Regeneration of the Aging Lung: A Mini-Review

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Abstract

Natural lung aging is marked by molecular changes that occur during development, maturation, and late-life decline. At the cellular and whole organ level, degenerative changes that are a hallmark of natural aging (shorter telomeres, increased expression of cellular senescence markers, increased DNA damage, oxidative stress, and apoptosis, accompanied by diminished elasticity) reach pathological levels in aging humans in the form of chronic respiratory disease. Aging strongly correlates with the development and incidence of chronic respiratory diseases, including cancer and idiopathic pulmonary fibrosis, but is most strongly linked with development of chronic obstructive pulmonary disease. Lung failure due to aging can be traced to loss of lung stem cell regenerative capacity within the distinctive stem cell niches found within each compartment of the lung. Current knowledge about the identity and function of these stem cell compartments has been largely drawn from a variety of transgenic and spontaneously mutated mouse models that are characterized by rapid rates of aging or have been used to examine regeneration from injury in the context of natural

or accelerated aging. While much work has focused on the failure of epithelial cell populations as a key component of the aging process, additional studies have shown that aging, as a global phenomenon in the lung, also impacts resident endothelial, mesenchymal, and immune cell populations. In this review, we examine aging as a process dependent on specific changes in molecular pathways within multiple lung cell populations.

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Introduction

The natural aging process is marked by molecular changes that occur during development, maturation, and decline. In this review, we will present current data on lung aging and lung regeneration, with specific attention paid to stem cell-driven regeneration within the context of aging. We will also examine how regenerative failure may contribute to development of aging-related, chronic lung disease.

At the cellular level, aging is marked by depletion of adult stem cell reservoirs, the inability to maintain baseline homeostasis, a reduced response to stress, an increased accumulation of damaged DNA leading to telo-

mere shortening, and mitochondrial dysfunction [1, 2]. In multiple organs, including the lungs, age-related tissue and organ dysfunction interferes with tissue regeneration, which requires functional stem cells. Stem cells are characterized by their ability to undergo self-renewal to maintain stem cell reserves, and, when required, to produce new, terminally differentiated cells. Stem cells of all organs – including the lung, which harbors distinct stem cells for each separate tissue that makes up the lung as a whole – reside in niches described as a microenvironment that supports and maintains the ‘stemness’ of cells as a critical reservoir for maintaining tissue homeostasis and responding to injury [3]. During aging, a decline in organ function can be traced to a loss of stem cell function due to increased cell turnover, depletion of stem cells, and alterations to the stem cell niche. This debilitation leads to an inability to replenish lost cells, resulting in overall diminished tissue and cellular regeneration. Consequences of aging include increased susceptibility to aging-associated degenerative disease(s) and a severely diminished ability to regenerate lost or damaged tissue.

Characteristics of the Aging Lung

The human lung reaches full function and maturation at the age of 20–25 years [4]. Lung function, as measured by forced expiratory volume, then progressively decreases even in the absence of disease, at a rate of 1% per year after the age of 25 years [5–7]. As function declines, the lung exhibits multiple age-associated changes, including reduced respiratory muscle strength, increased secretion of proinflammatory cytokines (IL-1 β , IL-6, and TNF- α) even in the absence of acute insult, a reduced immune response [8, 9], and alterations in structural extracellular matrix (ECM) proteins (collagen and elastin) [5–7]. Lung functional decline is accompanied by structural alterations characterized by enlarged airspaces, loss of surface area [4, 10], and a decrease in static elastic recoil [11], often accompanied by changes in compliance [6, 7].

Lung Aging and Lung Disease

Lung aging strongly correlates with the development and incidence of chronic respiratory diseases. The incidence of chronic obstructive pulmonary disease (COPD), a combination of emphysema and chronic bronchitis, increases with aging and is accelerated by smoking [12]. The pathological characteristics in COPD are enlarged

airspaces, destruction of the alveolar wall, chronic inflammation, fibrosis of the small airways, increased mucus production, and diminished lung function [8]. Once they appear, these pathologies occur at an accelerated rate, and the severity of the disease increases over late-middle to very late age. To decipher the mechanisms underlying the characteristic loss of lung parenchyma in emphysema/COPD, molecular changes in tissue from emphysema patients were analyzed [13–15]. Endothelial and alveolar cells from emphysema patients had shorter telomeres, increased expression of cellular senescence markers (p16 and p21), DNA damage (TUNEL), oxidative stress, apoptosis (BAX, BAD, and activated caspase-3), and proliferation (PCNA) compared to tissue from age-matched nonsmokers. These studies indicate that heightened alveolar cell turnover in response to the burden of maintaining homeostasis correlates with a loss of alveolar structures in the aged lung. Over time, the demand to replenish lost cells becomes impossible to sustain, leading to emphysema caused by adult stem cell depletion. The molecular pathways that contribute to the accelerated aging phenotype of COPD are still unclear and under intensive investigation.

The appearance of lung cancers, as well as the development of idiopathic pulmonary fibrosis (IPF), is also associated with aging, though the incidence of both within the total population is much lower than that of COPD. Of the 3 most prominent chronic respiratory diseases, IPF is diagnosed at a marginally earlier age, with a mean age at diagnosis of 66 years, versus 69–70 years for COPD and lung cancer. At middle age (35–40 years), the incidence of COPD is approximately 200/10,000, versus 0.3–0.9/10,000 for IPF. Cases rise on parallel tracks for both diseases with aging, though the incidence of IPF within the total population remains lower, with 4–17/10,000 diagnosed with IPF after the age of 75 years, versus 1,200/10,000 diagnosed with COPD after the age of 65 years [16–18]. While diagnoses of COPD and IPF continue to increase with age, rates for lung cancer peak at the age of 70 years at 70/10,000 cases, then decline with further aging, which may be a hallmark of diminished somatic cell turnover and increasing cellular senescence. COPD and natural aging have similar phenotypes, and thus could be mechanistically linked. In contrast to COPD pathologies, which result in a loose, overly compliant lung, IPF patients exhibit fibrotic lesions throughout the lung parenchyma, resulting in stiffening of the lung [19]. However, COPD and IPF share some common pathological characteristics that are probably driven by genetic predisposition, frequent and cumulative exposure to environmental toxins,

and/or the time and number of insults to the lung. Development of IPF has been linked to telomere shortening, cellular senescence, and stem cell exhaustion, all hallmarks of aging. Mutations in components of the telomerase holoenzyme and the shelterin complex that maintain telomeres have been found to be a factor common to cases of both familial and sporadic IPF, and have also been linked to development of COPD and emphysema [20, 21]. Some studies have speculated that IPF fibrotic lesions are a result of dysregulated wound repair, characterized by the presence of fibrocytes and myofibroblasts and an altered lung ECM [17]. In fact, intrinsic changes in ECM with age have been shown by studies in which seeded cells respond by activating very different pathways for proliferation, differentiation, and senescence depending on whether they are seeded on ECM derived from aged or from youthful lungs [22]. Thus, aging can intrinsically alter the lung at both the cellular and extracellular levels. There are clearly different combinations or degrees of activation of molecular pathways – which are not currently understood – that predispose susceptible individuals to onset of one chronic lung disease over the other, or allow other individuals to experience only natural lung aging.

Aging and Lung Regeneration

Maintenance of efficient lung function is critical for aging individuals. Failure of the cellular regenerative capacity leads to failure of both structure and function, but our understanding of how aging compromises the human lung regenerative capacity is not well understood. What is known is that older individuals who undergo a partial pneumonectomy (PNX) show a significantly reduced ability to repair damage and regenerate a healthy lung compared to younger patients. It has been hypothesized that the inability of aged lung tissue to reinitiate growth signaling may contribute to its slow, restricted, and even failed response to injury. Hence, it is critical to understand how aging impairs the mechanisms of regeneration of the human lung.

Stem Cell Response to Tissue Loss and the Drive to Regenerate

The respiratory system is composed of the conducting airways and millions of alveoli. Upon activation, stem cells within these distinct lung compartments undergo

asymmetric proliferation, with new cells possessing either the ability to self-renew or to transform into differentiated cell types for purposes of replacement [23]. In the lung, even in the absence of acute insult, aging can alter stem cell regenerative capacity, resulting in organ dysfunction and consequent manifestation of chronic lung disease [17]. At least 40 different types of cells, with new populations continuously being identified, contribute to the complex architecture of the lung [24]. Amongst these, lung stem cells are critical for the repair of injury, the generation of new tissue, and the reestablishment of homeostasis that together make up the process of regeneration. Thus, lung regeneration relies on the regenerative capacity of lung stem cells in both humans and experimental mouse models, and the identification and characterization of these specialized populations have been revealed by analysis of the response of each species to lung injury.

Airway Stem Cells

Multiple epithelial stem cell populations, such as club cells, basal cells, and neuroendocrine cells, reside in the trachea and primary bronchi of the mouse and small airways of humans. Cuboidal club cells function as stem cells of the airways [25], producing the marker secretoglobin family member 1a1 (Scgb1a1, also known as CCSP or CC10) in mice. Club cells respond to naphthalene injury experiments by self-renewal during repair and differentiate into ciliated cells [26], while the surviving ciliated cells transform into a squamous-like cell to protect and spread onto a denuded epithelium during repair [27]. During the repair phase, the club cells also differentiate into goblet cells, which secrete mucin glycoproteins (including Muc5ac) in response to an allergen challenge [28]. Basal cells are stem cells of the mouse tracheal epithelium, comparable to the human small airway, and express p63, keratin 5 (Krt5), keratin 14 (Krt14), and nerve growth factor receptor (NGFR) [29]. Their stem cell capacity is high, with the potential to self-renew, proliferate, and differentiate into club and ciliated cells at homeostasis or following sulfur dioxide injury [29] or naphthalene injury [30]. They can also proliferate and migrate to the alveoli to repopulate the denuded epithelium after a viral infection [31]. In these cells, the Notch pathway is activated for repair after injury, which can induce differentiation of basal cells into secretory cells [32]. Neuroendocrine cells cluster adjacent to the ciliated, goblet, and club cells [33], and stain positive for calcitonin gene-related peptide (CGRP). Their stem cell capacity has been observed after naphthalene injury, after which they self-renew and differentiate into club and ciliated cells [34]. It should be

noted that these data were all gathered from young animals, and, currently, no information is available for the response of airway stem cell populations to injury or tissue loss in the aged lung.

Non-Airway Parenchymal Stem Cells

Multiple other epithelial stem cells have so far been detected within the non-airway portion of the lung parenchyma. The bronchoalveolar stem cells (BASC) are located in the bronchoalveolar duct junction, designated as a putative stem cell niche, and coexpress Scgb1a1 and SPC [35]. Their stem cell capacity is remarkable, and they are multipotent depending on the type of injury incurred. BASC can expand *in vivo* after bleomycin-induced injury and differentiate into alveolar epithelial type 2 (AEC2) and type 1 cells (AEC1) [35, 36]. BASC also expand *in vivo* after hyperoxia-induced injury, but they differentiate into only Scgb1a1+ cells and not into alveolar epithelial cells [26]. The BASC population also rapidly expands in response to PNx, though the specific role played in the neoalveolarization that occurs in the remaining tissue following lung volume reduction is less clear [37].

Recently, Vaughan et al. [38] identified an injury-responsive, lineage-negative lung epithelial progenitor population that activates a splice variant of p63, as well as Krt5. These cells are exquisitely sensitive to Notch signaling, levels of which influence their ability to productively regenerate alveolar tissue. Dysregulated repair by these progenitors due to excessive Notch signaling, also observed in IPF tissue, points to the role developmental pathways play in maintaining homeostasis throughout the life span, via control of progenitor populations. It has been speculated that failure or alteration of these pathways with aging is one of the underlying factors of tissue breakdown and even development of disease.

Alveoli

AEC2 are cuboidal cells with distinct microvilli and lamellar bodies. They cover 5% of the alveolar surface area and are located at the corners of the alveoli. Their specialized function is the synthesis, storage, and exocytosis of lung surfactant via lamellar bodies [39], as well as to regulate surfactant catabolism [40] and lung ion exchange [41]. Unique markers used to identify AEC2 are surfactant proteins (SPA, SPB, SPC, and SPD) and a lipid transporter, ATP binding cassette A3 transporter (ABCA3), all of which are components of the lung surfactant and/or are involved in the maintenance of lamellar body formation. All are critical for maintaining efficient lung function and homeostasis. AEC2 are considered a

quiescent adult lung stem cell population with minimal turnover [42]. However, if the alveolar epithelium is damaged, AEC2 survivors – either resistant to the injury or capable of efficient self-repair – regenerate the distal lung epithelium by clonal expansion and differentiation into AEC1 [36]. These results are similar to our previous findings regarding the behavior of AEC2 after hyperoxic injury, indicative of damaged cells undergoing apoptosis while coexisting with a proliferative AEC2 subpopulation that presumably contributes to repair of the injury [42, 43]. Additional studies (including those by our laboratory) that use models of AEC2 ablation in mice show that varying levels of AEC2 loss can produce a variety of outcomes. These data indicate the existence of a wide spectrum of survival and regenerative capacities within the AEC2 population, with response most likely dependent on the context and severity of an injury [44]. Presumably, aging would have an impact on this response, but these studies have yet to be performed.

AEC1 are thin, flat squamous cells that cover 95% of the alveolar surface. They lie on top of the endothelial cells of lung capillary beds, with the 2 tissues separated by a thin basement membrane [45]. They are identified by expression of the markers aquaporin 5 (Aqp5), podoplanin (Pdpn), Rage, and Hopx. Their main function is to exchange carbon dioxide and oxygen by diffusion and to transport ions and water from the alveolar lumen across to the capillary beds. AEC1 have just recently been shown to self-renew and differentiate into AEC2 after PNx, but not during homeostasis [46]. While AEC1 have long been considered the most vulnerable target for distal lung injury and aging, these recent data also mark them as a critical population for efficient regeneration. Thus, the loss of AEC1, as well as of AEC2, that occurs with aging presumably has a considerable impact on the regenerative capacity of the aging lung, even though few studies to date have specifically addressed this hypothesis.

Lung Stem Cell Contributions to Regeneration

PNx is an acute insult that drives robust regeneration in younger individuals and is therefore an effective tool for understanding the mechanisms required for a return to lung functional capacity and homeostasis and for understanding the roles adult lung stem cells play in these processes. PNx can also reveal points of failure in the regenerative capacity of older lungs. During repair and regeneration, the remaining surviving cells must have the ability to reconstruct missing cellular and structural components of the functional lung. External signals that arise in response to the tissue loss and traumatic injury that

Table 1. Mouse aging models: lung repair and regeneration

Mouse strain	Age, months	Age-associated changes	Injury	Repair and regeneration outcome
Wild type – C57BL/6	2–3	No apparent changes	Hyperoxia [43]	Telomerase activation in AEC2
	2–3	No apparent changes	PNX [37]	Robust lung regrowth; AEC2 and BASC proliferation
	4	No apparent changes	PNX [59, 60]	EGFR-directed BASC, AEC2, and endothelial cell proliferation
	2–24	Lung functional deterioration, airspace enlargement [49]	PNX [56]	Differentiation of lung fibroblasts into myofibroblasts
	2–24	Lung functional deterioration, airspace enlargement [49]	PNX [67]	Increased collagen deposition
	2–24	Lung functional deterioration, airspace enlargement [49, 61]	None	Loss of AEC2 (SPC+) numbers and proliferation (Ki67+)
Telomerase null (terc ^{-/-})	3	Kyphosis, alopecia, sarcopenia, malocclusion, hair graying [52, 63], lung functional deterioration [61], AEC2 loss, airspace enlargement, increased apoptosis [53, 61]	None	Loss of AEC2 (SPC+) numbers and altered proliferation, DNA damage repair, and apoptotic signaling in AEC2
	3	Kyphosis, alopecia, sarcopenia, malocclusion, hair graying [52, 63], lung functional deterioration [61], AEC2 loss, airspace enlargement, increased apoptosis [53, 61]	PNX [37]	Poor lung regrowth; poor AEC and BASC proliferation
SAM	3–18	Destruction of the alveolar wall [55, 64]	Not performed	Unknown
Klotho	1–2	Airspace enlargement and increased compliance [54, 55]	Not performed	Unknown

acutely follow PNX stimulate dramatic changes in normally quiescent cells, including altered stem cell proliferation levels, a drive towards differentiation, and changes in polarity and positioning. How all these cellular events combine to regenerate complex lung structures, and why they fail with aging, is still unclear. While it is difficult to examine parameters other than survival, gross tissue changes, and lung function in humans following PNX, PNX in animal models – including models of lung aging – has been a highly useful experimental tool for interrogating mechanisms at the cellular and molecular levels.

Animal Models of Lung Aging

Aging is marked by molecular changes that occur throughout development, maturation, and decline. During the decline phase, natural aging of the lung is characterized by distinct structural and functional changes in humans [17]. Various animal models have been developed to better understand changes in the lung associated

with aging, and to define the molecular and cellular changes that lead to progressive loss and insufficiencies of regenerative stem cells (table 1). Models commonly used to study aging are telomerase null, Klotho null, and senescence-accelerated (SAM) mice, plus naturally aged, wild-type C57BL/6 mice, all of which have been used to identify signals and cell types that support lung homeostasis and drive repair and functional restoration during regeneration after lung injury. Recent data have shown that mutations that compromise the function of certain genes, which drives accelerated aging, also exhibit diminished expression in naturally aged humans and those diagnosed with COPD. These include components of the telomerase holoenzyme, the soluble form of klotho, Ku70, and sirtuin 1 [47, 48].

In healthy wild-type mice, including C57BL/6 mice, the decline in lung function and structure associated with aging is similar to the pattern observed in humans [49]. With age, progressively altered elastic properties of the lung structure and compromise of the cellular compartments are observed, including immune cells, the alveolar epithelium, and structural fibroblasts. The significant,

age-related deterioration in lung function in mice has been ascribed to an increase in AEC2 apoptosis, to increased elastase activity leading to airspace enlargement, and/or to fibroblast differentiation. The severity and prominence of each change and the rate at which they appear are strain dependent [50].

In adult mice, *in vivo* experiments have shown that telomerase activity in the lung decreases during maturation postnatally, but that it is activated following hyperoxic injury [43, 51]. High oxygen levels (>90%) lead to increased levels of toxic reactive oxygen species, which results in acute pulmonary inflammation and apoptosis of cells residing throughout the lung, and particularly in the alveolar epithelium. These studies demonstrated the replacement of damaged cells and a return to alveolar homeostasis by activation of telomerase in AEC2 stem cells. Although these studies have not been replicated in aged animals, there are data showing decreased telomerase activation with aging in many tissues, indicating that compromise of these molecular events may well underlie the poorer regenerative capacity of the aging lung.

Telomerase RNA component-deficient (telomerase null or *terc*^{-/-}) mice serve as a model of accelerated aging. Lack of telomerase activity causes telomeres to shorten in successive, inbred generations of *terc*^{-/-} mice [52]. Telomere elongation is thought to be important for the long-term survival and proliferative capacity of stem cell reserves for development, growth, and organ regeneration. *Terc*^{-/-} mice develop a premature aging phenotype, exhibiting kyphosis, alopecia, sarcopenia, malocclusion, and hair graying, which becomes more severe as telomeres shorten. Currently, our understanding of the contribution of telomerase function and stem cell capacity to regenerative lung repair during aging is limited, as only a few studies have utilized the *terc*^{-/-} model to study lung aging. Our laboratory has shown how *terc* loss impacts the lung at both whole organ and cellular levels, leading to a progressive deterioration in lung function [unpubl. data], as measured by changes in the lung structure and impairment of at least 1 population of adult lung stem cells (AEC2) [37, 53]. Our analysis has shown a significant degree of airspace enlargement in aging *terc*^{-/-} lungs, which resembles the structural changes observed in normal human lung aging. Other critical morphological abnormalities included decreased elastin deposition in the alveolar septa, suggesting a reduction in structural elasticity. *Terc*^{-/-} lungs also have significantly lower collagen content and synthesis, suggesting suppression of interstitial fibroblast-driven collagen production, which further contributes to alveolar enlargement. We have

used this model to study AEC2, known to be compromised in patients with COPD. In *terc*^{-/-} mice, degeneration in lung tissue with age is characterized by reduced numbers of AEC2 and a diminished capability to resolve lung injury. As a consequence of the *terc* deletion, there is an increased loss in AEC2 numbers and greater susceptibility to oxidative DNA damage in the lung parenchyma. Furthermore, AEC2 isolated from *terc*^{-/-} mice exhibit increased expression of markers of chronic stress, such as phosphorylated forms of SAPK, JNK, and c-Jun. These data show how exposure of AEC2 to the chronic stress of aging can have detrimental consequences due to a persistent inability to repair damage, even within a normal environment. Our evidence suggests that telomere shortening is an important factor that may contribute to the inability of aged AEC2 to maintain tissue homeostasis and/or regenerate tissue when necessary.

Other transgenic mouse models that exhibit pulmonary changes with accelerated aging consistent with normal aging of the human lung include the Klotho null mouse and the SAM mouse, both of which experience acutely shortened life spans. Both models exhibit progressive emphysematous changes accompanied by the destruction of alveolar walls and enlargement of airspaces [54, 55]. Additionally, Klotho null and SAM mice have increased compliance, a lower respiratory rate, and lower lung volumes, similar to the functional decline in aged humans. This aging-like phenotype is accompanied by a pattern of alveolar and AEC2 destruction in both models, and by abnormal, increased levels of surfactant proteins, collagen IV, and mitochondrial ATPase in Klotho null mice. Thus far, the lung regenerative capacity of these models has yet to be examined.

In vivo Models of Aged Lung Regeneration

A recent study by the Krasnow laboratory [42] used lineage labeling to examine AEC2 baseline behavior as mice aged over an 8-month period. These studies showed that AEC2 are a slowly renewing, adult lung stem cell population, with only 1% of AEC2 demonstrating cellular multiplication *in vivo*. A study by the Hogan laboratory [36] used lineage labeling technology to define the normal alveolar epithelial cell turnover under homeostatic conditions in *Sftpc*-CreER;Rosa-Tomato (Tm) or Confetti transgenic mice. These models allowed tracking of AEC2 activity for approximately 1 year to determine self-renewal potential and clonal expansion capacity. The results showed that at homeostasis, in transgenic mice with-

out any injury, AEC2 possess long-term self-renewal capacity with slow and constant proliferative (Ki67+) potential. Clonal expansion of lineage-labeled AEC2 was also observed for the duration of the experiment. These studies confirmed that AEC2 behave consistently at homeostasis over the first year of the wild-type mouse life span (approx. 2.5 years). However, data on the later, declining period of that span have yet to be generated.

The lung injury model of PNx – which in an experimental setting results in compensatory regrowth of the remaining right lung after the removal of the left lung – has been specifically used to better understand the mechanism(s) of injury response and resolution. In a few studies it has also been used to examine the reparative capacity in mice as it changes with aging. Techniques used to measure PNx response during repair and regrowth in both youthful and aged lungs include: analyses of cell morphological changes and changes in cell-specific marker expression; lineage tracing; clonal analysis; assessment of cell apoptosis, proliferation, and DNA damage and of repair marker expression; and analysis of cell cycle progression and DNA synthesis markers. The compensatory regrowth program following PNx depends on a joint effort by epithelial, mesenchymal, and inflammatory cells. Together, they regulate and respond to morphogens, growth factors, and transcription factors that are responsible for maintaining and activating resident lung stem cell populations for the purpose of regenerating functional lung tissue, particularly alveoli, in a process of neoalveogenesis.

A study by the Hoffman laboratory [56] investigated the regenerative capacity of the lung in 3-, 9-, and 24-month-old C57BL/6 mice following PNx. One key finding was that in aged, pneumonectomized mice, supportive and structural lung fibroblasts differentiated into a myofibroblast phenotype, characterized by increased mRNA levels of ECM proteins, including collagens III, IV, and V, elastin, and lysyl oxidase (the enzyme required for cross-linking structural fibers), and increased protein expression of α -smooth muscle actin. There was an increase in collagen content after PNx in both the 3- and the 9-month-old mice, which was not observed in the 24-month-old cohort, indicating that older mice had a reduced capacity for ECM remodeling. Also observed in this study was a gradual decline in the proportion of AEC2 within the parenchyma as measured by SPC immunostaining, demonstrating the loss of lung stem cell regenerative capacity in an age-dependent manner. Additionally, the proliferative capacity of AEC2, detected by Ki67 staining, was still robust at 9 months of age, but it

dramatically declined by 24 months of age. These data indicated a failure to replace the AEC2 population after 9 months of age. Aging in 9- to 24-month-old mice also led to an irreversible decline in respiratory function after PNx, reflecting a physiological deficit due to loss of regenerative capacity.

A second study conducted by the Hoffman group [57] evaluated cells other than AEC2 residing in the alveoli of aging mice at the ages of 3, 9, and 17 months. These results showed that after PNx in naturally aged mice there was no change in the endothelial compartment, but the proliferative capacity of lung mesenchymal stem cells was significantly reduced. Importantly, the lung mesenchymal stem cells from 12-month-old mice proliferated poorly in vitro and showed reduced telomerase activity, both of which have been associated with deterioration in the reparative capacity during lung regeneration in the *terc*^{-/-} mouse model [37]. Overall, these studies provided evidence that multiple lung cell populations contribute to regeneration following PNx, and that these populations are variably compromised by aging.

Our laboratory examined how *terc*^{-/-} mice respond to PNx [37]. Following surgical removal of the left lobe, *terc*^{-/-} mice showed a higher rate of mortality, diminished compensatory lung growth, diminished distal stem cell response and proliferation, and persistent DNA damage, all of which strongly correlated to telomere length. These data indicated that an aging phenotype, as marked by shortening telomeres, contributes to the suppression of repair, regeneration, and survival responses. In this study, *terc*^{-/-} mice that survived PNx displayed lower absolute numbers of both AEC2 and BASC in the distal lung parenchyma. AEC2 showed decreased proliferative capacity as measured by expression of the markers ERK1/2, Ki67, and PCNA, increased apoptosis (by increased PARP expression), cell cycle arrest (by increased p21 expression), increased DNA oxidation (by increased 8-OHdG expression), and decreased DNA repair (by decreased Ogg-1 and Gadd153 expression). Together, these data show that prematurely aged lungs from *terc*^{-/-} mice have overall diminished stem cell reserve levels, are more sensitive to apoptosis, and have compromised reparative capacity after a lung injury compared to the robust response of the youthful, wild-type mice used as controls. Our results show that accelerated aging of the lung is a consequence of telomere dysfunction and damage, with a gradual decline in the ability (1) to respond to injury, (2) to repair damage, and (3) to regenerate functional tissue. Because of the molecular insufficiency that occurs even at baseline during the process of aging, reserve stem cell

Table 2. Lung stem cells

Stem cell (marker)	Location	Injury	Repair and regeneration outcome (marker)
Club cells (Scgb1a1)	Trachea and bronchioles	Naphthalene [26]	Self-renew and differentiate into ciliated cells (acetylated tubulin+)
		Ovalbumin challenge [28]	Differentiate into goblet cells (Muc5+)
Basal cells (p63, Krt5, Krt14, and NGFR)	Trachea	Naphthalene [30]	Self-renew and differentiate into club (Scgb1a1+) and ciliated cells (acetylated tubulin+)
		Sulfur dioxide inhalation [29, 32]	Self-renew and differentiate into club (Scgb1a1+) and ciliated cells (FoxJ1+)
		H1N1 infection [31]	Differentiate into AEC1 (Pdpn+)
Neuroendocrine cells (CGRP)	Trachea	Naphthalene [34]	Self-renew and differentiate into club (Scgb1a1+) and ciliated (acetylated tubulin+) cells
BASC (Scgb1a1 and SPC)	Broncho-alveolar duct junction	Bleomycin [35]	Self-renew (Scgb1a1+ and SPC+)
		Hyperoxia [26]	Self-renew; no contribution to AEC2 (SPC+)
		Bleomycin [36]	Self-renew and differentiate into AEC2 (SPC+) and AEC1 (Aqp5+)
		PNX [37]	Self-renew (SPC+ cells at BADJ)
LNEP (NP63 and Krt5)	Alveoli	Bleomycin and H1N1 viral infection [38]	Differentiate into AEC2 (SPC+)
AEC2 [SPA, SPB, SPC, SPD, ABCA3, and lysozyme M (newly differentiated AEC2)]	Alveoli	Ablation by diphtheria toxin [36]	Self-renew (SPC+) and differentiate into AEC1 (Aqp5+)
		Hyperoxia [43, 51]	Self-renew (PCNA+ and telomerase+)
		Hyperoxia [42]	Self-renew (GFP+) and differentiation into AEC1 (Lys+)
		Ablation by ganciclovir [44]	Self-renew (SPC+ and PCNA+)
AEC1 (Aqp5, Pdpn, Rage, and Hopx)	Alveoli	PNX [46]	Self-renew (Hopx+) and differentiate into AEC2 (SPC+)

populations of the lung are compromised with age, along with the reparative capacity for regeneration.

A lung aging study by the Armanios laboratory [21] showed that *terc*^{-/-} mice exhibited airspace enlargement, an increase in the mean linear intercept, and a decrease in lung compliance only after chronic exposure to cigarette smoke. These results somewhat varied from previous observations that showed age-related structural change in the lung parenchyma at baseline, even without toxin exposure, and have been ascribed to differences between inbred telomerase null colonies. The Armanios study also showed increased DNA damage in small airway epithelial progenitor club cells, where telomere shortening induced persistent DNA double-strand breaks and cell cycle arrest by increased levels of p21, resulting in a slower reparative capacity. This outcome was similar to observations by our laboratory and others on AEC2. More recently, Armanios and colleagues [58] showed that AEC2 isolated from *terc*^{-/-} and *TRF*^{-/-}

(a component of the shelterin complex) mice, showed an inability to form alveolar spheres *in vitro* (a model for reconstitution of the stem cell niche by wild-type AEC2), which is indicative of the loss of the endogenous capacity for self-renewal and proliferation in telomerase-compromised lung stem cells.

Contributions of Mesenchymal and Immune Cell Populations to Lung Regeneration

In vitro experiments have demonstrated that murine lipofibroblasts that express platelet-derived growth factor- α (*Pdgfra*⁺) support AEC2 self-renewal and differentiation into AEC1 [36]. Thus, this study confirmed that additional cell populations, including those of nonepithelial lineages, probably support the stem cell niche for AEC2 self-renewal (table 2). As with the airway, the impact of aging on this niche is still unknown.

The Rafii laboratory [59] has investigated the role of pulmonary capillary endothelial cells (PCEC) in alveolar regeneration following PNX. Their studies have provided both cellular and molecular mechanisms for regulation of compensatory lung growth in adult mice. PCEC are a component of alveolar capillary beds. In the early regrowth period following PNX there was observed a significant proliferation of BASC, followed by AEC2 – and later PCEC – proliferation. As the repair and regrowth phase proceeded, isolated PCEC exhibited increased expression of phosphorylated and activated forms of VEGFR2 and FGFR1, both of which are angiocrine receptors, suggesting direct activation of PCEC. Validation experiments using knockout transgenic mice for VEGFR2 and/or FGFR1 demonstrated a diminished level of proliferation of the BASC, AEC2, and PCEC populations after PNX. This experiment indicated a critical role for PCEC in alveolarization during lung regeneration. To further decipher this mechanism, investigators identified an up-regulation of MMP14 expression in isolated PCEC, which can proteolytically cleave matrix components, providing evidence that PCEC secretion of MMP14 can enhance the proliferation of BASC and AEC2 and may enhance alveolar-capillary formation in alveolar spheres *in vitro*. This study concluded that PCEC are activated to eventually release the ectodomain of HB-EGF, a ligand for epidermal growth factor receptor (EGFR), which is known to stimulate epithelial compensatory lung regrowth. This study also showed that release of SDF-1 by platelets is critical for binding and activating CXCR4 and CXCR7 receptors on endothelial cells to drive the process of releasing MMP14 [60].

An additional *in vitro* study [42] showed that AEC2 express the receptors EGFR (ErbB1), ErbB2, and ErbB3, which increases their ability to proliferate but not to differentiate into AEC1. AEC2 exposed to EGF ligands, including transforming growth factor- α , HB-EGF, and neuregulin 1 exhibited proliferation with a sequential increase in response to each additional stimulus. In contrast, a selective blocking antibody to EGFR to inhibit the response to the external stimuli resulted in reduced AEC2 proliferation. Together, these studies indicate that the EGFR signaling pathway is involved in the stimulation of alveolar epithelial cell proliferation during compensatory lung regrowth, and that both endothelial and immune cell compartments play key roles in compensatory epithelial regrowth. As with other studies, the impact of aging on these interactions and the role they play in driving regeneration in an aging context has yet to be determined.

Conclusion

While the lung is a vastly resilient organ, with a structural design that features a substantial amount of redundancy, it is still vulnerable to environmental stressors and the constant burden of providing efficient gas exchange over the total life span of the organism. Genetic predispositions and constant exposure to a variety of environmental stressors combine to modulate individual rates of lung aging. In a substantial portion of the population, for underlying reasons still not well understood, lung aging occurs at an accelerated rate and results in chronic lung disease. One factor that may contribute to aging-related development of lung disease is the failure of multiple, endogenous lung stem cell populations to regenerate damaged tissue or even to maintain efficient homeostasis. Research to date – mainly using mouse models of natural and accelerated aging – has allowed identifying these critical populations. It is clear that research will be needed to address methods for prophylactically and/or therapeutically supporting lung stem cell populations if lung aging is to be managed, such that the lung can function efficiently into old age.

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Disclosure Statement

The authors declare no conflict of interest.

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