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Review

The aging lung: Physiology, disease, and immunity

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<https://doi.org/10.1016/j.cell.2021.03.005>

SUMMARY

The population is aging at a rate never seen before in human history. As the number of elderly adults grows, it is imperative we expand our understanding of the underpinnings of aging biology. Human lungs are composed of a unique panoply of cell types that face ongoing chemical, mechanical, biological, immunological, and xenobiotic stress over a lifetime. Yet, we do not fully appreciate the mechanistic drivers of lung aging and why age increases the risk of parenchymal lung disease, fatal respiratory infection, and primary lung cancer. Here, we review the molecular and cellular aspects of lung aging, local stress response pathways, and how the aging process predisposes to the pathogenesis of pulmonary disease. We place these insights into context of the COVID-19 pandemic and discuss how innate and adaptive immunity within the lung is altered with age.

INTRODUCTION

Aging is the major risk factor for death from all age-related adult chronic diseases. By 2034, for the first time in American history, older adults are projected to outnumber children. On a global scale, the number of adults over age 65 is projected to increase from 617 million today to over 2 billion by 2050 and will account for ~20% of the world's population (Dzau et al., 2019). This demographic transition poses several challenges from healthcare and socioeconomic perspectives as we observe precipitous rises in diseases that disproportionately afflict older individuals such as cancer, cardiovascular disease, pulmonary conditions, and dementia. Emerging evidence suggests that manipulating biological processes that deteriorate with age can ameliorate or delay many aging-related diseases. Pre-clinical models have demonstrated that aging is indeed a tractable and modifiable condition (de Cabo and Mattson, 2019), achieved through genetic manipulation, dietary intervention, or use of pharmacologic agents. Given the ability to extend healthspan in mammalian model organisms, including primates, there is enthusiasm to translate these findings to improve age-related disease outcomes in humans (Longo et al., 2015).

Cellular aging is defined as a multifactorial series of molecular alterations that leads to gradual deterioration of tissue function

and increased vulnerability to disease and death. Hallmarks of cellular aging can be grouped into different categories that include genome-based failures (genomic instability, telomere attrition, epigenetic dysregulation), signaling dysfunction (nutrient sensing aberrations, cell-cell communication anomalies), organelle compromise (mitochondrial abnormalities, loss of proteostasis), and cell phenotypic changes (stem cell exhaustion, cellular senescence) (López-Otín et al., 2013). Improving one cog in the wheel can positively impact other dysregulated pathways, as there is inter-dependency among them. For instance, selective restoration of autophagy in the liver of old mice leads to improvement not just in proteostasis but also in mitochondrial homeostasis, genomic stability, and organ function (Zhang and Cuervo, 2008). However, we are still in the nascent stages of understanding the selective pressures that occur with age and how aging impacts the fitness of specific organs.

Human lungs, the organ with the largest surface area in the body, represent a unique interface with the outside environment and are comprised of an array of cell types that face ongoing chemical, mechanical, biological, immunological, and xenobiotic stress over a lifetime. Advanced age causes progressive impairment of lung function in otherwise healthy individuals, marked by structural changes that impair gas exchange and immunologic changes that predispose to infections. Lung-resident cells rely on



robust stress response pathways to stave off cumulative damage, yet aging is characterized by a progressively handicapped ability to respond to environmental stressors. Developing a mechanistic understanding of how and why these adaptive mechanisms fail or become overwhelmed is integral in delineating their contribution to age-related diseases. The drivers of lung aging remain elusive and our understanding of why age increases the risk of primary lung cancer, respiratory infections, and obstructive and fibrotic lung disease is lacking (Budinger et al., 2017).

The unfolding of the COVID-19 pandemic underscores the urgent need to elucidate the changes that occur with age in the human lung and whether there are age-related molecular determinants that can be targeted for therapeutic purposes to mitigate morbidity and mortality in elderly populations (Koff and Williams, 2020). Although SARS-CoV-2 poses a mortality risk to young individuals, it is clear that respiratory illness is far more fatal in older adults, with mortality rates 20-fold higher or more for adults over 80 compared to those in their 50s (Williamson et al., 2020). Thus, it is possible that molecular and immunologic alterations with age provide a mechanistic basis for why elderly patients with COVID-19 fare worse. If there is indeed a fundamental difference in underlying pulmonary biology that drives the difference in mortality, this raises the question as to whether treatment paradigms should differ for elderly patients (Barzilai et al., 2020).

Although advanced age is the major risk factor for chronic lung diseases, the fundamental mechanisms that drive the aging process in the lung are largely unknown. Here, we discuss the cellular and molecular aspects of lung aging and failures in stress response pathway that contribute to development of pulmonary disease. We place these insights into context of the COVID-19 pandemic and discuss how innate and adaptive immunity within the lung is altered with age.

CELLULAR CHANGES IN THE AGING LUNG

Historically, lung tissue and resident cells were defined based on their immunohistochemical and morphologic features observed using light and electron microscopy (Crapo et al., 1982). Emerging technologies like single-cell transcriptomics and *in vivo* lineage tracing have provided an unparalleled view into the cellular complexity of the human lung. Today, we know the lung harbors at least 40 discrete cell types with single-cell molecular profiling helping to uncover cell type diversity and lineage hierarchy (Franks et al., 2008; Schiller et al., 2019). The identification of a new rare cell type, the cystic fibrosis transmembrane conductance regulator (CFTR)-expressing pulmonary ionocyte, highlights the evolving nature of the field (Montoro et al., 2018).

Despite these advances, the effects of aging on lung cellular composition and function have yet to be fully crystallized. Advanced age alone impairs pulmonary physiologic function even in the absence of disease. Lung maturation and function peak between 18–25 years of age, remain steady with minimal change up to 35 years of age, and gradually decline thereafter, manifested by progressive loss of alveolar surface area, dilation of air spaces, reduced mucociliary clearance, and changes in elasticity (Sharma and Goodwin, 2006). This is a direct result of underlying cellular changes that impact structural, functional,

and mechanical features of the respiratory system yet the precise molecular mechanisms are poorly understood. Below, we review the major compartments that are altered in the aged lung including (1) respiratory epithelium, (2) lung progenitor cells, (3) pulmonary immune cells, and (4) the interstitium.

Respiratory epithelium

The respiratory epithelium forms a vital barrier between the environment and internal human tissues, representing the first line of defense against inhaled pathogens, particulates, and other foreign material. This unique mucosal surface sees 10,000 L of inhaled air each day and is challenged with maintaining its structural and functional integrity over a lifespan. Upper airways are comprised of pseudostratified epithelium on the luminal mucosal surface and are predominantly made up of bronchial and bronchiolar epithelial cells including ciliated cells, goblet cells, secretory club cells, and basal progenitor cells, with less frequent cell types including neuroendocrine cells, ionocytes, and tuft cells. More distally, airways terminate in respiratory bronchioles, alveolar ducts, and alveolar sacs where gas exchange occurs in coordination with interwoven capillary beds. Type 1 alveolar epithelial cells (AT1) are flat, squamous cells that account for >95% of the surface area of the alveolar epithelium and play a prominent role in gas exchange. Type 2 alveolar epithelial cells (AT2) produce surfactant, maintain the fluid balance of the alveolar unit, and serve as progenitor cells for AT1 cells (Barkauskas et al., 2013). A complete catalog of cellular components of the human lung is reviewed in detail elsewhere (Franks et al., 2008; Schiller et al., 2019).

Cells that comprise the airway and alveolar respiratory epithelium exhibit quantitative and qualitative deficits with age (Figure 1). Aging is associated with reduced mucociliary clearance in both upper and lower airways (Proença de Oliveira-Maul et al., 2013; Svartengren et al., 2005). Age-dependent telomere shortening has been observed in normal human lungs, but which cell types are responsible for these changes is unclear (Everaerts et al., 2018). Single-cell transcriptome analysis from young and old wild-type mice under physiologic conditions reveals a global increase in transcriptional noise across most cell types in the murine lung with advanced age (Angelidis et al., 2019). This genetic signature of transcriptional variability is consistent with single-cell transcriptomics data collected from other aged human tissues, which may be due to a decline in epigenetic regulation with age.

Despite the accumulation of transcriptional noise across most pulmonary cell types with age, there are cell type-specific age-associated alterations. Single-cell transcriptional analysis shows that chronologic aging increases gene signatures associated with cholesterol biosynthesis in AT2 cells and lipofibroblasts, resulting in increased neutral lipid content in epithelial cells and fibroblasts with age (Angelidis et al., 2019). Aged AT2 cells also demonstrate altered antigen presentation, evidenced by increased major histocompatibility complex (MHC) class I expression. In aged murine lungs, ciliated cells are found in increased proportions, leading to an altered ratio of club-to-ciliated cells in mouse airways (Angelidis et al., 2019). Although single-cell technology has allowed the deconvolution of transcriptional profiles of distinct cell populations, further studies are

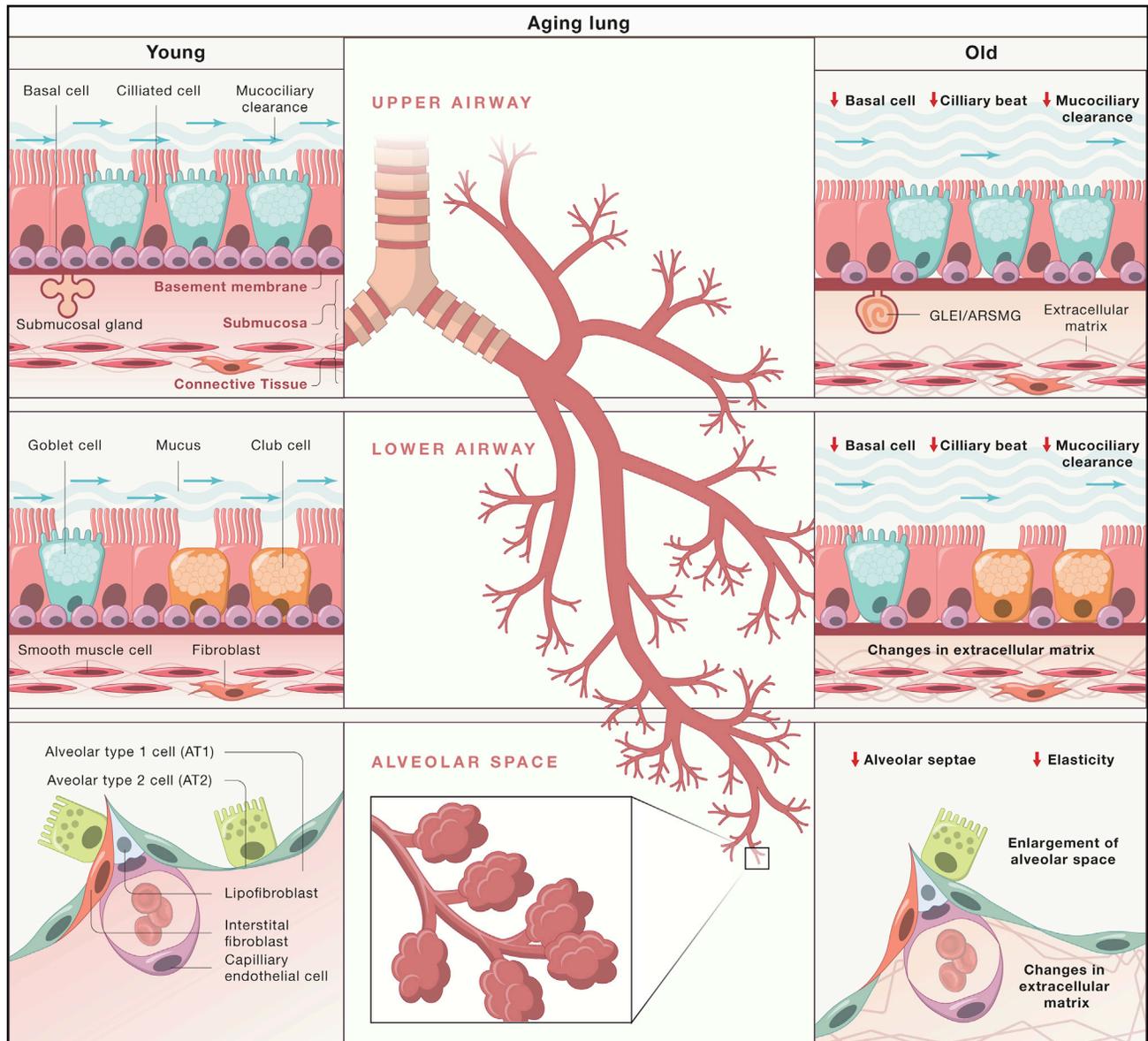


Figure 1. Cellular composition and functional changes in the aging lung

Schematic of changes in young (left) and aged (right) lung showing major changes in cellular composition and structure by anatomic region.

required to determine whether age-associated gene signatures are drivers of aging or a bystander effect.

Lung progenitor cells

The ability to repair, regenerate, and remodel the respiratory system is dependent on the functional capacity of adult progenitor cells. Under homeostatic conditions, the rate of lung cell turnover is low in comparison to other organs, as infrequent divisions of stem cells are sufficient to maintain the respiratory epithelium at baseline (Bowden, 1983; Rawlins and Hogan, 2008). Distinct pools of multipotent progenitor cells have been identified in the two main functional compartments of the lung: conducting airways and alveolar parenchyma. In con-

ducting airways, basal cells are multipotent stem cells that form part of the pseudostratified epithelium of the trachea and bronchi and have the capacity to maintain a homeostatic epithelial layer and restore the entire epithelium of the trachea in response to injury (Rock et al., 2009, 2010). In the smaller conducting airways, club cells are considered progenitor cells that differentiate into goblet and ciliated cells in response to stress (Hong et al., 2001; Rawlins and Hogan, 2005; Rawlins et al., 2009).

AT2 cells are the major progenitor cell population of the alveolar parenchyma. They regenerate after injury by proliferating and differentiating into AT1 cells that are critical to maintain gas exchange (Barkauskas et al., 2013). Recent data suggest

the existence of a subpopulation of dedicated AT2 progenitors that differ from classical AT2 cells based on expression of WNT signaling responsive molecules (Zacharias et al., 2018). This signaling activation after injury induces proliferative and self-renewal capacity as well as differentiation to AT1 cells (Nabhan et al., 2018). This newly described population implies the existence of an AT2 subpopulation specialized in the synthesis and secretion of the alveolar surfactant proteins and a dedicated progenitor AT2 population capable of self-renewal and differentiation into AT2 and AT1 cells (Travaglini et al., 2020).

One hallmark of aging is the depletion of adult stem cell reservoirs. Age-related quantitative and qualitative changes have been described for different progenitor populations in the lung (Table 1). Basal and club cells decrease in number with age, whereas AT2 cells are unchanged in quantity but exhibit deficits in self-renewal and differentiation capacity (Ortega-Martínez et al., 2016; Wansleben et al., 2014; Watson et al., 2020). Age-associated stem cell exhaustion has been ascribed to numerous mechanisms including oxidative stress response, mitochondrial dysfunction, shortened telomeres, and epigenetic changes (Navarro and Driscoll, 2017). Depletion and dysfunction of lung-resident stem cells with age compromises repair and regenerative capacity and contributes to pulmonary diseases like emphysema and pulmonary fibrosis (Kotton and Morrissey, 2014). Questions remain about how aging reshapes the lung stem niche and how advanced age alters the contributions of epithelial, mesenchymal, and immune cell populations to lung regeneration. One study highlighted the importance of cell non-autonomous processes in lung repair by showing lymphotoxin β -receptor signaling plays a role in airway inflammation, fibrosis, and regeneration in young and aged mice (Conlon et al., 2020). Whereas most data on pulmonary progenitors are derived from studies using rodent models, we have much to learn about how human lung stem cell populations change with age.

Pulmonary immune cells

The human respiratory tract is a critical immune interface that requires a tightly regulated response to the continuous exposure of environmental challenges. Pulmonary immune homeostasis is maintained by a complex network of airway-resident lung epithelial cell types, as well as by local and recruited immunocytes derived from myeloid and lymphoid lineages (Iwasaki et al., 2017). Through continual sampling of the antigen-rich external environment at the respiratory mucosal surface, pulmonary immune effector cells ensure robust responses against invading pathogens and foreign material. Although a coordinated immune response is critical to protect against pathogenic agents, tight control of pro-inflammatory mediators is necessary to prevent uncontrolled fibrotic deposition and scarring that can lead to deleterious tissue remodeling.

Within the respiratory epithelium, ciliated columnar cells, mucus-secreting goblet cells, tuft cells, and Club cells form a barrier that serves as the first line of defense against invading respiratory pathogens, pollutants, and allergens (Hiemstra et al., 2015). Airway epithelial cells themselves are immunologically active and capable of secreting cytokines that influence neighboring leukocytes (Lloyd and Marsland, 2017). They also

express pattern-recognition receptors such as Toll-like receptors (TLRs) that recognize pathogen-associated molecular patterns (PAMPs) derived from a variety of infectious agents. Direct secretion of anti-microbial mediators such as defensins, collectins, and lysozyme serves an important function in immune defense and represents one way that airway epithelial cells communicate with and recruit innate and adaptive immune effector cells.

In conjunction with airway-resident lung epithelial cells, alveolar macrophages (AMs) and peribronchial interstitial macrophages (IMs) (Gibbings et al., 2017) play a central role in orchestrating the pulmonary immune response. AMs are long-lived cells that colonize airways soon after birth and continue to self-renew in the local lung microenvironment over a lifetime (Gomez Perdiguero et al., 2015). They reside within the lumen, directly exposed to air and tethered to the alveolar epithelium via integrins, where they are responsible for sampling foreign material and engulfing pathogens that reach the distal airspaces (Tan and Krasnow, 2016). The epithelial-macrophage interaction serves as a specialized unit that integrates microbial-derived signals and responds by releasing various pro- or anti-inflammatory mediators. AMs sense external stimuli through a wide range of surface receptors that leads to either activation or suppression of downstream signaling cascades depending on the nature of the stimulus (Hussell and Bell, 2014).

AMs are the most abundant cellular fraction within bronchoalveolar lavage (BAL) fluid under healthy, homeostatic conditions (Srivastava et al., 2014). During aging, studies from mice and humans have documented a decline in concentration of AMs present in the respiratory tract (Wong et al., 2017) and multiple functional deficits including failures in phagocytosis and scavenging capacity, refractory activation to interferon (IFN)- γ signaling, impaired antiviral response, and maladaptive TLR signaling (Albright et al., 2016). Although the developmental origin of tissue macrophages is diverse, microenvironments are thought to be dominant over developmental origin in dictating the functionality of lung-resident macrophages (Tan and Krasnow, 2016). A recent study used genetic lineage tracing and transcriptomic profiling to elucidate the impact of the aged lung microenvironment on tissue-resident AMs. Indeed, physiologic aging directly modulates the expression profile of tissue-resident AMs in aged mice via cell non-autonomous mechanisms. Heterochronic adoptive transfer from young to old animals reverses age-related transcriptional changes as transferred cells adopt characteristics of the age of the recipient (McQuattie-Pimentel et al., 2019). Thus, the aged pulmonary microenvironment shapes the expression profile of AMs over the course of a mouse lifespan. In contrast, circulating factors from young rodents introduced via heterochronic parabiosis do not reverse the age-related transcriptional changes in tissue-resident AMs, demonstrating the importance of aged lung microenvironments.

Lung-resident neutrophils represent another myeloid-derived cell lineage that undergoes age-related quantitative and qualitative changes. Neutrophils are found in increased amounts in the lower respiratory tract in healthy, clinically normal, older adults where they play a role in age-associated inflammation (Meyer et al., 1998). Excessive neutrophil infiltration in the aged lung

Table 1. Summary of functions of pulmonary epithelial cells and age-related changes

Cell	Localization in human lung	Function	Age-related changes	References
Basal cells	Trachea, bronchi, bronchioles	Multipotent progenitor. Self-renewal and differentiation into club cells, goblet cells and neuroendocrine cells. Differentiation to ciliated cells after injury.	Decrease in the number of basal cells. Less proliferative phenotype (stem cell exhaustion).	Hong et al., 2004 ; Rock et al., 2009, 2011 ; Wansleeben et al., 2014
Club cells	Bronchi, bronchioles, respiratory bronchioles	Progenitor. Self-renewal. Differentiation into goblet and ciliated cells. De-differentiation into basal cells after injury. Club cells secrete primary components of the fluid lining the respiratory bronchioles, they play a key role in the biotransformation of xenobiotics, oxidative stress reduction, and immunomodulation.	Reduced self-renewal and differentiation. Increased apoptosis.	Hong et al., 2001 ; Ortega-Martinez et al., 2016 ; Rawlins et al., 2009 ; Watson et al., 2020
Ciliated cells	Trachea, bronchi, bronchioles	Ciliated cells have a critical biomechanical role as a component of mucociliary clearance. There is increasing recognition that airway ciliated cells sense and respond to both mechanical and irritant stimulation.	Slow ciliary beat frequency. Decrease in the number of ciliated cells.	Bailey et al., 2018 ; Rawlins et al., 2007 ; Wansleeben et al., 2014
Glandular-like epithelial invaginations	Trachea, bronchi	Arise from basal cells during aging and contribute to homeostasis and repair.	Appearance of age-related GLEI/ARSMG.	Aros et al., 2020 ; Wansleeben et al., 2014
SMG myoepithelial cells	Trachea, bronchi	SMG-derived myoepithelial cells display multipotency and contribute to basal and luminal cell types of the SMGs and surface airway epithelium.	Not described.	Lynch et al., 2018 ; Tata et al., 2018
Alveolar type 2 cells	Alveolar parenchyma	Progenitor AT2: self-renewal and differentiation into AT1 cells. "Mature" AT2: synthesis and secretion of surfactant proteins and phospholipids. Innate immune response.	Impaired differentiation into AT1 cells. Senescence. Endoplasmic reticulum stress. Increase of MHC class I genes. Altered lipid metabolism, increased cholesterol biosynthesis.	Angelidis et al., 2019 ; Barkauskas et al., 2013 ; Borok et al., 2020 ; Nabhan et al., 2018 ; Travaglini et al., 2020 ; Zacharias et al., 2018
Alveolar type 1 cells	Alveolar parenchyma	Gas exchange	Reduced number of AT1, uniform airspace enlargement.	Schulte et al., 2019 ; Verbeken et al., 1992

GLEI/ARSMG, glandular-like epithelial invagination/glandular related submucosal gland; MHC, major histocompatibility complex; SMG, submucosal gland.

has been implicated in the pathogenesis of increased influenza-related mortality among elderly patients ([Kulkarni et al., 2019](#)). Formation of neutrophil extracellular traps (NETs), a response enacted to seclude rapidly dividing pathogens and neutralize virulence factors, is impaired in aged mouse models and in older adults ([Hazeldine et al., 2014](#)). Reduced chemotaxis and inaccurate migration of neutrophils leads to bystander tissue damage and misdirected inflammation, an effect mediated by aberrant PI3K signaling in neutrophils from older patients ([Sapey et al., 2014](#)). The degree of neutrophil migratory inaccuracy correlates

with clinical indices of frailty in humans and is partially reversed using PI3K inhibitors *ex vivo* ([Wilson et al., 2020](#)).

If the epithelial-macrophage first line of defense fails, a second-tier immune response is elicited that is mediated by terminally differentiated lung-resident lymphocytes including innate lymphoid cells, natural killer (NK) cells, mucosal-associated invariant T cells, and tissue-resident memory T cells (T_{RM}) ([Iwasaki et al., 2017](#)). Similar to alterations in myeloid-derived immune cells with age, lung-resident lymphocytes exhibit a wide array of age-related malfunctions. Decades of characterization

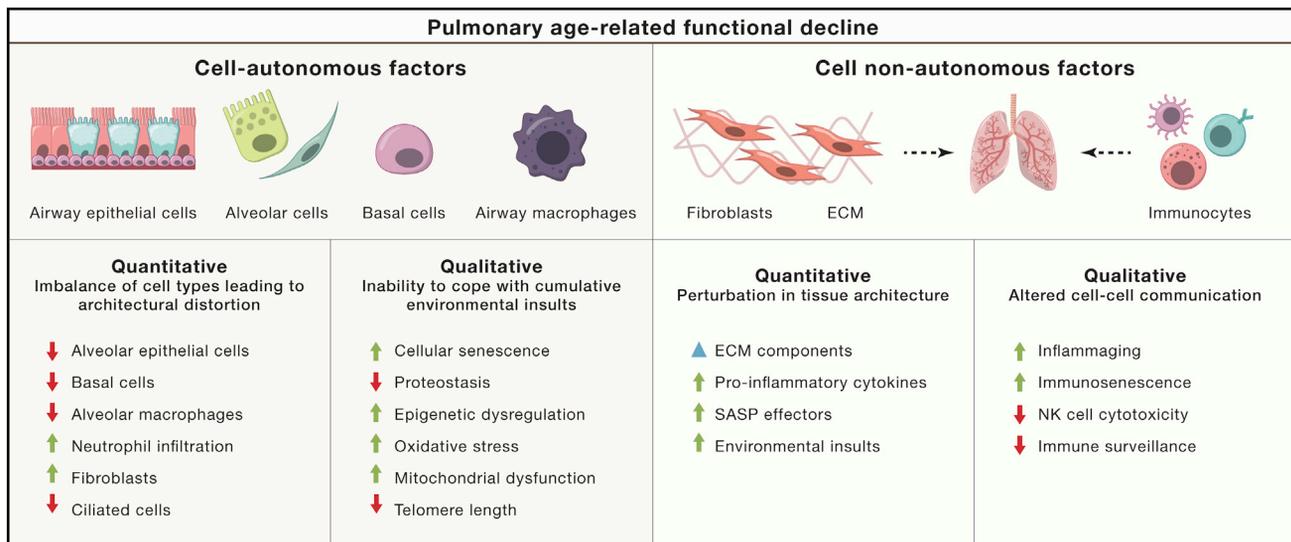


Figure 2. Contribution of cell-autonomous and non-autonomous factors to pulmonary age-related functional decline

Cellular function is a composite of intrinsic biological processes and extrinsic interactions with other cell types and bioactive molecules. Left: both qualitative and quantitative changes have been described in specific cellular compartments in the aged lung. Red arrows: decrease in cell number or in pathway activity. Green arrows: increase in cell number or pathway activity. Right: lung-resident cells interact with neighboring cells and acellular structures in a way that alters their function with advancing age. Age-related changes have been described for extracellular matrix (ECM) components, tissue and circulating cytokines, senescent-associated secretory phenotype (SASP), and environmental insults which contribute to alterations in tissue architecture. Qualitative deficits during aging lead to abnormal cell-cell communications mechanisms and are manifested by aberrant interactions with microbial pathogens and skewing of innate and adaptive immunity toward heightened inflammation, disrupted adaptive immune responses, and impaired immunosurveillance.

have highlighted age-related systemic deficits in T cells including oligoclonal expansions in cytotoxic CD8⁺ T cells leading to constrictions in the T cell repertoire (Jiang et al., 2011; Messaoudi et al., 2004). Single-cell RNA sequencing of CD4⁺ T cells from young and old animals shows increased frequencies of four distinct CD4⁺ T cell populations (Elyahu et al., 2019). Analogous age-related B cell dysfunctions have also been uncovered and defined by inability to undergo class switch recombination, decreased antibody production and avidity to certain antigens, and narrowed clonotypic diversity (Frasca and Blomberg, 2009).

Interstitial compartment

Cellular aging must be considered in the context of the lung's unique microenvironment since the decline of pulmonary physiologic function is a consequence of failures in both cell autonomous and non-autonomous processes (Figure 2). Cells that inhabit the lung are supported by a scaffold comprised of extracellular matrix (ECM) components and resident interstitial cells. The integrity of this interstitial compartment is critical to maintain gas exchange between ventilated alveolar sacs and penetrating capillaries beds. Moreover, cross-linked, highly post-translationally modified proteins in the interstitial compartment bind bioactive molecules in the extra-cellular space, serving as a reservoir for growth factors, cytokines, and metabolites.

Studying the molecular foundations of the pulmonary ECM can be challenging given the biochemical and biophysical complexities of proteins that typically are large, covalently cross-linked, and insoluble. Early studies using microarray technology from bulk RNA extraction from humans and rodent models yielded conflicting results with some data showing age-associ-

ated increases and other showing decreases in collagens and other ECM proteins (Gruber et al., 2006; Misra et al., 2007). One limitation of this approach is the reliance on bulk gene expression to inform on protein composition. Moreover, ECM proteins are long-lived and heavily post-translationally regulated, which may contribute to the decoupling of protein and mRNA levels.

To resolve alterations in ECM components, groups have turned to detergent solubility profiling to maximize yield and to liquid chromatography-mass spectrometry (LC/MS)-based approaches to probe the ECM composition using deep tissue proteomics (Decaris et al., 2014; Schiller et al., 2015). Quantitative detergent solubility profiling from rodent lung tissue shows an increase in collagen IV and XVI and a decrease in collagen XIV and Fraser syndrome complex with age (Angelidis et al., 2019). Further functional studies are needed to investigate the physiologic significance of the 32 matrisome proteins that were identified to be significantly altered with age. Interestingly, side-by-side analysis of mRNA and protein levels did not reveal concordant genetic changes, highlighting the importance of post-transcriptional regulatory pathways in controlling protein levels.

Understanding how ECM components change with age is critical because variations in elastic properties of the human lung are known to be important determinants of lung function. Using atomic force microscopy, investigators identified age-related increases in stiffness of parenchymal and vessel compartments (Sicard et al., 2018), highlighting the changes in biomechanical properties in the aged lung. This is concordant with data showing that the degradation of elastin, a key protein in the lung connective tissue that provides elasticity and resilience, is enhanced

with age and in chronic obstructive pulmonary disease (COPD) (Godin et al., 2016; Huang et al., 2016). However, the precise mechanisms that drive this phenotype are unclear.

Pulmonary fibroblasts comprise another important part of the interstitial compartment. Fibroblast senescence has been associated with pulmonary remodeling and linked to age-related respiratory diseases such as pulmonary fibrosis (Yanai et al., 2015). Several groups have studied pulmonary fibroblast senescence in the context of aging to understand whether there is a causal link. Proteomic and microarray analysis in “young” versus senescent human fibroblasts demonstrates alterations in ECM protein expression (Yang et al., 2011). Similarly, single-cell RNA sequencing identified that aged interstitial fibroblasts express significantly less collagen XIV along with its binding partner, Decorin (Angelidis et al., 2019). *In vivo* studies comparing young and old mouse lungs reveal a correlation between ECM changes and increased cellular senescent markers in aged animals (Calhoun et al., 2016). Although both cell-autonomous and cell non-autonomous processes undergo alterations with age, understanding how aging disrupts the interplay between cell-intrinsic and cell-extrinsic factors is an area of active investigation.

CELLULAR STRESS RESPONSE IN THE AGING LUNG

Human lungs are chronically exposed to an array of cellular stressors including particulate matter, aerosols, infectious agents, allergens, pollutants, ionizing radiation, pneumotoxic chemicals or medications, biomechanical injury, hyperoxia, and cigarette smoke (Figure 3). To cope with biological, chemical, and physical stress, lung-resident cells enact a variety of robust stress response pathways. However, the key in stress response biology is homeostatic balance: under-responding can lead to persistence of the insult without the achieving full repair, while over-responding can lead to aberrant cellular activation, fibrotic scarring, and lung remodeling.

Given the cellular complexity of the lung, it is unsurprising that there is cell-type specificity in stress response pathways with age. Because respiratory epithelial cells bear the brunt of exogenous environmental stimuli, many investigations have focused on the molecular changes in AT2 cells and how age alters their ability to respond to stress. Thus, there is an under-representation of studies on other cell types, exposing gaps in our knowledge about pulmonary cellular health during aging. In this section, we review how stress response pathways involved in proteostasis, oxidative stress, metabolic adaptations, and senescence change with age and how this impacts the ability of the lung to respond to exogenous and endogenous stressors.

Proteostasis in the aging lung

Proteostasis refers to the dynamic process by which cells regulate various aspects of protein handling including synthesis, folding, trafficking, post-translational modification, and degradation. Collectively, this integrated network of pathways helps preserve the stability and functionality of the cellular proteome by preventing accumulation of aggregated, misfolded, or damaged proteins (Balch et al., 2008). Diminished proteostasis occurs during aging, as studies have demonstrated that protein quality control deteriorates with age even in the absence of

underlying disease (Morimoto and Cuervo, 2014). Aging tissues exhibit impaired proteotoxic stress responses, which rely on induction of molecular chaperones to preserve protein folding and stability (Calderwood et al., 2009). In addition, the activities of two major proteolytic systems decline with age: the ubiquitin-proteasome system (UPS) and the autophagy-lysosome system (Rubinsztein et al., 2011). Although there is some redundancy in that a single protein can, in some cases, undergo degradation through either pathway, studies have shown that malfunction of one proteolytic system impacts the other through a variety of cross-talk mechanisms (Korolchuk et al., 2010).

The lung faces perpetual biochemical perturbations over a lifetime, leading to the accrual of deleterious post-translational modifications, protein misfolding, and disrupted protein-protein interactions that necessitates a robust response to resultant proteotoxic stress. Yet, there is a relative paucity of studies dedicated to the examination of proteostasis in the lung during healthy aging compared to other tissue types. A recent proteomic analysis of AT2 cells isolated from young and old mice revealed maladaptive collapse of the proteostasis network with age and an important role for the co-chaperone adaptive response (CARE) network in handling chronic misfolded proteins in the aging lung (Loguercio et al., 2019), paving the way for further investigation into manipulating proteostasis pathways to rejuvenate pulmonary health.

In addition to alterations in the chaperone network in aged lungs, failures in protein degradation pathways such as autophagy have also been identified (Schneider and Sanchez, 2016). Autophagy is the process by which intracellular macromolecules and organelles are targeted to and degraded by lysosomes. It plays a fundamental role in the maintenance of protein and organelle quality control and is integral in carrying out an adaptive response to a variety of cellular insults. A decline in macroautophagy and chaperone-mediated autophagy activities with age has been reported in a variety of tissue types and has been implicated in the pathogenesis of progressive fibrotic lung disease. In alveolar epithelial cells, inhibition of macroautophagy promotes epithelial-to-mesenchymal transition (EMT) through stabilization of p62 and resultant transactivation of EMT transcription factor Snail2 (Hill et al., 2019). Moreover, aged mice subjected to chemical lung injury with bleomycin have reduced ability to upregulate autophagic activity compared to their younger counterparts and exhibit compromised mitochondrial clearance via mitophagy (Sosulski et al., 2015).

Several genetic respiratory diseases are attributable to protein misfolding due to underlying genetic mutations. This may provide insight into the importance of protein homeostasis during aging, because clinical disease does not manifest for decades despite culprit genes being transcribed from an early age. Thus, understanding how aging exacerbates proteostasis failure is critical, even in the context of young-adult diseases like cystic fibrosis and alpha-1 antitrypsin deficiency. In contrast, mutations in surfactant protein C gene (SFTPC) (Lawson et al., 2004) and in the MUC5B promoter region (Seibold et al., 2011) are linked to pulmonary fibrosis, a disease that typically manifests much later in life in the sixth or seventh decade. This suggests that fallout from mutations that cause aggregation-prone or misfolded proteins first require age-related proteostasis collapse to drive

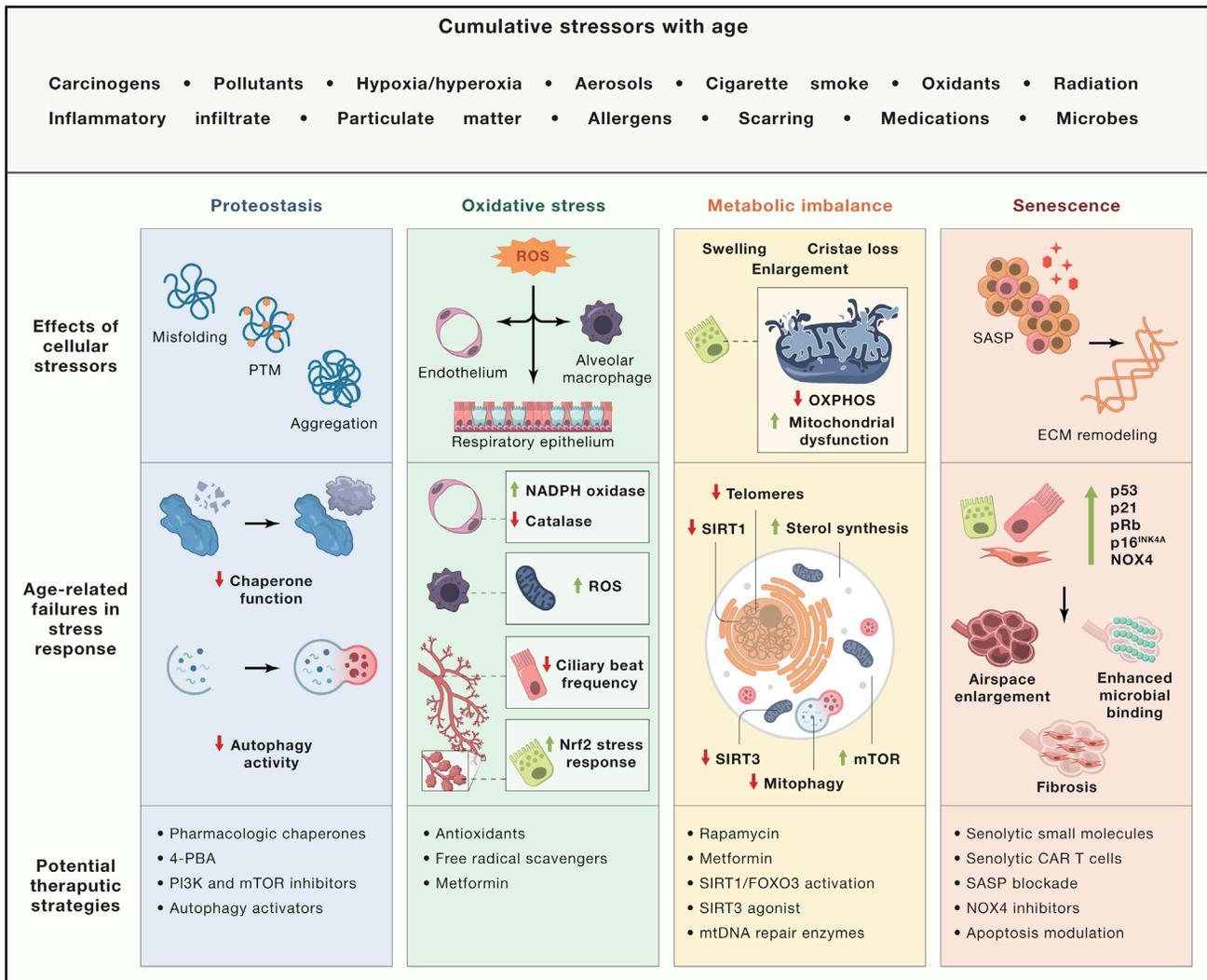


Figure 3. Stress response pathways in the aging lung and potential therapeutic strategies

Top: exogenous and endogenous insults have the potential to accrue in lung tissues throughout the lifespan of an organism. Bottom: various mechanisms that are critical to maintaining cellular homeostasis have been shown to decline with age in the lung. Decreasing chaperone and proteolytic capacity results in compromised protein homeostasis leading to a proteome plagued by misfolded, aggregated, or covalently modified proteins. Oxidative stress responses are hampered in endothelial, inflammatory and epithelial compartments of the aged lung, resulting in compromised mucociliary clearance in upper airways and compensatory Nrf2-mediated stress response in alveolar epithelial cells. Metabolic imbalance with age stems from the convergence of abnormal signal transduction pathways and mitochondrial dysregulation. The increasing burden of senescent cells in the lung with age impacts ECM remodeling which ultimately disrupts airway architecture and contributes to fibrogenesis and altered interactions with microbial pathogens. ROS, reactive oxygen species; NOX, NADPH oxidase; OXPHOS, oxidative phosphorylation; SASP, senescent-associated secretory phenotype; ECM, extracellular matrix.

disease (Balch et al., 2014). This raises the question as to whether there is a gradual age-associated loss of the proteostatic network that results in the inability to compensate for protein misfolding burden. Younger organisms benefit from having a fully functional chaperone and proteolytic stress response whereas older organisms may have exhausted their capacity. Age-related declines in Hsp70 and Hsp90 chaperones, co-chaperones, as well as in macroautophagy and chaperone-mediated autophagy, may create a tipping point for the system to become overwhelmed and succumb to disease. This postulation is supported by evidence that stimulating autophagy and UPS to clear intracellular inclusions in alpha-1 antitrypsin

deficiency has therapeutic benefit (Lomas, 2018). Further research is needed to define the mechanisms that drive the loss of proteostasis with age and delineate the extent to which environmental exposures, such as cigarette smoking, exacerbate proteome compromise.

Mitochondrial dysregulation in the aging lung

Mitochondrial dysfunction is also recognized as a hallmark of cellular aging. Age-related changes include accumulation of mtDNA mutations and deletions, increased oxidation of mitochondrial structural and functional proteins, alterations in the lipid composition of mitochondrial membranes, destabilization

of electron transport chain (ETC) complexes, and stress-induced permeabilization of the membrane (reviewed elsewhere) (Haas, 2019). Strikingly, AT2 cells contain ~50% of the total lung mitochondrial mass and are particularly susceptible to age, manifested by mitochondrial enlargement, loss of cristae, inner membrane destruction, and impaired respiratory capacity (Bueno et al., 2015; Massaro et al., 1975). Senescent lung epithelial cells also exhibit features of mitochondrial dysfunction due to alterations in mTOR/PGC-1 α / β signaling (Summer et al., 2019).

To sustain a healthy pool of cellular mitochondria, both mitochondrial biogenesis and degradation need to be tightly regulated. However, the integrity of both processes is compromised with age. Mitochondrial biogenesis is controlled by transcriptional coactivator PGC-1 α , activation of NRF-2, AMPK signaling, and SIRT1 regulation, all of whose functions are attenuated with age. Telomere attrition in telomerase-deficient mice leads to p53-mediated repression of PGC-1 α and PGC-1 β and consequent reduction in mitochondrial biogenesis (Sahin and DePinho, 2012), an effect that can be partially reversed by telomerase activation in old mice (Bernardes de Jesus et al., 2012). A recent study uncovered a new role for telomerase-mediated mitochondrial impairment in the pulmonary innate immune response. *Terc* null mice that harbor short telomeres exhibit exaggerated lung inflammation and increased mortality from pneumonia upon infection with respiratory pathogens (Kang et al., 2018). Telomere dysfunction specifically in AMs leads to disruption of PGC-1 α /ERR α transcriptional programs with a wide array of effects on mitochondria including reduced expression of ETC genes, decreased ATP, and increased reactive oxygen species (ROS). This results in activation of the NLRP3 inflammasome and an over-exuberant innate immune response, similar to the proinflammatory state observed in advanced age. Interestingly, telomerase mutations have been identified in patients with familial and sporadic forms of idiopathic pulmonary fibrosis (IPF) (Calado and Young, 2009), but the mechanism of how aging exacerbates this fibrotic phenotype and whether it is mediated by mitochondrial dysfunction is unclear.

Selective removal of damaged mitochondria via mitophagy also plays an important role in lung aging and age-related pulmonary disease (Cloonan and Choi, 2016). Mitophagy is a process that is regulated by a variety of signaling and effector molecules, with one such pathway relying on the coordinated effort of PINK1 and Parkin. Under stress, PINK1, a serine/threonine kinase, accumulates on the outer membrane of depolarized mitochondria and recruits Parkin, an E3 ubiquitin ligase, to catalyze the polyubiquitination of several substrates. This triggers recruitment of p62/SQSTM1, mitochondrial engulfment by autophagosomal vesicles, and fusion with lysosomes where mitochondria are degraded and constituent components recycled. Strikingly, AT2 cells from IPF patients exhibit marked accumulation of dysfunctional mitochondria that was found to be associated with low expression of PINK1 (Bueno et al., 2015). Suppression of PINK1 in AT2 cells *in vitro* leads to mitochondrial depolarization and increased expression of pro-fibrotic factors, transforming growth factor β (TGF- β)1 and TGF- β 2. Accordingly, PINK1-deficient mice are more susceptible to development of lung fibrosis, even in young animals. When aged wild-type mice are challenged with bleomycin, they fail to induce mitophagy, which

impairs mitochondrial ETC complex activities (Liu et al., 2011; Sosulski et al., 2015). Furthermore, in fibroblasts, diminished mitophagy is mediated by constitutive mTORC1 activation during aging, an effect that is reversible with rapamycin treatment (Romero et al., 2016). Although failure of mitophagy is a hallmark of lung fibrosis, other lung diseases such as COPD (Mizumura et al., 2014) exhibit upregulation of both PINK1 and mitophagy, cautioning that age-related lung diseases may have molecular drivers that are distinct from one another and from those that occur during physiologic healthy aging.

Metabolic adaptations in the aging lung

Metabolic imbalance occurs during aging and is characterized by altered nutrient sensing, energy imbalance, and signaling dysregulation. Although lung tissue is not typically characterized as an organ involved in whole-body energetic balance, AT2 cells possess robust metabolic activity and the ability to rapidly acclimate to changes in oxygen availability. In a normal lung, the alveolar epithelium resides in an exceptionally well-oxygenated environment with O₂ levels around 100 mm Hg, higher than in most other organs (Schumacker, 2011). In response to acute or chronic hypoxic stress, AT2 cells preserve ATP despite low oxygen tension, highlighting their inherent resilience and ability to maintain bioenergetic homeostasis under limitations on aerobic respiration (Lottes et al., 2014). Signaling pathways that mediate response to hypoxia (HIF2 α , AMPK, and mTOR) are also dysregulated during aging. Although aging is associated with the convergent disruption of numerous nutrient sensing pathways, questions remain regarding how lung-resident cells rewire their metabolic networks with age and whether therapeutic modulation of metabolism alleviates age-related respiratory illness.

Sirtuins, a highly conserved family of nicotinamide adenine dinucleotide (NAD)⁺-dependent protein deacetylases, have emerged as players in lung aging and disease. Mammalian sirtuins localize to various subcellular compartments including the nucleus (SIRT1 and SIRT6–SIRT7), cytosol (SIRT2), or mitochondria (SIRT3–SIRT5), and serve as metabolic sensors by gauging NAD levels and post-translationally modifying substrates to enact a downstream cytoprotective response (Haigis and Sinclair, 2010). SIRT1 is reduced in the lungs of COPD patients, and genetic ablation of SIRT1 in mouse airway epithelial cells leads to airspace enlargement, impaired lung function, and reduced exercise tolerance via loss of deacetylation of FOXO3 transcription factor (Yao et al., 2012). Genetic and pharmacologic restoration of SIRT1 attenuates premature cellular senescence in the lung in a FOXO3-dependent manner that protects from smoking-induced emphysematous changes, making the SIRT1/FOXO3 axis a potential therapeutic target (Yuan et al., 2015).

SIRT3 binds and deacetylates numerous substrates to activate mitochondrial oxidative pathways including those in the tricarboxylic acid (TCA) cycle, ETC, and fatty acid β -oxidation (Yang et al., 2016). SIRT3 expression is reduced in lungs of aged mice as well as in IPF murine models and fibrotic areas of affected human lungs. SIRT3 loss leads to increased acetylation and inactivation of antioxidant SOD2 and TCA enzymes such as IDH2, impairing mitochondrial function in the aged lung (Sosulski et al., 2017). Additionally, SIRT3 regulates

mitochondrial bioenergetics in AMs where its inactivation decreases maximal oxygen consumption rates, increases proton leak, and predisposes mice to lipopolysaccharide (LPS)-induced acute lung injury (Kurundkar et al., 2019).

Moving forward, unbiased investigations into metabolic networks that change with physiologic age in the lung will be crucial for uncovering previously unappreciated mechanisms. For example, single-cell transcriptomics from young and old murine lungs identified AT2-specific alterations in key lipid regulators: *Sreb2*, a sterol-response element binding protein (SREBP) critical for lipogenesis and surfactant production, and *Insig1*, a negative regulator of SREBPs. This age-related gene expression profile mimics that seen in genetic mouse models of *Insig1/2* deletion that feature constitutive activation of SREBP, accumulation of neutral lipids in AT2s, and lipotoxicity-related lung inflammation (Plantier et al., 2012). Tightly controlled lipid metabolism is essential in AT2 cells, because they serve as the synthetic hub for pulmonary surfactant. However, the functional implications of why sterol regulatory transcriptional factors are altered with age and whether this is a driving force (or bystander effect) during aging is unknown. More broadly, gaps remain in our understanding of how cellular metabolism and fuel preferences are altered in the aged lung.

Oxidative stress in the aging lung

Oxygen is transformed through enzymatic and non-enzymatic reactions to yield intermediates termed ROS, including superoxide anions (O_2^-), hydroxyl radicals ($\cdot OH$), and hydrogen peroxide (H_2O_2). There is a long history linking reactive oxygen species with aging, which has its roots in the free radical theory of aging (Harman, 1956) and the mitochondrial theory of aging (Alexeyev, 2009). These theories posit that age-associated cellular deterioration is a direct consequence of the accumulation of ROS-induced damage over a lifetime, particularly in mitochondrial DNA. Despite decades of studies showing that constitutive increases in ROS levels accompany aging, recent work has called into question whether ROS may actually be beneficial for extending lifespan. The concept of hormesis in relation to oxidative stress refers to a favorable biological response to low levels of free radicals that primes cells to cope with larger burdens of ROS during times of stress. Experiments in *S. cerevisiae* and *C. elegans* show that mitochondrial oxidant release can induce cytoprotective programs that actually lead to extension of chronological lifespan (Pan et al., 2011; Yang et al., 2007). However, the benefits of the hormetic response against oxidative stress have not borne out in the context of the mammalian lung as the majority of studies link elevated ROS to respiratory illnesses including lung fibrosis and lung cancer (Liu and Chen, 2017).

Given high oxygen tension, airway cells must contain the machinery to protect themselves from byproducts of aerobic metabolism. Respiratory epithelial, endothelial, and inflammatory cells such as AMs and neutrophils express several ROS-generating enzymes including NADPH oxidases (NOX enzymes) and contain antioxidant defenses including superoxide dismutases, catalases, and glutathione peroxidases to neutralize ROS (Hecker, 2018). Under normal conditions in the respiratory tract, it is now appreciated that ROS play a variety of physiological roles in signal transduction, injury-repair response, ECM remod-

eling, and pathogen clearance (Holmström and Finkel, 2014). As long as ROS levels are kept in check through regulation of antioxidant enzymes, cells can avoid deleterious effects of aerobic byproducts. When ROS production exceeds antioxidant capacity, as during hyperoxia, proteins, lipids, and nucleic acids become oxidized and often trigger an adaptive stress response.

Intracellular ROS can originate from endogenous sources, such as mitochondrial metabolism, or exogenous sources, such as cigarette smoke, particulate matter, and noxious gases. Whole lung analyses from humans (Lee et al., 1999) and rats (Braidly et al., 2011) show increased lipid peroxidation, protein carbonylation, and DNA damage with advancing age. Accumulation of H_2O_2 selectively upregulates microRNA-34a in bronchial epithelial cells in a PI3K α -dependent fashion, leading to reductions in SIRT1 and SIRT6 levels (Baker et al., 2016). Single-cell transcriptional analysis and imaging techniques that spatially define lung tissue at single-cell resolution can deconstruct the heterogeneity in the degree to which different pulmonary cell types experience redox imbalance and how redox programs are altered with age. For example, single-cell sequencing from aged murine lungs reveals an increase in *Nrf2*-mediated oxidative stress responses specifically in AT2 cells compared to club cells (Angelidis et al., 2019).

Oxidants slow ciliary beat frequency in the upper respiratory epithelium which impairs mucociliary clearance and predisposes to infection. Cilia slowing is mediated by an age-dependent increase in expression and activity of PKC ϵ in response to oxidative stress, an effect that is reversed by PKC ϵ inhibition or treatment with the antioxidant *N*-acetylcysteine (NAC) (Bailey et al., 2018). Cells that comprise the pulmonary vasculature (endothelial and smooth muscle cells) are also susceptible to oxidative stress in aging. A comparison between the long-lived naked mole rat (maximum lifespan >28 years) and the laboratory rat (maximum lifespan ~3 years) found increases in superoxide and H_2O_2 production in the vasculature of shorter-lived rodents, suggesting longer-lived animals may harbor protective mechanisms in endothelial cells (Csiszar et al., 2007). Indeed, aged rats exhibit endothelial dysfunction in pulmonary arteries as a result of increased activity of NOX enzymes gp91, Nox1, and Nox4, as well as decreased expression of catalase (Podlutzky et al., 2010). Nox4 is induced in human lung endothelial cells with hyperoxic stress (Pendyala et al., 2011), but it is unclear whether this response diminishes with age.

Oxidative stress plays an important role in the pathogenesis of age-related pulmonary diseases including pulmonary hypertension (Huetsch et al., 2019), fibrotic lung disease (Cheresh et al., 2013), and COPD (Matera et al., 2016). Therapeutic modulation of ROS has been extensively evaluated both in pre-clinical models and clinical trials for a variety of disease states. One antioxidant, NAC, is broadly viewed as protective against mutagenic effects of free radicals and is currently Food and Drug Administration (FDA)-approved for acetaminophen overdose. Attempts have been made to repurpose this medication for respiratory illnesses believed to be driven by oxidative stress. In a recent study, NAC was tested as a chronic treatment in an aged *JunD*-deficient mouse model that exhibits oxidative stress in the lung (Breau et al., 2019). Although systemic NAC administration had the expected effects of reducing pulmonary oxidative

damage and cellular senescence, it also led to induction of lung adenocarcinomas.

Although antioxidants have historically been viewed as cytoprotective from malignancy, recent experimental and clinical data support the idea that antioxidants can increase the risk of tumorigenesis (Bjelakovic et al., 2007), including cancers originating in the lung. The first hint of this relationship was borne out in a landmark randomized double-blind, placebo-controlled primary prevention study that showed that antioxidant supplements beta carotene (vitamin A precursor) and alpha-tocopherol (vitamin E) failed to reduce the incidence of lung cancer among male smokers. Unexpectedly, the trial raised the possibility as to whether these antioxidants may be harmful, as higher rates of lung cancers were reported among those who received beta carotene (Alpha-Tocopherol/Beta Carotene Cancer Prevention Study Group, 1994). Different murine models of lung cancer have shown that antioxidants can lead to lung tumor development (Sayin et al., 2014) and increased metastasis (Wiel et al., 2019), despite having the intended effect of reducing oxidative stress. Murine lung tumor models harboring *Kras*^{G12D} or *Braf*^{V600E} that were treated with NAC or vitamin E showed hastened tumor cell proliferation, cancer progression, and reduced survival (Sayin et al., 2014). Furthermore, antioxidants stimulate *Kras*-driven lung cancer metastasis through a stabilization of BACH1 that activates a transcriptional program to induce glucose catabolism by increasing expression of glycolytic enzymes and enhancing glucose uptake (Wiel et al., 2019). Nrf2 accumulation in lung cancers causes stabilization of BACH1 in a Ho1-dependent manner, providing a mechanistic link between antioxidant supplementation and Nrf2-mediated activation of a metastatic program (Lignitto et al., 2019).

Ultimately, the key to safe therapeutic manipulation of ROS may rely on how it is modulated. Given the potential beneficial effects of a hormetic response to physiological levels of ROS combined with data suggesting antioxidant supplementation can promote tumorigenesis, a cautious approach is needed in considering how to manipulate ROS levels with the goal of lung tissue rejuvenation.

Cellular senescence in the aging lung

Cellular senescence is a hallmark of aging characterized by cell-cycle arrest, distinct cellular morphologic and transcriptional alterations, and a unique secretory phenotype that modulates the local tissue microenvironment through autocrine and paracrine signaling. Human lung cells were first observed to undergo replicative arrest after serial passaging in culture by Hayflick and Moorehead in the 1960s. Since that time, various stimuli have been implicated in senescent cell accumulation in the lung *in vivo*, including chronological aging, chemotherapy, radiation, supplemental oxygen, oxidative stress, and cigarette smoke (Childs et al., 2015). Senescent cells accrue with age in epithelial, endothelial, fibroblastic, and immunological compartments within human lungs.

Despite replicative arrest, senescent cells remain metabolically active and release a variety of factors, termed the senescence-associated secretory phenotype (SASP). The senescence-associated increase in cytokines, matrix remodeling proteases, and growth factors is particularly detrimental for

cells in the lung niche that require tight regulation of ECM remodeling and inflammatory responses to prevent abnormal tissue repair. Teleologically, the irreversible state of cell-cycle arrest was thought to serve as a protective mechanism to avoid malignant transformation of damaged cells. However, it is now appreciated that senescent cells contribute to the pathogenesis of cancer and age-related diseases, including COPD and pulmonary fibrosis. Thus, selective targeting of senescent cells and molecular effectors of SASP has been suggested as therapeutic avenues to rejuvenate pulmonary health in aging. Excitingly, the implementation of senolytics as a means to deplete senescent cells in tissues *in vivo* has helped to elucidate their causal role in aging and demonstrate efficacy in delaying deterioration and disease (van Deursen, 2019).

Early studies in mouse models of premature aging uncovered a role for senescence in accelerating a structural, functional, and biomechanical decline of the murine lung. Young mice with induced premature cellular senescence develop features of aging, such as airspace enlargement and disruption of lung cellular architecture (Kurozumi et al., 1994). Cumulative damage in lung-resident cells induces key senescence markers including p53, p21, p16^{Ink4a}, and pRB, leading to blockade of cell-cycle progression. Aged wild-type mice and aged human lungs exhibit increased senescence markers in parenchymal alveolar and bronchiolar cells as well as in the pulmonary vasculature compared to young counterparts. In both rodents and humans, senescent markers colocalize with ECM components, linking senescence and interstitial remodeling with age (Calhoun et al., 2016).

Given the connection between SASP and interstitial remodeling, the role of senescence in mediating fibrotic pulmonary diseases has been explored. p16, p21, and β -galactosidase are up-regulated in fibroblasts and epithelial cells in lung tissue from IPF patients and p16 transcript abundance correlates with fibrotic disease severity (Schafer et al., 2017). Inducing senescence in the lung worsens pulmonary function while conversely, clearing senescent cells mitigates fibrotic lung disease, demonstrating a causal role for senescence in lung diseases (Schafer et al., 2017). Conditioned media collected from senescent fibroblasts from murine lungs contains markedly increased levels of profibrotic and proinflammatory factors which is consistent with the model that the SASP secretome promotes fibrosis. Interestingly, conditioned media collected from senescent bronchiolar epithelial cells in culture does not activate a fibrotic response, suggesting there may be cell-type-specific SASP contributions.

Senolytics selectively deplete senescent cells from tissue microenvironments to suppress the release of deleterious SASP factors. In light of compelling evidence in transgenic mouse models that killing senescent cells attenuates progression of age-related disorders in various tissues and extends overall healthspan (Baker et al., 2011), small molecules that target senescent cells have been developed. The first senolytics reported, the combination of dasatinib and quercetin (D+Q), preferentially ablate senescent cells over proliferating or differentiated cells through inhibition of key pro-survival transcriptional programs (Zhu et al., 2015). In aged mice, clearance of senescent cells by treatment of D+Q increased late-life survival and physical activity without compromising morbidity

(Xu et al., 2018). To evaluate the effect of senolytic agents specifically in the lung, D+Q was tested in experimental models of lung fibrosis. *Ex vivo*, D+Q decreases fibrotic markers and increases epithelial markers, with reduction of SASP factors and ECM components (Lehmann et al., 2017). Administration of D+Q *in vivo* enhances senescent cell clearance from murine lungs and alleviates IPF-related dysfunction in bleomycin-challenged animals (Schafer et al., 2017). A small open-label pilot study carried out in patients with IPF showed that D+Q senolytics improved physical metrics such as walk distance and gait speed, warranting larger randomized clinical trials for IPF (Justice et al., 2019).

Other approaches have emerged to selectively eliminate senescent cells in the lung. Chimeric antigen receptor (CAR) T cells engineered to target a senescence-associated surface protein extend survival of mice with orthotopic Kras^{G12D} p53^{-/-} lung adenocarcinoma (Amor et al., 2020). Lung tumors show a decrease in senescent cancer cells and an increase in infiltration of activated T cells, highlighting the potential of senescent cells to suppress anti-tumor immunity in the lung. Modulation of microRNAs to manipulate senescence has also been shown to rejuvenate lung tissue (Baker et al., 2019). Thus, continued exploration of *in vivo* use of senolytic agents will further elucidate the mechanistic contributions of senescent cells in the healthy aging lung and in pulmonary disease.

AGE-RELATED PULMONARY DISEASE

Advanced age is the major risk factor for developing a chronic lung condition such as COPD, interstitial fibrotic lung disease (ILD), and lung cancer. Changes that occur in the respiratory system during aging show a striking resemblance to the pathophysiological changes that occur in younger patients afflicted by lung parenchymal disease (Mercado et al., 2015). Although exposure to air pollutants, cigarette smoke, asbestos, and other environmental insults promote the development of chronic pulmonary conditions, it is unclear why it takes several decades for disease to manifest and whether age-related cellular changes underlie and potentiate these disease phenotypes. Because both obstructive and fibrotic lung diseases are defined by several of the same cellular hallmarks that are known to be dysregulated during aging, there is interest in illuminating how age-associated molecular alterations predispose to disease pathogenesis and how they can be targeted to restore lung fitness.

Age-related physiologic changes in the respiratory system

As early as the 1980s, it was appreciated that lung function plays an integral role in healthy aging, as decreased pulmonary reserve is a risk factor for morbidity and mortality for both respiratory and non-respiratory illness (Beatty et al., 1982). The aged lung is characterized by a decrease in the density of bronchioles and an increase in their diameter. In the absence of overt disease, aging leads to loss of alveolar surface area accompanied by enlargement of alveolar and airspace size (Fain et al., 2005). Lungs gradually lose their elasticity and become more rigid, an effect postulated to be secondary to altered expression of ECM proteins lamin, elastin, and fibronectin (Godin et al., 2016). To investigate

how age-related changes in the ECM influence cellular phenotypes, decellularized lungs from aged rodents that were reconstituted with bronchial epithelial cells and lung fibroblasts exhibited altered gene expression of scaffolding structural proteins when compared to young animals, namely in lamins $\alpha 3$ and $\alpha 4$. Thus, changes in the ECM may potentiate age-related phenotypes in the lung by directly impacting gene expression.

Several pulmonary physiologic parameters change with age, including an increase in functional residual capacity and a decrease in forced expiratory volume in 1 s/forced vital capacity (FEV₁/FVC) ratio (García-Río et al., 2004). The latter is based on spirometric measurements that reflect the degree of airflow obstruction and are critical to diagnosing and surveilling patients with lung disease. Both FEV₁ and FVC decline with age, albeit at different rates. FVC alone has been shown to be a strong predictor of mortality among the general population (Lee et al., 2010). Genome-wide association studies have uncovered associations between FEV₁, FEV₁/FVC, and FVC clinical measurements and genomic variations at specific loci (Loth et al., 2014). However, it remains unclear whether these genetic variants predispose to accelerated lung aging.

COPD in the aged lung

Although obstructive and fibrotic diseases of the lung affect different anatomic compartments and have distinct pathogenic drivers, one commonality is that the likelihood of developing COPD or ILD increases exponentially with age. Although cigarette smoking is the most important risk factor for COPD, only 10%–15% of smokers develop the disease. This implies there are modifying genetic, epigenetic, or post-translational factors that increase risk for certain individuals. Several molecular features of COPD overlap with those that occur during aging and may act as sensitizers to disease. These include perturbations in telomere maintenance, cellular senescence, proteostasis, and genomic stability.

COPD is characterized by progressive airflow obstruction due to destruction of lung parenchyma. There are striking similarities between features of normal aging in the lung and those observed in COPD, prompting the postulation that cigarette smoke may cause disease by accelerating baseline molecular alterations that occur during normal aging (Mercado et al., 2015). Mice deficient in *klotho* (KL^{-/-}), a β -glucuronidase, exhibit significantly shortened lifespan and early development of numerous age-related pathologies, including emphysema. Similar to certain changes seen in COPD, lungs of KL^{-/-} mice exhibit airspace enlargement, destruction of alveoli, decreased elastic recoil, and increased apoptosis in the airway epithelium due to suppression of mitogenic signaling (Ishii et al., 2008). *Klotho* expression is decreased in airway epithelial cells in smokers and is further suppressed in patients with COPD, resulting in increased sensitivity to cigarette smoke-induced inflammation and oxidative stress (Gao et al., 2015). Strikingly, clinical features of premature lung aging can predict the likelihood of being diagnosed with COPD later in life (Lange et al., 2015). Individuals less than 40 years of age with low FEV₁ have a risk of midlife COPD that is more than 3 times higher compared to those with normal FEV₁, raising intriguing questions about what drives premature lung dysfunction in early adulthood.

One mechanism through which chronic cigarette exposure hastens premature aging and predisposes to COPD is through telomere attrition. Smokers with COPD have accelerated telomere shortening with some studies positing that circulating leukocyte telomere length is a biomarker of COPD progression (Córdoba-Lanús et al., 2017). The telomerase null mouse model that harbors short telomeres develops severe emphysema after chronic cigarette smoke exposure. Familial germline deletion of the RNA component of telomerase results in early onset emphysema implicating telomere length as a genetic susceptibility factor in COPD. When examined in larger cohorts of individuals with severe COPD, a subset of patients was found to carry deleterious mutations in the essential telomerase gene TERT. Harboring this genotype results in compromised catalytic activity of telomerase and shortened telomeres, demonstrating that germline mutations in telomere regulatory genes are a risk factor for COPD susceptibility (Stanley et al., 2015).

Lung tissue from COPD patients also exhibits more senescent cells compared with control populations, and COPD-derived lung fibroblasts secrete higher levels of SASP proteins, effects that may be exacerbated by advancing age (Woldhuis et al., 2020). However, although aging increases the severity of cigarette smoke-induced inflammatory infiltrate and upregulates key SASP genes in a COPD mouse model, aging is not the sole contributor to senescence induction by cigarette smoke (Rashid et al., 2018). Premature cellular senescence in COPD impacts distinct pulmonary progenitor cell populations such as mesenchymal stem cells, ultimately leading to stem cell exhaustion and impaired lung regenerative capacity. This fits with the postulation that COPD is a consequence of aberrant parenchymal remodeling and progressive dilation of alveolar spaces without the ability to sustain adequate connective and epithelial tissue repair pathways.

Chronic exposure to cigarette smoke also disrupts autophagy, with conflicting reports about whether cigarette smoke exposure induces or impairs autophagy activity. One study demonstrated that autophagy is upregulated in lung tissue from COPD patients (Chen et al., 2008), whereas other experimental models of COPD have demonstrated a defect in autophagic flux resulting in accumulation of autophagic vacuoles, poly-ubiquitinated proteins, and aggresome bodies (Vij et al., 2018). Although the precise mechanisms remain elusive as to which step in autophagy is disrupted, a recent study revealed suppressed activity of the master transcriptional regulator of autophagy and lysosomal biogenesis, TFEB, in a COPD mouse model (Bodas et al., 2017). Another central regulator of autophagy induction, Beclin 1, has higher circulating levels in healthy centenarians (Emanuele et al., 2014) and decreased levels in smokers and in COPD patients (Schlemmer et al., 2018). Although these studies are correlative and rely on serum Beclin 1 levels as a surrogate of autophagy activity, augmentation of Beclin 1-mediated autophagy in mice directly extends healthspan and lifespan (Fernández et al., 2018). Interestingly, the genetic mouse model with disruption of Beclin 1 interaction with its negative regulator, Bcl-2, rescues several age-related phenotypes observed in Klotho-deficient mice. Because upregulation of autophagic activity promotes longevity and improves tissue health in several model organisms, groups are actively investigating

pharmacologic augmentation of autophagy for COPD treatment (Bodas and Vij, 2017).

Changes in the epigenome have been implicated in the pathogenesis of COPD and in aging, including disrupted DNA methylation patterns, decreased levels of deacetylases leading to altered histone modification, and reduced expression of microRNAs (Schamberger et al., 2014). An altered epigenomic landscape in aging may be compounded by other dynamic epigenetic modifications that occur in the bronchial epithelium and airway inflammatory cells due to cigarette smoke, thus opening the possibility of targeting the COPD epigenome for treatment purposes. If COPD is representative of accelerated lung aging, it stands to reason that rescuing cellular functions that decline with age may prove beneficial for COPD.

Pulmonary fibrosis and aging

Fibrotic lung disease is a catchall term that refers to a collection of conditions characterized by interstitial remodeling, destruction of tissue architecture, irreversible scarring, and compromised lung function. IPF is the most common type of idiopathic interstitial pneumonia and is a chronic, progressive, fibrosing interstitial lung disease of unknown etiology with a median survival of less than 3 years from diagnosis. Fibrotic lung diseases like IPF are increasing in incidence as the world's population ages at an unprecedented rate (Raghu et al., 2016). Epidemiologic studies show that IPF prevalence is estimated at 4.0 per 100,000 persons for age group 18–34 years old and 227.2 per 100,000 persons for age group 75 years and older, representing over a 50-fold increase in elderly adults. In contrast to COPD in which cigarette smoke plays a major role in pathogenesis, aging confers the greatest risk of IPF beyond any other known environmental or genetic factor (Ley et al., 2012). Several molecular hallmarks of aging have been associated with pulmonary fibrosis (Gulati and Thannickal, 2019) but pathogenic mechanisms involved in the heightened susceptibility of aged individuals to fibrosis remain unknown.

Lung fibrosis is characterized by matrix deposition, tissue remodeling, and formation of fibrotic scars. This cascade is perpetuated by biochemical mediators, such as TGF- β , that stimulate myofibroblast differentiation and metabolic reprogramming. IPF is thought to be a consequence of repetitive injury to the respiratory epithelium and dysregulated repair that leads to activation and proliferation of myofibroblasts and resultant ECM deposition (Lederer and Martinez, 2018). Older organisms are predisposed to tissue scarring and irreparable organ damage due to diminished capacity for wound healing and fibrosis resolution.

Evolutionary theories of aging and IPF have posited that fibrogenic pathways evolved as a tradeoff for early life fitness over a later-in-life predisposition to developing fibrosis, a concept inspired by the antagonistic pleiotropy theory of aging (Thannickal, 2010). Although it is clear that changes during age influence susceptibility to lung fibrosis in older adults, the precise mechanisms are still being clarified. Studies have focused on features of the aged lung including telomere attrition, accelerated senescence, mitochondrial dysfunction, and impaired oxidative and proteostatic stress responses that impede its ability to cope with repetitive epithelial injury. Current FDA-approved

therapies for IPF include anti-fibrinogenic agents nintedanib and pirfenidone, but targeting upstream pathways involved in cellular decline during aging may hold promise in counteracting development of IPF and age-associated lung fibrosis.

Telomeropathies are a class of genetic defects in telomerase and telomere-regulatory genes that confer increased risk of IPF and other interstitial lung disease (Snetselaar et al., 2015). In fact, IPF is the most common clinical manifestation in telomere-related disorders. Mutations in telomerase components were initially identified as drivers of accelerated aging from studies of dyskeratosis congenita, a multisystemic inherited disorder that causes premature mortality due to bone marrow failure and pulmonary fibrosis (Vulliamy et al., 2001). Subsequently, short telomeres having been identified in ~25% of sporadic and ~15% of familial IPF cases (Armanios et al., 2007). Although germline mutations in *hTERT* and *hTR* underlie the inheritance of a subset of families with IPF, individuals with idiopathic interstitial lung diseases also have evidence of short telomeres in both peripheral blood and in the lung (Alder et al., 2008). These findings emphasize the role for telomere shortening in IPF pathogenesis beyond familial telomerase mutations and suggest that short telomere signatures may underlie genetic susceptibility of age-related fibrotic disease. However, it is not known how dysfunctional telomeres directly contribute to the development of lung fibrosis.

One way that telomere attrition limits renewal capacity in the lung is by inducing cellular senescence. Telomere dysfunction in AT2 cells is sufficient to drive ECM remodeling via collagen deposition and accumulation of senescent lung epithelial cells in aged telomere repeat binding factor 1 (*TRF1*)-deficient mice (Naikawadi et al., 2016). However, regardless of telomere status, IPF human lungs exhibit an increase in both epithelial and fibroblast senescent cells that directly contribute to development of fibrotic pulmonary disease (Schafer et al., 2017). The secretome of senescent pulmonary fibroblasts is pro-fibrotic, thus skewing the release of ECM components toward fibrotic deposition via TGF- β , interleukin (IL)-6, and MMP12, an effect abrogated with senolytic agents *in vivo* (Schafer et al., 2017). Intratracheal delivery of bleomycin induces molecular signatures of senescence and results in age-dependent accumulation of senescent myofibroblasts, key effector cells in fibrotic disease (Waters et al., 2018). Early intervention in these mice with suicide-gene-mediated senescent cell ablation improves pulmonary function but does not reverse established lung fibrosis. Senescent fibroblasts are also problematic because they appear resistant to oxidative stress-induced apoptosis through elevated expression of NOX4 (Hecker et al., 2014), underscoring why senolytics hold promise as a therapeutic avenue for IPF treatment.

Protein misfolding is particularly critical for AT2 cells, which are professional secretory cells and require robust ER machinery to support synthesis, folding, assembly, and release of surfactant. Mutations in surfactant-associated proteins A (SFTPA2), surfactant protein C (SFTPC), and mucin 5B (MUC5B) account for ~5% of IPF cases and link age-related failure of proteostasis to disease (Romero and Summer, 2017). Genetic variants in SFTPC, for instance, result in misfolding, misrouting, misprocessing of the affected protein, activating ER stress, the unfolded protein response (UPR), and compensatory upregula-

tion of autophagy. Induction of ER stress and UPR is observed in AT2 cells that express mutant SFTPC and in lungs of patients with both sporadic and familial IPF (Lawson et al., 2008). Even in the absence of a surfactant-associated mutation, ER stress mediators such as ATF6, ATF4, CHOP, and XBP-1 are elevated in AT2 cells in IPF-affected lungs (Korfei et al., 2008). UPR activation in rodent and human AT2 cells triggers pro-apoptosis signaling cascades, ultimately leading to loss of respiratory epithelium and development of fibrosis (Zhong et al., 2011). The chemical chaperone mimetic 4-phenyl butyric acid (4-PBA) has shown promising activity in bleomycin-induced lung fibrosis models by diminishing ER stress and TGF- β 1-induced myofibroblast differentiation (Plate et al., 2016). Thus, it is currently being investigated in IPF to overcome age-related failures in proteostasis.

Defects in autophagy and mitophagy also play a role in IPF. Inhibition of ATG5 in human bronchial epithelial cells and lung fibroblasts accelerates senescence and myofibroblast *trans*-differentiation (Araya et al., 2013). Fibroblastic foci in IPF-affected human lungs contain elevated ubiquitinated proteins and p62, suggesting impaired autophagy. Animals with dysfunctional autophagy due to loss of the critical gene *Atg4b* exhibit an exaggerated acute pro-inflammatory response after injury and a delayed manifestation of severe fibrosis marked by collagen accumulation and dysregulated ECM remodeling (Cabrera et al., 2015). mTOR inhibition by rapamycin and its analogs derepress autophagy and extend both mean and maximum lifespan in a variety of model organisms including mammals (Lamming et al., 2013). Although early trials with the mTOR inhibitor everolimus failed to improve disease in IPF patients (Malouf et al., 2011), next-generation PI3K and mTOR inhibitors such as omipalisib, show promise in preclinical models and are being tested in patients (Lukey et al., 2019). Thus, emerging therapies for IPF that target age-related perturbations have the potential to ameliorate age-associated fibrotic diseases that affect a wide array of organs (Mora et al., 2017).

PULMONARY IMMUNITY AND AGING

Inflammaging

Advancing age leads to immune dysfunction and a heightened inflammatory state. Inflammaging, a term coined two decades ago (Franceschi et al., 2000), is a hallmark of aging that refers to a skewing toward systemic inflammation which is paradoxically coupled to a suppressed ability to respond to immunological threat. Older adults exhibit an elevation in circulating and tissue pro-inflammatory cytokines including IL-1 β , IL-6, IL-8, and TNF- α . This smoldering proinflammatory phenotype is a feature of human aging and carries functional implications for an organism's ability to mount an immune response and respond to vaccines. Furthermore, chronic sterile inflammation has been linked to the pathogenesis of several human diseases including diabetes (Barzilai et al., 2012), atherosclerosis (Tabas, 2010), and COPD (John-Schuster et al., 2016).

Inflammaging results from a variety of stimuli that induces aberrant activation of the innate immune system with age. Pro-inflammatory signals originate from foreign pathogens, endogenous cell debris, and damage-associated molecular patterns

(DAMPs) released by dying cells. In addition to the cumulative exposure to immunogenic material over time, impaired clearance of such debris through deficient xenophagy with age further exacerbates this process. In aged tissues, a gradual increase in senescent cells, both of immune and non-immune origin, contributes to a proinflammatory milieu via the SASP secretome (Basisty et al., 2020). Together, these stimuli activate the NLRP3 inflammasome and lead to synthesis of proinflammatory mediators that perturb local microenvironments and are released into the circulation to influence distal sites.

Although there are several resident immune cells that inhabit the lung, the precise sources of inflammation that drive inflamming is unclear. AMs, thought to be a culprit, reside in the epithelial lining fluid where they adhere to the alveolar surface. They serve as a first line of defense against pathogens and particulate matter by continually sampling foreign material that reach alveoli. When challenged, AMs induce a robust pro-inflammatory signature through the LPS-TLR4-mitogen-activated protein kinase (MAPK) signaling axis (Albright et al., 2016) but should return to a quiescent anti-inflammatory state once apoptotic debris is cleared (Watanabe et al., 2019). Pulmonary macrophages isolated from aged mice by bronchoalveolar lavage express elevated pro-inflammatory cytokines including CCL2, IFN- β , IL-10, TNF- α , and MIF (Lafuse et al., 2019). Similarly, AMs analyzed by single-cell sequencing from murine lungs exhibit differential expression of over 100 genes in old mice compared to young (Angelidis et al., 2019). This includes upregulation of *Cebp*, a transcription factor that controls a variety of inflammatory response genes, and other genes that may contribute to inflamming in the respiratory tract. Furthermore, identification of an AM subpopulation that is enriched in lungs from old mice suggests that the proinflammatory signature may originate from a distinct population of airway macrophages that undergoes clonal expansion with age (Lafuse et al., 2019).

Innate immune response in aged lungs

Dysregulation of pulmonary innate immunity with age represents a critical failure because it plays a key role in protecting the lung's mucosal surface from airborne pathogens. Although the aging innate immune system augments release of proinflammatory cytokines thus contributing to inflamming, it is simultaneously handicapped in its ability to recognize pathogens, signal through TLR cascades, present antigens, undergo chemotaxis, and phagocytose foreign material (Shaw et al., 2013). Even prior to the term "inflamming" being coined, there was already an appreciation that cytokines and neutrophils are found in increased amounts in the lower respiratory tract of healthy, clinically normal, older adults (Meyer et al., 1998). Alveolar fluid collected from aged mice and elderly humans contains elevated levels of TNF, IL-6, and complement components, reflective of a dysregulated innate immunity (Moliva et al., 2014).

Age-associated immunosenescence in elderly humans also results in quantitative and qualitative defects in TLR signaling. Monocytes isolated from aged individuals have reduced transcript levels of TLRs 1–9 (Renshaw et al., 2002), whereas stimulation of aged monocytes leads to defective production of proinflammatory cytokines TNF- α and IL-6 when compared to young controls (van Duin et al., 2007). Diminished TLR expression is

exacerbated by defective signal transduction via adaptor molecule MyD88, which also has reduced expression in aged rodents (Chelvarajan et al., 2006). Furthermore, aging disrupts primary and secondary RIG-I signaling pathways that control expression of type I interferon genes, leading to impaired antiviral responses (Molony et al., 2017).

Other arms of innate immunity are negatively impacted with age such as engulfment of microbes via phagocytosis, pathogen destruction via ROS production, and trafficking to sites of infection by neutrophils, macrophages, and dendritic cells (DCs) (Boe et al., 2017). Because these cells reside at the environmental interface of the respiratory mucosal surface, a reduced local immune response to invading microorganisms may potentiate the systemic spread of an otherwise contained infection in aged hosts. DCs, a critical intermediary in innate and adaptive immunity, engulf microorganisms and migrate to lymph nodes where they prime adaptive immune responses via the activation of T cells. Aged DCs display impaired phagocytosis, migration to lymph nodes, cytokine production, costimulation, and antigen presentation (Agrawal et al., 2007; Zhao et al., 2011). This failure of innate immune response in the lung results in the less robust priming of adaptive immune cells and has implications for vaccine responsiveness in older adults (Panda et al., 2010).

Alveolar epithelial cells from aged mice produce higher levels of chemokines CXCL1/2 resulting in increased neutrophil trafficking to the lungs during influenza infection (Kulkarni et al., 2019). The presence of these neutrophils is responsible for the increased mortality observed in these animals as neutrophil depletion restores the likelihood of survival to frequencies observed in young mice. Similarly, infection with the respiratory pathogen *S. pneumoniae* in aged mice is associated with decreased TLR expression, TNF- α production, NF- κ B expression, and increased mortality (Hinojosa et al., 2009). These defects extend to other innate immune cell types such as aged macrophages that exhibit diminished cytokine production, NF- κ B activity, and NLRP3 inflammasome assembly during *S. pneumoniae* infection (Cho et al., 2018). These studies highlight the effects of aging on pulmonary innate immune dysfunction and the defective response to invading microorganisms. Immunomodulatory strategies that prevent these age-related failures will be critical in restoring proper immune function to mitigate the morbidity associated with infectious and inflammatory lung conditions.

Adaptive immune response in aged lungs

The adaptive arm of the immune system provides sterilizing immunity to foreign microbes, maintains immunologic memory to pathogens and vaccines, and surveils for neoantigens generated during malignant transformation. Adaptive immunity undergoes significant alterations with age including skewed hematopoiesis toward myeloid production, suppression of lymphopoiesis, decreased production of naive lymphocytes, increased T regulatory cells, and diminished B cell adaptability and antibody avidity (Nikolich-Zugich, 2018) (Figure 4).

Advancing age compromises the generation of naive lymphocytes from hematopoietic stem cells (HSCs) that is essential to maintain the pool of diverse naive T and B cells. Aged mice contain less HSC populations with low ROS activity compared to young

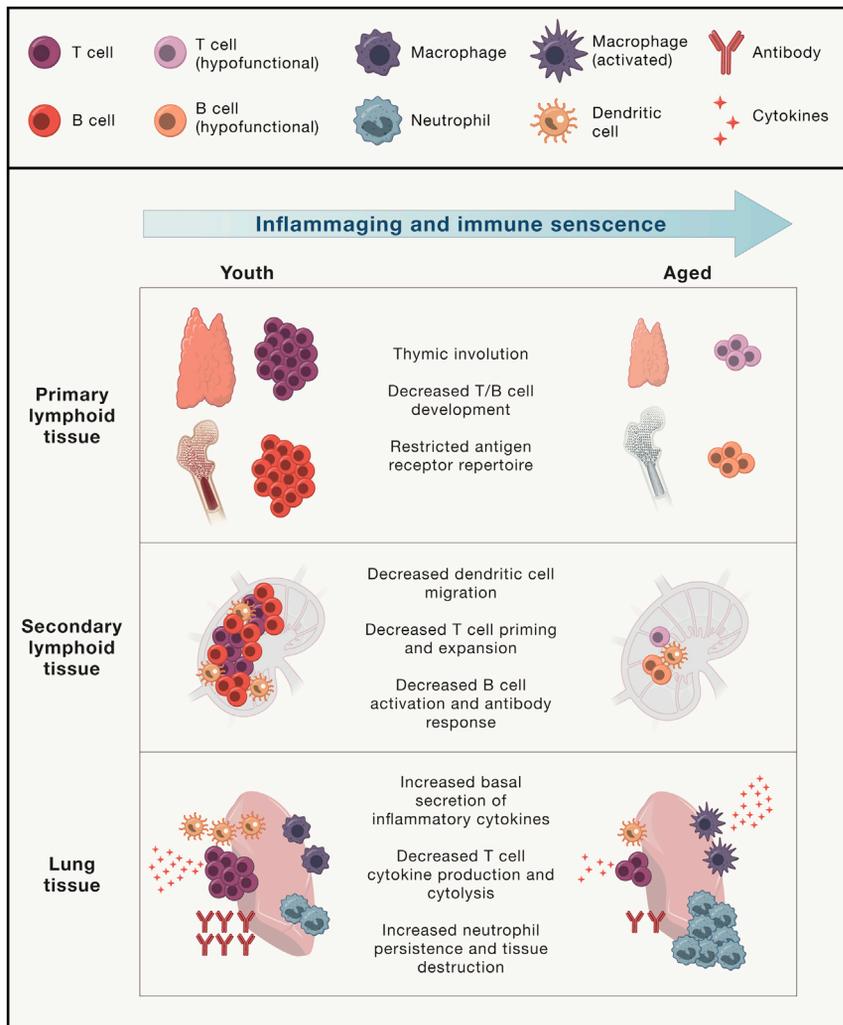


Figure 4. Age-related changes in systemic and pulmonary immunity

Advanced age leads to immunosenescence and inflammaging through effects on primary lymphoid, secondary lymphoid, and target tissues. Top: thymic involution and bone marrow fibrosis decreases circulating levels of naive T/B lymphocytes. Center: loss of lymph node architecture disrupts dendritic cell (DC) priming of T cells and B cell activation. Bottom: defects in T/B cell priming and activation results in loss of effector T cell protection, decreased antibody production. Defects in neutrophil migration from lung tissue results in increased immune pathology. Inflammaging results in the increased production of inflammatory cytokines from macrophages in the absence of infection.

TCR signaling decreases, in part, due to aged-associated loss of miR181a that results in failure to repress dual-specific phosphatase (DUSP)6 (Li et al., 2012). Aging also skews the ratio toward inhibitory T follicular regulatory cells (Sage et al., 2015) and promotes early T follicular helper cell (Tfh) differentiation via expression of the transcription factor RBPJ (Webb et al., 2021). The increase of pre-Tfh cells that are phenotypically similar to mature Tfh cells may explain conflicting reports in observed frequencies of circulating Tfh cells in elderly humans and blunted antibody response to influenza vaccination (Herati et al., 2014; Zhou et al., 2014). Aged mice have defective pathogen-specific CD8⁺ T cell expansion (Richner et al., 2015), thus reducing diversity and functionally impairing antiviral immunity.

animals (Jang and Sharkis, 2007), leading to diminished HSC self-renewal capacity. B cell differentiation is also reduced in aged rodents, stemming from decreased pre-B cell E47 expression and loss of IL-7 production from supporting stromal cells (Van der Put et al., 2004). These signals are essential in driving expression of RAG2 that is necessary for recombination of the B cell receptor (BCR), leading to restricted BCR diversity in aged mice (Labrie et al., 2004). Similarly, naive T cell generation is reduced in aged individuals from thymic involution leading to restriction of the T cell receptor (TCR) repertoire diversity (Griffith et al., 2012). Loss of naive cell antigen receptor diversity with age impairs the ability of adaptive immune cells to identify antigens from novel infectious agents and malignantly transformed cells, leading to compromised immunosurveillance (Wang et al., 2020).

T cells become progressively hypofunctional and hyporesponsive with advancing age as a result of defective priming from innate immune cells and inherent cell-autonomous molecular changes. Naive CD4⁺ T cells from aged animals have reduced expression of the pro-apoptotic factor Bim (Tsukamoto et al., 2010) that leads to decreased proliferative potential, IL-2 production, and CD4⁺ T cell-dependent antibody responses.

T cells from aged mice also undergo shifts in cellular metabolism that impact activation and function. Increased expression of ATPase CD39 on CD4⁺ cells with age renders them more susceptible to apoptosis and mitochondrial dysregulation (Fang et al., 2016). Moreover, CD4⁺ T cells from aged mice have decreased oxygen consumption via mitochondrial respiration in addition to defective upregulation of enzymes involved in the one-carbon metabolism resulting in decreased IL-2 production and proliferative potential (Ron-Harel et al., 2016).

B lymphocyte populations also demonstrate decreased functionality with age. Protective antibody titers following influenza vaccination are present in 30%–50% of elderly compared with 65%–80% of young individuals (Kogut et al., 2012). This is attributed to the fact that elderly adults have less *de novo* somatic hypermutation in immunoglobulin variable genes resulting in diminished adaptability in their antibody response (Henry et al., 2019). The defects in antibody production are in part driven by decreased expression of Blimp1 that prevents differentiation into plasma cells but spares memory B cell formation (Frasca et al., 2016). Additionally, aged mice and humans exhibit decreased expression of AID and E47 that are key for affinity

maturation and whose alterations result in a sub-optimal vaccine response (Frasca et al., 2008). Aged mice exhibit an expansion of B cell-repressive T follicular regulatory (T_{FR}) cells that are phenotypically distinct from those in young animals and occurs in conjunction with increased PD-1 expression (Sage et al., 2015). Together, these age-related changes limit germinal center B cell maturation and B cell function, resulting in defective antibody production and compromised humoral immunity.

Although studies have characterized age-associated changes in innate and adaptive immunity, questions remain about how a distorted immunological landscape translates to lung disease and mortality risk. Systems biology approaches have uncovered predictors of immune response across aging populations (Poland et al., 2014), but individual genetic and environmental variation creates challenges for defining immunoaging dynamics at high resolution. To address this question, a multi-immunomics approach was used to longitudinally profile various features of individuals' immune systems over a decade. This led to the development of an "immune aging" score that describes a person's immune status better than chronological age and was shown to be predictive of all-cause mortality (Alpert et al., 2019). This study and others highlight the important concept that chronological age is not necessarily a reliable indicator of biological age. This decoupling of chronological and physiological age has been borne out in data showing that biological aging metrics, such as immunological (Martínez de Toda et al., 2016), epigenetic (Horvath, 2013), and clinical (Clegg et al., 2013) phenotypes, can be superior predictors of longevity.

Lung cancer, immunotherapy, and aging

As the world's population ages, the incidence of cancer diagnoses is rising at an alarming rate, yet our understanding of the connections between cancer and aging is lacking. Lung cancer remains the leading cause of cancer deaths, killing more people annually than breast, prostate, colorectal, and brain cancers combined (Siegel et al., 2020). Early detection strategies, decreased smoking rates, and development of targeted therapeutics have led to a recent decline in lung cancer mortality. However, lung cancer is still responsible for a staggering 25% of all cancer deaths with one of the worst 5-year survival rates of all cancer types.

Adenocarcinomas, the most common histologic type of non-small cell lung cancers (NSCLC), are subdivided by their molecular characteristics. It has become clear that distinct subsets of NSCLCs are caused by expression of oncogenic drivers. Interestingly, these molecular profiles of lung adenocarcinomas stratify by age. For instance, the frequency of a lung cancer containing a targetable genomic alteration, such as EGFR mutation, ALK or ROS1 fusion, or ERBB2 insertion, is higher among patients younger than 50 years of age (Sacher et al., 2016). Conversely, other oncogenic drivers such as Kras mutations, BRAF V600E, and MET exon 14 skipping are found in adenocarcinomas at a higher rate in patients of older age (Lee et al., 2017; Sacher et al., 2016). Notably, these data are based on information obtained from clinical genotyping panels that analyze a predetermined set of genes in tumors. Although studying the relationship between age and tumor genotype is challenging given confounding factors, studies using untargeted sequencing to under-

stand whether age segregates with other genomic alterations would be insightful.

Age acts as a modifier in lung tumorigenesis, as activation of $Kras^{G12D}$ -driven lung tumors in old mice results in more numerous adenomas and higher-grade lesions compared to younger animals (Parikh et al., 2018). Lung tumors in aged mice exhibit impaired p53-dependent DNA damage response and result in shorter survival. Other age-related modifiers such as *Bin1* and *SIRT4* also increase susceptibility to lung tumorigenesis in old animals (Chang et al., 2007; Jeong et al., 2013). The majority of studies that model lung cancers in rodents are performed in young animals, thus, further studies are needed to clarify how the aged microenvironment predisposes to lung cancer development.

Historic theories on cancer and aging posit that malignancies are caused by sequential multistep genetic lesions arising in somatic cells (Nordling, 1953). Consistent with this theory, smoking-related lung cancer subtypes including small cell carcinoma and squamous cell carcinoma increase in incidence with age and are associated with DNA damage, distinct genomic alterations, and high mutational burden (Willis et al., 2019). However, next generation ultra-deep sequencing from normal human tissue in skin (Martincorena et al., 2015) and esophagus (Martincorena et al., 2018) show astonishingly high somatic mutational burden in non-transformed cells. In sun-exposed normal skin cells, several mutations known to drive the growth of cutaneous squamous cell carcinomas were already under strong positive selection at a young age. Likewise, in aged normal esophageal epithelial cells, there is a striking increase in number of mutations which correlates with age. These studies underscore the shortcomings in theories that attribute age-related cancer development to cell autonomous genetic events. Non-cell autonomous processes such as age-dependent changes in the tumor microenvironment likely also play a critical role in tumorigenesis and cancer progression (Figure 5).

For treatment of advanced lung cancers, there are currently five FDA-approved immunotherapy agents including pembrolizumab, atezolizumab, durvalumab, nivolumab (that targets the PD-1 pathway), and ipilimumab (that targets CTLA-4). Worse survival outcomes and decreased efficacy of immunotherapy agents in elderly populations with lung cancer have been reported, but data are challenging to collect and interpret given the paucity of older individuals included in prospective clinical trials (Elias et al., 2016; Whelehan et al., 2018). Cytotoxic CD8⁺ T cells, the target of many immune checkpoint inhibitors (ICI) through the PD-1/PD-L1 axis, have decreased TCR diversity, reduced proliferative capacity, and a lower threshold for apoptosis in older adults (Elias et al., 2018). Interestingly, whereas expression of PD-1 has been shown to be increased on T cells of older adults, signaling inhibition with ICI did not restore T cell activity to the same degree as in younger patients (Lages et al., 2010). Moreover, transcriptional profiling of circulating PBMCs shows impaired upregulation of PD-1 on T cells in older adults in addition to other age-associated changes including hampered response to innate immune agonists and reduced T cell proliferation (Metcalf et al., 2015). Thus, PD-1 expression levels in elderly individuals may be tissue-dependent and may be uncoupled from T cell activation. This may be related

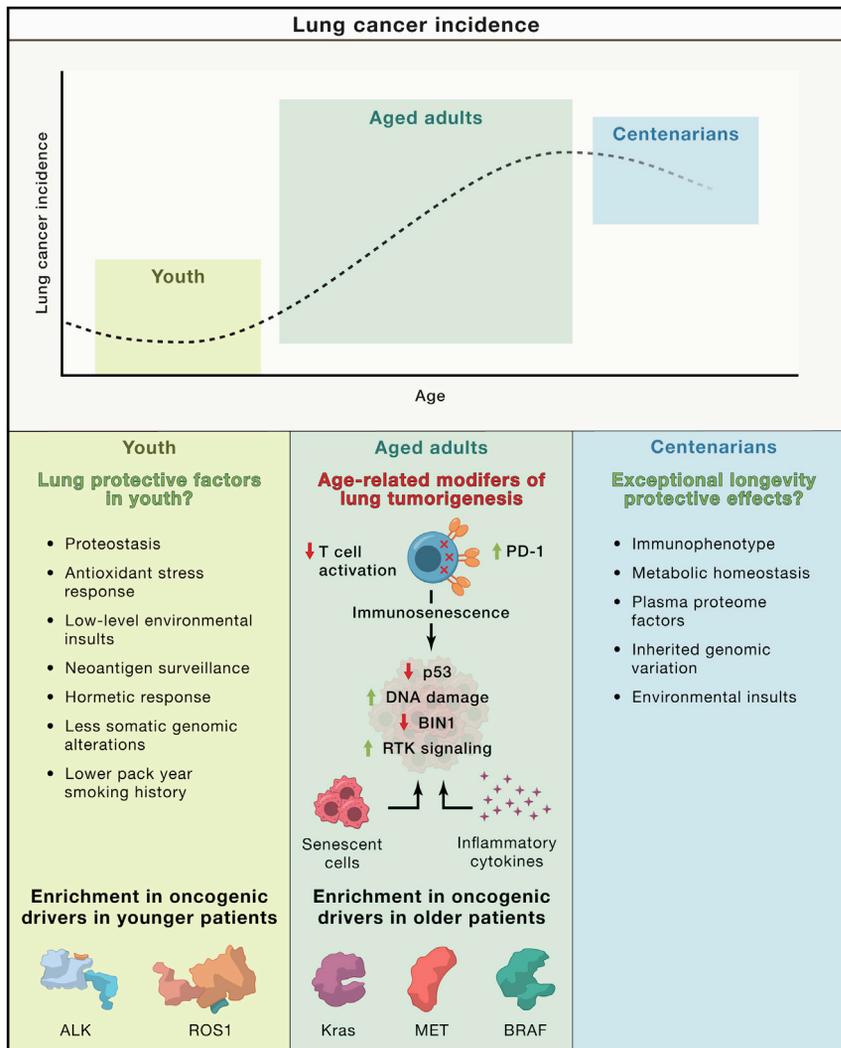


Figure 5. Age-related modifiers of lung tumorigenesis

Left: in youth, protective factors are in place to thwart the development of a primary lung cancer. However, there are certain molecular subtypes of lung adenocarcinomas that are enriched in younger adults (i.e., ALK and ROS1 translocations) which challenge the notion that advanced age drives tumorigenesis in these patients. Middle: as humans age, there is a precipitous rise in the incidence of lung cancer diagnosis. There are certain molecular subtypes of lung adenocarcinomas that are enriched in elderly patients. The increase in lung-resident senescent cells and the decrease in immunosurveillance may potentiate other cell-intrinsic pro-mitogenic alterations that may ultimately result in lung tumorigenesis. Right: in centenarians, rates of lung cancer incidence and prevalence surprisingly decreases, but why this occurs is not fully understood.

eries for how we can effectively modulate the immune system in an age-appropriate context.

While lung cancer rates rise with age, there is a paradoxical downtick in cancer rates in individuals greater than 85 (Nolen et al., 2017). Large datasets demonstrate that cancer incidence decreases by more than 3-fold after age 90 and plateaus between 0% and 4% above age 100 (Pavlidis et al., 2012). Although there could be a diagnostic bias inherent in this epidemiologic cohort of the “oldest old,” studies in nonagenarians and centenarian have revealed a unique biology in the form of functional genetic variations (Ryu et al., 2016) and plasma proteome signatures (Lehallier et al., 2019) that correlate with

to increased T regulatory cells in older individuals, as recent work has shown that PD-1 expression balance between effector and regulatory T cells in the tumor microenvironment can predict the response to PD-1 cancer immunotherapy (Kumagai et al., 2020).

Notably, T cells experience gradual loss of co-stimulatory CD28 expression with age, leading to an expansion of CD8⁺CD28⁻T cells in elderly individuals (Chen et al., 2018). Yet, PD-1⁺ CD8⁺ T cells that proliferate in the peripheral blood in NSCLC patients following PD-1-targeted therapy are predominantly CD28-positive (Kamphorst et al., 2017). Such age-related immune remodeling may explain the diminished efficacy of checkpoint blockade in the elderly. The expression of PD-1 and co-stimulatory molecules in lung tumor-infiltrating lymphocytes in older patients has yet to be defined, and it remains unclear whether this would impact response to ICI. Thus, modeling lung cancer will be important to decipher if aged tumor microenvironments contain different molecular determinants and whether this has implications for treatment of older patients. Uncovering how age influences the tumor immunological milieu can serve as a platform for future discov-

exceptional longevity. Some of these variants are associated with protection from various age-related diseases, including cancers, but how they confer a protective effect remains to be determined.

Respiratory infections in elderly adults

Respiratory bacterial and viral pathogens are a major cause of morbidity and mortality in the elderly. In fact, respiratory infections are the leading cause of death from any infectious etiology among older adults. In the pre-COVID-19 era, 85% of all pneumonia deaths occurred in individuals over 65 years with only ~3% in those under 45 (American Lung Association, 2015). Several mechanisms of age-related pulmonary dysfunction have been proposed as explanations for increased predisposition to respiratory tract infections. Age-related changes in lung-resident cells and alterations in innate and adaptive immunity underlie the severity of pneumonias in hosts with aged lungs.

Bacterial microbes *S. pneumoniae*, *H. influenzae*, *S. aureus*, and *gram-negative bacilli* are the major pathogens that cause

pneumonias in the elderly population at a rate disproportionate to younger age groups. Pulmonary pneumococcal infection is a leading cause of death worldwide among people over 65 years of age (van der Poll and Opal, 2009), highlighting the susceptibility of older adults across the globe. Features of the aged respiratory system that predispose to pneumococcal infection include impaired cough reflex, mucosal barrier function, and mucociliary clearance, which are important for mechanical clearance of aspirated pathogens. Accumulation of senescent cells in the aged lung also account for age-related vulnerability to pneumococcal pneumonias since senescent lung epithelial cells bind more *S. pneumoniae* antigen due to increased expression of host proteins that are co-opted for bacterial adhesion (Shivshankar et al., 2011). This coupled with impaired ability to regenerate respiratory epithelial cells with age results in a slower healing in older adults and longer hospital stays.

The macrophage, a cell with potent antimicrobial properties that resides in distal airways and interstitial compartments, is a critical mediator of the pulmonary anti-bacterial inflammatory response. Chronic exposure to TNF- α in aged mice results in abnormal maturation of monocytes, premature egress from the bone marrow, and altered migratory potential (Puchta et al., 2016). When challenged with antigen, Ly6C⁺ monocytes from elderly mice produce more IL-6 and TNF- α compared to young animals, contributing to the positive feedback loop of pro-inflammatory signatures seen during aging. Although the Ly6C⁺ monocyte population is elevated in the nasopharynx in old mice colonized with *S. pneumoniae*, bacterial clearance is impaired in an age-dependent manner (Puchta et al., 2016). Although AMs in young murine lungs exist mainly in a quiescent state, AMs in aged animals express more proinflammatory cytokines at baseline. However, they are more refractory to activation by IFN- γ (Canan et al., 2014) and exhibit dysregulated NF- κ B and MAPK activation downstream of TLR stimulation (Boyd et al., 2012). Robust lung-specific and age-dependent induction of A20, a deubiquitinase that regulates TRAF6 and suppresses downstream NF- κ B signaling, is one explanation for dysregulated TLR signaling in AMs with age (Hinojosa et al., 2014).

Pulmonary-resident macrophages demonstrate features of mitochondrial dysfunction with age. AMs from aged mouse lungs exhibit decreased ATP production, enhanced ROS generation, and diminished antioxidant response in response to *S. pneumoniae* infection. This is abrogated with pre-treatment with the anti-fibrotic agent pirfenidone that improved mitochondrial function and decreased oxidative stress (Plataki et al., 2019). Additionally, telomere dysfunction has been implicated in the dysfunction of AMs as TERC^{-/-} mice demonstrate heightened susceptibility to bacterial pneumonia and lung inflammation as a result of metabolic perturbations in AMs (Kang et al., 2018). PGC-1 α and TNFAIP3 are homeostatic effectors of telomere length which directly influence mitochondrial function in macrophages, thus uncovering a new link between telomere shortening and the pulmonary innate immune response.

In addition to bacterial pneumonias, viral influenza is a cause of significant mortality in elderly populations. Individuals between 65–74 years of age have a 30-fold higher risk of death from influenza A infection compared with those aged 25–49

(Ortiz et al., 2013). Immunosenescence enhances the susceptibility of elderly patients to viral pathogens and renders prophylactic vaccination approaches less effective (Henry et al., 2019). Because disease severity depends on the properties of the host, research has focused on elucidating specific factors that change with age that directly influence antiviral immunity. Proteomic profiling demonstrates a maladaptive collapse in the proteostasis network in AT2 cells and AMs in aged animals infected with influenza (Loguercio et al., 2019). These age-associated changes in proteostasis effectors are most pronounced in Hsp70 family members and co-chaperones. In addition to AMs, age-related deficits in phagocytosis and proteostasis have been described in tissue-resident skeletal muscle macrophage populations in old mice, leading to hampered recovery of skeletal muscle function after influenza pneumonia (Runyan et al., 2020).

Immunosenescence of lung-resident adaptive immune effector cells increases vulnerability to viral infections. In murine models of RSV or influenza, aged mice have reduced expansion of virus-specific CD8⁺ T cells and production of protective IFN- γ (Fulton et al., 2013; Sant et al., 2018). Although these studies may reflect the impact of deficits in T cell priming and localization to the lung, the effect of aging on tissue-resident populations of T cells in the lung is still unclear. The human lung contains a large population of resident memory T cells (T_{RM}) that persist in the interstitium following recovery from respiratory viral infections (Pizzolla et al., 2018). Although T_{RM} cells coordinate the response to secondary challenges with pathogens and are distinct from CD8⁺ effector memory T cells, it is unknown how aging affects this population. In humans, single cell transcriptomics performed over six decades elucidated unique spatial and temporal changes in T cell compartmentalization with advancing age (Thome et al., 2014). Human lungs harbor a unique CD8⁺ T cell population, TEMRA cells, suggesting a pulmonary niche for this subset of effector memory T cells that accrues with age. The role of this cell population in protecting aged individuals from secondary infections is undefined but represents an important area for future investigation.

Although most studies have focused on age-related dysregulation of innate and adaptive immunity as explanations for susceptibility to pulmonary infectious agents, other host-specific factors are being explored. Investigations of the commensal human microbiome in the respiratory system have revealed that lungs are not sterile as once presumed but rather, they harbor a distinct microbial landscape whose composition changes in the context of pulmonary diseases (Moffatt and Cookson, 2017). Studies are ongoing to determine whether a perturbed pulmonary microbiome directly influences pulmonary disease pathogenesis and whether there are age-associated changes in individual microbial species or broader commensal ecosystems that impact age-related disease and susceptibility to infections. Likewise, another host-specific factor that has the potential to confer host-response heterogeneity to pulmonary pathogens is CHIP, the term used to describe the presence of clonally expanded hematopoietic cells with acquired somatic mutations that are present in the circulation (Steensma, 2018). The prevalence of CHIP increases with age due to the gradual accumulation of mutations that occur throughout an individual's

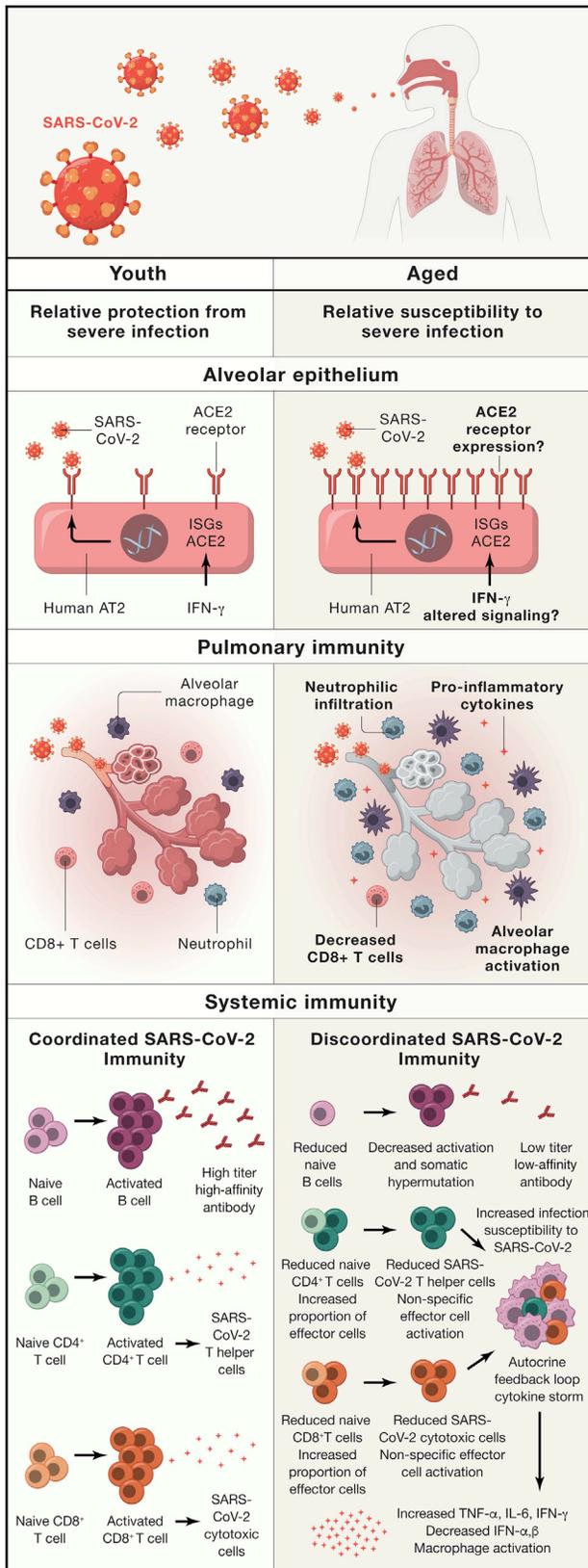


Figure 6. Age-related molecular and immunological determinants that may contribute to worse outcomes in elderly patients infected with SARS-CoV-2

Putative molecular and immunologic alterations with age may provide a biological basis for why elderly patients with COVID-19 have increased mortality. Top: SARS-CoV-2 infects AT2 cells where the angiotensin-converting enzyme 2 (ACE2) serves as a receptor for cellular entry. ACE2 is an interferon-stimulated gene (ISG) in lung tissue. It is unknown how ACE2 levels change with age and whether altered interferon signaling cascades with age impact the expression dynamics of the SARS-CoV-2 receptor. Middle: multiple pulmonary immune effector cells undergo changes in the aging lung including neutrophil infiltration and alveolar macrophage (AM) activation. An over-exuberant proinflammatory response triggered by SARS-CoV-2 in the background of inflammaging and an already skewed population of aged lung-resident macrophages may be a lethal combination leading to increased mortality in infected older adults. Bottom: reduction in naive T/B lymphocytes in aged individuals leads to decreased antigen-specific T cell responses and reduced antibody titers. The increased frequency of terminally differentiated T cells and cytokine dysregulation from inflammaging leads to a T cell auto-inflammatory loop with macrophages resulting in cytokine release syndrome and severe systemic inflammation with end organ dysfunction.

lifetime. Although CHIP has been implicated in hematologic malignancies and cardiovascular disease, the impact of CHIP on age-related pulmonary immunity is not yet known.

COVID-19 in the elderly population

As the novel SARS-CoV-2 virus continues to infect millions around the globe, society faces a pressing urgency to understand the pathogenesis of the severe acute respiratory syndrome that has disproportionately impacted the elderly. Epidemiologic data reveal that age is strongly associated with COVID-19-related mortality, with people aged 80 or over having more than 20-fold-increased risk compared to those in their 50s (Williamson et al., 2020). The simplest explanation is that older adults have preexisting medical conditions that increase their susceptibility (Guan et al., 2020a) or certain medications prescribed at higher frequency to elderly patients put them at greater risk (Mancia et al., 2020). However, there remains the possibility that molecular and immunologic alterations with age may provide a biological basis for why elderly patients with COVID-19 fare worse (Figure 6).

SARS-CoV-2 infects AT2 cells where it propagates and releases large numbers of viral particles. The angiotensin-converting enzyme 2 (ACE2) is a receptor for the SARS-CoV-2 virus on AT2 cells, with cellular entry mediated by the viral spike protein and the host protease TMPRSS2. Interestingly, the ACE2 receptor is an interferon-stimulated gene in human nasal epithelia and lung tissue, suggesting that SARS-CoV-2 may exploit this regulatory axis to augment receptor expression to facilitate entry into cells. One hypothesis that emerged early in the course of the pandemic postulated that older adults have increased expression of the ACE2 receptor in airway epithelial tissue that would lead to enhanced viral entry, higher viral loads, and increased pathogenicity. An increase in ACE2 expression has yet to be definitively proven in non-cancerous human aged lung tissue but older studies in rat lungs surprisingly found reduced expression of ACE2 with advancing age (Xie et al., 2006). However, it is unclear how reliable rodent models are for SARS-CoV-2 given the structural differences between mouse and human ACE2 (Hassan et al., 2020) and data showing that mouse epithelial ACE2 is not regulated by interferon signaling as occurs in humans, raising

concerns about disease modeling (Ziegler et al., 2020). Because interferon signaling is altered in older adults, it is also possible that changes in ACE2 expression with age have gone unappreciated in mouse models, as studies performed in young rodents with robust interferon signaling could mask dynamic regulation of ACE2.

To identify additional genetic factors involved in the development of severe respiratory failure due to COVID-19 in humans, a GWAS revealed a novel susceptibility locus with coverage of six genes, including two chemokine receptors, *CCR9* and *CXCR6*, the latter that regulates the localization of lung-resident memory CD8⁺ T cells to the airways in response to infection with viral infection (Ellinghaus et al., 2020; Wein et al., 2019). The novel coronavirus is co-expressed with genes involved in innate immune functions such as interleukin signaling (*IRAK3*), phagocytosis (*NOS2*), interferon modulation (*MX1*), and inhibition of viral replication (*OAS1*) (Sunngnak et al., 2020). Thus, although age-related molecular determinants in the lung such as changes in ACE2 may play a role in dictating illness severity, the role of immunosenescence has garnered attention as an explanation for the susceptibility of aged populations to the virus (Mueller et al., 2020). For instance, the identification of mutations in interferon response genes and the presence of neutralizing autoantibodies that inactivate interferon signaling have recently been implicated in patients with severe COVID-19 (Bastard et al., 2020; Zhang et al., 2020). However, whether this plays a role in heightened age-related vulnerability is unknown.

It is important to note that in the study of human immune aging, most samples are collected from peripheral blood and thus reflect circulating immunocytes and soluble mediators. This leaves a gap in our understanding of how age impacts the function of tissue-resident immune cells, such as those that reside in the lung. Studies in non-human primates with other betacoronaviruses that induce acute lung injury reveal worse multifocal pulmonary consolidation, immune infiltration, and diffuse alveolar damage in aged macaques (Smits et al., 2010). This is due to an overly robust innate immune response within the lung, with transcriptomics revealing a central role for activation of NF- κ B in driving expression of pro-inflammatory cytokines and interleukins, an effect not observed in younger animals. Moreover, tissue-resident AMs from aged mice shows upregulation of inflammatory response genes including those involved in chemotaxis, cell adhesion, and interferon signaling compared to young mice (McQuattie-Pimentel et al., 2019), highlighting the fact that lung-resident immune cells can undergo molecular changes in response to the aged local pulmonary environment without input from the systemic immune system. Taken together, it is plausible that an over-exuberant proinflammatory response triggered by SARS-CoV-2 in the background of inflammaging and an already skewed population of aged lung-resident macrophages may be a particularly lethal combination leading to increased mortality in infected older adults.

Mortality from SARS-CoV-2 is influenced not only by age but also by gender. Sex-disaggregated data from twenty-six countries indicate that men have more severe disease and higher mortality rates (Guan et al., 2020b; Sharma et al., 2020). Analysis of SARS-CoV, a closely related coronavirus that caused the 2003 SARS epidemic, revealed that male mice had enhanced suscep-

tibility to infection that became more pronounced with advancing age (Channappanavar et al., 2017). Male rodents infected with SARS-CoV demonstrate elevated viral titers, increased alveolar edema, and accumulation of AMs and neutrophils in the lungs. An augmented innate immune response in male rodents is independent of T and B cell responses. Intriguingly, human PBMCs across age ranges 20–90 exhibit a significant sexual dimorphism in the human immune system with age (Márquez et al., 2020). ATAC-seq, RNA-sequencing, and flow cytometry (available at <https://immune-aging.jax.org>) reveal that genomic and epigenomic differences between genders were more pronounced after age 65, with men having more robust innate and pro-inflammatory activity with concomitant lower adaptive immune activity. Moreover, a scoring metric for immunological aging in humans also demonstrated that men exhibit significantly higher immune-aging scores compared to women (Alpert et al., 2019), again supporting the notion of increased perturbations of the male immune system with age. Overall, whether sex-specific differences in COVID-19 outcomes are attributable to distinct age-related immunophenotypes, hormonal landscapes, pulmonary molecular determinants, or epidemiologic characteristics remains to be determined.

Although elderly individuals arguably have the most to gain from a SARS-CoV-2 vaccine, they represent a part of the populace that is least likely to mount an effective immune response, as vaccines are generally less immunogenic in aged hosts (Sasaki et al., 2011). For instance, the influenza vaccine response in the elderly is blunted owing to reduced *de novo* somatic hypermutations in immunoglobulin variable genes and diminished adaptability in their antibody response (Henry et al., 2019). This necessitates that individuals over 65 years receive a high-dose quadrivalent vaccine or a formulation that includes the MF59 adjuvant to augment their immune response (Tsang et al., 2020). As vaccines against SARS-CoV-2 are being developed, approved, and distributed at an unprecedented speed, we are still learning about their efficacy in the elderly (Anderson et al., 2020).

CONCLUDING REMARKS

For centuries, humans have been preoccupied with age-old folklore of Ponce de León's Fountain of Youth and what it means to stay young. However, there must be a shift in our calculus as we appreciate the importance of elucidating the drivers of the aging process and what it means to be old. A core question that has emerged is whether we can harness our ability to modulate aging in a meaningful way in order to delay, prevent, or reverse aging phenotypes that are tightly linked to pathogenesis of an array of human disease.

Lung disease accounts for millions of deaths across the globe each year and disproportionately affects elderly individuals. Morbidity and mortality from pulmonary conditions are expected to worsen as the human population ages, underscoring the dire need to pinpoint mechanisms that explain age-related pulmonary decline. This review summarizes our current understanding of cellular features of lung aging. We describe both quantitative and qualitative changes in cellular and extra-cellular compartments that occur with age in the respiratory tract. Despite

exposure to environmental insults that accrue over a lifetime, aging is linked to failure of multiple cellular stress response pathways, leading to higher rates of parenchymal lung disease in older patients. Developing a mechanistic understanding of how and why these adaptive mechanisms fail or become overwhelmed is integral in delineating their contribution to physiological age-dependent decline and age-related diseases. Furthermore, we describe how cell non-autonomous factors play a major role in age-related lung dysfunction, with alterations in the immunological landscape being a key contributor to the development of age-associated inflammation, lung cancer, and infectious processes including SARS-CoV-2 in older adults.

Technological advances in single-cell transcriptional profiling and genetic lineage tracing have revolutionized the field, shedding light on how specific cell types and microenvironments evolve during aging. High-throughput, layered, multi-omics approaches also have the potential to further revolutionize our understanding of the mechanisms of age-related pulmonary diseases. For instance, initiatives such as the Human Lung Cell Atlas consortium aim to collect samples from the entire adult age range to comprehensively analyze the effect of aging on lung-resident cells (Schiller et al., 2019). Such large-scale approaches will be critical to distinguish natural variation from subclinical and overt disease. However, an emphasis on mechanism and less on descriptive associations is needed to fully illuminate causal drivers of physiologic age. Questions still remain about cell type heterogeneity, because not all cells in the lung age at the same rate. Understanding the interplay between cell-autonomous and non-autonomous features of aging is critical, as recent studies have highlighted the importance of the extracellular milieu and local immunological niche in modulating local cellular functions.

The collective amalgamation of age-related changes in innate and adaptive immunity results in failure of older adults to respond to immunological threat as robustly as younger adults. Although extensive characterization of age-associated changes in primary and secondary lymphoid tissue has been carried out over several decades, more investigations are needed to fully crystallize how systemic failures in innate and adaptive immunity with age impact the end-organ, such as the lung. Although studies have shed light on how the aged immune system predisposes to bacterial and viral pulmonary infections, the real challenge lies in parsing out which age-related molecular alterations are targetable and which would have therapeutic benefit. Exploiting age-related changes in immunity will be critical to construct prophylactic and immunomodulatory strategies to improve outcomes in elderly patients.

Unfortunately, recent events have cast a spotlight on the vulnerability of our elderly populace. The unfolding of the COVID-19 pandemic and the numerous tragic deaths incurred in its wake have exposed the deficiencies in our understanding of how immunologic features of the respiratory system are altered with age and whether there are age-related molecular determinants that can be targeted with therapeutic intent. Although immune aging scoring metrics have been shown to predict all-cause mortality, is not yet known whether an individual's immunophenotype correlates with response to infection with SARS-CoV-2 and whether centenarians who have survived COVID-19

harbor these favorable biological profiles. Elucidating the principles of effective immunity in the elderly may also hold promise for furthering our understanding across all populations. Perhaps the silver lining is that the exposed gaps in our knowledge will catalyze a wave of scientific research dedicated to clarifying the molecular basis of aging in the lung.

ACKNOWLEDGMENTS

We apologize for all the primary literature and work we could not cite due to space limitations. We thank Dr. Jiska Van Der Reest and Dr. Kiran Kurmi for their discussions on the manuscript. J.L.S. is supported by the National Cancer Institute (T32CA071345). J.H.R. is supported by the Division of Pediatric Oncology at Dana Farber (T32CA136432) training grant, and Cannonball Kids Cancer Young Investigator Award. C.G.R. is supported by the Mexico in Harvard Foundation-Mexican Council of Science and Technology Fellowship, and Bristol-Myers Squibb. C.F.K. is supported by the National Heart, Lung, and Blood Institute of the NIH under award number R35HL150876, Bristol-Myers Squibb, and Longfonds Stichting. A.H.S. is supported by NIH P01 AI056299 and U54CA224088. M.C.H. is supported by the Ludwig Center at Harvard Medical School, the Paul F. Glenn Foundation for Medical Research, and NIH grants R01CA213062 and U54CA224088. Some figures were created with BioRender.com.

DECLARATION OF INTERESTS

C.F.K. is a member of a consortium funded by Bristol-Myers Squibb. A.H.S. is on advisory boards for Surface Oncology, Elstar, SQZ Biotechnologies, Elpisience, Selecta, Monopteros, and Bicara and consults for Novartis. A.H.S. has received research funding from Novartis, Roche, UCB, Ipsen, Quark, Merck, and AbbVie outside the submitted work. She also is on the scientific advisory boards for the Massachusetts General Cancer Center, Program in Cellular and Molecular Medicine at Boston Children's Hospital, and the Human Oncology and Pathogenesis Program at Memorial Sloan Kettering Cancer Center and is a scientific editor for the Journal of Experimental Medicine. A.H.S. has patents/pending royalties on the PD-1 pathway from Roche and Novartis. Her spouse has patents/pending royalties on the PD-1/PD-L1 pathway from Roche, Merck MSD, Bristol-Myers-Squibb, Merck KGA, Boehringer-Ingelheim, AstraZeneca, Dako, Leica, Mayo Clinic, and Novartis. He has served on advisory boards for Roche, Bristol-Myers-Squibb, Xios, Origimed, Triursus, iTeos, NextPoint, IgM, Jubilant, Trillium, and GV20 and has equity in Nextpoint, Triursus, Xios, iTeos, IgM, GV20, and Geode. M.C.H. has received research funding from Roche. She is on the advisory board for Pori Therapeutics.

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