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TELOMERASE DEFICIENCY DOES NOT ALTER BLEOMYCIN INDUCED FIBROSIS IN MICE

Amber L. Degryse^a, Xiaochuan C. Xu^a, J. Luke Newman^a, Daphne B. Mitchell^a, Harikrishna Tanjore^a, Vasiliy V. Polosukhin^a, Brittany R. Jones^a, Frank B. McMahon^a, Linda A. Gleaves^a, John A. Phillips III^b, Joy D. Cogan^b, Timothy S. Blackwell^{a,c,d,e}, and William E. Lawson^{a,e}

^aDepartment of Medicine, Division of Allergy, Pulmonary and Critical Care Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee

^bDepartment of Pediatrics, Division of Medical Genetics, Vanderbilt University School of Medicine, Nashville, Tennessee

^cDepartment of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, Tennessee

^dDepartment of Cancer Biology, Vanderbilt University School of Medicine, Nashville, Tennessee

^eDepartment of Veterans Affairs Medical Center, Nashville, Tennessee

Abstract

Introduction—Idiopathic pulmonary fibrosis (IPF) is characterized by interstitial lung infiltrates, dyspnea, and progressive respiratory failure. Reports linking telomerase mutations to familial interstitial pneumonia (FIP) suggest that telomerase activity and telomere length maintenance are important in disease pathogenesis.

Methods—To investigate the role of telomerase in lung fibrotic remodeling, intratracheal bleomycin was administered to mice deficient in telomerase reverse transcriptase (*TERT*) or telomerase RNA component (*TERC*) and wild type controls. *TERT* deficient and *TERC* deficient mice were interbred to the F6 and F4 generation, respectively, when they developed skin manifestations and infertility. Fibrosis was scored using a semiquantitative scale and total lung collagen was measured using a hydroxyproline microplate assay. Telomere lengths were measured in peripheral blood leukocytes and isolated type II alveolar epithelial cells (AECs). Telomerase activity in type II AECs was measured using a real time PCR based system.

Results—Following bleomycin, *TERT* deficient and *TERC* deficient mice developed an equivalent inflammatory response and similar lung fibrosis (by scoring of lung sections and total lung collagen content) compared to controls, a pattern seen in both early generation (F1) and later

Address correspondence to: William E. Lawson, M.D., Assistant Professor of Medicine, Division of Allergy, Pulmonary and Critical Care Medicine, Vanderbilt University School of Medicine, T-1218 MCN, Nashville, TN 37232-2650, william.lawson@vanderbilt.edu, Phone: (615) 322-3412 Fax: (615) 343-7448.

DECLARATION OF INTEREST

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generations (F6 *TERT* and F4 *TERC*). Telomere lengths were reduced in peripheral blood leukocytes and isolated type II AECs from F6 *TERT* and F4 *TERC* mice compared to controls.

Conclusions—Telomerase deficiency in a murine model leads to telomere shortening, but does not predispose to enhanced bleomycin induced lung fibrosis. Additional genetic or environmental factors may be necessary for development of fibrosis in the presence of telomerase deficiency.

Keywords

familial interstitial pneumonia; idiopathic pulmonary fibrosis; lung

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease characterized by bilateral interstitial infiltrates, restriction on pulmonary function testing, and progressive dyspnea leading to respiratory failure [1]. Estimates for prevalence suggest that approximately 20 per 100,000 males and 13 per 100,000 females have IPF [2]. Unfortunately, no effective treatments are yet available and mortality from disease remains quite high. While the cause of IPF remains unknown, recent studies in familial interstitial pneumonia (FIP) have begun to shed light on potential pathogenic mechanisms [3;4]. FIP is defined when two or more individuals in a family have proven idiopathic interstitial pneumonia [4–6]. Based on available information, mutations in genes in the telomerase complex may account for up to 15% of cases of FIP.

The telomerase complex is responsible for maintaining the integrity of telomeres, the tandem repeats of TTAGGG sequences that are responsible for providing protection of the end of chromosomes. Telomeres and their specific telomere binding proteins prevent DNA repair mechanisms from recognizing chromosome ends as DNA breaks. During DNA replication, telomere shortening occurs, and with repetitive cell divisions, the resultant telomere erosion can lead to arrest of growth and cell death [7–11]. To offset the effect of telomere shortening, the telomerase complex, a specialized DNA polymerase, adds the telomeric repeat sequences to the chromosome ends. Telomerase has two essential components, the catalytic component telomerase reverse transcriptase (TERT), and the RNA template telomerase RNA component (TERC) [12–14].

Recently, mutations in both *TERT* and *TERC* have been linked to cases of FIP. The mutations resulted in decreased telomerase activity in *in vitro* evaluations, and mutation carriers had marked telomere shortening in peripheral blood leukocytes compared to age matched controls [15;16]. Since those initial evaluations, it has been hypothesized that these families developed pulmonary fibrosis because of progressive telomere shortening in the alveolar epithelium [4]. To further investigate this potential relationship, we evaluated *TERT* deficient and *TERC* deficient mice to determine if telomerase deficiency and telomere shortening predisposes to bleomycin induced lung fibrosis.

METHODS

Animal model

TERT heterozygous deficient, *TERC* heterozygous deficient, and wild type C57BL/6J mice were obtained from Jackson Laboratories, Bar Harbor, Maine. For these studies, mice maintained in the heterozygous state are identified as generation F0. To generate mice deficient in each gene, F0 males were crossed to F0 females, with the pups identified as F1. Serial homozygous deficient matings were performed to generate subsequent generations down to F4 for *TERC* deficient and F6 for *TERT* deficient. Both males and females were used in experiments. All mice for these experiments were in a C57BL/6J background and entered experiments at greater than 8 weeks of age, with ages, body weights, and sex distribution similar among groups for individual experiments. All mouse experiments were approved by the Vanderbilt Institutional Animal Care and Use Committee.

Bleomycin (Bedford Laboratories, Bedford, Ohio) was injected intratracheally (IT) in wild type and gene targeted mice using an intubation procedure as previously described [17;18]. Both single dose IT bleomycin injections and repetitive dose IT bleomycin injections (dose given every two weeks for 4 or 8 doses) were performed as previously described [17]. At baseline and at designated time points post bleomycin, lungs were harvested for histology and frozen tissue as previously described [17–22].

Histology and microscopy

For formalin fixed lung sections, lungs were formalin-fixed and paraffin embedded, sectioned, and stained with hematoxylin and eosin (H&E) or Masson's trichrome as previously described [19;20]. Light microscopy was performed using an Olympus IX81 Inverted Research Microscope (Olympus, Tokyo, Japan).

Semiquantitative fibrosis scoring

Quantification of lung fibrosis on histological specimens was performed by an investigator blinded to the group using a semiquantitative score on 10 sequential, nonoverlapping fields (magnification $\times 300$) as previously described [17–19;22].

Collagen content

Frozen lung tissue samples were hydrolyzed in 6N HCl, and hydroxyproline content quantitated using a microplate assay based on the Ehrlich's reaction as previously described [18;22;23]. Lung collagen content was calculated from these results as hydroxyproline accounts for approximately 13.3% of collagen by weight.

Lung lavage and cell counts

Bronchoalveolar lavage (BAL) was performed as detailed previously [18]. After euthanasia, three 800 μ l lavages of sterile saline were performed using a 20g blunt tipped needle inserted into the trachea. Samples were centrifuged at 400 $\times$ g for ten minutes and the supernatant discarded. Cell counts were performed by manual counting under light microscopy using a hemocytometer. Approximately 30,000 cells from each specimen were loaded onto slides using a Cytospin 2 (Shandon Southern Products, England). These slide preparations were

then stained using a modified Wright stain and reviewed under light microscopy for differential white blood cell counts.

Telomere length measurements

Blood was collected from the mice at the time of harvest, and peripheral blood leukocytes were isolated from blood as previously described [24]. DNA was isolated from the peripheral blood leukocytes using a Qiagen DNAeasy Blood and Tissue Kit (Qiagen, Valencia, CA) per manufacturer instructions. Telomere length measurements on this DNA were performed using a real time PCR based protocol [25]. Briefly, 90ng of DNA was aliquoted for use on both the telomere and single copy gene plates with water added to bring the final volume to 90 ul. A cocktail consisting of 2.5mM DTT, 50nM ROX, 1X SYBR greener super mix (Invitrogen, Carlsbad, CA) was thoroughly mixed and 96 ul added to each of the DNA aliquots. Each aliquot was divided between 2 PCR plates containing either primers for telomeres or the single copy gene, each at 450nM final concentration. All samples were run in triplicate with a final volume for all wells of 30ul. Primer sequences for the telomeres were Tel1b CGGTTTGGTTGGGTTT GGGTTTGGGTTTGGGTTTGGGTT and Tel2b GGCTTGCCTTACCCTTACCCTTACCCTT ACCCTTACCCT [24]. Single copy gene primer sequences were m36B4 F ACTGGTCTAGG ACCCGAGAAG and m36b4 R TCAATGGTGCCTCTGGAGATT [26]. A five point standard curve was generated for each plate using oligonucleotides as follows: Tel-84 oligo 1E9 (kb), 3E8, 1E8, 3E7 and 1E7; m36B4-79 oligo 1E6 (equivalent to 2.5E6 diploid copies per reaction), 3E5, 1E5, 3E4, 1E4. Oligos were as follows: Tel 84-mer: ACCCCATTCTATCATCAACGGGT ACAACGAGTCCTGGCCTTGTCTGTGGAGACGGATTACACCTTCCCACTTGCTG AA; m36B479-mer: ACTGGTCTAGGACCC GAGAAGACCTCCTTCTTCCAGGCTTTGGGCATCACC ACGAAAATCTCCAGAGGCACCATTTGA. A 15ng/ul solution of pBR322 plasmid was used to dilute the oligo DNAs. Cycling parameters for the telomere plates were 5 min at 50° C, 10 min at 95° C followed by 40 cycles of 95° C for 15 seconds, 54° C for 1 min. Conditions for the single copy gene plate were 5 min at 50° C, 10 min at 95°C followed by 40 cycles of 95° C for 15 seconds, 60° C for 1 min. Data was generated using the Applied Biosystems 7500 Real Time PCR System (Carlsbad, CA) with version 2.0.4 software. Telomere length is calculated as the telomere signal per sample divided by its companion single copy gene signal to give the average telomere length per diploid genome. Type II AECs were isolated from adult mice using techniques as previously described [20;21], with DNA isolated and telomere lengths analyzed as with the leukocytes above.

Telomerase activity measurements

Type II AECs were isolated from adult mice using techniques as previously described [20;21]. Telomerase activity was determined on isolated type II AECs using the TRAPEZE RT Telomerase Detection Kit (Chemicon, Temecula, CA), a PCR-based telomeric repeat amplification protocol method, per manufacturer recommendations. With this kit, Amplifluor primers are designed to emit a fluorescence signal only when they are incorporated into PCR products, with the net increase of fluorescence in the reaction vessel directly correlating to the amount of amplified DNA produced in the reaction. Briefly, type 2 cells pellets were resuspended in CHAPS lysis buffer and protein levels were evaluated

using the Bradford method (Bio-Rad, Hercules, CA). Equal amounts of protein were combined with the Trapeze RT reaction mix, which contained TS primer, RP tailed primer, Amplifluor primers and dNTP, in RNase-free PCR tubes, adding Taq polymerase. Positive and negative controls provided by the manufacturer were examined accordingly. PCR amplification was performed using the Applied Biosystems 7500 Real Time PCR System (Carlsbad, CA) per manufacturer recommendations. Telomerase activity data was reported as activity relative to the kit specified negative control.

Statistics

Statistical analyses were performed using GraphPad InStat (GraphPad Software, San Diego, CA). Differences among groups were assessed using one-way analysis of variance (ANOVA) with a Student-Newman-Keuls multiple comparison post test. Differences between pairs were assessed using Student's t-test. Results are presented as mean $\pm$ standard error of the mean. P values < 0.05 were considered significant.

RESULTS

Telomerase deficient mice do not have enhanced lung fibrosis following intratracheal bleomycin

In initial experiments, 8–10 week old *TERC* deficient and *TERT* deficient mice in the F1 generation and littermate F1 wild type controls were given intratracheal bleomycin and harvested at 3 weeks post bleomycin with trichrome blue stained slides analyzed for lung fibrosis. No differences in appearance of the lung sections or semiquantitative fibrosis scoring were seen among these three groups with 0.04 units of bleomycin (Figure 1A,B), a finding in contrast to prior work by Liu et al, in which early generation *TERT* deficient mice were protected from bleomycin induced lung fibrosis using a similar bleomycin dose [27]. After this initial observation and given the fact that effects of telomerase deficiency might be accentuated with aging and repeated injury, we then evaluated 8–12 month old F1 *TERC* $-/-$ mice and littermate controls with repetitive bleomycin 0.04 units every other week for 4 doses with lung harvest at 2 weeks after the final dose, but again no difference was noted in fibrosis between the two groups (Figure 1C).

Next, *TERC* deficient and *TERT* deficient mice were crossed in successive breedings to give later generation mice (Figure 2A). *TERC* deficient mice could be generated down to the F4 generation and *TERT* deficient mice to the F6 generation. At those generations, the mice could not breed any further and also displayed the skin manifestations previously noted in prior studies with telomerase deficiency [28;29]. On histologic evaluation, lung sections from untreated *TERC* deficient and *TERT* deficient mice appeared similar to wild type controls with no evidence of lung fibrosis (Figure 2B–D). Multiple bleomycin studies were performed across different generations, and even down to the F4 *TERC* $-/-$ and F6 *TERT* $-/-$ mice, the degree and distribution of fibrosis was similar to control wild type mice. Representative trichrome blue stained lung sections are shown from F4 *TERC* $-/-$, F6 *TERT* $-/-$, and wild type controls at 3 weeks following bleomycin in Figure 3A–F. Furthermore, semiquantitative scoring of lung fibrosis in young mice (8–10 weeks) whether they received 0.04 units or 0.08 units of bleomycin was similar among the three groups (Figure 3G,H).

Again, because of the possibility that aging might contribute to effects of telomerase deficiency, we evaluated older mice (8–12 months) with 0.04 units bleomycin and again noted no difference in fibrosis among the three groups as determined by both semiquantitative scoring (Figure 3I) and lung collagen content (Figure 3J). We also performed repetitive bleomycin studies in later generation *TERT* deficient mice and controls, where mice received IT bleomycin 0.04 units every 2 weeks for 8 doses with harvesting at 2 weeks after the last dose [17], and as shown in Figure 3K, lung fibrosis was similar between the two groups. Furthermore, other experiments were performed in intermediate generations without any difference in lung fibrosis among *TERC* $-/-$, *TERT* $-/-$, and controls. In addition to these fibrosis measurements, lung inflammation as measured by total and differential cell counts in bronchoalveolar lavage (BAL) was similar among F4 *TERC* deficient, F6 *TERT* deficient, and wild type mice at 2 weeks following single dose bleomycin (Figure 4).

Telomerase deficient mice have absence of telomerase activity and telomere shortening

Type II AECs were isolated from F4 *TERC* deficient, F6 *TERT* deficient, and wild type controls, and telomerase activity evaluated on these cells using a real time PCR approach. AECs from both of the telomerase deficient lines did not have telomerase activity present (results were at the level of the kit specified negative control), while wild type AECs had telomerase activity that was 2.3 logs greater than the negative control. To determine if telomere lengths were different between telomerase deficient and wild type control mice, telomere lengths were measured on peripheral blood leukocytes using a real time PCR approach. Telomere lengths in both F4 *TERC* deficient and F6 *TERT* deficient mice were significantly shorter than in wild type controls (Figure 5A). Furthermore, telomere lengths from isolated type II AECs were shorter in F4 *TERC* deficient and F6 *TERT* deficient mice compared to wild type controls (Figure 5B).

DISCUSSION

In these studies, telomerase deficiency, either through absence of the *TERT* or *TERC* component, did not result in enhanced lung fibrosis following intratracheal bleomycin. Even with interbreeding these mice until telomere shortening was prominent and the mice could not reproduce easily, these mice did not develop a spontaneous lung phenotype and there was no difference in bleomycin induced fibrosis compared to wild type controls.

The most compelling evidence that telomerase has a prominent role in the pathogenesis of lung fibrosis comes from studies of human FIP cases in which mutations in either *TERC* or *TERT* are present [15;16]. With these mutations, decreased telomerase activity is noted and telomere shortening is severe with many mutation carriers having peripheral blood leukocyte telomere lengths less than 10% (and some less than 1%) of age matched controls [15;16]. In fact, subsequent studies of cases of IPF indicate that telomere shortening is prominent even in the absence of *TERC* or *TERT* mutations [30;31], suggesting the potential for a common role for telomerase dysfunction in IPF [4]. With these human findings, studies are needed to better define the mechanisms involved in telomerase mutation associated FIP. With this in mind, an attractive model to consider was to evaluate *TERC* and *TERT* deficient mice with

experimentally induced lung fibrosis, and thus the rationale for the studies we have performed here.

Tissues affected most by decreased telomerase activity (due to mutations or deficiency) would be predicted to be those subject to significant proliferation, including bone marrow, areas of epithelial turnover such as the skin and gastrointestinal tract, and the germline. Similarly, murine models of telomerase deficiency result in abnormalities in tissues with high rates of proliferation [28;29;32–34]. Most research mouse strains (including C57BL/6) have long telomeres, and studies to date delineate that telomere shortening is critical to developing a disease phenotype. In fact, in early generation C57BL/6 *TERC* deficient mice, no phenotype is observed because telomere shortening is not at a critical level to affect cell survival. With successive generations of breeding into a maintained homozygous deficient line, *TERC* deficient mice have progressive telomere shortening and begin having disease manifestations by the fourth generation [28;32;33]. In later generation *TERC* deficient mice (generations 4–6), an increased incidence of skin lesions, alopecia, and hair graying is noted, which we also observed in these studies. Decreased survival, atrophy of villi and focal adenomas in the gastrointestinal tract, hematologic abnormalities, and aberrancies in the seminiferous tubules are found in later generation *TERC* deficient mice [28]. An increased incidence of genomic instability and spontaneous cancer are noted in aging *TERC* deficient mice [33;34]. *TERT* deficient mice have not been studied as extensively as *TERC* deficient mice, but they also have telomere shortening across generations and phenotypes similar to *TERC* deficient mice [29].

Until recently, studies analyzing telomerase expression in the lung were relatively limited. Rodent studies have shown that telomerase expression occurs during development, is normally quiescent during adulthood, and can be induced in type II AECs in response to injury [35–37]. A recent study evaluating telomerase activity in wild type murine AECs delineated that after a single dose of intratracheal bleomycin, telomerase activity was increased in isolated type II AECs, but telomere lengths were unchanged [38]. Prior studies by Driscoll and colleagues observed that telomerase expression could be observed in a subpopulation of type II AECs [35;36], suggesting that these cells may be an important stem cell population in the lung. Subsequently, this group has demonstrated that telomerase deficiency impacts lung growth as well as lung regenerative capacity. Lee et al. reported that when bred into the F4 generation, *TERC* deficient mice had alveolar simplification with alveolar wall thinning, increased alveolar size, and an absolute reduction in the number of type II AECs [39]. We did not observe any gross lung abnormalities in late generation *TERC* deficient or *TERT* deficient mice, although we did not employ the morphologic measurements those investigators used. In a partial pneumonectomy study, F3 *TERC* deficient mice had decreased survival (no F4 mice survived), a marked attenuation of the compensatory lung growth typically noted in this procedure, and a diminished stem/progenitor cell response [40], demonstrating an important role for telomerase in lung repair mechanisms. Furthermore, recent studies by Alder et al. revealed that mice with telomerase deficiency and telomere shortening had increased susceptibility to cigarette smoke induced emphysema [41]. Finally, in 2007, Liu et al analyzed early generation *TERT* deficient mice and noted a relative protection from bleomycin induced fibrosis with *TERT* deficiency, an

outcome opposite what might have been predicted based on the FIP genetic associations, and that the effect appeared to be mediated from bone marrow progenitor cells [27]. No telomere length data were reported in that manuscript, so it is not known whether telomere length differences contributed to their observations or if the results stemmed from potential non-telomere length effects of *TERT*. In contrast, we did not observe any differences in lung fibrosis post bleomycin with telomerase deficiency with early or late generation *TERC* deficient and *TERT* deficient mice. The reason for this discrepancy between our results presented here and those from the Liu et al study remains unclear, even though we used similar gene targeted mice (from the *TERT* standpoint) and a similar dose of bleomycin.

Given the FIP data and the growth arrest data in the partial pneumonectomy model [40], it may have been expected that these *TERC* or *TERT* deficient mice, with appropriate telomere shortening, would have had greater bleomycin induced fibrosis. However, across many different experiments with these *TERC* $-/-$ and *TERT* $-/-$ mice, we did not find evidence that telomerase deficiency affected lung fibrosis in the bleomycin model (in either direction). The reason for this observation remains unclear, but some possibilities should be considered. Given that the effects of telomerase dysfunction or deficiency may increase with aging, we did some of our experiments in older mice (8–12 months), but it is possible that considerably greater age (18–24 months) in the mice might be necessary to unmask a profibrotic tendency. Furthermore, our single dose experiments were done with measurements at the peak point of fibrotic remodeling in the bleomycin model without an analysis at later time points, where there could possibly be an issue with resolution of fibrosis in the telomerase deficient mice. However, this possibility is tempered considerably given the fact that no differences were noted in the repetitive bleomycin model where the lungs are exposed to multiple episodes of injury and repair over a longer period of time. Another possibility is that, though quite prominent, the degree of telomere shortening in the alveolar epithelium, which was limited by the ability to continue breeding the mice in successive generations, did not reach a level that precluded normal repair responses. Additionally, it is possible that the bleomycin model is simply not the optimal model for detecting such a propensity to fibrosis with these mice.

CONCLUSIONS

In summary, our experiments suggest that telomerase deficiency and telomere shortening do not impact bleomycin induced lung fibrosis. Nonetheless, the findings that telomerase mutations are associated with FIP and that telomere shortening occurs in IPF are clear indications that telomerase dysfunction may play a prominent role in lung fibrosis and provide justification for an ongoing search into improved animal models to investigate this relationship.

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Figure 1.

F1 *TERC* deficient and F1 *TERT* deficient mice had similar levels of lung fibrosis following intratracheal bleomycin compared to their respective littermate controls. A) Semiquantitative scoring of lung fibrosis from 8–10 week old F1 *TERC* deficient (F1 *TERC* $-/-$) and F1 littermate wild type controls (F1 *TERC* $+/+$) at 3 weeks following 0.04 units of intratracheal bleomycin. N = 6 for F1 *TERC* $-/-$ and 6 for F1 *TERC* $+/+$. Experiment survival was 6/6 for F1 *TERC* $-/-$ and 6/6 for F1 *TERC* $+/+$. B) Semiquantitative scoring of lung fibrosis from 8–10 week old F1 *TERT* deficient (F1 *TERT* $-/-$) and F1 littermate wild type controls (F1 *TERT* $+/+$) at 3 weeks following 0.04 units of intratracheal bleomycin. N = 5 for F1 *TERT* $-/-$ and 5 for F1 *TERT* $+/+$. Experiment survival was 5/5 for F1 *TERT* $-/-$ and 5/5 for F1 *TERT* $+/+$. C) Semiquantitative scoring of lung fibrosis from 8–12 month old F1 *TERC* $-/-$ and F1 *TERC* $+/+$ littermate controls at 2 weeks following 4 biweekly repetitive doses of 0.04 units of intratracheal bleomycin. N = 9 for F1 *TERC* $-/-$ and 10 for F1 *TERC* $+/+$. Experiment survival was 9/14 for F1 *TERC* $-/-$ and 10/14 for F1 *TERC* $+/+$.

Figure 2.

F4 *TERC* deficient (F4 *TERC* -/-) and F6 *TERT* deficient (F6 *TERT* -/-) mice did not develop spontaneous lung fibrosis. A) Schematic illustrating breeding strategy to develop F4 *TERC* deficient and F6 *TERT* deficient mice. Trichrome blue stained lung sections from B) F4 *TERC* deficient, C) F6 *TERT* deficient, and D) wild type (WT) control mice. Magnification x200.

Figure 3.

F4 *TERC* deficient, F6 *TERT* deficient, and wild type control mice had similar levels of lung fibrosis following intratracheal bleomycin. A–F) Trichrome blue stained lung sections from A,D) F4 *TERC* deficient, B,E) F6 *TERT* deficient, and C,F) wild type (WT) control mice. Magnification: A–C x40; D–F x200. G) Semiquantitative scoring of lung fibrosis from 8–10 week old F4 *TERC* deficient (F4 *TERC*^{-/-}), F6 *TERT* deficient (F6 *TERT*^{-/-}), and wild type (WT) control mice at 3 weeks following 0.04 units of bleomycin. N=11 for F4 *TERC*^{-/-}, 11 for F6 *TERT*^{-/-}, and 13 for WT. Experiment survival was 11/13 for F4 *TERC*^{-/-}, 11/13 for F6 *TERT*^{-/-}, and 13/15 for WT. H) Semiquantitative scoring of lung fibrosis from 8–10 week old F4 *TERC*^{-/-}, F6 *TERT*^{-/-}, and WT control mice at 3 weeks following 0.08 units of bleomycin. N=5 for F4 *TERC*^{-/-}, 9 for F6 *TERT*^{-/-}, and 7 for

WT. Experiment survival was 5/8 for F4 TERC $-/-$, 9/12 for F6 TERT $-/-$, and 7/10 for WT. I) Semiquantitative scoring of lung fibrosis and J) total lung collagen content as assessed by a microplate hydroxyproline assay from 8–12 month old F4 TERC $-/-$, F6 TERT $-/-$, and WT control mice at 3 weeks following 0.04 units of bleomycin (bleo) or saline. N=9 for F4 TERC $-/-$ saline treated, F6 TERT $-/-$ saline treated, WT saline treated, and F4 TERC $-/-$ bleomycin treated; 12 for F6 TERT $-/-$ bleomycin treated; and 10 for WT bleomycin treated. Experiment survival post bleomycin was 9/10 for F4 TERC $-/-$, 12/13 for F6 TERT $-/-$, and 10/10 for WT. For collagen data in J, $p < 0.05$ between individual genotype columns for saline versus bleo. K) Semiquantitative scoring of lung fibrosis from 8–10 week old F4 TERT $-/-$ and WT controls at 2 weeks following 8 biweekly repetitive doses of 0.04 units of intratracheal bleomycin. N = 12 for F4 TERT $-/-$ and 9 for WT. Experiment survival was 12/14 for F4 TERT $-/-$ and 9/11 for WT.

Figure 4.

Lung inflammation as assessed by bronchoalveolar lavage (BAL) cell counts was similar among F4 *TERC* deficient (F4 *TERC* ^{-/-}), F6 *TERT* deficient (F6 *TERT* ^{-/-}), and wild type (WT) control mice at 2 weeks following bleomycin. Graphs depict total BAL A) leukocytes, B) macrophages, C) neutrophils, and D) lymphocytes. N = 7 for F4 *TERC* ^{-/-}, 5 for F6 *TERT* ^{-/-}, and 8 for WT.

Figure 5.

Telomere lengths were shortened in F4 *TERC* deficient (F4 *TERC* $-/-$) and F6 *TERT* deficient (F6 *TERT* $-/-$) mice compared to wild type (WT) controls. A) Real time PCR telomere lengths from peripheral blood leukocytes. N = 14 for F4 *TERC* $-/-$, 13 for F6 *TERT* $-/-$, and 9 for WT. * $p < 0.01$ compared to other columns. B) Real time PCR telomere lengths from isolated type II AECs. N = 3 for F4 *TERC* $-/-$, 4 for F6 *TERT* $-/-$, and 4 for WT. * $p < 0.01$ compared to other columns.