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14. ABSTRACT

Chronic exposure to tobacco smoke (TSE) causes irritation of and damage to the lung epithelium. This can lead to chronic obstructive pulmonary disease (COPD), the third leading cause of death in the United States. In COPD, chronic inflammation leads to alveolar wall destruction leading to emphysema. Recent literature clearly suggest that telomere dysfunction is a determinant of emphysema susceptibility in mice and humans. Further, telomere dysfunction due to TSE induces replicative senescence and death of progenitor type II alveolar epithelial cells (A_2Cs) leading to alveolar wall damage and alveolar injury. We found that increased expression of the tumor suppressor protein p53, plasminogen activator inhibitor-1 (PAI-1) and caveolin-1 (Cav1) by A_2Cs are linked to TSE lung injury. Interleukin-17A (IL-17A) levels were also significantly increased in the lungs of patients with severe COPD (GOLD stage III and IV). We found elevated levels of IL-17A in lungs of wild-type (WT) mice exposed to TS for 20 weeks, while IL-17A$^{-/-}$ mice resisted TSE lung injury. Our findings also indicated that IL-17A augments Cav1, p53 and PAI-1 expression, and senescence and apoptosis in A_2Cs. The contribution of these newly recognized interactions to telomere dysfunction, and to the pathogenesis of TSE lung injury or COPD is unclear. We will address this critical gap of knowledge, using transgenic mice as well as range of molecular and novel interventional approaches that targets TSE or IL-17A induced p53 and downstream PAI-1 expression in both human and mouse A_2Cs, human COPD tissues and in mice with TSE lung injury. **Hypothesis:** TSE and IL-17A increase p53 and PAI-1 expression to promote telomere dysfunction in A_2Cs, which is central to the pathogenesis of TSE lung injury, including COPD. Our data further support the postulate that inhibition of p53 and PAI-1-positive feedback by CSP7, a seven amino acid peptide derived from Cav1, mitigates TSE lung injury. **Objectives:** To establish how IL-17A induced p53-PAI-1-positive feedback in A_2Cs induces TSE lung injury and determine if targeting of this pathway with CSP7 delivered *via* airway is protective. **Specific Aims**: **1) To elucidate the mechanism by which CSP7 mitigates telomere dysfunction in mice model of TSE-induced lung injury. 2) To decipher the efficacy of CSP7 delivered by airways to mitigate TSE-induced lung injury in mice.**

This project will define how IL-17A-induced p53-PAI-1-positive feedback in A_2Cs modulates the outcome of lung injury due to TSE using WT, IL-17A$^{-/-}$, conditional-knockout mice that lack p53 and PAI-1 expression in A_2Cs. The proposed airway delivery of CSP7, may define a new, pharmacologically targeted intervention, to improve clinical outcomes of patients with TSE lung injury, including COPD.

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1. **INTRODUCTION:** Chronic exposure to tobacco smoke (TSE) can lead to COPD. In COPD, chronic inflammation leads to narrowing of small airways and alveolar wall destruction/emphysema. TSE contributes to telomere shortening, which limits the proliferative recovery in lung epithelium especially in progenitor type II alveolar epithelial cells (A_2Cs) and promotes cellular senescence and emphysema susceptibility. The mechanisms by which telomere defects provoke lung disease are not completely understood, but several observations have pointed to lung-intrinsic factors and epithelial cell dysfunction as candidate events. For example, in telomerase-null mice, DNA damage preferentially accumulates in the air-exposed epithelium after environmentally induced injury, such as with TSE. The additive effect of environmental injury and telomere dysfunction has been suggested to contribute to the susceptibility to emphysema seen in these mice. Abnormally short telomeres in A_2Cs have also been noted in patients with pulmonary emphysema. Our findings demonstrate that exposure of wild-type (WT) C57BL/6J mice to tobacco smoke (TS) for 20 weeks (wks) results in telomere dysfunction, senescence, and death of A_2Cs. This also occur in lungs of patients with COPD. Curative therapy for COPD has been unavailable and surgical lung volume reduction remains an option to compensate the loss of elastic recoil. This project aims to elucidate the factors contributing for telomere shortening in mice model of TSE-induced COPD/emphysema. Further test whether caveolin-1 (Cav1) scaffolding domain peptide, CSP7 delivered by intraperitoneal (IP) injection or *via* airways mitigates lung injury and telomere dysfunction in A_2Cs using mouse model of TSE-induced lung injury. We hypothesize that targeting of Cav1-mediated induction of p53 and downstream plasminogen activator inhibitor-1 (PAI-1) using systemic or inhalation delivery of CSP7 resolves TSE-induced lung injury by concurrently inhibiting telomere dysfunction, senescence and apoptosis in A_2Cs. Our objective is to define how interleukin-17A (IL-17A), p53 and PAI-1 affect TSE-induced telomere dysfunction in A_2Cs and emphysema, and test an intervention (CSP7) that allows us to dissect the contributing mechanisms and identify new targets that could be exploited to improve clinical outcomes. The overarching goal is to develop CSP7 as a safe and potentially effective treatment for patients with COPD/emphysema.

2. **KEYWORDS:** p53, Cav1, PAI-1, telomere dysfunction, CSP7, emphysema, A_2Cs, TSE, lung injury, IL-17A, systemic/local delivery, liquid nebulization, dry powder inhalation.

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

1. To elucidate the mechanism by which CSP7 mitigates telomere dysfunction in mice model of TSE-induced lung injury.

2. To decipher the efficacy of CSP7 delivered by airways to mitigate TSE-induced lung injury in mice.

What was accomplished under these goals?

CSP7 inhibits TSE-induced chronic lung injury in mice. We first investigated whether CSP7 delivered systemically by IP injection mitigates TSE-induced lung injury. To test this possibility, WT mice (n=10/group) were exposed to TS for 4 h/day 5 days/wk. Sixteen wks later, TSE WT mice were left untreated or IP injected with CSP7 (1.5 mg/kg) in 0.2 ml of PBS daily 5 days/wk for 4 wks. Control TSE mice were similarly IP injected with control peptide (CP) of scrambled sequence or left untreated (None). Mice were continued to expose to TS for 4h/day 5 days/wk while undergoing CSP7 or CP treatment. Twenty wks post-TSE, mice were subjected to quantitative chest micro-computer tomography (μCT) (**Fig. 1A**). Lung volumes were calculated from μCT renditions at full inspiration (**Fig. 1B**), and compliance (**Fig. 1C**), and elastance (**Fig. 1D**) were measured. Pulmonary function tests by SCIRAQ suggested alveolar damage reflected by increased lung volume and compliance, and reduced elastance in TSE WT mice. These changes were significantly reversed in TSE mice treated with CSP7 while those IP injected with CP failed to exhibit improvement in lung function parameters. To further independently confirm that CSP7 mitigates TSE-induced alveolar damage, lung sections of these mice were subjected to Hematoxylin and Eosin (H&E) staining (**Fig. 2A**), and mean linear intercepts (MLI) were measured using H&E stained lung sections (**Fig. 2B**). We found that exposure of WT mice to 20 wks of TS caused alveolar wall or septal damage revealed by a significant increase in MLI, which was markedly reduced in CSP7 treated TSE mice. However, TSE WT mice exposed to CP still showed a significant increase in MLI suggesting alveolar damage.

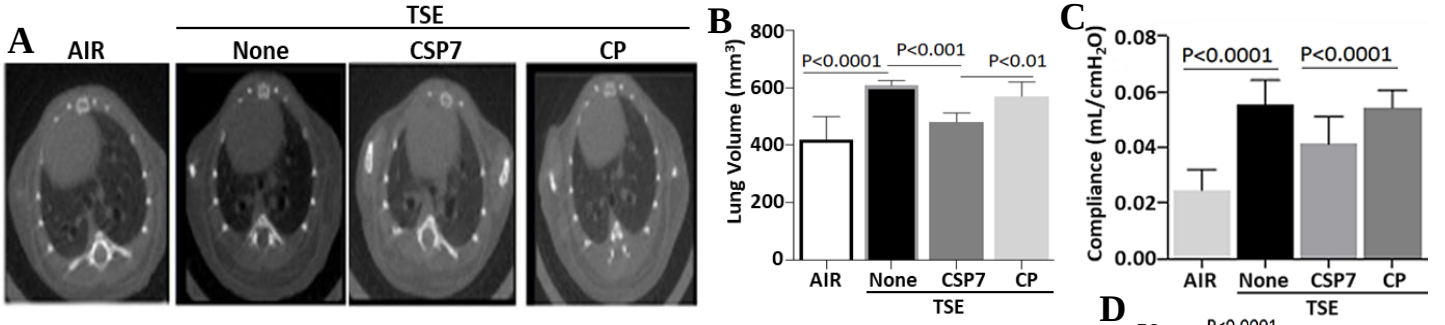


Figure 1. Systemic delivery of CSP7 by IP injection mitigates TSE-induced lung injury in mice. WT C57BL/6J mice (n=10/gp) were kept in ambient AIR or TSE for 4h/day 5days/wk. After 16 wks, TSE WT mice were left untreated (None) or IP injected with 1.5 mg/kg of CSP7 or CP daily 5 days/wk for 4 wks. All mice were subjected to μCT (**A**), lung volume (**B**), lung compliance (**C**) and lung elastance (**D**) measurements 20 wks after TSE. Our results show that systemic (IP) administration of CSP7 reduces lung volume and improves lung function, which are otherwise significantly increased in TSE WT mice. The beneficial effects of systemically delivered CSP7 against TSE-induced lung injury provide strong rationale and basis for its further development as an intervention.

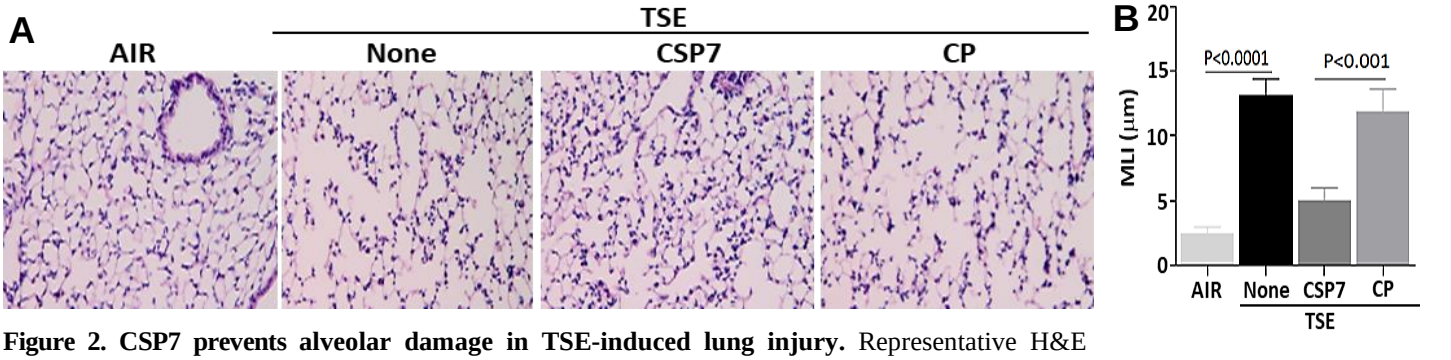


Figure 2. CSP7 prevents alveolar damage in TSE-induced lung injury. Representative H&E staining of lung tissue sections of ambient AIR kept-, TSE-, TSE+CSP7- and TSE+CP-treated WT mice (**A**), and alongside bar graph showing increased MLI (**B**) observed in TSE WT mice *vs* ambient AIR-kept controls, indicating emphysema-like condition due to TSE. This was significantly reduced in TSE WT mice treated with CSP7 while CP treated TSE mice showed increase in MLI.

TSE reduces A_2C viability by inducing senescence, and apoptosis, which has been implicated in alveolar damage often seen in the lungs of patients with COPD/emphysema and mice with TSE lung injury. Cav1 induces p53 while p53 augments PAI-1 expression, and PAI-1 is a downstream mediator of p53-induced senescence and apoptosis, suggesting that induction of p53 and PAI-1 by increased Cav1 could contribute to senescence, and death of A_2Cs and alveolar damage. Therefore, we isolated A_2Cs from the lungs of mice kept in ambient AIR or TSE mice and left untreated (None) or treated with CSP7 or CP by IP injection and tested for p53, acetylated p53 (^{Ac}p53) and phosphorylated p53 (p53^{S15}), which contributes to inhibition of mdm2-mediated degradation of p53, thereby leading to increased expression of p53, PAI-1, senescence and apoptosis. As shown in **Fig. 3A**, TSE induced p53 and PAI-1 with remarkable elevation of p53^{Ac} and p53^{S15}, senescence and apoptosis (**Fig. 3B**), suggesting reduced A_2C viability in TSE WT mice. This was prominently inhibited in TSE mice treated with CSP7 in contrast to CP treated mice that did not respond.

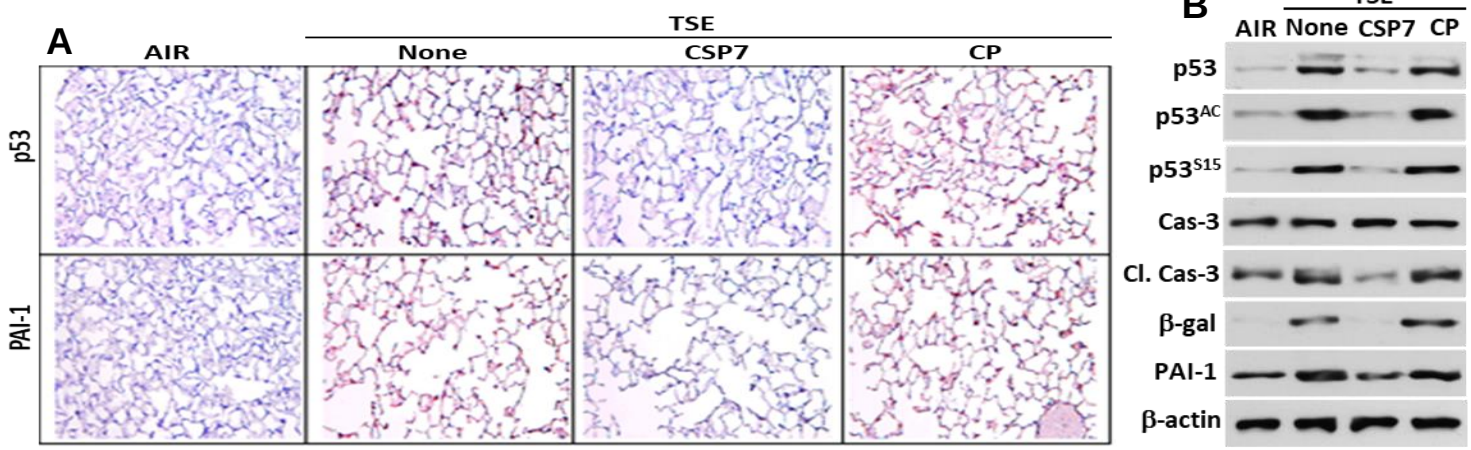


Figure 3. Inhibition of TSE-induced p53^{AC}, p53^{S15} and p53, PAI-1, apoptosis and senescence by CSP7 in WT mice. Sixteen wks after initiation of TSE as in **Fig. 1**, WT mice were IP injected with CSP7 or CP daily for 5 days/wk for 4 wks along with TSE. Lung sections from there mice were subjected to immunohistochemical (IHC) analysis for p53 and PAI-1 that revealed prominently increased expression of p53 and PAI-1 in the lungs of TSE and TSE+CP mice *vs* TSE+CSP7 and Air-kept WT mice (**A**). Immunoblotting of A_2Cs lysates isolated from the lungs of TSE and TSE+CP mice showed increased expression of p53, p53^{AC}. p53^{S15}, PAI-1, cleaved caspase-3/total caspase-3 (Cl. Cas-3/Cas-3, apoptosis marker) and beta-galactosidase (β-gal, senescence marker), which are abolished upon CSP7 treatment. β-actin serves as loading control (**B**).

Premature shortening of the telomere, increased expression of p53, p53^{Ac}, p53^{S15} and PAI-1, senescence and apoptosis were observed in A_2Cs isolated from the lungs of COPD patients. These indicate that telomere dysfunction reduces A_2Cs viability through induction of p53 and PAI-1 during TSE-induced lung injury. Therefore, we tested telomere length in A_2Cs isolated from mice exposed to TS for 20 wks. qPCR analysis of genomic DNA (**Fig. 4A**) and Trapeze enzymatic assays (**Fig. 4B**) of A_2Cs isolated from TSE mice showed a significant reduction in A_2Cs' telomere length and telomerase enzyme activity respectively in TSE WT mice *vs* ambient AIR-kept WT mice. This was significantly improved in TSE WT mice treated with CSP7 by IP injection while those treated with CP still showed significant shortening of telomere due to TSE-induced lung injury. Thus, consistent with the changes observed in A_2Cs of COPD tissues, A_2Cs from the WT mice exposed to 20 wk of TS has also showed significant reduction in telomere length when analyzed by qPCR, even though the extent of the telomere reduction was less severe than that observed in COPD lungs.

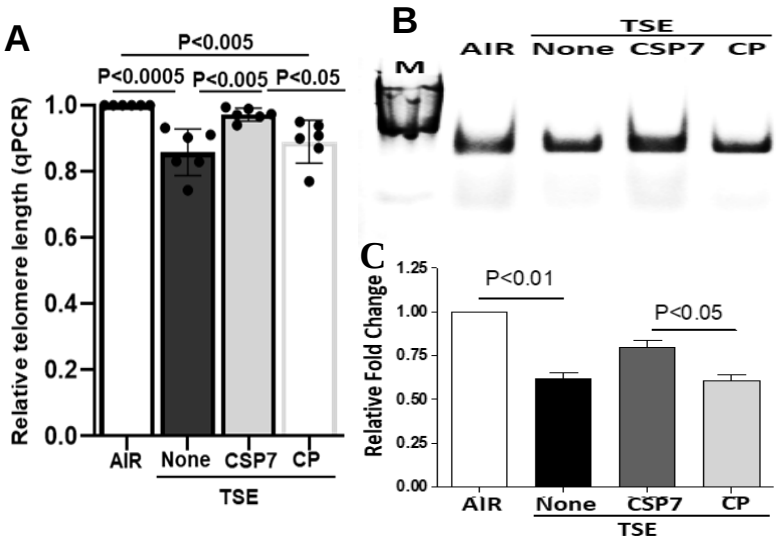


Figure 4. CSP7 inhibits TSE-induced telomere dysfunction in A_2Cs of WT mice. Genomic DNA was extracted from A_2Cs isolated from AIR -kept-, TSE- (None), TSE+CSP7- and TSE+CP-treated WT mice and relative telomere length was analyzed by qPCR. Bar graph shows significantly improved telomere length in A_2Cs of CSP7 treated TSE mice *vs* TSE and TSE+CP treated mice (**A**). Gel image shows prominently increased telomerase enzyme activity in A_2Cs of CSP7 treated TSE mice as analyzed by TRAPEZE enzyme assay (**B**). Bar graph showing quantitation of the TRAPEZE enzyme activity that reflects significantly improved telomerase activity in A_2Cs of CSP7 treated TSE WT mice(**C**).

To gain mechanistic insight, we analyzed A_2Cs lysates for various telomeric proteins such as shelterin component telomere-repeat binding proteins (TRF1, TRF2), SIAH-1, a p53-inducible E3 ubiquitin ligase that is known to down regulate the expression of TRF2, Protein phosphatase 1 nuclear targeting subunit (PNUTS) and telomerase reverse transcriptase (TERT). Consistent with TSE-induced increase in p53 and telomere shortening, we observed marked increase in SIAH1/2 and TRF1 expression with concurrent down-regulation of TRF2, PNUTS and TERT

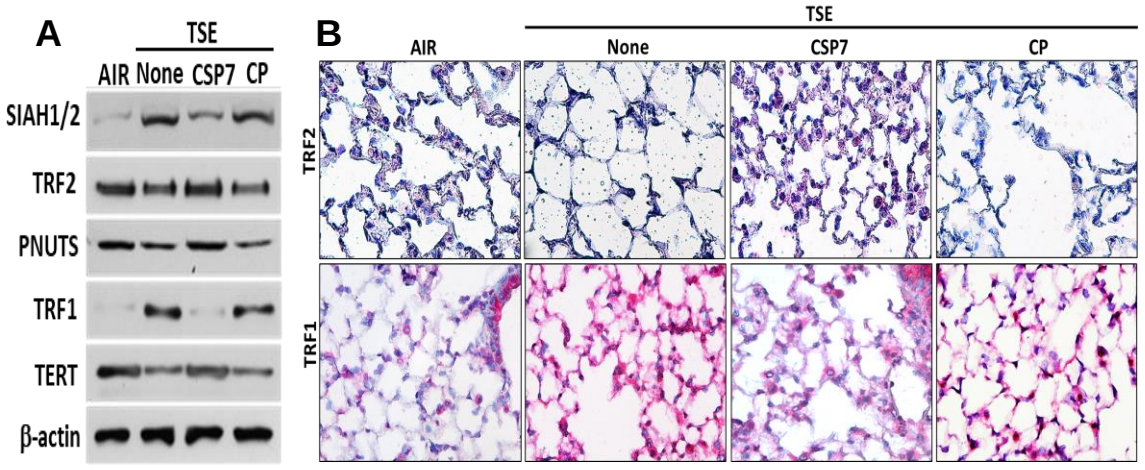


Figure 5. CSP7 abrogates telomere dysfunction in A_2Cs of TSE WT mice. A_2Cs isolated from the lungs of AIR-kept-, TSE (None)-, TSE+CSP7- and TSE+CP treated WT mice were immunoblotted for TRF2, SIAH1/2, PNUTS, TRF1 and TERT. β-actin serves as loading control. Increased expression of SIAH1/2 and TRF1, and decreased expression of TRF2, PNUTS and TERT was observed in A_2Cs of TSE WT mice, which was reversed by CSP7 treatment (**A**). IHC analysis of lung tissue sections of AIR-kept-, TSE-, TSE+CSP7- and TSE+CP treated WT mice for TRF2 and TRF1 proteins also revealed similar pattern of expression in TSE *vs* TSE+CSP7 mice (**B**).

in A_2Cs of TSE mice in contrast to A_2Cs from AIR kept control mice. This was reversed upon CSP7 treatment (**Fig. 5A**). In line with this, IHC analysis of the lung tissue sections also revealed decrease in TRF2 expression and increase in TRF1 in TSE- and TSE+CP treated WT mice compared to TSE+CSP7- and AIR-kept WT mice (**Fig. 5B**). Thus, it is evident that CSP7 treatment restores TRF2, PNUTS and TERT expression in A_2Cs of TSE mice, thereby averting telomere dysfunction and TSE-induced lung injury.

We found that IL-17A levels are significantly increased in TSE mice, while those lacking IL-17A resist TSE-induced lung injury. Further, IL-17A augments Cav1, p53 and PAI-1 expression in A_2Cs during TSE-induced lung injury in WT mice. Therefore, we wanted to test if IL-17A$^{-/-}$ mice could resist TSE induced telomere dysfunction. Similar to C57BL/6J WT mice, we subjected IL-17A$^{-/-}$ mice to 20 wks of TSE for 4h/day and 5 days/wk. 16 wks after TSE, TSE mice were either left untreated (None) or IP injected with CSP7 or CP daily for 5 days/wk for 4 weeks. Telomere length analysis of A_2Cs' genomic DNA by qPCR isolated from these mice lungs revealed no significant change in telomere length in TSE IL-17A$^{-/-}$ *vs* Air-kept IL-17A$^{-/-}$. CSP7 and CP treatment also didn't show any difference (**Fig. 6A**). Further, immunoblotting analysis of A_2Cs' lysate demonstrated no difference in the expression of TRF2, TRF1, TERT, SIAH1/2 ad PNUTS between Air-kept-, TSE-, TSE+CSP7- and TSE+CP treated IL-17A$^{-/-}$ mice. This was in line with inability of TSE to induce p53, p53^{Ac}, p53^{S15}, Cas-3, Cl.cas-3 and β-gal protein in IL-17A$^{-/-}$ mice (**Fig. 6B**). Confirming this, IHC analysis of the lung tissue sections also displayed no difference in expression of TRF2 and TRF1 between AIR-kept-, TSE, TSE+CSP7- and TSE+CP-treated IL-17A$^{-/-}$ mice (**Fig. 6C**). Thus, it is evident that IL-17A$^{-/-}$ mice resists TSE-induced lung injury by resisting premature telomere dysfunction, senescence, and apoptosis in A_2Cs.

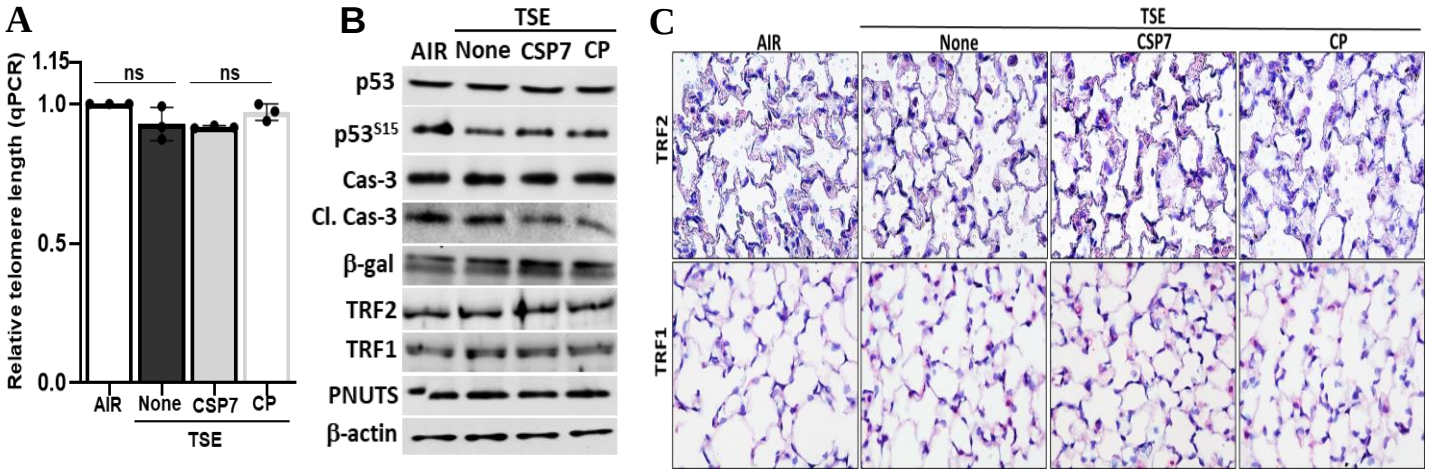
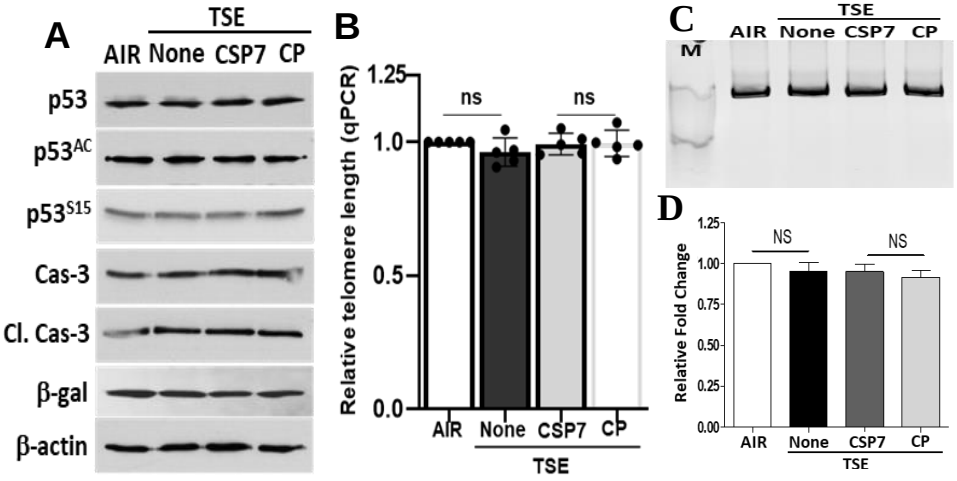


Figure 6. IL-17A$^{-/-}$ mice resists TSE-induced telomere dysfunction in A_2Cs. Genomic DNA was extracted from A_2Cs isolated from AIR-kept-, TSE (None)-, TSE+CSP7- and TSE+CP-treated IL-17A$^{-/-}$ mice and relative telomere length was analyzed by qPCR. Bar graph shows no significant difference in telomere length of A_2Cs of these mice (**A**). Immunoblot images show similar level of p53, p53^{S15}, Cas-3, Cl-cas.3, β-gal, TRF2, TRF1, and PNUTS expression in A_2Cs of various groups of IL-17A$^{-/-}$ mice. β-actin serves as loading control (**B**). IHC analysis of lung tissue revealed no prominent difference in the expression of TRF2 and TRF1 between AIR-kept-, TSE (None)-, TSE+CSP7- and TSE+CP-treated IL-17A$^{-/-}$ mice (**C**).

We next exposed mice lacking PAI-1 expression to TSE with or without CSP7 or CP treatment as described above. We then euthanized the mice at 20 wks and isolated the lungs for further analysis. A_2Cs of these mice resisted the activation of p53, senescence and apoptosis, which was evident from the immunoblot analysis showing similar levels of expression of p53, p53^{Ac}, p53^{S15}, Cas-3, Cl. Cas-3, and β-gal



between AIR-kept, TSE, TSE+CSP7 and TSE+ CP treated mice (**Fig. 7A**). Telomere length analysis by qPCR (**Fig. 7B**) and Trapeze enzymatic assays (**Fig. 7C** and **7D**) of A_2Cs did not show significant changes in telomere length and telomerase enzyme activity respectively in AIR-kept, TSE, TSE+CSP7 or TSE+CP treated mice. Further, analysis for proteins directly involved in regulation of telomere such as SIAH1/2, TRF2, PNUTS, TRF1 and TERT from the treated group also did not show any significant change with respect to the AIR-kept control mice (**Fig. 7E**). IHC analysis also revealed no notable difference in the expression of TRF2 and TRF1 expression between the various groups of mice lacking PAI-1 expression. Taken together, these results prove that TSE of mice lacking PAI-1 resists telomere dysfunction in A_2Cs, and CSP7 or CP treatment has no effect on these mice. We also have bred enough mice lacking p53 expression in A_2Cs and are currently exposing them to TS 4h/day 5 days/wk.

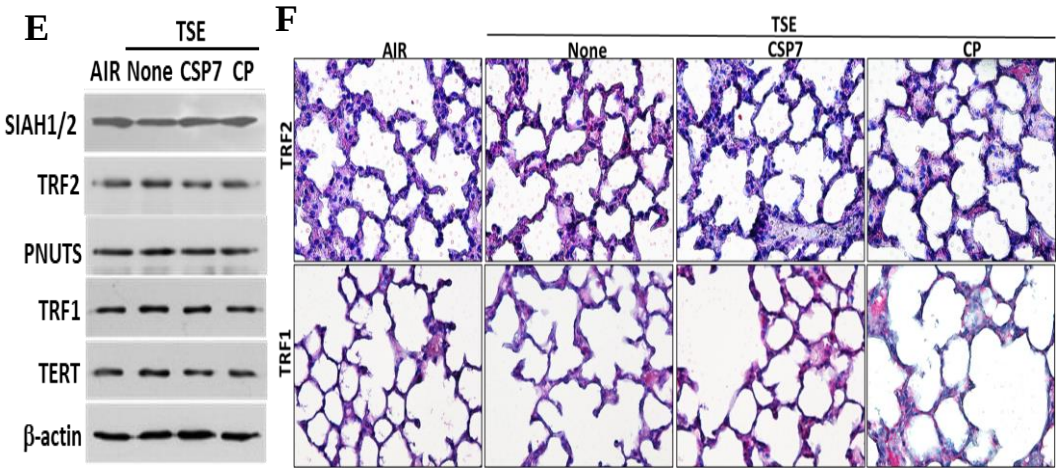
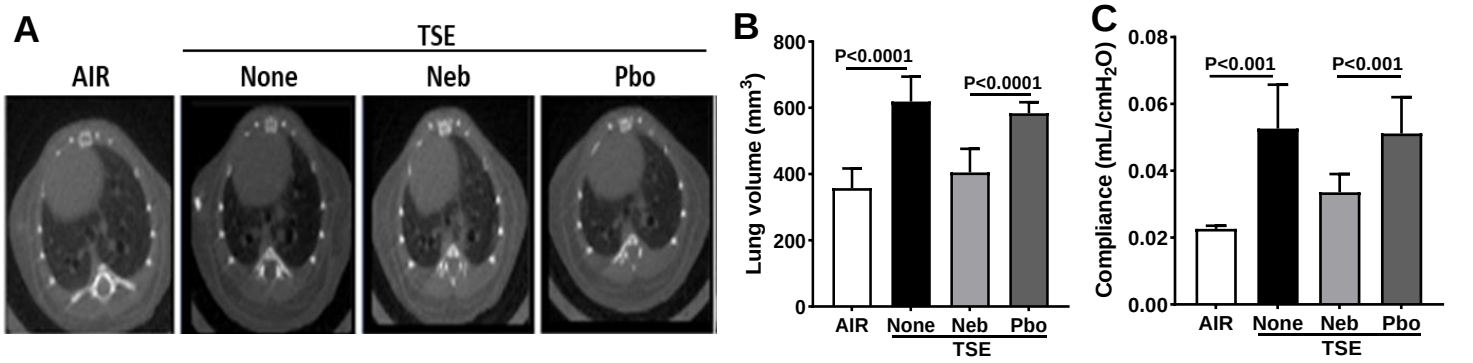


Figure 7. PAI-1$^{-/-}$ resists TSE-induced telomere dysfunction in A_2Cs. Immunoblotting images of A_2Cs lysates of AIR-kept-, TSE (None)-, TSE+CSP7- and TSE+CP treated PAI-1$^{-/-}$ mice showed no difference in the expression of p53, p53^{AC}, p53^{S15}, Cas-3, Cl-cas.3 and β-gal. (**A**). Bar graph shows insignificant changes in telomere length of A_2Cs of PAI-1$^{-/-}$ mice subjected to AIR, TSE, TSE+CSP7 and TSE+CP treatment (**B**). Gel image (**C**) and bar graph (**D**) showing unaltered telomerase enzyme activity analyzed by TRAPEZE enzyme assay in A_2Cs of all groups of PAI-1$^{-/-}$ mice. Immunoblotting images showing no significant changes in the expression of telomeric proteins such as SIAH1/2, TRF2, PNUTS, TRF1 and TERT in A_2Cs of TSE-, TSE+CP PAI-1$^{-/-}$ mice *vs* AIR-kept- and TSE+CSP7 PAI-1$^{-/-}$ mice (**D**). IHC images showing no prominent difference in the expression of TRF2 and TRF1 between various groups of PAI-1$^{-/-}$ mice.

Airway delivery of CSP7 inhibits TSE-induced lung injury in mice. Local delivery of a drug often minimizes target dose requirements and lessens the chance for off-target effects associated with systemic administration. Local delivery can also be more convenient for patients with chronic diseases such as COPD. Therefore, we investigated whether CSP7 delivered *via* airways in liquid formulation mitigates TSE-induced lung injury. To test this route of delivery, WT mice were exposed to TS for 4 h/day 5 days/wk for 20 weeks. After 16 wks, TSE WT mice were left untreated (None) or exposed to formulated CSP7 (5.8 mg) in 30 ml of PBS containing lactose monohydrate (154 mg) (Neb) or placebo (Pbo) alone for 2 h daily 5 days/wk for 4 wks using a nebulization tower. TSE WT mice exposed to Pbo and ambient AIR-kept WT mice will serve as controls for comparison. Twenty wks post-TSE, mice were subjected to quantitative chest μCT (**Fig. 8A**). Lung volumes were calculated from CT renditions at full inspiration, and compliance, elastance, and resistance were also measured. Pulmonary function tests by SCIRAQ suggested alveolar damage reflected by increased lung volume (**Fig. 8B**) and compliance, and reduced elastance (**Fig. 8C-E**). These changes were significantly improved in nebulized CSP7 treated TSE WT mice in contrast Pbo treated TSE WT mice. Likewise, analysis of H and E stained lung tissue sections of these mice (**Fig. 8F**), and measurement of MLI (**Fig. 8G**) revealed reduced alveolar damage after treatment of TSE WT mice with nebulized CSP7 *vs* to Pbo treated TSE mice.



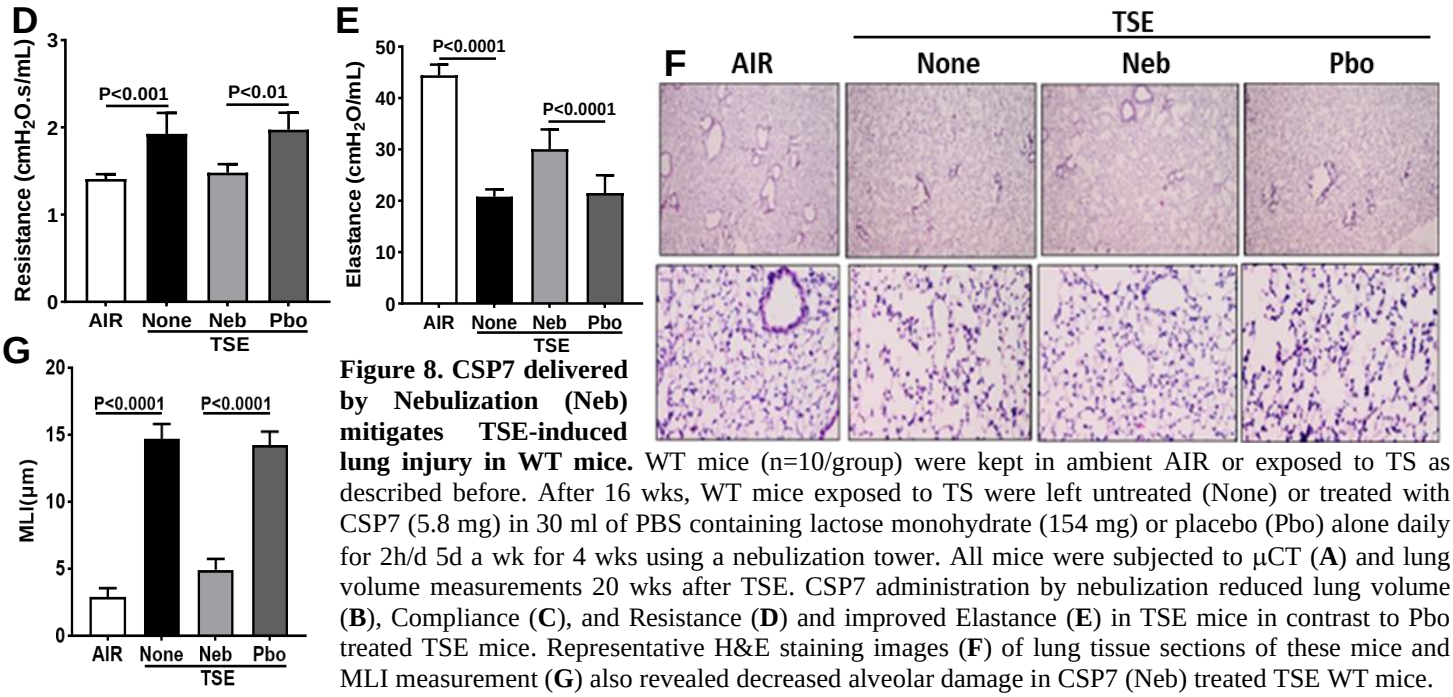


Figure 8. CSP7 delivered by Nebulization (Neb) mitigates TSE-induced lung injury in WT mice. WT mice (n=10/group) were kept in ambient AIR or exposed to TS as described before. After 16 wks, WT mice exposed to TS were left untreated (None) or treated with CSP7 (5.8 mg) in 30 ml of PBS containing lactose monohydrate (154 mg) or placebo (Pbo) alone daily for 2h/d 5d a wk for 4 wks using a nebulization tower. All mice were subjected to µCT (**A**) and lung volume measurements 20 wks after TSE. CSP7 administration by nebulization reduced lung volume (**B**), Compliance (**C**), and Resistance (**D**) and improved Elastance (**E**) in TSE mice in contrast to Pbo treated TSE mice. Representative H&E staining images (**F**) of lung tissue sections of these mice and MLI measurement (**G**) also revealed decreased alveolar damage in CSP7 (Neb) treated TSE WT mice.

What opportunities for training and professional development has been provided?

In this project, we are training a US citizen from the minority community as a postdoctoral fellow in research related to TSE-induced lung injury and mechanism contributing to TSE-induced telomere dysfunction. The research training from this project will enable him to develop his career as an independent investigator in research related to pathogenesis of COPD and/or telomere dysfunction and explore funding from DoD or NIH.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

Our studies show that systemic delivery of CSP7 inhibits IL-17A- and TSE-induced lung injury through suppression of p53 and downstream PAI-1 expression, which subsequently blocks telomere dysfunction, senescence, and apoptosis of A_2Cs thereby preventing emphysema development. In the upcoming year, we will continue our work to establish how IL-17A-mediated p53- and PAI-1-positive feedforward induction in A_2Cs regulates telomere dysfunction and TSE-induced lung injury. Further, test the efficacy of CSP7 intervention delivered in liquid and dry powder formulations for nebulization (neb) and dry powder inhalation (DPI) against TSE-induced telomere dysfunction and lung injury. We will further explore whether mice lacking p53 and PAI-1 expression in A_2Cs could similarly resist TSE-induced telomere shortening, senescence and apoptosis. In addition, we will breed knockout mice lacking Trf2 expression in A_2Cs and test the role of Trf2 in A_2Cs on CSP7-mediated protection against TSE-induced telomere dysfunction and lung injury. As we described in the statement of work of the original application, we will complete all the remaining experiments proposed in both Specific Aims to test the hypothesis of whether targeting of Cav1-mediated induction of p53 and downstream PAI-1 expression using inhalation delivery of CSP7 resolves TSE-induced lung injury by concurrently inhibiting telomere dysfunction, senescence and apoptosis in A_2Cs.

4. IMPACT:

What was the impact of the development of the principal discipline(s) of the project?

We are developing liquid and dry powder formulations of CSP7 for local delivery *via* nose to improve treatment outcomes in patients with COPD/emphysema. The results from these studies will provide key insights into the

beneficial effects of CSP7 and may lead to development of novel therapeutic candidate, nebulized or dry powder inhaled CSP7 to mitigate the damaging effects of TSE in the lungs and for the treatment of COPD.

What was the impact on other discipline(s)?

Efficacy of systemic and airway delivered CSP7 is also being tested against lung injury using a mouse model of COVID-19.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on Society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Nothing to Report.

6. PRODUCTS:

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

No change.

Has there been a change in the active other support of the PD/PI or senior/key personnel since the last reporting period?

Nothing to Report.

What other organization were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

Not Applicable.

9. APPENDICES:

Not Applicable.