Review Article

Chronic obstructive pulmonary disease and the hallmarks of aging

Shweta P. Kukrety, Jai D. Parekh, Kristina L. Bailey[1,2]

Department of Internal Medicine, Creighton University, [1]Department of Internal Medicine, University of Nebraska Medical Center,
[2]Research Service, Veterans Affairs Nebraska-Western Iowa Health Care System, Omaha, NE, USA

ABSTRACT

Aging is characterized by progressive deterioration of physiological integrity, decline in homeostasis, and degeneration of the tissues that occurs after the reproductive phase of life is complete, leading to impaired function. This deterioration is an important risk factor for chronic lung pathologies such as chronic obstructive pulmonary disease (COPD). COPD is a disease that develops gradually. Emphysematous changes in the lung take years to develop after exposure to cigarette smoke; hence, the vast majority of patients are elderly. There has been a dramatic increase in the life expectancy of the general population, resulting in an increased burden of chronic lung diseases. There is growing evidence that molecular mechanisms involved in aging may also play a role in COPD pathogenesis. Recently, the nine hallmarks of aging were identified. In this article, we will review the nine hallmarks of aging and how each hallmark contributes to the pathogenesis of COPD.

KEY WORDS: Cellular senescence, dysregulated nutrient sensing, emphysema, epigenetic alterations, genetic instability, loss of proteostasis, mitochondrial dysfunction, smoking, stem cell exhaustion and altered intercellular communications, telomere attrition

Address for correspondence: Dr. Kristina L. Bailey, 985910 Nebraska Medical Center, Omaha, NE 68198-5910, USA. E-mail: kbailey@unmc.edu

INTRODUCTION

Aging is a complex, heterogeneous process that can vary from organism to organism and from organ system to organ system. Despite this heterogeneity, several "hallmarks of aging" have been identified.[1] These include genetic instability, telomere attrition, epigenetic alterations, loss of proteostasis, dysregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communications.

Older age is an established risk factor for the development of chronic obstructive pulmonary disease (COPD),[2] in part, because COPD develops gradually. Most exposures such as cigarette smoking, biomass smoke, or occupational exposures take time to induce COPD. However, there is some overlap between the hallmarks of aging and cellular processes that lead to COPD. Hence, it is also possible that normal aging facilitates the development of COPD.

In this review, we will discuss physiologic changes that occur with healthy aging that are similar to or contribute to those that occur in COPD. We will then examine each hallmark of aging and how it contributes to COPD pathology.

Physiological changes in the lung with aging
Several physiological changes occur in the lung as a part of the normal aging process. Pulmonary function naturally declines as we age. In normal healthy subjects, there is

an annual decrease in forced expiratory volume in 1 s of approximately 20 mL/year in those aged 25–39 years and up to 38 mL/year in those 65 years or older.[3] Compounding those changes, there is a decrease in the static elastic recoil of the lung, which occurs because of progressive dilatation of the alveolar ducts along with loss of supporting tissues for the peripheral airways. This leads to a condition termed senile emphysema.[4] However, senile emphysema leads to homogeneous airspace dilation, compared to the irregular distribution of airspace dilatation in emphysema.[5] Another important distinguishing characteristic is the absence of alveolar wall destruction in the normal aging process.[5] In addition, there is also a decrease in chest wall compliance and a decline in the strength of the respiratory muscles.[4] This decrease in chest wall compliance and loss of elastic recoil of the lung results in increased residual volume (air trapping) and increased functional residual capacity in the elderly.[4]

Telomere attrition

Telomeres are repetitive nucleotide sequences on the ends of chromosomes, which protect chromosomes from deterioration and fusion with neighboring chromosomes. This helps protect chromosomal stability. With each deoxyribonucleic acid (DNA) replication, there is a progressive shortening of the telomeres due to the inability of DNA polymerase to replicate completely the ends of the DNA molecule. Natural aging results in telomere shortening.

This progressive telomere shortening is responsible for "replicative senescence." Replicative senescence was described for the first time by Hayflick. He demonstrated that human cells *in vitro* could undergo a limited number of cell divisions and then arrest.[6] Telomere length is not only related to aging but also it is influenced by oxidative stress and inflammation.[7]

Savale *et al*. demonstrate that peripheral leukocytes of COPD patients have excessive telomere shortening as compared to age-matched controls with normal lung function. They did not, however, find any association between telomere length and tobacco use. Telomere length did not vary among smokers, nonsmokers, current smokers, or former smokers. They also did not find any relationship between telomere shortening and the degree of airway obstruction.[8] One of the major drawbacks of this study was its small size. However, other studies show that smoking increases telomere shortening in peripheral leukocytes. In contrast to the smaller study, Valdes *et al*. demonstrate a relationship between shorter telomere length and increasing number of cigarettes smoked. Each pack-year smoked is equivalent to a loss of an additional 5 bp from the telomere.[9] Type II alveolar and endothelial cells in patients with emphysema have telomere length significantly shorter than asymptomatic nonsmokers.[10] Telomere length has also been shown to be related to mortality. COPD patients with shorter telomeres are at an increased risk of all-cause and cancer mortality.[11]

Cellular senescence

Cellular senescence is strongly associated with aging and the development of COPD. Cellular senescence is a process in which cells stop dividing and undergo phenotypic changes. The goal of senescence is to prevent propagation of damaged cells and to enhance their immune clearance. It is also thought to help prevent unchecked replication associated with cancer.[12] Senescent cells often show an enlarged and flattened morphology, histochemical staining positive for senescence-associated B-galactosidase (SA B-gal),[13] and an increased expression of p16^{INK4a}.[14] Another important characteristic of senescent cells is the senescence-associated secretory phenotype, which includes various pro-inflammatory cytokines, which may promote chronic inflammation in tissues.[15] Cells undergo senescence in response to several stimuli, including telomere attrition (replicative senescence) and stress-induced senescence in response to genomic damage (stress-induced premature senescence).[16] Due to this, cellular senescence is associated with several other hallmarks of aging including telomere attrition and DNA damage. Once telomeres reach a critical length, they elicit a persistent DNA damage response (DDR). This DDR activates p53, a tumor suppressor, thereby resulting in cell cycle arrest and senescence.[17,18] Many cells also undergo senescence in response to DNA damage such as those induced by oxidative stress.[19] These stimuli induce senescence through activation of two major tumor suppressor pathways such as p53/p21 and p16^{INK4a}/pRB.[20]

Cigarette smoking, the most important risk factor for COPD, is known to induce senescence in alveolar epithelial cells. Tsuji *et al*. demonstrate that cigarette smoke extract exposure of normal human type II alveolar epithelial cells induces changes associated with senescence. The cells demonstrate a flat, enlarged morphology, increased expression of SA B-gal, and irreversible growth arrest.[21] Patients with emphysema have been shown to have accelerated senescence of Type II alveolar and endothelial cells as compared to asymptomatic smokers and nonsmokers.[10]

Tsuji *et al*. propose cellular senescence as a potential pathogenic mechanism of COPD.[10] Smoking results in apoptosis of the alveolar cells. To compensate for this loss, there is an increased proliferation of the alveolar cells. This compensatory increase in cell turnover causes telomere attrition and induces replicative senescence. In addition to this, increased oxidative stress caused by cigarette smoking results in stress-induced premature senescence. When senescence occurs, regenerative capacity is lost, but the chronic damage continues resulting in the formation of emphysematous lesions.[10]

Genomic instability

Genomic instability is defined as an increased susceptibility to DNA mutations. Throughout life, we accumulate genetic damage. This is related to defects in DNA repair that have been strongly linked to the aging process.

Studies in humans and mice show that deficiencies in DNA repair mechanisms result in accelerated aging in mice and are also responsible for several human progeria syndromes such as Bloom and Werner syndromes.[22] Bloom syndrome is characterized by short stature, predisposition to early development of cancers, infertility, and immunodeficiency. Patients with Werner syndrome demonstrate premature aging: growth retardation, short stature, wrinkling, and alopecia. Bloom syndrome is caused by mutations in the BLM gene, which encodes RecQ helicase. RecQ helicase unwinds the DNA, which is necessary to initiate DNA repair. Werner syndrome is caused by mutations in the WRN gene, which encodes Werner protein. Werner protein assists in the replication of DNA in preparation for cell division. Low levels of Werner protein have been measured in fibroblasts isolated from emphysema patients, along with increased senescence.[23] Cigarette smoke extract also decreases Werner's protein in cultured fibroblasts and epithelial cells. In addition, fibroblast with low levels of Werner's protein was more susceptible to cigarette smoke-induced cellular senescence. In contrast, exogenous overexpression of Werner's syndrome protein attenuated the cigarette smoke effects.

Smoking is shown to cause DNA damage in the form of DNA double-strand breaks (DDSBs).[24] DDSBs play a role in the pathogenesis of COPD by inducing apoptosis, cellular senescence, and pro-inflammatory changes.[25] COPD patients have increased foci of DDSBs in Type I and Type II alveolar and endothelial cells as compared to asymptomatic smokers and nonsmokers. This DNA damage has been shown to extend beyond the lungs into peripheral blood. Ceylan *et al*. found that peripheral blood leukocytes of COPD patients had a significantly higher number of DDSBs as compared to healthy controls.[26]

Microsatellites are very short nucleotide repeats found scattered throughout the human genome. Microsatellite instability (MSI) results from impaired DNA mismatch repair and correlates with a high mutational rate and has been linked to various malignancies. MSI has been detected in the sputum of COPD smokers when compared to non-COPD smokers.[27] MSI did not seem to be related to the severity of illness. MSI appears to be specific for the target organ of COPD, i.e., the lungs. Although inflammation is present in the nasal mucosa of COPD patients, no MSI was noted in the nasal cytological sample of COPD patients.[28] These mutations persevere even after individuals have quit smoking,[29] possibly explaining why inflammation in COPD continues even after smoking cessation.

Epigenetic alterations

Epigenetic alterations are heritable alterations to the genetic code that are not due to changes in DNA sequence. Epigenetic marks alter DNA accessibility and chromatin structure. There are three main classes of epigenetic marks: DNA methylation, histone tail modifications, and noncoding microRNA. Aging is associated with global

DNA hypomethylation as well as hypermethylation of various tumor suppressor genes, increasing susceptibility to malignancies as we age.[30] Histone acetylation and methylation induce epigenetic changes that contribute to the aging process.[31]

Acetylation of histones opens the chromatin structure, initiating gene transcription. Histone acetylation is reversed by histone deacetylases (HDACs) inducing gene repression. In COPD patients, HDAC 2 activity is decreased in peripheral lung tissue, airway epithelia, and alveolar macrophages. This causes an increased acetylation of histones in the promoter region of pro-inflammatory genes, resulting in increased production of cytokines and chemokines, which are responsible for chronic inflammation.[32,33] In addition to HDAC2, HDAC 3, 5, and 8 are also decreased in lung tissue and macrophages.[32] There is also a link between the increasing disease severity and reduction in HDACs.[32] Nuclear factor erythroid 2-related factor 2 (Nrf2) plays a crucial role in inducing the expression of antioxidant genes. Mercado *et al*. demonstrate that reduced HDAC2 activity in COPD may be responsible for the increased acetylation of Nrf2, resulting in impaired antioxidant defenses, playing a role in the pathogenesis of COPD.[34] Corticosteroids reduce inflammation by recruiting HDAC2 to the activated NF-kB-stimulated inflammatory gene complex, thus silencing them. Reduction of HDAC2 activity in COPD may account for the corticosteroid insensitivity.[33]

Sirtuins are HDACs that are important in the regulation of acetylation of DNA. In mammals, the sirtuin family has been implicated in aging. Sirtuin 1 (SIRT1) is thought to be an "antiaging" molecule.[35] Deficiencies in SIRT1 are associated with increased oxidative stress, as well as COPD onset[36] and progression.[37] Lungs of patients with COPD have been shown to have decreased levels of SIRT1.[38]

Likewise, the large and small airways have decreased levels of SIRT1.[39] Exposure of monocyte-macrophages to cigarette smoke extract results in decreased SIRT1 and increased acetylation of nuclear factor-kappa B (NF-κB). NF-κB is the master regulator of inflammation and increased acetylation results in release of IL8 from the cells.[38]

DNA methylation may also play a role in the pathogenesis of COPD. There are variable changes in DNA methylation in peripheral leukocytes from patients with and without COPD. The majority of the genes that show differential methylation are hypomethylated in COPD patients. The gene segments affected includes several genes responsible for immune and inflammatory pathways. This is similar to the global hypomethylation seen in aging. DNA isolated from small airways of COPD patients have also been shown to have aberrant DNA methylation affecting hundreds of genes. This results in altered expression of genes and pathways involved in small airway remodeling, wound healing, and in mediating cellular response to polycyclic

aromatic hydrocarbon (a component of cigarette smoke) exposure.[40]

Loss of proteostasis

Proteostasis, or protein homeostasis, refers to the systems in place to help maintain functional proteins in the cell. This involves stabilization of correctly folded proteins and degradation of misfolded proteins by the proteasome or the lysosome. Two main systems exist to perform this task, chaperones such as heat shock proteins (HSP) and proteolytic systems such as the ubiquitin-proteasome and the lysosome-autophagy system. Both systems function to prevent accumulation of damaged proteins.

HSPs are strongly upregulated in aging to preserve proteostasis.[41] Genetically modified mice deficient in HSP demonstrates accelerated aging.[42] Function of the proteolytic systems also diminishes with aging.[43] Similar to aging, several HSPs are upregulated in COPD. Serum HSP27 and HSP70 have been shown to be elevated in COPD, compared to healthy smokers.[44] Airway epithelial cells also have higher levels of HSP70 in chronic bronchitis/COPD.[45] Sputum HSP70 and HSP90 have also been shown to be elevated in COPD.[46]

Cigarette smoking may be one of the early triggers for the dysregulation of proteostasis in COPD. Oxidative stress created by cigarette smoke induces protein damage and causes accumulation of misfolded proteins in the endoplasmic reticulum (ER) resulting in the ER stress response. The unfolded protein response (UPR) is a compensatory cellular response to ER stress. It attempts to restore homeostasis by decreasing protein synthesis, increasing production of chaperones, and enhancing degradation of misfolded proteins by the ubiquitin-proteasomal pathway. If UPR fails to resolve the stress, apoptosis may be initiated.[47] In addition to protein damage, cigarette smoke also reduces proteasomal activity in the alveolar epithelial cells,[48] resulting in poor clearance of damaged proteins. Prolonged accumulation of dysfunctional proteins eventually causes apoptosis and triggers chronic inflammation, possibly contributing to the pathogenesis of COPD.[49]

Aggresome bodies are formed when the protein degradation system of the cell is overwhelmed by misfolded and damaged proteins. Cigarette smoke-induced accumulation of aggresome bodies and ubiquinated proteins in human bronchial epithelial cells is demonstrated by numerous studies.[50,51] Vij *et al*. found a significant increase in the number of aggresome bodies in COPD patients as compared to nonsmoker controls. The level of aggresome bodies statistically correlated with the severity of COPD. They concluded that smoking exposure accelerated lung aging by impairing autophagy.[50]

Autophagy defects are also identified in the alveolar macrophages of smokers.[52] Decreased autophagy results in dysfunctional mitochondria and impaired bacterial lysis by the lysosomes, which in turn may be responsible for the repeated infections in smokers.[52]

Dysregulated nutrient sensing

Nutrient sensing is the cell's ability to recognize and respond to fuel sources such as glucose. A rapid, efficient response to fluctuations in nutrient levels is essential for cell survival. With aging, cells lose their ability to sense and respond to these changes. A central figure in nutrient sensing is the kinase, mammalian target of rapamycin (mTOR). It has the ability to sense a variety of essential nutrients and respond by altering cellular metabolism. In aging, mTOR is upregulated. Inhibition of mTOR by rapamycin is also shown to extend lifespan in mammals.[53]

Like aging, increased mTOR signaling is measured in the lungs of COPD patients as compared to controls. In addition, inhibiting mTOR *ex vivo* decreases cellular senescence in COPD.[54] Yoshida *et al*. demonstrate that rtp801, an mTOR inhibitor, is increased in the lungs of patients with COPD as compared to controls. Upregulation of rtp801 is associated with increased activation of NF-KB, resulting in increased inflammation, while mice with rtp801 deficiency were resistant to cigarette smoke-induced lung injury.[55]

Mitochondrial dysfunction

Mitochondrial dysfunction plays a role in aging, independent of increased production of reactive oxygen species (ROS).[56,57] As we age, mitochondria acquire mitochondrial DNA damage and have diminished the capacity to produce energy through oxidative phosphorylation.[58] In some cases, uncoupling of mitochondrial oxidative phosphorylation can lead to increased production of ROS and oxidative stress.

DNA damage of the mitochondria is associated with COPD. In normal cells, mitochondria have 2–10 copies of their genome.[59] Leukocytes from COPD patients are shown to have lower mitochondrial DNA copy numbers compared to healthy smokers and controls. This also correlates with a lower glutathione level.[60]

Airway smooth muscle (ASM) cells from COPD patients and smokers demonstrate mitochondrial dysfunction when compared to healthy controls. This mitochondrial dysfunction was related to increase mitochondrial ROS. Mitochondria-specific antioxidants such as MitoQ, directed against ROS, reduce the cytokine secretion and ASM proliferation.[61]

Bronchial epithelial cells in COPD patients demonstrate fragmented mitochondria. Cigarette smoke extract induced mitochondrial fragmentation and mitochondrial ROS production, both of which resulted in the acceleration of cellular senescence in human bronchial epithelial cells.[62] Abnormal mitochondrial function has also been seen in skeletal and respiratory muscles of COPD patients.[63]

Stem cell exhaustion

Telomere attrition, accumulation of DNA damage, and other types of aging-associated changes all result in stem cell exhaustion causing a decline in the regenerative potential of tissues. Many different types of progenitor populations are seen in the lungs. Basal cells found in the airway epithelium can self-renew and differentiate into ciliated and secretory cells.[64] Club cells in the bronchioles can give rise to ciliated and secretory cells; however, they do not contribute descendants to the alveoli.[65] In the distal lung, alveolar Type II epithelial cells can give rise to alveolar Type I cells and function as progenitor cells.[66]

Stem cell population size depends on an equilibrium between self-renewal and cell differentiation. When the rate of differentiation is greater than self-renewal, the population declines. Increased ROS forces the stem cell into replication.[67] Persistent oxidative stress in COPD may result in excessive differentiation of stem cells causing stem cell exhaustion.[68] Teixeira *et al.* demonstrate that the rate of progenitor cell replacement and loss is increased in the airway of smokers, most likely due to rapid turnover of smoker's airway.[69] Senescence has been demonstrated in the alveolar Type II cells.[70]

Circulating progenitor cells have also been implicated in COPD. Increased DNA damage and senescence were observed in endothelial progenitor cells isolated from peripheral blood, in smokers and COPD patients. These dysfunctional endothelial progenitors displayed impaired angiogenesis and increased apoptosis.[71]

Altered intercellular communication

Aging also leads to changes in intercellular communication including endocrine, neuroendocrine, and neuronal signaling. Inflammation is one way that cells communicate with each other. "Inflammaging" is an important aging-associated alteration in intercellular communication. Chronic low-grade inflammation associated with aging occurs due to an increased secretion of pro-inflammatory cytokines by senescent cells and progressive dysfunction of the immune system, which fails to clear pathogens and senescent cells.[72]

Chronic inflammation plays a key role in the pathogenesis of COPD. Cigarette smoking, the most important risk factor for COPD, is known to induce senescence in the alveolar epithelial cells.[21] These senescent cells release increased amounts of pro-inflammatory cytokines,[15] resulting in chronic inflammation in the lungs. COPD patients also have increased levels of circulatory inflammatory markers which may contribute to the extrapulmonary manifestations of the disease.[73]

A general decline in the function of various immune cells is seen with aging, which results in increased susceptibility to infection and cancer.[74] This decline in immune function is termed as "immunosenescence." COPD patients and smokers have suppressed innate immunity, which is like the immunosenescence phenotype associated with aging.[75] This suppression in the immune response results in the defective elimination of viruses and bacteria, a common cause of COPD exacerbations.

CONCLUSIONS

COPD and aging share many overlapping hallmarks of aging, which has led to the hypothesis that COPD is a disease of accelerated aging. Much more research is needed to help identify how the lung changes with normal aging and how that process is altered in COPD. COPD is projected to become the third leading cause of death worldwide in 2030.[1] This coupled with the fact that nearly 25% of the world's population in 2030 is projected to be over the age of 65[76] provides an urgency to better understand the intersection between aging and lung disease.

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