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REVIEW

Aging and induced senescence as factors in the pathogenesis of lung emphysema

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Summary
Classically, the development of emphysema in chronic obstructive pulmonary disease is believed to involve inflammation induced by cigarette smoke and leukocyte activation, including oxidant-antioxidant and protease-antiprotease imbalances. While there is substantial evidence for this, additional aspects have been suggested by a number of clinical and experimental observations.

Smokers exhibit signs of premature aging, particularly obvious in the skin. The link between aging and chronic disease is well-known, e.g., for the brain and musculoskeletal or cardiovascular system, as well as the clinical link between malnutrition and emphysema, and the experimental link to caloric restriction. Interestingly, this intervention also increases lifespan, in parallel with alterations in metabolism, oxidant burden and endocrine signaling.

Of special interest is the observation that, even in the absence of an inflammatory environment, lung fibroblasts from patients with emphysema show persistent alterations, possibly based

Abbreviations: α_1-AT, alpha-1-antitrypsin; Akt, serine-threonine-kinase, member of the protein kinase B (PKB) family; ALT, alternative mechanisms of telomere lengthening; Bad, Bcl-2-associated death promoter, member of the B-cell leukemia/lymphoma 2 (Bcl-2) family; Bax, member of the Bcl-2 family; CDK, cyclin-dependent kinase; COPD, chronic obstructive pulmonary disease; DHEAS, dehydroepiadrosterone sulphate; DNA, deoxyribonucleic acid; FOXO, forkhead box O; GM-CSF, granulocyte/macrophage-colony-stimulating factor; H_2O_2, hydrogen peroxide; HAT, histone acetyltransferase; HDAC, histone deacetylase; IL, interleukin; IGF-1, insulin-like growth factor 1; MMP, matrix metalloproteinase; PCNA, proliferating cell nuclear antigen; PI3K, phosphatidylinositol 3-kinase; pRb, retinoblastoma protein; PPAR-γ, peroxisome proliferator-activated receptor gamma; PTEN, phosphatase and tensin homolog, tyrosine and lipid phosphatase; RNA, ribonucleic acid; ROS, reactive oxygen species; SA-β-gal, senescence-associated β-galactosidase; SIPS, stress-induced proliferative senescence; Sir2, silent information regulator 2; SIRT1, homolog of Sir2 in mammals; TNF-α, tumor necrosis factor alpha; mTOR, mammalian target-of-rapamycin kinase; UV, ultraviolet.

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on epigenetic mechanisms. The importance of these mechanisms for cellular reprogramming and response patterns, individual risk profile and therapeutic options is becoming increasingly recognized. The same applies to cellular senescence. Recent findings from patients and experimental models open novel views into the arena of gene-environment interactions, including the role of systemic alterations, cellular stress, telomeres, CDK inhibitors such as p16, p21, pRb, PI3K, mTOR, FOXO transcription factors, histone modifications, and sirtuins.

This article aims to outline this emerging picture and to stimulate the identification of challenging questions. Such insights also bear implications for the long-term course of the disease in relation to existing or future therapies and the exploration of potential lung regeneration.

Contents

Introduction

Lung emphysema is a major phenotype of COPD[1] and represents a significant health burden. Even more so since no causal therapy is available to restore lung architecture. A formidable body of evidence has been accumulated regarding the role of inflammatory factors in the pathogenesis of the disease. It comprises the now classical concept of protease-antiprotease and oxidant-antioxidant imbalances.[2,3] Tissue destruction driven by neutrophils and macrophages via these compounds undoubtedly plays an important role.[4] This is supported by data for hereditary α_1-antitrypsin (AT)-deficiency, which typically leads to severe emphysema relatively early in life, particularly in the presence of noxious agents.[5] Moreover, instillation of elastase into the lung is a well-known technique for inducing experimental emphysema in animals.[6] Intimately linked to the protease-antiprotease disturbance, oxidative stress originating from compounds of cigarette smoke or inflammatory cells can overcharge the antioxidative capacity of pulmonary tissue and further diminish the antiprotease defense.[7]

In combination with proinflammatory cytokine production and a host of other responses, these influences lead to apoptosis, necrosis, compensatory proliferation and an imbalance in the maintenance of cells, ultimately resulting in alveolar destruction and airway remodeling. While the importance of inflammation is undeniable, clinical and cell biological observations suggest mechanisms beyond inflammation, albeit linked to it, to play a critical role as well (Fig. 1). These mechanisms include cellular senescence and epigenetic control, which appear of particular interest in view of the observed systemic alterations and chronicity of the disease.

The present article aims to provide the clinician with a comprehensive overview of the multiple facets presenting from the viewpoint of senescence and epigenetics, as well as to delineate major mechanistic aspects of this novel view. In order to truly appreciate the complexity of the molecular networks involved we refer to specialized reviews. A large number of missing links still need to be identified in this area, while the generation of clinically tractable research questions remains an exciting challenge.

Aging and senescence: basic characteristics

Biological aging involves a variety of cellular, molecular and structural alterations based on several mechanisms.[8] Although normally linked to chronological age, biological aging can occur earlier in life, being partially independent from an individual's chronological age (premature aging). Interestingly, many markers that are used to describe biological aging are related to (chronic) inflammation, e.g., the serum levels of IL-6, IL-1β or TNF-α.[9] As a result, there seems to be no single, comprehensive or easily available marker of biological age.

In this article, the term ''senescence'' is used to describe aging on the cellular level (cellular aging), which

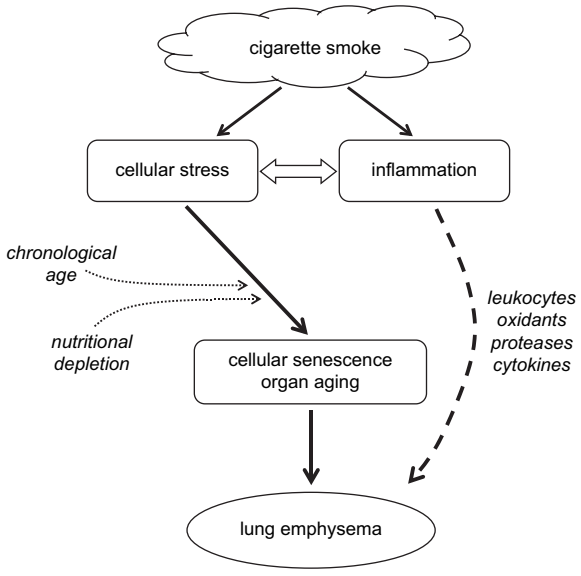

Figure 1 Diagram illustrating major factors of aging and senescence that are involved in the development of lung emphysema. The contribution originating as a more or less direct result of inflammation is summarized by the dashed line.

comprises a series of cell morphological and functional alterations including the loss of proliferative activity in otherwise viable cells (Table 1). This loss is observed *in vitro*[10,11] and is in all likelihood also of relevance *in vivo*.[12,13] To distinguish the cellular process from the aging of whole organs or organisms, it is denoted more specifically by ''proliferative/replicative senescence.'' The cell arrest not only occurs after exhausting the predetermined proliferative capacity (intrinsic senescence), but is also inducible by external stressors administered in sublethal doses (extrinsic or stress-induced proliferative senescence, SIPS).[14] Thus, there are different routes leading to a senescent phenotype, though potentially sharing common signaling pathways. It should be kept in mind that senescence can be interpreted as an evolutionary protective mechanism against tumor development[15] which is particularly relevant in pre-malignant cells.[16] It circumvents eventual irreversible structural losses due to apoptosis, while avoiding the risk associated with the generation of replacement cells from aged, potentially damaged progenitors. This survival strategy, however, may still come at the price of impaired organ function.

Table 1 Major molecular and cellular mechanisms associated with cellular senescence and aging

Mechanisms of aging
Telomere attrition[243]
Cumulative DNA damage[244]
Impairment of DNA repair[245]
Epimutations in nuclear DNA[246]
Mutations in mitochondrial DNA[112]
Increased rigidity of cytoskeleton[247]
Increased cross-linking of extracellular matrix[248]
Protein damage[249,250]
Increased production of free radicals[251]
Accumulation of waste products[252]

For each of the listed factors, a reference is given that provides either a comprehensive overview or an experimental example.

Aging as a contributor to chronic disease

Age represents a risk factor for the development of many diseases, including cardiovascular and metabolic disorders. Moreover, if a chronic disease is already established, it constitutes a factor contributing to mortality, e.g., in COPD and chronic hypercapnic respiratory failure.[17]

Aging comprises the accumulation of damage from exogenous causes as well as intrinsic, systemic susceptibilities determining the responses. These factors also determine its relation to the development of chronic diseases. Aging is known to be involved in neurodegenerative disorders[18] and cardiovascular diseases in which accelerated vascular aging and senescence of endothelial cells[19] seem to play a role through both telomere-dependent (see below) and -independent mechanisms.[20] Osteoarthritis[21] and the impact of aging on bone marrow-related therapies[22] are further examples. However, time-dependent losses might occur dissociated from age, as demonstrated for periodontal disease as a chronic, age-related disorder,[23] again emphasizing the concept of biological *versus* chronological age.

For a variety of diseases, mechanistic evidence already exists which indicates a link to (induced) aging. Moreover, patients with diabetic nephropathy showed signs of aging of skin fibroblasts.[24] In COPD, some of the comorbidities[25] can be considered as consequences of the lung disease, whereas others might be based on common susceptibility traits and linked to aging. COPD itself is a risk factor for other disorders including cardiovascular disease,[26] type II diabetes,[27] or cognitive and functional deteriorations,[28] all of which are age-related. Especially the association between arterial stiffness, osteoporosis and the severity of airflow obstruction[29] has provided supportive evidence on premature aging in COPD.[30] Moreover, reductions in lung function have been shown to be associated with systemic inflammation *per se* und thus potentially with aging, in addition to smoking.[31]

Aging and COPD

Structure and function of the human lung show a variety of alterations as part of the normal aging process (Table 2).[32,33] Of particular interest seems to be the rarification of alveolar structures that is known to occur in older never-smokers.[34] Although the structural changes of the senile lung[32] are considered to be nondestructive[35] and are rather homogeneous compared to the more focal alterations in emphysema, the overall result appears to be similar with regard to the loss of tissue renewal and regenerative potential. Additionally, it should be noted that diffuse (senile) emphysema is difficult to diagnose by lung function indices and its occurrence rate might therefore be underestimated.

Genetically modified mice have provided additional support for a relationship between aging and emphysema, while at the same time demonstrating differences between phenotypes of the disease, e.g., homogeneous *versus* focal alterations.[36] The

Table 2 Changes in structure and function of the human lung and respiratory system that occur with age

Age-related alterations of the lung
Rarification of alveolar architecture/enlargement of air spaces
Vascular remodeling
Altered composition of extracellular matrix
Reduced strength of respiratory muscles
Impaired respiratory mechanics/increased stiffness of chest wall
Reduction of lung function reserves (volumes, flows)
Heterogeneity of ventilation
Impaired gas exchange capacity

Detailed accounts of the functional aspects can be found in excellent review articles.[32,33]

parallel between both processes has been emphasized before,[37] in view of defects in vascular maintenance in patients with emphysema [38] and genetically modified rodents. When interpreting the findings, particularly animal data, it seems prudent to keep in mind that aging is a multi-faceted process involving many intimately interwoven factors (Table 1) and that it might not be evaluated from a single aspect.[37,39]

The major extrinsic factor in COPD is smoking, promoting alterations of tissue and organ architecture that resemble those of aging. Prominent manifestations are cardiovascular or cerebrovascular diseases[40] and premature skin aging[41,42] as compared to normal[41] or UV-induced aging.[43] Skin aging includes skin wrinkling[44] which, interestingly enough, has been reported to exhibit a weak but significant association with pulmonary emphysema.[45] It also involves an increased proportion of elastic fibers,[46,47] associated with lung function impairment.[48] Intriguingly, there are associations between COPD and periodontitis,[49] the latter also being a disease of connective tissue mediated by inflammation and promoted by smoking, as in COPD. The association between periodontitis and age *per se* is not particularly strong,[50] but this seems to be analogous to the discrete alterations occurring in the senile lung in the absence of noxious agents.

Cultured lung parenchyma fibroblasts from patients with emphysema also showed elevated expression of the senescence-associated β-galactosidase (SA-β-gal) compared to control smokers.[51] SA-β-gal, a common marker of cellular senescence,[52] is regularly expressed in senescent cells, though probably as an indicator of stress in general.[53] Corresponding to this finding, *in vitro* exposure of human cells to cigarette smoke extract led to increased expression of SA-β-gal,[54,55] similar to exposure of primary lung fibroblasts.[56] Available data suggest that cellular senescence is limited to lung fibroblasts and not present in skin fibroblasts of patients with lung emphysema,[57] which might underline the importance of local exposure levels.

In addition, proliferation rate and capacity, as major markers of senescence, were reduced in parenchymal lung fibroblasts of patients with emphysema compared to control smokers, although cells were grown under standardized conditions in the absence of inflammation.[51,58,59] While in these experiments the culture medium contained serum and thus a mixture of growth factors, the response to defined stimulation by TGF-β and IL-1β was also found to be altered in emphysema.[60] Fibroblasts also showed a dysregulation of decorin production,[61] a molecule involved in collagen assembly and related to aging.[62]

Mechanisms of cellular senescence and evidence in COPD

Telomere loss

For protective purposes, the ends of chromosomes carry noncoding DNA repeats called telomeres. In each DNA reduplication, about 35—100 base pairs are lost (end replication problem) implying a countdown mechanism and loss of replicative potential as telomeres shorten. Telomere shortening is a major determinant of cellular senescence, although currently it is not clear whether by the shortest telomere or mean telomere length.[63]

Many studies have demonstrated that telomere length of human skin fibroblasts[64] and blood leukocytes[65] decreases with age, with considerable variability inter- and intra-individually[66—68] and within cell populations.[69] The rate of telomere erosion can also differ between organs or cell populations, such as lymphocytes,[70] as well as between males and females.[67] Since telomere length appears to be a heritable trait,[71] it could be a key factor for the individual rate of aging and the disposition to develop age-related diseases.[72] Importantly, short telomeres can limit tissue renewal capacity[73] and thus are likely to affect the maintenance of organs. Based on this, telomeres rank among the most suitable markers of biological age, which integrate both intrinsic and extrinsic aging.

In addition to the end replication problem and sporadic telomere deletion, telomeres can be directly damaged by free radicals that target G-triplets[74] and induce single strand breaks. Apparently, oxidants enhance telomere loss primarily during mitosis[75]; it is not clear whether this also occurs in the absence of cell division.

Immortalized cells often, though not always, show an increase in the activity of telomerase, a ribonucleoprotein that can restore telomeres.[76,77] Alternative mechanisms of telomere lengthening (ALT) exist, and segments of telomeres can be copied from neighboring DNA strands.[77,78] Moreover, proteins involved in chromosome recombination were detected in telomerase-negative tumor cells, which obviously utilized this mechanism to gain unlimited replicatice capacity. Conversely, senescent fibroblasts often though not always show shortened telomeres.[79]

Regarding their association with disease, telomere length in blood leukocytes has been found to be related to disease activity or chronicity e.g., in kidney diseases[12] including chronic renal insufficiency.[80,81] Their dysfunction also seems to be a predisposing factor for renal cancer.[82] Telomere dysfunction may also affect the immune system[83] and thus have implications beyond a single organ. It is also closely linked to other mechanisms that control cellular aging (see below). As a result of premature aging induced by external factors, telomere length can be reduced in response to accumulated stress, as shown for blood leukocytes and the oxidative stress of smoking,[84] or chronic psychological stress.[85]

With specific regard to COPD, telomere length in alveolar type II cells and endothelial cells *in situ* has been found to differ between emphysema and control patients.[86] In contrast, cultured parenchymal lung fibroblasts from patients with emphysema did not show altered telomere lengths despite unequivocal signs of cellular senescence.[51] Thus, as a point calling for methodological caution, different mechanisms of senescence could be active in the majority of cells found in histological sections of lung parenchyma as opposed to cells obtained by outgrowth cultures from such samples. Hence, the contribution of local or cell-specific telomere shortening to senescence in emphysema is currently difficult to quantify. It is also unknown whether telomere shortening is directly due to cellular stress and to what extent it is a consequence of increased cellular turnover due to inflammatory processes.

Inflammation, role of proteases and oxidative stress, mitochondrial dysfunction

According to the common view, protease-antiprotease imbalances are involved in the development of COPD.[2,3,87] In line with this, smokers show upregulation of the fibroblast collagenase MMP-1 in the skin[88] which can mediate the degradation of interstitial collagen. In addition to the observations in patients, exposures of animals and cell cultures provided valuable insights. The upregulation of MMP-1 could be mimicked by *in vitro* exposure to cigarette smoke[89,90] or UV light, inducing a senescent cellular phenotype.[91] Cigarette smoke elicited further effects in cultured human lung fibroblasts, such as an increase in the activity of MMP-2,[92] as well as induction of cyclooxygenase-2 and microsomal prostaglandin E_2 synthase.[93] It also affected fibroblasts in terms of their ability to contract[94] and deteriorated epithelial cell repair capacity,[95] leading to the hypothesis that a disturbance in repair underlies the development of emphysema.[96] There is a close link to aging in which prostanoids[97,98] are involved, as well as MMPs which are implicated, e.g., in the age-related remodeling of vascular walls.[99] Age also implies a general decrease in the ability for tissue repair, as demonstrated, in liver regeneration for example.[100]

In addition to protease-antiprotease imbalance, oxidative stress originating from reactive oxygen species (ROS) is believed to drive chronic obstruction and emphysematous changes.[87,101] Oxidants arise from cigarette smoke and from inflammatory cells which might be additionally stimulated by recurrent respiratory tract infections.[102] From *in vitro* exposures, it is well established that oxidants such as H_2O_2 can induce proliferative senescence in fibroblasts,[103,104] which is, however, not necessarily driven by telomeres.[105] Parallel results have been obtained for cigarette smoke exposure, showing a reduction in proliferation rate or capacity as one requisite of cellular senescence.[106] This can be induced *in vitro* by continuous or repeated,[54] or even a single, temporary exposure of human primary lung fibroblasts.[56] Hence, there is evidence from various sources that cigarette smoke-induced effects observed *in vitro* or *in vivo* resemble those of aging. Moreover, cigarette smoke might exert parallel effects in different organs, possibly on the basis of an intrinsic susceptibility that differs between individuals, since only a minority of smokers develop clinically relevant emphysema.

ROS or ultraviolet (UV) radiation are known to particularly affect DNA integrity[107] and DNA damage signaling cascades.[108] They can also induce multiple other changes,[109] including cellular reprogramming and epigenetic mechanisms. For example, oxidative stress can induce cellular senescence via forkhead box O (FOXO) transcription factors and the deacetylase SIRT1, but the balance in this response can also be turned towards apoptosis[110] (see below). Notably, systemic inflammation and oxidant-antioxidant imbalance could favor changes in cellular phenotype throughout the organism, implying impaired maintenance in more than one organ.

Mitochrondrial dysfunction involves the production of ROS within the respiratory chain. These can directly damage proteins, RNA, and genomic or mitochondrial DNA,[111] which is generally considered an important contributor to aging.[112] Moreover, mitochondria exert indirect effects on cell survival, e.g., by mediating apoptosis. Though considered particularly important in neurodegenerative disorders, they are also probably involved in the aging of other organs, and accumulation of mutations in mitochondrial DNA leads to a decline in respiratory chain function.[113]

It is, however, not fully clear to what extent the loss of mitochondrial fidelity is causative for aging and to what extent it results from a decline in other functions. A causal contribution is suggested by the existence of mouse strains bearing mutations of mitochondrial DNA and showing the phenotype of premature aging. Currently, there seem to be no detailed data regarding mitochondrial dysfunction in COPD. Interestingly, *in vitro* exposure of lung epithelial cells to the supernatants from senescent lung fibroblasts reduced their protective capacity against mitochondrial dysfunction and increased ROS production.[114] This suggests that senescent cells can exert detrimental effects on other cells, a finding which is underscored, for example, by the observation that senescent fibroblasts can enhance the formation of tumors in mice.[115]

Major cell cycle regulators

The progress of the cell cycle is basically controlled via cyclins, cyclin-dependent kinases (CDK) and their inhibitors, as well-known from tumor biology. Senescent dermal fibroblasts, and even more so lung fibroblasts, exhibit increased expression of the CDK inhibitor $p16^{INK4a}$ and the effector protein pRb. During the normal cell cycle, $p16^{INK4a}$ seems to serve as a constant braking mechanism.[116] Prior to senescence, cells exhibit an increase in $p21^{Cip1/Waf1}$ expression, another important CDK inhibitor. This is controlled by the tumor suppressor and transcription factor p53, which plays a major role in the induction of cellular senescence (Fig. 2). When reaching senescence, $p21^{Cip1/Waf1}$ expression decreases, while that of $p16^{INK4a}$ increases. In accordance with this, primarily $p16^{INK4a}$ and its pathway are considered to be responsible for the final irreversible proliferation stop.[117,118]

Thus, $p21^{Cip1/Waf1}$ can initiate senescence — primarily telomere-dependent — which is then maintained and established by $p16^{INK4a}$. Additionally, pRb is involved in the

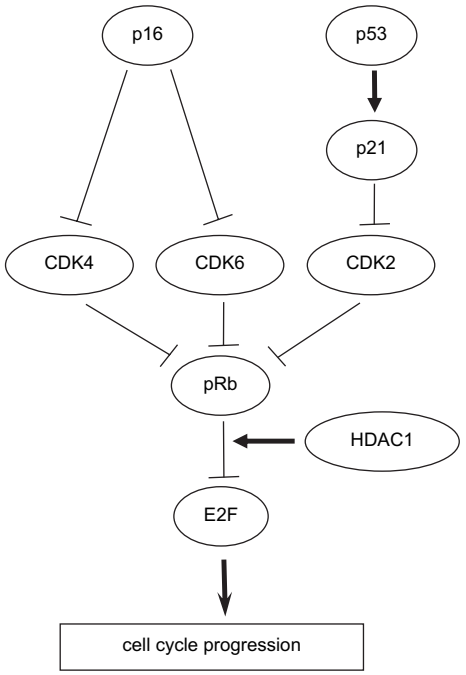

Figure 2 Links between major cell cycle regulators involved in cellular senescence, with emphasis on cyclin-dependent kinases (CDK) and their inhibitors such as p16 and p21. Details on the multiple relationships and an explanation of abbreviations can be found in the text.

control of genes responsible for cell cycle progression and other functions, by recruitment of the histone deacetylase (HDAC) 1[119] and HDAC complexes[120] (see below). This is apparently related to heterochromatin formation as an epigenetic mechanism that permanently suppresses crucial growth-promoting genes.[121] Conversely, suppression of $p16^{INK4a}$ expression can increase proliferative capacity, provided that cells contain functional $p16^{INK4a}$.[122] Further regulators of $p16^{INK4a}$, e.g., transcription factors,[122] can act directly on the $p16^{INK4a}$ promoter.

Another protein controlling mitotic activity and encoded by the gene locus *INK4a*, ARF ($p14^{ARF}$), can prevent p53 degradation and act as a link between diverse pathways of senescence. Accordingly, enhanced expression of $p16^{INK4a}$ and $p14^{ARF}$ was found in aging mammals including humans,[123] e.g., in pancreas islands, kidney, spleen, skin and lung.[121] While playing a central role in senescence induction, *INK4a* itself is subject to various control mechanisms, and there are links to metabolic status, since caloric restriction (see below) could, e.g., increase the expression of another CDK inhibitor, $p27^{kip1}$.[124] The involvement of $p16^{INK4a}$ and p53 in maintaining senescence is underscored by the observation that pre-malignant lesions show a loss of these and other senescence markers when turning into malignancy.[125]

CDK inhibitors might well be involved in the development of cellular senescence in emphysema. Evidence on this has been provided by the observation that endothelial cells and alveolar type II cells from patient lungs showed increased expression of $p16^{INK4a}$ and $p21^{Cip1/Waf1}$. Moreover, $p16^{INK4a}$ expression was opposite to that of the proliferation marker PCNA.[86] These markers were also linked to the impairment of lung function, as a cumulative result of destruction and remodeling. Similar evidence arose from exposures of mice *in vivo* and human cells *in vitro* to cigarette smoke causing increased expression of $p21^{Cip1/Waf1}$.[55] The expression of $p16^{INK4a}$ and $p21^{Cip1/Waf1}$ could also be increased by cigarette smoke extract in a human fibroblast cell line.[54] Such effects might, however, not be specific to cigarette smoke, since e.g., alveolar epithelial type II cells of rats exposed to bleomycin also exhibited signs of cellular senescence, e.g., $p21^{Cip1/Waf1}$ and SA-β-gal expression.[126] It might be of interest that induced cellular senescence, probably by reperfusion ischemia, can also affect the function of transplanted organs,[127,128] and increased expression of $p16^{INK4a}$ has been observed in transplanted kidneys.[129] Cellular senescence seems to be a part of many disorders, as underlined by the fact that bleomycin is established for the induction of fibrotic lesions but not emphysema.[130] It might be speculated that an overshooting injury repair after bleomycin originates from a subpopulation of resistant cells which lack control by the other, now senescent, cells.

This also underlines a further important issue in studying cellular senescence, namely the heterogeneity of cell populations.[131] Single cell analysis has revealed that cell populations exhibit a broad range of activities at a single time point. Critical decisions, such as between apoptosis and senescence, depend on a balance between opposing factors that is likely to result in stochastic behavior of cells within the population (see, e.g., the discussion on FOXOs below). Even more importantly, the known heterogeneity of pulmonary fibroblasts[132] is likely to imply different responses to environmental stress.[133] It is therefore not unexpected that emphysema, as lung fibrosis, often starts as a spatially heterogeneous disorder which only attains a more homogeneous pattern of damage in later stages.[134] Heterogeneity in early stages might also have implications for regenerative therapy by being linked to the reversibility of structural changes.

Role of PI3K and mTOR

Throughout a wide range of cell types, including pulmonary cells, phosphatidylinositol 3-kinase (PI3K) is one of the key regulators of survival and mitosis. PI3K has stimulated much interest, particularly in the understanding of tumor development. It controls longevity and robustness by phosphorylating the proapoptotic enzyme Bax.[135,136] Correspondingly, apoptosis is inducible via inhibition of the PI3K/Akt pathway,[137] which is mediated by FOXO type transcription factors (see below). A further level of control is exerted by the proapoptotic Bad,[138] a relative of Bax, which is influenced by cytokines such as GM-CSF and TNF-α. Interestingly, in lung epithelial cells PI3K could be activated by low concentrations of nicotine,[139] and the concomitant anti-apoptotic effect might be linked to malignant neoplasia. Importantly, PI3K is antagonized by the phosphatase PTEN, acting as a tumor suppressor.[140]

PI3K plays a central role in the increase in cell size that typically precedes mitosis, and in cell cycle initiation.[141] Among the various effects of PI3K[142] those mediating the combination of cellular hypertrophy and blocked mitotic activity appear to be especially relevant for senescent cells.[143] In the absence of mitogenic signals cellular growth

leads to cellular hypertrophy.[144] With regard to PI3K, the relation between senescence, aging and longevity is extremely complex and might depend on the species studied.[145] It is, however, clear that cell cycle arrest and a senescent phenotype can be induced by PI3K inihibitors.[146,147]

The Janus-faced position of PI3K in the control of mitosis is evident in the distinction between its anti-apoptotic action potentially entailing abnormal mitotic activity and tumorigenesis, and the induction of cellular senescence as characterized by abolished mitotic activity. Thus, switching of PI3K from metastable cellular states to opposing directions could also provide a link between senescence and tumorigenesis, particularly since cell populations are probably more heterogeneous in the disease rather than the healthy state.[131] This also appears interesting in view of the association between emphysema and lung cancer.[148]

Moreover, since PI3K is activated via the insulin receptor,[149] it offers a direct link to cell metabolism and nutrition which are known to be relevant factors in emphysema and COPD.[150] Interaction between the insulin pathway and PI3K or their homologs is essential for metabolic homeostasis.[151,152] PI3K also mediates mechanisms by which insulin adjusts the activity of FOXO transcription factors[153] (see below), thereby affecting senescence induction on many levels.[154]

A further important player in growth and cell cycle control is the kinase mTOR.[155] It is related to CDK inhibitors (see above), and its inhibition causes increased expression of $p16^{INK4a}$.[156] Of particular interest among its multiple functions seem to be its action on adipocyte differentiation via the transcription factor PPAR-γ[157] and its involvement in cellular stress responses including ROS.[158] These mechanisms could provide links between local lung disease and systemic alterations such as cachexia, a common phenomenon in emphysema.[159] Blocking of mTOR has been proposed as a therapeutic option to attenuate age-related malfunction,[160] but at present it is not clear whether this is feasible in emphysema, or whether interference with such a central enzyme results in significant negative side-effects.

Epigenetic mechanisms and senescence

Epigenetics is increasingly recognized as a key to the understanding of gene-environment interactions, including the ontogenetic and (partially) transgenerational memory of gene expression patterns. The impact of epigenetic mechanisms on the senescent phenotype and altered regulation of mitotic activity has not yet been illuminated in much detail, but current evidence suggests that this regulation might, at least in part, rely on such mechanisms.

In its proper sense, the term ''epigenetics'' designates heritable changes in gene expression without changes in DNA sequence. In a broader sense, it is often used to describe a variety of regulatory mechanisms, basically including histone modifications and DNA methylation irrespective of their degree of heritance. While DNA sequences are essentially identical in all cells of an individual, patterns of epigenetic modification occur in multiple variants, some of them short-lived, some of them long-lived, e.g., in the determination of cell type or in imprinting.[161] Much research is currently devoted to a detailed understanding of these modes of control and the corresponding epigenetic codes.[162]

There are different types and levels of epigenetic control. On its lowest level, DNA methylation represents a mechanism of gene expression regulation[163] that is thought to be particularly important in tumor cells characterized by virtually unlimited mitotic capacity. Correspondingly, in lung tumors suppression of $p16^{INK4a}$ expression (see above) via methylation of the promotor DNA is regularly found.[164] DNA methylation and histone modification are intimately intertwined.[165] For example, silencing of $p16^{INK4a}$ could be reversed through cooperative action between histone deacetylase (HDAC) inhibitors and inhibitors of DNA methylation,[166] and cell proliferation could be antagonized.[167]

DNA is arranged on nucleosomes in a form resembling a series of pearls on a bead. The building blocks of nucleosomes are histones, while the entire chromatin scaffold comprises many other proteins. Each nucleosome is an octamer assembled from histones H2A, H2B, H3 and H4. The DNA double-helix is wound around this histone core, and nucleosomes are connected by so-called ''linker DNA.'' The tails of the histones that protrude from the globular nucleosome can be altered by post-translational modifications, such as acetylation, methylation, phosphorylation, and their multiple combinations.[168] Their complex local state controls the binding of regulator proteins and the accessibility of DNA for transcription, probably being the key for short- and long-term gene regulation.[169]

For example, acetylation of lysine residues in histone tails generally facilitates the access to DNA, while deacetylation corresponds to a nonaccessible, silenced state. Specific enzymes mediate the different modifications, such as histone acetyltransferases (HAT) and HDACs which can partially control each other. According to the present view, histone acetylation represents a dynamic balance between HATs and HDACs, while it seems that certain methylations have the greatest potential for mediating persistent alterations.[170]

The involvement of histone modifications in induced senescence is currently not fully understood. Links are provided by the finding that the expression of $p16^{INK4a}$ and $p21^{Cip1/Waf1}$ is, at least partially, controlled through histone acetylation within promoter regions.[171,172] For example, increased $p21^{Cip1/Waf1}$ transcription through HDAC inhibition is linked to increased H3 acetylation in that region.[173–175] Such effects are thought to play a role in the anti-tumor effects of HDAC inhibitors, which are currently under investigation.[176]

In addition to nucleosomes, there are further levels of control, such as chromatin remodeling machines and complexes,[177] which can interact with HAT complexes to mediate gene expression.[178] This also seems to play a role in senescence by controlling both $p16^{INK4a}$ and $p21^{Cip1/Waf1}$ expression regardless of telomere shortening.[178,179] Regulation is also mediated by histone variants[180] substituting for other histones. All of these factors contribute to the partitioning between transcriptionally silent heterochromatin and transcriptionally competent, though not necessarily active, euchromatin. Silencing of DNA in cellular senescence can even be recognized macroscopically in terms of condensed, senescence-associated heterochromatic foci (SAHF) which comprise genes relevant for proliferation.[181]

HDAC inhibitors arrest the cell cycle not as a result of telomere shortening or uncapping. Their effect requires

functional p16^{INK4a}, but is hardly influenced by p53.[182] Possibly, stabilization of DNA damage signals underlies this arrest. The reason is that telomere uncapping occurs physiologically in every mitosis, where it might induce transient activation of DNA damage signals. These are controlled by histone acetylation and could be perpetuated in the presence of HDAC inhibitors. In accordance with this, cells often resume proliferation after removal of the HDAC inhibitor.

Since various agents including cigarette smoke can induce a persistent cell cycle arrest associated with increased acetylation, the high degree of reversibility of acetylation calls for further lock-like mechanisms. While *in vivo* persistence might be supported through chronic inflammation, this would not explain increased acetylation in cultured cells outside the inflammatory environment.[183] It is currently not clear according to which timeframes senescence-relevant modifications of histones take place and which of them survive the disassembly of nucleosomes upon DNA replication, thus being transmitted to daughter cells.

Irrespective of these uncertainties, the involvement of epigenetic memory mechanisms is strongly suggested by the persistently altered gene expression profiles of experimental animals exposed to cigarette smoke[184] as well as airway of epithelial cells of ex-smokers.[185] Correspondingly, elevated global acetylation of H3 has been detected in the lung epithelium of ex-smokers with COPD and of H4 in current smokers,[186] specifically covering the IL-8 promoter region in COPD. [187] The same is true for cultured lung fibroblasts of patients with emphysema.[183] These findings are supplemented by data from rats after cigarette smoke exposure[188] and from cultured human lung fibroblasts studied immediately after *in vitro* exposure to cigarette smoke extract,[183] although the acetylation had disappeared 2 days after exposure. At present, the observed patterns of histone modifications do not allow safe conclusions as to whether they represent an intermediate state and cellular senescence is maintained by other mechanisms, or whether they basically underlie the persistence of senescence.

Sirtuins and FOXOs as integrators of multiple pathways

Within the group of HDACs, sirtuins maintain a special position since they are structurally different from other HDACs and are inhibited by different compounds.[189] Sirtuins act on histones similarly to other HDACs thereby mediating gene silencing. Of particular interest in mammals is SIRT1 with its yeast homolog Sir2 that is known to deacetylate defined lysines in histones for gene silencing.[190,191] Importantly, SIRT1 also targets other proteins than histones, especially transcription factors. It can, for example, down-regulate p53-mediated senescence via deacetylation of p53.[192] Such effects seem relevant, since depletion of the pool of renewable cells by p53-mediated apoptosis and senescence could contribute to organismal aging.[193] Deactivation of p53 by Sir2 was also capable of raising cellular resistance to oxidative stress. However, cells without functional p53 still showed increased resistance after Sir2 activation, e.g., by resveratrol.[194,195] Obviously, there are several different mechanisms of stress response regulation controlled by sirtuins.

Sirtuin action depends on NAD^+ and is therefore coupled to the energy or redox state. This link might be relevant for emphysema, which is associated with metabolic imbalances. In addition to its supply function, NADH plays major regulatory roles. This is reflected in the fact that its energy is not needed for deacetylation, but probably spent for chromatin remodeling.[196] At present, the question remains open whether NAD^+ itself is decisive in the control of sirtuins, as studies have not detected appreciable changes in the NAD^+/NADH ratio in senescent cells or cells studied under various stress conditions.[191] Nicotinamide, a source of NAD^+ metabolism, is an inhibitor of Sir2, suggesting that possibly the overall put-through rate of NAD^+ processing is crucial.[189]

Among the most noteworthy recent findings appears to be the link between SIRT1 and FOXO transcription factors which are key players in the determination of cell fate. Depending on the type of activation, FOXOs can exert diverse, even opposite, effects including induction of cell cycle arrest, cell differentiation, removal of ROS, activation of DNA repair, and induction of apoptosis.[197,198] FOXOs are negatively regulated by PI3K (see above), which activates the kinase Akt that phosphorylates FOXOs. This is accompanied by the export of FOXOs from the nucleus and loss of their transcriptional activity.[199] SIRT1 can reduce FOXO3a and FOXO4 activity and their ability to induce apoptosis.[198] It intervenes with FOXO3a on different levels, either indirectly suppressing the induction of proapoptotic genes,[197] or directly by deacetylating histones at the promotor regions of FOXO3a target genes.[198] At the same time SIRT1 can inhibit growth through p27^{kip1} (see above) in connection with FOXO-induced stress responses, such as induction of manganese superoxide dismutase.[195] For this reason, SIRT1 may occupy a critical position in switching stress responses executed by FOXOs, ranging from upregulation of cellular defense through cell cycle arrest towards eventual apoptosis.[197] Pathways involving critical, possibly stochastic, decisions between divergent outcomes are of particular interest in COPD when considering the heterogeneity of the disease within the lung and between patients, as well as the systemic manifestations.

Recent data have indicated a role for SIRT1 in the regulation of inflammatory cells, in particular macrophages, when exposed to components of cigarette smoke[200] and SIRT1 activity in lung tissue has been found to be reduced in COPD and smokers.[201] Thus sirtuins, particularly SIRT1, are of great interest not only as general integrators of aging, longevity, stress responses, metabolic state, insulin signaling, and epigenetic mechanisms but also specifically due to their altered expression in COPD. Moreover, their regulation by specific compounds — e.g., resveratrol[202] — might provide an opportunity to move cells, and possibly organisms, into a state of higher stress resistance and increased lifespan. The multiple links also suggest that sirtuins may be effective candidates for mediating the relationship between lung disorder and systemic alterations, which are of major clinical importance.[203]

Links to energy metabolism/caloric restriction

The term ''caloric restriction'' designates reduced energy intake without malnutrition, a condition of great interest as

it is linked to longevity, aging and disease development or progression. At least in humans, however, the underlying molecular network is still far from being elucidated in sufficient detail to draw safe conclusions. Caloric restriction elicits multiple changes in terms of a regulated response of cellular metabolism and signaling. Among other effects, it leads to an increase in SIRT1 activity[204] and attenuates insulin or insulin-like growth factor (IGF-1) signaling, while promoting longevity in many species including probably primates.[205,206] These effects are intimately linked to the action of FOXO transcription factors, PI3K and Akt (see above, Fig. 3).[207] Conversely, enhanced insulin signaling is thought to be associated with aging.[208]

On the cellular level, caloric restriction is capable of antagonizing the loss of replicative capacity that occurs with increasing age both *in vivo* and *in vitro*.[209,210] Moreover, it can provide cellular protection by interfering with basic mechanisms involved in senescence, such as oxidative damage, telomere shortening, and changes in the hormone system.[211] However, the question, whether and under which conditions the effects of caloric restriction can enhance instead of attenuate those of cigarette smoke, has not yet been clarified.

Despite its ability to increase lifespan, caloric restriction has long been known to lead to alveolar rarification within a short time in rodents,[212,213] although it is not fully clear to what extent this is equivalent to diffuse lung emphysema.[213] Remarkably, the findings in animals are in agreement with the CT-morphological rarification of pulmonary tissue reported in young patients with *Anorexia nervosa*.[214,215] Despite unresolved issues regarding the interpretation of this observation,[216] it remains challenging, since it does not easily fit into the concept of the inflammatory origin of emphysema. Even more interesting is that the emphysema-like alterations induced by caloric restriction may disappear in mice within a short time after refeeding.[217]

The fact that caloric restriction can lead to reversible emphysema in rodents emphasizes the distinction between temporary, potentially reversible and chronic, in all likelihood, irreversible effects. The similarity — at least superficially — between pulmonary alterations in hunger and cigarette smoke-induced emphysema raises the question about shared *versus* divergent pathways. In humans, there are currently no comparative data on this; results from mice suggest that at least elastase- and hunger-induced emphysema bear different biochemical and functional characteristics.[218] Intriguingly, this difference parallels that between cellular quiescence, as a reversible state of cell arrest, and cellular senescence, understood as an irreversible state. One of the most challenging issues appears to be whether the quiescent state associated with enhanced stress resistance can be converted into senescence by an unfortunate combination of external factors including inflammation. Possibly, such conversion occurs in emphysema, thus turning a somewhat beneficial response into a deleterious one. In addition, it might be that the inevitable chronological aging facilitates the process, as indicated by the observation that clinically relevant emphysema is generally a disease of the elderly.

Although the progression of emphysema in humans seems to be accelerated under malnutrition,[219] the predictive power of body weight for mortality in severe COPD[17,220] primarily reflects the overall physical reserves. The same is true for fat-free mass,[221] a phenomenological measure of body composition[222,223] that is associated with lung function.[224] Cachexia is often viewed as a consequence of systemic processes including TNF-α-driven inflammation,[225] not as a factor which itself might promote lung destruction. In advanced disease, it is certainly extremely difficult to disentangle the threads corresponding to cause and effect. In this regard, it is, however, striking that smokers with slightly elevated body weight have been reported to exhibit a lower relative risk for developing COPD.[226] Energy depletion can be caused by starving or by wasting, and energy consumption might be raised in smokers, at least during light activity.[227] The evidence on a raised total energy expenditure in COPD is mixed, but the wasting of skeletal muscles and other tissues is likely to induce an imbalance between energy intake and need.[228] Sirtuins are more likely to be induced by starving than by wasting. Under caloric restriction, a reduction in cell cycle-inhibiting compounds could counteract an extrinsically induced senescence but

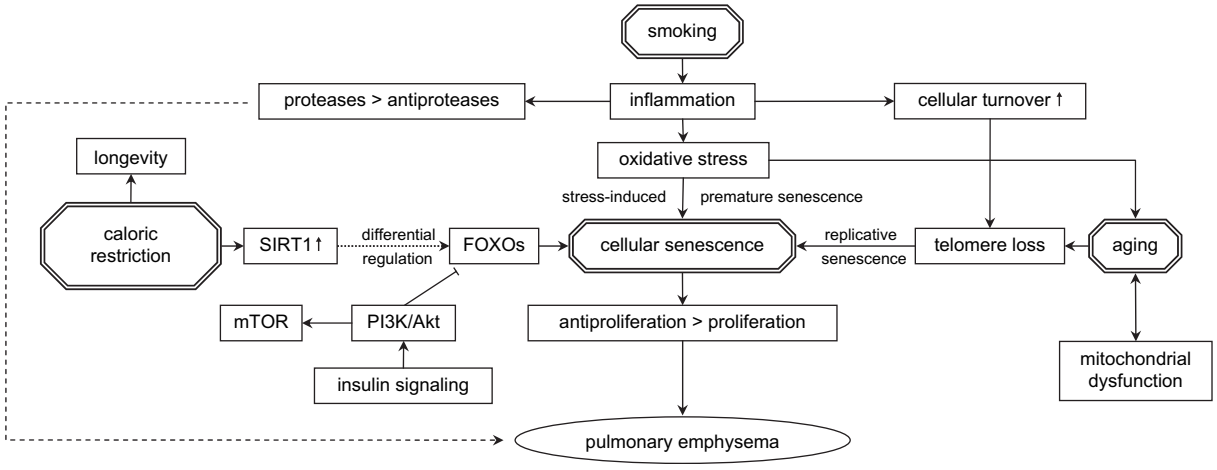

Figure 3 Diagram providing details on the links between important regulators of cellular senescence. Information on specific interactions and an explanation of abbreviations are given in the text.

this mechanism seems to be secondary in the lung,[123] which might therefore be especially susceptible to the effects of malnutrition.

Converse to caloric restriction, in overweight persons accelerated aging and shortened telomeres have been described,[229] potentially indicating an elevated rate of cell turnover. Among the factors linked to the metabolic (catabolic *versus* anabolic) state, especially in aging and disease, are dehydroepiadrosterone sulphate (DHEAS) and cortisol levels.[230,231] Particularly for DHEAS, multiple associations with longevity and age-related changes have been reported.[232,233] The interplay between nutrition, body weight, aging and disease risk also seems to be manifest in the serum level of leptin, a multipotent adipokine.[234] Adipokines are of interest with regard to their pleiotropic effects beyond a single organ, and it is meanwhile recognized that adipose tissue can influence lifespan and aging through cell non-autonomous regulation.[235] Noteworthy is that the differentiation and function of adipocytes is partially controlled by SIRT1,[236] and that adipocyte performance might be hampered by cigarette smoke.[237]

Conclusion and outlook

In recent years, aging and cellular senescence have come into focus as contributors to disease development and organ dysfunction that could provide considerable insight into the processes involved. The parallel between clinical signs of aging in smokers or patients with emphysema and findings on the cellular and biochemical level is striking, although the database is still limited. Animal experiments and *in vitro* exposure of human cells, including cells from the lung, have provided evidence that cigarette smoke compounds are capable of exerting effects that bear the signature of cellular senescence. Corresponding findings are available from immunohistological examinations of samples obtained from patients with emphysema, or from the analysis of lung cells taken in culture. The altered expression of key cell cycle regulators and mediators of growth arrest or proliferative responses, and the reduction in proliferative capacity indicate multi-faceted changes in the maintenance of cell and organ integrity. It is important to acknowledge that the notion of cellular senescence covers a multitude of causes and consequences, requiring a careful analysis in each individual condition.

Although it is impossible to draw a coherent picture at present, the multiple links between aging, longevity, stress responses, metabolic state, insulin signaling, sirtuins and epigenetic mechanisms render it likely that senescence based on these factors adds to, if not even underlies the pathogenesis of emphysema. Owing to its integrative capacity, the concept of senescence will substantially improve the understanding of the development and progression of this chronic disease. This is especially true since there seems to be a close link between senescence and epigenetic mechanisms. Such mechanisms are, among others, also of eminent importance in lung cancer, whose risk is associated with COPD. It is also worth considering the potential interference between cellular senescence and the action of pharmacological compounds. This should be one of the keys for unravelling their impact on long-term structural changes in the lung. Recent work, for example, on the anti-inflammatory efficacy of corticosteroids in relation to gene expression control by histone acetylation[238] has the potential to be extended in the direction of senescence and aging. This fascinating perspective is reinforced by the fact that multipotent molecular players are involved, providing a link between environment, individual disposition, local lung disease, and systemic alterations.

These factors might also bear implications for the regenerative biology of the lung. Whether targeted interference with cell cycle and differentiation control, either by activation of resident cells or by indirect effects of stem cells, is a therapeutic option in lung emphysema remains a topic for future investigations, particularly since additional morphogenetic guidance is likely to be needed. Interventions involving epigenetic, senescence-related mechanisms might at least be an option to help slow down the disease progression, and sirtuins are particularly interesting in view of their broad networking abilities. If regenerative therapies would be feasible in future, it is likely that the (partial) reconstitution of lung architecture has to deal not only with unfavorable inflammatory, structural and mechanical[239] conditions in the diseased organ, but also with a potential inherent resistance of the resident cells to support reconstitution. Some of these limitations might be of evolutionary origin and manifested as genetic module-dependent ontogenetic constraints, and some might be due to (induced) cellular senescence. That regeneration in terms of recallable complex morphogenetic programs, in contrast to simple growth, is not entirely switched off in humans, is illustrated by the possibility of nearly complete restoration of fingertips in children, which also provides valuable insight into the contrast between wound healing and regeneration.[240] It is noteworthy that some species exhibit impressive persistent abilities of morphogenesis throughout much of their life, as demonstrated by the growth of antlers in deer, or of limbs[241] and even complete lung lobes in newts.[242] In this regard, it seems sensible to perform detailed comparisons between the morphogenetic programs and regulatory modules across species of different evolutionary position. This could provide crucial information for the induction of regeneration *in situ* in humans and for the (extracorporal) generation of replacement organs, as well as for counteracting the constraints caused by cellular senescence.

Conflict of interest

The authors declare that none of them has any conflict of interest related to the article or the research described.

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