

Mitochondria in the spotlight of aging and idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a chronic age-related lung disease with high mortality that is characterized by abnormal scarring of the lung parenchyma. There has been a recent attempt to define the age-associated changes predisposing individuals to develop IPF. Age-related perturbations that are increasingly found in epithelial cells and fibroblasts from IPF lungs compared with age-matched cells from normal lungs include defective autophagy, telomere attrition, altered proteostasis, and cell senescence. These divergent processes seem to converge in mitochondrial dysfunction and metabolic distress, which potentiate maladaptation to stress and susceptibility to age-related diseases such as IPF. Therapeutic approaches that target aging processes may be beneficial for halting the progression of disease and improving quality of life in IPF patients.

Linking aging and chronic lung diseases

One of the most remarkable medical accomplishments in the last century has been the increasing longevity of the human population. A decrease in birth rates, accompanied by a reduction in mortality among the elderly population, has collectively increased the percentage of the aged population, particularly in developed countries. Globally, it is estimated that the proportion of the population over the age of 60 will increase from 11% to 22% by 2050, and 28% by 2100. Currently, 18% of the US population is over 60 years of age (57 million), which is predicted to rise to 27% (107 million) in 2050 and up to 31% (149 million) by 2100 (1). The aging population has propelled an increase in the overall prevalence of chronic conditions. Several lung diseases, including acute lung injury and asthma, and some infectious diseases, such as pneumonia, increase in severity and mortality with age. Additionally, there has been a significant increase in incidence of chronic lung diseases—including chronic obstructive pulmonary disease, most forms of lung cancer, and idiopathic pulmonary fibrosis (IPF)—resulting in aging-related increases in prevalence.

IPF is the most common form of idiopathic interstitial lung diseases. Its overall incidence in the US is 7 to 17 per 100,000 persons with a prevalence between 13.2 and 63 per 100,000 persons (2). A 1996 study initially demonstrated a higher incidence and prevalence of IPF in patients over 65 (3). These observations were confirmed more recently by Raghu et al. (4) and others, using both broad and narrow case-finding criteria for IPF diagnosis. These studies estimated that in the US, the prevalence of IPF increases with age, ranging from 4 per 100,000 for population aged between 18 and 34 years old to 227.2 per 100,000 among those 75 or older (4). This aging-related increase in cumulative incidence and prevalence of IPF has been corroborated by several studies that include populations in Europe and Asia (5–9). Accordingly, the median age

at diagnosis of sporadic IPF ranges between 50 and 85 years old, and patients younger than 50 are more commonly associated with familial, rather than sporadic, forms of the disease (2). Mortality rates from IPF are steadily increasing worldwide, and a positive association has also been observed with advanced age (10). Examination of US government health insurance data for people aged 65 and older shows an increasing prevalence of IPF among older age groups (11). These studies confirm the growing concern that aging, and consequently age-related diseases, will drive up health care expenditures. As the elderly population in developed countries is expected to double in the next 25 years, there is an urgent need to understand the pathogenesis of IPF and develop interventions to attenuate or reverse lung fibrosis.

The physiology of aging and the lung

Evolutionary theories of aging include the concept of selection shadow that permits the accumulation of mutations with late deleterious effects, and the theory of antagonistic pleiotropy, which supports the selection of genes that benefit early reproductive life even if they have a deleterious effect at later ages. The disposable soma theory proposes that to maximize reproduction and survival, the organism optimally allocates its metabolic resources. This comes at the cost of limited resources for somatic maintenance and repair, allowing for accumulation of molecular and cellular damage. This concept supports the observation that there is individual plasticity, and the aging process is stochastic in nature (12, 13). Despite the identification of conserved longevity pathways and major environmental factors, the potentially major role of stochastic factors remains poorly defined. Exploring these factors has the potential to increase our knowledge of the variability in the aging process between cells, tissues, and organisms, and even identify new interventions in the aging process (14).

Lung maturity peaks between 20 and 25 years of age. Thereafter, an age-progressive decline in lung function occurs, which is often marked by diminution of forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC). In addition, the resid-

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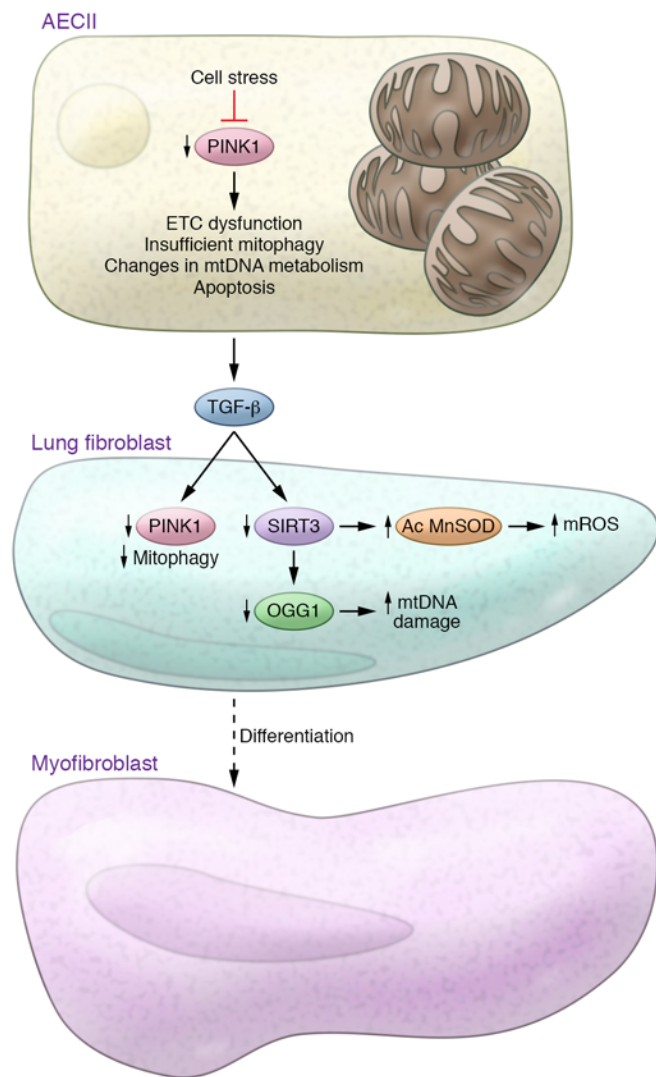


Figure 1. Mitochondrial dysfunction and lung fibrosis. Aging and ER stress cause mitochondrial dysfunction in type II alveolar epithelial cells (AECIIs) by diminishing the expression of the mitochondrial homeostasis regulator PINK1. Deficiency of PINK1 causes mitochondrial dysfunction, which is characterized by low activity of the electron transport chain (ETC) complexes I and IV, alterations in mtDNA metabolism, and insufficient mitophagy, leading to increased susceptibility to apoptosis and induction of TGF- β . TGF- β stimulation of lung fibroblasts has been shown to decrease PINK1 levels and promote insufficient mitophagy and myofibroblast differentiation. Additionally, aging and TGF- β stimulation reduce the expression of SIRT3. SIRT3 deficiency increases levels of acetylated (Ac) MnSOD and consequently increases mitochondrial ROS levels and mtDNA damage, which are also related to low expression of the DNA repair enzyme 8-oxoguanine-DNA glycosylase-1 (OGG1).

ual volume increases and the transport of respiratory gases across the alveolar wall declines, affecting the arterial oxygen tension. A study conducted with a limited number of healthy elderly subjects showed a high frequency of basal subpleural reticular patterns and cysts on CT, as well as bronchial dilation and bronchial wall thickening (15). However, systematic imaging and functional studies in asymptomatic elderly patients have not been conducted to determine the spectrum of normal aging in the lung, which leaves the clinical relevance of these findings unclear.

Table 1. Aging mitochondria in the lung

| Characteristic | Finding | References |
|------------------------------------|--|------------|
| Increase of mROS | Increased formation of oxidatively modified proteins in lung of aging rats; reduced complex I, II, III, and IV activities in lung of aging rats | 18 |
| Impaired respiration | Decreased basal and maximal mitochondrial respiration in aged murine AECIIs | 17 |
| mtDNA deletions | Accumulation of 4,977 bp deleted mtDNA in human lung tissues >40 years old | 92 |
| | Higher mtDNA content and oxidative damage in human aging lung | 93 |
| Compromised mitochondrial dynamics | Inhibition of mitochondrial fission and elevation of mitochondrial fusion markers in aged murine AECIIs; increased area in human alveolar epithelial cells | 17 |
| Mitophagy | Decreased mitophagy | 17, 19 |
| Low sirtuins | Low expression of SIRT3 in aging mouse and rat lungs | 18, 94 |

mROS, mitochondrial reactive oxygen species; AECII, type II alveolar epithelial cell; mtDNA, mitochondrial DNA.

At a cellular level, only limited knowledge of the factors that define *healthy* aging of different lung cells is available, and the majority of these studies use animal models. Reporter mouse models that monitor critical factors in the induction and maintenance of senescence, such as p19^{ARF}, demonstrate that accumulation of senescence cells in the lung becomes apparent at 12 months of age associated with decreased lung function characterized by higher compliance or lower capacity to expand at a given pressure and lower elastance, a measure that captures the increase in rigidity and stiffness of the aging lungs (16). In the same set of studies, elimination of p19^{ARF}-expressing cells using a toxin receptor knockout mouse model shows reduction in the loss of elastic fibers in the aging lung tissue, and lower levels of MMP10 and MMP12, two matrix metalloproteinases with elastase activity, and the concomitant improvement in parameters of lung function (16). Altered mitochondrial homeostasis is found in the healthy aging lung (summarized in Table 1). Our group has described a higher frequency of enlarged mitochondria with a bias toward mitochondrial fusion and an increased mitochondrial area in aging type II alveolar epithelial cells (AECIIs) (17). In addition, impaired respiration has been shown in lungs from aged rats (18) and mice (17), with a concomitant increase in mitochondrial ROS (18). Analyses of autophagy activity in aging murine lungs show markers of autophagy flux blockade (17, 19). Most recently, we have shown that dysfunctional autophagy activity also occurs in aging lung fibroblasts from healthy humans (20). Senescence, mitochondrial dysfunction, and insufficient autophagy in the aging lung might have implications in maladaptive responses to stress. For instance, elderly mice are more vulnerable to fibrosis after injury or stress (17, 21, 22) and have a lower capacity for normal repair after damage (23). In this context, our group has shown that the lung's protective mechanisms against injury, such as mesenchymal stem cell differentiation, are also impaired with age (24).

Cellular perturbations in the IPF lung

Genomic instability. Throughout life, DNA is exposed to a wide variety of exogenous and endogenous damaging stimuli. Genetic

Table 2. DNA and chromatin changes associated with pulmonary fibrosis

| Characteristic | Finding | References |
|---------------------------|--|----------------|
| Genetic instability | Multiple susceptible genomic loci for pulmonary fibrosis | 27, 28 |
| | Microsatellite instability in IPF population | 29–31 |
| Epigenetic changes | DNA methylation changes in IPF | 38 |
| | Changes in noncoding RNAs in lung fibrosis | 45, 46, 50, 51 |
| RTEL1 mutations | RTEL1 mutations are associated with familial pulmonary fibrosis | 59, 61 |
| | TERT mutations associated with IPF | 63 |
| TERT mutations | Premature graying and subclinical lung diseases | 58, 60 |
| | Telomere shortening in IPF | 55–57, 62 |
| | Telomere dysfunction causes alveolar stem cell failure | 64 |
| Murine models of fibrosis | Telomerase induction in fibroblasts | 66 |
| | Mice with <i>Trf1</i> deletion in type II alveolar cells develop lung fibrosis | 65 |

IPF, idiopathic pulmonary fibrosis; RTEL1, regulator of telomere elongation helicase 1; TERT, telomerase reverse transcriptase; *Trf1*, telomeric repeat factor 1.

stability has to be preserved to ensure proper cell replication, and even the smallest imbalance between DNA damage and repair can typically lead to cell death (25). With age, the activity of the cellular DNA repair systems diminishes, allowing accumulation of errors (26) that, when perpetuated as genetic anomalies, can destabilize the genome and dysregulate homeostasis of the cell bearing it.

Several studies have reported genome instability in the IPF patient population (27, 28). Half of sputum samples from IPF patients displayed genetic alterations, either microsatellite instability (MSI) or loss of heterozygosity (LOH), when compared with matched controls (29). A follow-up study including peripheral blood analysis determined that LOH was located in key cell cycle genes such as p16 (30). Moreover, MSI was found in the TGF- β receptor II gene from AECIIs isolated from honeycomb structures in IPF lungs (31). Collectively, these data suggest that DNA damage can actually play a significant role in susceptibility to IPF. Recently, a comparative proteomic analysis of lung tissue found higher expression of DNA damage proteins in IPF lungs (32).

Epigenetic changes. Epigenetic mechanisms are able to modulate gene activity in the absence of DNA sequence changes, including DNA methylation, histone modification, and expression of noncoding RNAs (microRNAs [miRNAs] and long noncoding RNAs [lncRNAs]). Epigenetic changes that are associated with age (33, 34) seem to be highly conserved in mammals (35, 36), especially the global change of DNA methylation patterns, also known as the “epigenetic clock.” This particular loss of methylation and the subtle differences between changes in healthy aging and in age-related pathological processes (37) could be the key to identifying novel biomarkers in IPF, since it exhibits its own particular methylation profile (38).

Histone posttranslational modifications add another layer of regulation to DNA transcription. Age can modify histone acetylation (39), and it has been proposed that sirtuins, a family of deacetylases whose expression patterns are altered with age, could be one of the culprits (40). For example, SIRT1 suppresses the expression of senescence-associated secretory phenotype (SASP) factors via

histone deacetylation in their promoter regions (41). Recently, Sanders et al. (42) reported that pharmacological regulation of histone acetylation ameliorated bleomycin-induced pulmonary fibrosis in mice, suggesting a new therapeutic avenue for IPF.

Noncoding RNAs in small or long forms establish a complex network of transcriptional regulators that can control and modulate different cell programs. Several miRNA target genes are associated with age-related pathways, such as p16 and p21 (43). Studies in IPF lungs have identified significant pathogenic changes in the expression of miRNAs (44). Some of those changes are related to fibrotic response and TGF- β 1 pathways, such as decreases in the miRNA let-7 (45), while others target senescence pathways (46). In high contrast, many of the miRNAs dysregulated in IPF recapitulate the fingerprint of developmental cell programming (47).

The notion of lncRNAs (over 200 bp in length) is still young (48), but they are the subject of an emerging body of work to uncover their role in aging and pathological processes (49). It is hypothesized that they can function as competing endogenous miRNAs (ceRNA). Huang et al. elegantly showed that the lncRNA n341773 might function as a ceRNA for miR-199–induced increases in collagen expression in IPF lung fibroblasts (50). Also, the expression of miR-29 and that of MRAK088388 were strongly correlated in lung tissue from murine fibrosis models (51).

Telomere attrition. Familial IPF and sporadic IPF have also been associated with mutations in the telomerase genes *TERC* and *TERT* (52, 53). Around 8%–15% of the familial pulmonary fibrosis and 1%–3% of sporadic IPF are associated with germline mutations in telomerase genes (54). Absence of telomerase function leads to telomere shortening and induction of senescence. In addition, telomere attrition is a common finding in patients with IPF, even in the absence of telomerase gene mutations (55–63) (Table 2).

Our knowledge is still limited regarding the link between telomerase genetic defects and development of lung fibrosis. It is postulated that telomerase mutations could cause senescence of AECIIs by directly affecting their regenerative capacity, leading to increased susceptibility to lung injury (64). In mice, even just accumulation of telomeric damage through telomere dysfunction in the AECIIs is enough to promote a fibrotic phenotype (65). In sharp contrast, human IPF fibroblasts do not always exhibit short telomeres, even when attrition is detected in matching peripheral blood samples. Furthermore, Liu et al. showed induction of telomerase activity in fibroblasts from bleomycin-treated mice and IPF lungs (66). Taking all these findings into account reminds us that cell type and biological context could also affect how telomerase changes can drive the aging process. Finally, it is worth noting that both *TERC* (the telomerase RNA subunit) and *TERRA* (telomeric repeat-containing RNA), important regulators of the telomerase heterochromatin, are transcribed as lncRNAs (67).

Cellular senescence and SASP. Diminution of the replicative capacity of lung cells is a well-known phenomenon in IPF. Early studies performed in the senescence-accelerated mouse strain SAMP8 demonstrated that mice with a senescence phenotype are more susceptible to lung injury and fibrosis (68). The age-related increase in fibrosis and a defective repair capacity after lung injury have been successively confirmed in aged wild-type mice (21). Senescence can affect the lung’s distinct cell types quite

Table 3. Cellular senescence and SASP in fibrotic models

| Cell type | Finding | References |
|-------------|---|------------|
| Macrophages | SASP is mediated by NLRP3 | 75 |
| | PAR-1 regulates cellular senescence | 70 |
| | SASP profile depends on type of stimuli | 74 |
| Fibroblast | Senescence in primary IPF fibroblast | 71 |
| | Epigenetic control of senescence | 72 |
| | Bone marrow-derived cells alter the outcome of lung injury in aged mice | 68 |
| Epithelial | Senescence caused by telomere deficiency | 69 |
| | Caveolin-1 deficiency protects from pulmonary fibrosis | 77 |
| | Accelerated epithelial cell senescence in IPF | 78 |

SASP, senescence-associated secretory phenotype; NLRP3, NLR family, pyrin domain containing 3; PAR-1, protease activated receptor 1; IPF, idiopathic pulmonary fibrosis.

differently (Table 3). For example, senescence can be responsible for exhaustion of stem cells (69), secretion of inflammatory mediators in immune cells and fibroblasts (70), and increased myofibroblast differentiation in lung fibroblasts (71). Stress-induced senescence has been observed in isolated mouse lung fibroblasts from fibrotic lungs, although their proliferative capacity vary between studies. Hecker et al. described that the senescence response was transitory and self-limited in young fibroblasts, in contrast to aging lung fibroblasts that have sustained senescence to resist apoptosis (23). Mechanistic analyses indicate that altered redox balance in aging, controlled by NOX4 and NRF2, promoted myofibroblast senescence. High expression of NOX4 in aging fibroblasts was associated with histone modifications secondary to epigenetic changes with enrichment of H4K16Ac and depletion of H4K20Me3 (72).

It has long been recognized that senescent cells have alterations in the secretome with an increased production of secretory proteins including PAI-1, and a range of cytokines, chemokines, and proteases (73). In the lung, replicative senescence of mouse lung fibroblasts has been shown to induce expression of several inflammatory mediators and growth factors that have the ability to induce proliferation in adjacent cells (74). SASP provokes ER stress and reinforces senescence through autocrine activity rather than by spreading the senescence phenotype to healthy neighboring cells. Activation of the inflammasome has been shown to be relevant in development of lung fibrosis in aging mice, since deficiency of the inflammasome component NLRP3 diminishes bleomycin-induced lung injury in older animals (75). Another important component in the modulation of SASP is the abundance of caveolae and their components, which increases with senescence (76). As a result, mice deficient in caveolin 1 are protected from bleomycin-induced lung injury with a low profile of SASP (77).

Consistent with studies in mouse models, fibroblasts and lung epithelial cells from IPF lungs express markers of senescence, including positive β -gal staining and p21 expression (23, 71, 78). However, the majority of the current studies with human lung fibroblasts lack appropriate age-matched controls to determine whether the senescent and profibrotic phenotype is related to advanced age.

Mitochondrial dysfunction. Aging appears to particularly affect mitochondria, and mitochondrial abnormalities including enlargement, loss of cristae, and destruction of inner membranes (79, 80) are often observed with aging. Moreover, ATP production and respiration in mitochondria from older animals are lower than those in mitochondria from younger counterparts, and often, aging mitochondria produce greater amounts of ROS (81). Consequently, mice with more mitochondrial DNA (mtDNA) mutations display accelerated signs of aging. Despite these findings, genetic interventions that impair the expression and function of the electron transport chain components and mitochondrial function have resulted in an increased lifespan. These disparities are most likely associated with the concept of mitohormesis, where mild mitochondrial impairment of function might increase longevity, but severe mitochondrial damage shortens lifespan.

Dysfunctional mitochondria accumulate with age and are a driving aging mechanism in postmitotic cells including muscle cells, neurons, and cardiomyocytes (82, 83). Less is known about mitochondrial dysfunction as a driving mechanism of age-related disease in mitotic tissues. We discovered that AECIIs from human IPF lungs have accumulation of dysmorphic and dysfunctional mitochondria associated with upregulation of markers of ER stress compared with age-matched controls. These findings were replicated in aging mice (20–24 months old) in response to ER stress stimulation. Dysfunctional mitochondria in AECIIs from IPF and aging lungs were found to be related to a low expression of the regulator of mitochondrial homeostasis PTEN-induced putative kinase 1 (PINK1), a kinase linked to age-related neurodegenerative disease. As a result, PINK1-deficient mice exhibited increased susceptibility to apoptosis and spontaneous TGF- β -driven lung fibrosis, developing similar dysmorphic and dysfunctional mitochondria in the AECIIs (17) and an increased susceptibility to lung injury and subsequent fibrosis. Other research groups have also confirmed the role of mitochondrial dysfunction in lung fibrosis. Hawkins and collaborators recently demonstrated that mutations within a surfactant protein that is linked to familial pulmonary fibrosis cause defective clearance of mitochondria (84). Studies by Patel and collaborators confirmed that PINK1-deficient mice have higher susceptibility to lung fibrosis associated with increased apoptosis (85). In addition, studies focused on lung fibroblasts demonstrated that TGF- β induces amelioration of PINK1 expression and low expression of PINK1 resulted in promotion of myofibroblast transformation (19). Similarly, enhanced myofibroblast differentiation and pro-fibrotic signaling pathways were induced by Parkin deficiency, a well-known regulator of mitophagy (86).

New mitochondrial signaling pathways are being studied in relation to increased vulnerability to lung injury, particularly SIRT3. Mitochondrial SIRT3 is reported to control the transformation of fibroblasts into myofibroblasts via suppression of TGF- β 1 signaling (87, 88). In concordance, SIRT3-deficient, aging mice were found to develop tissue fibrosis of multiple organs, including heart, liver, kidney, and lungs (87). In the lung, SIRT3 deficiency augmented pulmonary fibrosis after asbestos and bleomycin exposure (88, 89), and SIRT3 expression is attenuated in skin and lung of systemic sclerosis patients (90). It is also proposed that SIRT3 modulates mtDNA damage by regulating 8-oxoguanine-DNA glycosylase-1 (OGG1) acetylation (91, 92). OGG1 is a DNA repair

Table 4. Dysregulation of proteostasis in IPF

| Characteristic | Related to | Reference |
|---|--|--------------|
| Insufficient/impaired autophagy in IPF | Controlled by TGF- β upregulation | 19, 114, 117 |
| | Increased S1P | 117 |
| | Reduced FOXO3A | 116 |
| | Decreased PINK1 | 17, 19 |
| | ATG4B-dependent | 118 |
| | Aberrant PTEN/Akt/mTOR axis | 113, 114 |
| | TGF- β upregulation | 17, 99 |
| ER stress and unfolded protein response | Induction of M2 phenotype in macrophages | 99 |
| | Decreased PINK1 | 17 |
| | Viral infections | 96, 108 |
| | Aberrant SPC processing | 97, 98, 108 |
| | Exacerbated with age | 22 |
| Proteasome | Apoptosis in AECII | 22, 107 |
| | Alterations in 20S/26S content | 100, 101 |

IPF, idiopathic pulmonary fibrosis; S1P, sphingosine-1-phosphate lyase; FOXO3A, forkhead box O3a; PINK1, PTEN-induced putative kinase 1; ATG4B, mammalian homolog of yeast ATG4 (autophagy-related gene 4); PTEN, phosphatase and tensin homolog; Akt, protein kinase B; mTOR, mammalian target of rapamycin; SPC, surfactant protein C; AECII, type II alveolar epithelial cell.

enzyme that hydrolyzes oxidized guanine residues that are modified as a result of excessive ROS production, and deficiency of OGG1 increases susceptibility to pulmonary fibrosis induced by asbestos (89, 92–94). Accumulation of mutations and the deletion of mtDNA can accelerate aging. There is evidence that the aging lung contains a higher proportion of mutated and damaged mtDNA (95, 96), as well as low expression of SIRT3 (97). It has been suggested that the premature aging phenotype of mtDNA mutator mice accumulates high levels of point mutations due to a proofreading deficiency of the mtDNA polymerase POLG (98). Interestingly, expression of SIRT3 diminished significantly with age in these mice, suggesting that this pathway could also regulate aging-associated mitochondrial dysfunction with vulnerability to lung fibrosis. These findings are summarized in Figure 1.

Defective proteostasis. The efficiency of proteostasis declines with age in parallel to defective translation, missense mutations, and oxidative modification. Changes in proteostasis lead to an increase of nonfunctional proteins, accumulation of cytotoxic proteins, and/or aggregation of misfolded proteins. In IPF lungs, evidence of altered proteostasis has been found at different levels, including protein misfolding, markers of ER stress (99–102), defective autophagy, and impaired proteasome activity (103, 104) (Table 4). For instance, familial pulmonary fibrosis is associated with several mutations in surfactant A and C genes (105–108). In addition, recent studies show that genetic alterations outside the coding region of *MUC5B* can alter *MUC5B* protein secretion and contribute to cell stress in IPF (109). Alterations in the normal function of the unfolded protein response (UPR), the ubiquitin-proteasome system, or the autophagy-lysosome pathway can increase the pathology caused by impaired proteostasis. For instance, autophagy plays a critical role in antagonizing proteostatic stress by allowing the cellular reorganization and remodeling required for tissue repair.

The details of the molecular mechanisms associated with the age-related modifications in proteostasis are not yet clearly understood; however, it is known that younger individuals can enhance proteostasis to compensate for some protein misfolding mutations. Moreover, with advanced age there is an accumulation of cell stress due to environmental exposure (e.g., infections and cigarette smoke) or comorbidities (e.g., metabolic syndrome and sleep deprivation) that might have a negative impact in proteostasis and activate UPR responses in the ER.

Markers of ER stress are found in lungs from sporadic IPF (110, 111). Particularly, AECII from areas with dense fibrosis and fibroblast foci have the most notable expression of ER stress markers that colocalize with markers of apoptosis. The cause of ER stress in sporadic IPF is not well understood. Some potential factors involved in induction of ER stress in lung epithelium have been previously associated with IPF, such as herpesvirus infection and exposure to cigarette smoke (112, 113). Our studies using the herpesvirus-induced lung fibrosis murine model have shown that individuals with advanced age respond differently to lung injury, preferentially experiencing lung cell apoptosis and activation of profibrotic pathways (22). We also have shown that ER stress influences mitochondrial function in alveolar epithelial cells by down-regulating the critical regulator of mitochondrial homeostasis PINK1 (17). It is possible that additional mechanisms are involved in this crosstalk between ER and mitochondria influencing susceptibility to age-related lung diseases.

Reduced autophagy. Autophagy, a highly conserved process that participates in maintaining cellular energy resources and quality control by degrading unnecessary proteins and organelles, is an essential anti-aging pathway (25). As an adaptive response, autophagy is able to relieve stressful conditions in the cell, such as starvation, hypoxia, ER stress, oxidative stress, etc. (114). Conversely, IPF lungs have been reported to have a reduced rate of autophagy activity, which may contribute to rapid and sustained myofibroblast differentiation and resistance to apoptosis in lung fibroblasts that perpetuate cell senescence in lung epithelial cells (115–118). Deficient autophagy in IPF lungs has been associated with persistent activation of the serine/threonine protein kinase mammalian target of rapamycin (mTOR), potentially due to low levels of PTEN and consequent hyperactivation of Akt in response to collagen-rich matrices. The transcription factor forkhead box O3a (FOXO3A) is a proposed regulator of autophagy, and it contains conserved Akt phosphorylation sites that create a docking site for 14-3-3, preventing its nuclear translocation (119). Another potential mechanism for the inhibition of autophagy in IPF is related to the high concentration of TGF- β . TGF- β has been found to mediate activation of mTORC1 expression (115). TGF- β stimulation of lung fibroblasts has also been associated with regulation of the expression of LC3 and beclin through a mechanism that includes TGF- β -mediated induction of sphingosine-1 phosphate (120). Controversially, TGF- β also plays a role in the regulation of damaged mitochondria-specific autophagy or mitophagy. In lung fibroblasts, TGF- β has been shown to reduce the expression of PINK1, an important modulator of mitophagy (19), yet it has the opposite effect on PINK1 expression within the lung's epithelial cells (85).

A more general approach to determine how inhibition of autophagy modulates the fibrotic response has used mice deficient

in ATG4B, an essential autophagic factor for autophagosome formation (121). ATG4B expression increases during bleomycin-induced lung fibrosis, but most importantly, its deficiency causes more severe apoptosis, inflammation, and fibrotic responses, confirming that autophagy plays a protective role against lung fibrosis. Finally, we analyzed autophagic activity in young, old, and IPF human lung fibroblasts. Old and IPF lung fibroblasts have dysfunctional autophagy activity with increased LC3 puncta, p62 accumulation, and persistent activation of the mTOR pathway under starvation conditions. However, the activation of mTOR was further enhanced in IPF fibroblasts and associated with greater resistance to apoptosis (20). Altogether, these studies suggest that aberrant activation of mTOR and the resulting defective autophagy in aging IPF fibroblasts play a role in the development of lung fibrosis.

Metabolic dysregulation as a convergent event in the aging cell

One of the most fundamental alterations observed in aging cells is metabolic dysregulation. Mitochondria are the organelles that not only manage the cell's energy production but can also detect danger and control cell death. Fibrosis results from abnormal tissue repair, and exaggerated remodeling is frequently associated with persistent and/or severe injury and cell apoptosis. Current evidence suggests that mitochondrial dysfunction plays a critical role in the vulnerability to fibrotic conditions (17, 19, 84, 85, 87, 122, 123). Tissue- and cell-specific pathways regulate or respond to mitochondrial function to meet ever-changing physiological needs. During cellular stress, cells adapt to protect mitochondria by activating different signaling pathways to maintain cellular homeostasis. Growing evidence suggests that DNA damage can cause mitochondrial dysfunction by activating nucleus-to-mitochondria signaling (124). Activation of DNA-damage sensors, particularly poly(ADP)ribose polymerase 1 (PARP1), leads to loss of NAD⁺ and acetyl-CoA, important molecules that regulate the activity of sirtuin and cellular metabolism (125). Loss of sirtuin activity mediates an increase in mitochondrial ROS and inactivation of AMPK, FOXO proteins, and PGC1- α . These alterations dysregulate mitochondrial biogenesis, mitophagy, metabolism, and the expression of mitochondrial antioxidant mechanisms (including superoxide dismutase and uncoupling proteins like UCP2) (126). Additionally, SIRT1 and SIRT6 are required for regulation of DNA repair pathways (127). A vicious cycle of PARP1 activation, NAD⁺ deficiency, and dysregulation of SIRT1 leads to increases in oxidative stress, and reduced DNA repair capacity might contribute to aging and susceptibility to age-related diseases such as IPF. In concordance, fibroblasts isolated from IPF patients exhibit significantly increased PARP1 expression and activity relative to fibroblasts isolated from control subjects, and PARP1-deficient mice show attenuated pulmonary fibrosis in the bleomycin model (128).

It is also recognized that mitochondrial metabolism can influence and modulate nuclear DNA and histone methylation. Existing evidence supports a redox regulation of epigenetics. Consequently, nuclear DNA methylation patterns change with copy number of mtDNA (129), mtDNA variants with different oxidative phosphorylation efficiencies (130), and the content of intracellular ROS (131). In addition, recent studies show the presence of epigenetic

modifications in the mtDNA (132–136). Limited information exists about a possible relationship between aging and mitochondrial epigenetic modifications. Preliminary studies show that fibroblasts from old donors exhibit higher levels of mtDNA methylation (137).

Supporting the concept of a bidirectional signaling pathway between the nucleus and mitochondria, telomere attrition has been found to activate p53 with further regulation of mitochondrial function and metabolism by repression of PGC1- α and - β (important regulators of mitochondrial biogenesis). Consequently, generation 4 of mice deficient in TERT exhibit telomere shortening, and reduced number and function of mitochondria (138). Similarly, mitochondrial dysfunction can lead to telomere attrition. Studies using the uncoupling agent FCCP suggest that enhanced production of ROS induces genomic instability and influences telomere length (139). A more recent concept is the interplay between mtDNA and nuclear DNA. Using conplastic mice, mtDNA variants were shown to be sufficient in promoting differences in mitochondrial function and activation of adaptive cellular responses that can lead to alterations in glucose and lipid metabolism, telomere shortening, and even reduced lifespan. This evidence supports the concept that mitochondrial homeostasis is negatively regulated by aging-associated cell perturbations such as genomic instability, deficiency in proteostasis, and impaired autophagy.

Notably, mitochondrial dysfunction can drive aging cell phenotypes like senescence (140). Potential mechanisms of mitochondrial dysfunction-induced senescence include enhanced levels of ROS, sustained activation of AMPK, and alterations of the NAD⁺/NADH ratio (141–143). Importantly, NAD has been linked to both senescence and aging, and mitochondrial sirtuins such as the NAD-dependent proteins SIRT3 and SIRT5 can suppress senescence and modulate SASP (18, 140). Additional mitochondrial alterations that promote cellular senescence are aberrant mitochondrial dynamics, defective oxidative phosphorylation, and calcium homeostasis (144).

An additional mechanism is the mitochondrial unfolded protein response (UPR^{mit}) that is activated by mitochondrial proteotoxic stress. UPR^{mit} monitors not only the degradation of defective proteins in the mitochondria but also mitochondrial protein import as an indirect indicator of mitochondrial fitness, which is linked to transcription of quality control components (145, 146). As a consequence, synthesis of mitochondrial proteases and mitophagy are adapted accordingly to allow cell survival during UPR^{mit}. In parallel, the dysregulation of mitochondrial proteases has been associated with aging and age-related lung diseases (147).

A possibility exists that mitochondrial dysfunction is a convergent point for aging-related cellular perturbations, eventually becoming a “point of no return” for aging cells and a critical contributor to the pathogenesis of chronic lung diseases such as IPF (Figure 2).

Perspective on aging regulation in IPF pathology and treatment

Current efforts exist to integrate the normal physiology of aging with the pathobiology of age-related chronic diseases like IPF. A new understanding of how biological systems change with age offers novel pathways that might impact not only the human lifespan but also the extension of human healthspan. Studies in the IPF lung strongly support the concept that aging promotes this

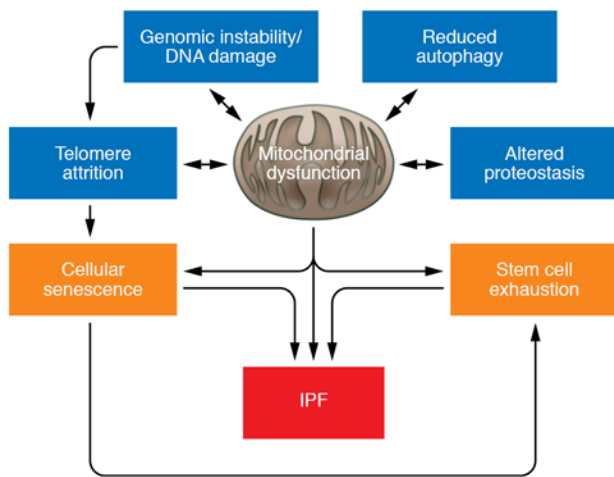


Figure 2. Mechanisms of aging and the pathogenesis of IPF. Several of the aging cell perturbations associated with IPF lungs converge to produce mitochondrial dysfunction, including DNA damage/genomic instability, altered proteostasis, reduced autophagy, and telomere attrition. Additionally, mitochondrial dysfunction can lead to cellular senescence and stem cell exhaustion. We propose that mitochondrial dysfunction plays a central role in the process of aging and the pathogenesis of IPF.

disease. As we described above, multiple pathways associated with aging are activated in lung epithelial cells, fibroblasts, and progenitor cells in the IPF lung. Imperative goals in the field are the incorporation not only of aged mice as animal models of lung fibrosis, but of the use of primary human cells and tissues from healthy aging controls in order to clearly illustrate the dynamic connection between aging and disease.

In this new era of gerosciences and with the identification of common pathways in age-related diseases, one of the ultimate goals is to develop preventive and therapeutic approaches to multiple age-related diseases. New data suggest that despite the apparent inevitability of this aging process, interventions such as intermittent fasting, sustained calorie restriction, exercise, and pharmacological treatments (i.e., rapamycin and metformin) will eventually extend the human lifespan (148–154). Additionally, approaches to target specific aging pathways are under development, such as senolytic drugs to preferentially induce apoptosis in senescent cells (155). Our studies support the idea that mitochondrial dysfunction can be an important factor in susceptibility to IPF. Mitochondria-targeted therapies have been developed to target (a) specific mitochondrial diseases, or (b) general mitochondrial homeostasis processes that can be applied to a wide spectrum of diseases (156). The second group of strategies includes regulation of mitochondrial biogenesis, activation of mitophagy, inhibition

of mitochondrial apoptosis, scavenging of toxic compounds, and bypass of electron transport chain defects. For instance, the peroxisome proliferator-activated receptor (PPAR) and its coactivator PGC1- α play important regulatory role in biogenesis, and this pathway is a potential target for mitochondrial therapy. AMPK agonists that can indirectly activate PGC1- α , or bezafibrate (a PPAR agonist), have been used to stimulate mitochondrial biogenesis and mediate protective effects in myopathy and neurodegenerative disease models associated with mitochondrial dysfunction (157, 158). For instance, a transgenic mouse model of mitochondrial catalase mediates protection against asbestos, and bleomycin induced lung fibrosis associated with prevention of mitochondrial DNA damage (159). Hexafluoro, a novel honokiol derivative, has been shown to induce SIRT3 expression in lung fibroblasts and protect from TGF- β -mediated SIRT3 depletion and ameliorate bleomycin-induced lung fibrosis in mice (90). Our studies on mitochondria homeostasis have shown preferential mitophagy dysregulation, and alterations in fusion/fission dynamics are deregulated in the fibrotic lung. Inhibitors of mitochondrial fission such as the specific DRP1 inhibitor Mdivi-1 have been shown to rescue abnormal mitochondrial dynamics in models of chronic obstructive pulmonary disease (160). Induction of selective mitochondrial turnover of defective mitochondria has been achieved by overexpression of parkin in a model of heteroplasmic cybrid cells (161). Interestingly, studies in humans support the concept that aerobic exercise might increase proteins involved with mitochondrial biogenesis and mitochondrial dynamics (162). Additionally, the fact that mitochondrial dysfunction can be the result of other aging-related cell perturbations, including changes in proteostasis and reduced autophagy, might suggest that therapies targeting these cell dysfunctions can also beneficially affect the mitochondria and promote lung repair. These novel approaches have the potential to be transformative. In the future, innovative drugs may be able to prevent, delay, or reverse multiple or specific aging-related chronic diseases, including IPF, ultimately improving not only an individual's lifespan but also, and most notably, quality of life.

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