibodies were used to determine the association of TIMP-1 with MMP-9 and SIRT1, respectively.

**MMPs activity assay by zymography.** The zymography was performed to determine the activity of MMPs in mouse lungs as described previously (46, 47). Briefly, the lung tissue was homogenized with 50 mM Tris·HCl (pH 7.4) on ice, and lung homogenates that contained 200 µg of protein were mixed with an equal volume of sample buffer [80 mmol/l Tris·HCl (pH 6.8), 4% SDS, 10% glycerol, 0.01% bromophenol blue]. For electrophoresis, 7.5% SDS-polyacrylamide resolving gels that contained 1 mg/ml gelatin were overlaid with 5% stacking gels, and the samples were loaded and run at 4°C (25 mA/gel). The gels were rinsed briefly with distilled water and washed three times (15 min each) with 150 ml of 2.5% Triton X-100 solution on a rotary shaker after electrophoresis. The gels were then incubated at 37°C for 18 h in 250 ml of 50 mmol/l Tris·HCl (pH 7.5) that contained 10 mmol/l CaCl2, 1% Triton X-100, and 0.02% NaN3. After incubation, the gels were stained with 100 ml of 0.5% Coomassie blue solution in 10% acetic acid/50% methanol for 1 h. The gels were destained with 10% acetic acid/50% methanol until clear bands appeared and the gels were dried on a flat bed of Mylar. The gels were then scanned and band intensity was quantified by ImageJ software (http://rsbweb.nih.gov/ij/). The densitometric intensity of the bands was quantified by ImageJ and data were expressed as fold change relative to the control group.

**Fig. 2.** SIRT1 inhibits CS-induced increase of MMP-9 activity in mouse lungs. SIRT1+/−, SIRT1 Tg, and WT mice were exposed to CS for 6 mo, and the activities of MMPs in the lungs were determined by zymography. CS exposure increased the activity of MMP-9, which were attenuated by SIRT1 overexpression. However, SIRT1 did not exhibit any effect on the level or activity of MMP-2 or MMP-12. Gel pictures shown are representative of at least 3 separate mice. Fold change is indication of the alteration of MMP-2, MMP-9, and MMP-12 activities compared with air-exposed WT mice. The reassembly of noncontiguous gel lanes is demarcated by white spaces/boxes that are from the same gel but are aligned based on the orders as presented in other figures. Data are shown as means ± SE (n = 3 to 4 per group). **P < 0.01, ***P < 0.001, significant compared with the corresponding air-exposed mice; +++P < 0.01, significant compared with CS-exposed WT mice.
50% methanol, 10% acetic acid, and 0.1% Coomassie blue R-250 for 3 h, then destained with 50% methanol and 10% acetic acid. After being destained, the gels were immersed in distilled water for 20 min and scanned immediately.

LC-MS/MS analysis for specific TIMP-1 residue acetylation. Immunoprecipitated TIMP-1 samples resolved by SDS-PAGE followed by band excision were digested with trypsin overnight (43). Peptides were twice extracted with 50% acetonitrile containing 5% trifluoroacetic acid. For LTQ (linear trap quadrupole) analysis, 2 μl of each sample was loaded onto a home-pulled, and home-packed C18 analytical column. The tip was pulled to ~10 μm with a Sutter Laser puller. Columns were packed to 10 cm with C18 AQ 5 μm 200 Å media (Michrom) by use of a pressure bomb. The internal diameter of the columns used was 75 μm. Peptides were eluted with the following chromatographic profile: 5% solvent B [liquid chromatography (LC)/mass spectrometry (MS) grade methanol, Burdick & Jackson] for 6 min, ramping to 20% solvent B over 1 min then to 60% solvent B over 113 min, washing at 95% solvent B for 3 min, and finally returning to initial run conditions with solvent A as LC/MS grade water (Burdick & Jackson) plus 0.1% formic acid (Pierce) and solvent B plus 0.1% formic acid. The flow rate was 350 nl/min. Instrument specific parameters included analysis by data-dependent MS/MS mode, where a survey scan was performed followed by MS/MS analysis of the top seven analytes in each survey scan. Once fragmented, each analyte was placed on an exclusion list for 45 s to avoid repetitive identifications. Helium was used as collision gas, with an activation Q of 0.25, activation time of 30 ms, and a normalized collision energy of 35%. For data processing, LTQ files were converted from .raw files to .mgf files by use of BioWorks Browser (Thermo). These files were imported into ProteinScape (Bruker Daltonics) for database searching. MASCOT was used to search the mouse database (available for download from GPM at ftp://ftp.thegpm.org/fasta/eukaryote/mouse_chromosomes/) (35). For LTQ data set searches, tolerances were 1.5 Da for MS and 0.8 Da for MS/MS. All instruments included 1 for #13C as part of the search parameters. All matched spectra were manually verified by use of BioTools (Bruker Daltonics).

Statistical analysis. Statistical analysis of significance was calculated by one-way ANOVA followed by Tukey’s post hoc test for multigroup comparisons using StatView. The results are shown as means ± SE. *P < 0.05 is considered as statistically significant.

RESULTS

Overexpression of SIRT1 decreases the protein level and activity of MMP-9 in mouse lungs with emphysema. Imbalance of MMP/TIMP plays an important role in the pathogenesis of COPD (4, 7, 17, 29). Hence we determined the protein levels and activities of MMP-2, MMP-9, and MMP-12 in lungs of SIRT1−/− and SIRT1 Tg mice as well as their WT littermates exposed to CS by immunoblotting and zymography. Chronic
CS exposure reduced the protein level of SIRT1 in mouse lung (55). As shown in Figs. 1 and 2, chronic CS exposure increased the levels and activities of MMP-2, MMP-9, and MMP-12 in lungs of WT mice. Interestingly, SIRT1 deficiency increased the MMP-9 level and activity, whereas the level and activity of MMP-9 were reduced in lungs of SIRT1 transgenic mice compared with their WT littermates in response to CS exposure. However, the protein level or activity of MMP-2 or MMP-12 was not affected by SIRT1 in lungs of 6-mo CS-exposed mice. These results suggest that SIRT1 specifically reduces increased abundance and activity of MMP-9 in mouse lungs with emphysema.

Overexpression of SIRT1 prevents the TIMP-1 reduction in lungs of emphysematous mice. It is well known that the activities of MMPs are regulated by TIMPs through their NH2-terminal inhibitory domain (34, 39, 48, 50, 52). Hence the levels of TIMPs were determined in lungs of SIRT1+/− and SIRT1 Tg mice as well as WT littermates in response to CS exposure. The protein level of TIMP-1 was significantly decreased in lungs of SIRT1-deficient mice compared with WT littermates exposed to CS, and these effects were attenuated by SIRT1 overexpression (Fig. 3). However, the protein level of TIMP-2, TIMP-3, or TIMP-4 was not altered either by CS exposure or by loss or gain of SIRT1 function in mouse lungs (Fig. 3). Both genetic overexpression and pharmacological activation of SIRT1 have been shown to protect against pulmonary emphysema (55). Therefore, redressing the TIMP-1/MMP-9 imbalance by SIRT1 overexpression may contribute to its protection against pulmonary emphysema in mice.

Fig. 4. TIMP-1/MMP-9 imbalance and SIRT1 reduction occurs in lungs of smokers and chronic obstructive pulmonary disease (COPD) patients. The levels of TIMP-1, MMP-9, and SIRT1 were determined in lungs from nonsmokers, smokers, and patients with COPD by immunoblotting. The level of MMP-9 was increased, whereas TIMP-1 was reduced in smokers and COPD patients compared with nonsmokers. Similar to our previous findings (36, 55), the level of SIRT1 was reduced in lungs of smokers and COPD patients when compared with nonsmokers (Fig. 4). All these data suggest that SIRT1 reduction is associated with TIMP-1/MMP-9 imbalance in lungs of smokers and patients with COPD. To further study the association of TIMP-1/MMP-9 imbalance with SIRT1 reduction, the levels of TIMP-1, MMP-9, and SIRT1 were determined in lungs from nonsmokers, smokers, and COPD patients by immunoblotting. As shown in Fig. 4, the level of TIMP-1 was decreased, whereas the level of MMP-9 was increased in lungs of smokers compared with nonsmokers. Furthermore, the imbalance of TIMP-1/MMP-9 was more prominent in lungs of patients with COPD compared with smokers. Similar to our previous findings (36, 55), the level of SIRT1 was reduced in lungs of smokers and COPD patients when compared with nonsmokers (Fig. 4). All these data suggest that SIRT1 reduction is associated with TIMP-1/MMP-9 imbalance in lungs of smokers and patients with COPD.
suggest that SIRT1 reduction is associated with imbalance of TIMP-1 and MMP-9 in smokers and COPD patients.

TIMP-1 acetylation was increased in mouse lungs exposed to CS and in lungs of COPD patients. TIMPs can be posttranslationally modified, resulting in alteration of their ability to inhibit MMPs (34, 39, 48, 50, 52). We therefore hypothesized that CS exposure caused TIMP-1 protein posttranslational modifications, such as acetylation. First, the LC-MS/MS technique was employed to identify the specific TIMP-1 residues that undergo acetylation in mouse lungs. The fragment ions for the peptides YIKIKMTKMLK and MMAPFASLASGILLLLSLIASSK were observed in samples from air- and CS-exposed mouse lungs, which were used for the representation of final spectrum data (Yao et al., data not shown). The basal acetylation on lysines 68 and 71 of TIMP-1 was shown in mouse lungs regardless of air or CS exposure (Fig. 5, A and B). The lysine 33 of TIMP-1 was identified to be acetylated in CS-exposed, but not in air-exposed, mouse lungs (Fig. 5, A and B). TIMP-1 immunoprecipitation also showed that CS exposure increased the acetylation of TIMP-1 on lysine residues in mouse lungs (Fig. 6A). This was associated with decreased interaction of TIMP-1 with MMP-9 (Fig. 6B). Similarly, the level of TIMP-1 acetylation on lysine residues was increased in lungs of smokers and COPD patients compared with nonsmokers (Fig. 6C). These results demonstrate that TIMP-1 acetylation was increased in mouse lungs with emphysema and in lungs of patients with COPD.

SIRT1 reduces TIMP-1 acetylation in mouse lungs exposed to CS. To determine whether SIRT1 reduction contributes to the increased TIMP-1 acetylation in response to CS exposure, we performed TIMP-1 immunoprecipitation and found that the association of TIMP-1 with SIRT1 was reduced in CS-exposed mouse lungs (Fig. 7A). Importantly, the levels of TIMP-1 acetylation were increased in SIRT1−/− mice in response to CS compared with WT littermates, which was significantly attenuated by SIRT1 overexpression (Fig. 7B). These results suggest that, in addition to SIRT1 reduction, CS-induced disruption of SIRT1’s association with TIMP-1 increases TIMP-1 acetylation in mouse lungs.

DISCUSSION

An imbalance of protease-antiprotease is a key perpetuating factor in tissue remodeling and development of COPD/emphysema (4, 7, 14, 16, 17, 21, 29, 41). Recent studies have shown the regulation of TIMPs and MMPs by SIRT1 (10, 23, 31–33). However, the role of SIRT1 in redressing the TIMP/MMP imbalance leading to the protection of pulmonary emphysema is not known. We therefore determined the effect of loss and gain of SIRT1 function on tissue remodeling via TIMPs/MMPs in the development of COPD/emphysema. We proposed that redressing the TIMP/MMP imbalance would be one of the mechanisms underlying the protection of SIRT1 against emphysema in mice. Consistent with previous studies (16, 56), CS exposure caused MMP/TIMP imbalance, as reflected by increased levels/activities of MMP-2, MMP-9, and MMP-12, as well as reduced levels of TIMP-1 in mouse lungs with emphysema. This is in agreement with the findings that the level of MMP-9 was increased, whereas TIMP-1 level was reduced in lungs of smokers and COPD patients compared with nonsmokers. However, the level of TIMP-2, TIMP-3, or TIMP-4 was...
not altered by CS exposure. Moreover, it is interesting to note that the level of MMP-2 or MMP-12 was not altered, whereas MMP-9 level and activity were negatively regulated by SIRT1 in mouse lungs with emphysema. Furthermore, SIRT1 protected against reduction of TIMP-1 protein in mouse lungs exposed to CS. Overall, these observations suggest that the protection against emphysema by SIRT1 is associated with its ability to redress TIMP-1/MMP-9 imbalance in mouse lung.

Accumulating evidence has shown that acetylation of TIMPs impairs their ability to inhibit the activity of MMPs when they are expressed in bacteria (48, 50). Furthermore, TIMP-1 can be accumulated into the nucleus, although it mainly localized in cytoplasm (25, 40, 60). In light of the deacetylase activity and nuclear localization of SIRT1, we proposed that TIMP-1 protein would be acetylated by CS exposure, which can be regulated by SIRT1 in mouse lung. As expected, CS exposure led to TIMP-1 acetylation on lysine 33, despite the basal TIMP-1 acetylation on lysines 68 and 71 was observed in mouse lungs. Similarly, TIMP-1 acetylation on lysine residues was increased in lungs of smokers and COPD patients. Furthermore, the interaction of TIMP-1 with MMP-9 was impaired by CS exposure in mouse lungs. Thus CS-induced TIMP-1 acetylation may disrupt its association with MMP-9 or impair its MMP-inhibitory activity leading to activation of MMP-9. Further study is required to investigate whether alteration of TIMP-1 acetylation affects CS-induced development of emphysema. The possibility of other posttranslational modifications, such as carbonylation and nitration, by CS cannot be ruled out, since both oxidation and nitration modifications regulate TIMP-1’s ability to inhibit MMP activities (34, 39, 52). Importantly, CS-induced TIMP-1 acetylation was increased in lungs of SIRT1-deficient mice, and this effect was significantly attenuated by SIRT1 overexpression compared with those in WT littermates. In addition, TIMP-1 and SIRT1 association was significantly disrupted by CS exposure in mouse lungs. These findings suggest that CS exposure causes

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**Fig. 6.** TIMP-1 acetylation on lysine residues is increased in mouse lungs with emphysema as well as in lungs of smokers and COPD patients. Lung lysates (250 μg of proteins) from CS-exposed mouse lungs (A and B) and lungs of COPD patients (C) were used for TIMP-1 immunoprecipitation, and the blots were probed with antibodies specific to acetylated lysine (Ac-K, A and C) and MMP-9 (B). Gel pictures shown are representative of at least 3 separate mice. Fold change is indicative of the alteration of acetylated lysine and MMP-9 compared with corresponding air or nonsmoker group after normalization to TIMP-1. Data are shown as means ± SE (n = 3 per group). *P < 0.05, **P < 0.01, significant compared with the air or nonsmoker group. IP, immunoprecipitation; IB, immunoblotting.
SIRT1 reduction/nucleocytoplasmic shuttling and its disassociation with TIMP-1 leading to TIMP-1 acetylation on specific lysine residues, which may reduce its MMP-9-inhibitory activity (9). Further study is required to determine whether this modification has any impact on intracellular proMMP-9 maturation (e.g., glycosylation), TIMP-1/MMP-9 extracellular secretion, TIMP-1 nuclear-cytoplasmic shuttling and degradation, as well as whether SIRT1 activation by pharmacological mean reverses TIMP-1 acetylation. In contrast to immunoblotting, we noticed a similar level of control TIMP-1 in some immunoprecipitation experiments among groups (i.e., between air- and CS-exposed groups, or among nonsmokers, smokers, and COPD patients). This may be due to the pull-down of comparable amount of TIMP-1 protein among samples using the same amount of the antibody.

It is well known that MMP-9 is involved in the pathogenesis of COPD/emphysema (3, 18, 26). Animal studies have demonstrated that MMP-9 overexpression in macrophages causes adult onset of emphysema, whereas IL-13-induced air space enlargement and lung function decline were attenuated in MMP-9 knockout mice (14, 20). This is in agreement with our findings that the level and activity of MMP-9 were increased in mouse lungs with emphysema and in lungs of COPD patients that MMP-9 inhibitor significantly attenuated elastase-induced air space enlargement and abnormal lung mechanics in mice (Yao H et al., unpublished data). However, some other studies have shown that MMP-9-deficient mice were not protected from the development of chronic LPS inhalation and CS exposure-induced emphysema (2, 8). These findings suggest the role of MMP-9 in the development of pulmonary emphysema depends on the mouse model of this disease. This may also be due to the different roles of MMP-9 produced by inflammatory cells (leading to the destruction of lung architecture) or by lung epithelial cells (promoting reepithelization and repair of lung) after injury (5, 11, 15, 19, 24, 51). In addition to activity, the level of MMP-9 was also increased by CS exposure, which may be associated with abnormal NF-κB or FOXO3 activity due to SIRT1 reduction, since both NF-κB and FOXO3 can regulate the production of MMP-9 and TIMP-1 (22, 31, 32, 44, 53, 55, 58). The increased levels and activities of MMPs may also be due to increased recruitment of inflammatory cells (e.g., neutrophils and macrophages) in mouse lungs with emphysema as well as in lungs of smokers and COPD (12, 26).

Both TIMP-1 and MMP-9 play an important role in cellular senescence, proliferation, and apoptosis, which are the pivotal contributing factors in driving the progression of COPD/emphysema (1, 13, 27, 30, 37, 38, 42, 45, 49). This provides a possibility that the protection against experimental emphysema by SIRT1 is due to reduced cellular senescence and apoptosis via redressing of TIMP-1/MMP-9 imbalance in mouse lungs by a mechanism involving dampening the senescence-associated secretory phenotype.

In conclusion, TIMP-1 level was decreased, whereas the level of MMP-9 was increased in mouse lungs with emphysema and in lungs of smokers and patients with COPD, which was associated with SIRT1 reduction. Furthermore, SIRT1 redressed the imbalance of TIMP-1 and MMP-9 in mouse lungs with emphysema. The mechanism underlying these findings is associated with TIMP-1 deacetylation on specific lysine residues.
residues by SIRT1, which may increase its ability to inhibit MMP-9 activity. Redressing TIMP-1/MMP-9 imbalance in lungs partially contributes to the ability of SIRT1 to protect against COPD/empysema. Hence these findings not only highlight the importance of SIRT1 in the development/progres-
sion of COPD/empysema but also provide the rationale for a key and specific therapeutic target via pharmacological SIRT1 activation in ameliorating the progression of this diverse and complex debilitating disease.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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SIRT1 REGULATES TIMP-1 AND MMP-9 IMBALANCE


