The role of accelerated ageing in aberrant lung tissue repair and remodelling in COPD

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DOI:
10.33612/diss.155044507

CHAPTER 2

Lung ageing and COPD: is there a role for ageing in abnormal tissue repair?

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Eur Respir Rev. 2017 Dec 6;26(146):170073.

ABSTRACT

COPD is the fourth leading cause of death worldwide with increasing prevalence, in particular in the elderly. COPD is characterized by abnormal tissue repair resulting in (small) airways disease and emphysema. There is accumulating evidence that ageing hallmarks are prominent features of COPD. These ageing hallmarks have been described in different subsets of COPD patients, in different lung compartments, and also in a variety of cell types, and thus might contribute to different COPD phenotypes. A better understanding of the main differences and similarities between normal lung ageing and the pathology of COPD may improve our understanding of the mechanisms driving COPD pathology, in particular in those patients that develop the most severe form of COPD at a relatively young age, i.e. severe early onset COPD patients. In this review, after introducing the main concepts of lung ageing and COPD pathology, we focus on the role of (abnormal) ageing in lung remodelling and repair in COPD. We discuss the current evidence for the involvement of ageing hallmarks in these pathologic features of COPD. In the last part, we highlight potential novel treatment strategies and opportunities for future research based on our current knowledge of abnormal lung ageing in COPD.

What is ageing?

As the world indicates, ageing is a process that mainly affects elderly people. With the quickly growing elderly population, the negative aspects of ageing are becoming increasingly apparent. Ageing is defined as the progressive decline in homeostasis after the reproductive phase is complete, which results in increased risk of disease or death (1). As such, ageing is one of the main driving forces of the development and increasing burden of non-communicable diseases (NCDs), i.e. chronic diseases. Worldwide, NCDs are the leading cause of mortality and responsible for 38 million deaths each year. Of these deaths, 4 million can be attributed to respiratory diseases (2). In many of the NCDs including ischaemic heart disease, diabetes, Alzheimer's disease and chronic obstructive pulmonary disease (COPD), it is proposed that acceleration of the normal ageing process is involved in the disease pathogenesis (3).

In this review we will first describe the processes involved in the normal ageing lung and the disease pathology of COPD and then summarize the similarities and the differences. We will specifically focus on the role of abnormal ageing in lung remodelling and repair in COPD and discuss the current evidence for ageing hallmarks in the pathologic features of COPD. Finally, we will discuss potential novel treatment strategies based on the current evidence for lung ageing in COPD.

The ageing lung

On average, the human lung is growing until 10-12 years of age and further matures until it reaches its maximum function at approximately 20 years of age for females and 25 years of age for males (4). From then on lung function progressively declines with increasing age as a consequence of structural and physiological changes of the lung (4).

To start with the structural changes of the ageing lung, we can broadly divide these structural changes in three categories: changes in lung structure, changes in the chest wall and changes in respiratory muscles (5). The changes in the structure of the lung are mainly attributed to an increase in the size of the alveolar space without any inflammation or alveolar wall destruction, so called 'senile emphysema'. This microscopic emphysema increases in a linear fashion with age in non-smokers whereas when smoking a progressive increase in alveolar space size can be observed in specific (susceptible) individuals only (6-8). Senile emphysema might be a consequence of loss of the supporting structure of the lung parenchyma (4,5). Additionally, it has been observed that the elastic recoil of the lung reduces with increasing age. It has been postulated that this phenomenon is rather caused by reduced surface tension forces from the alveoli due to increased individual diameter size than by changes in elastin and collagen in the lung parenchyma (5). Upon increasing age, the compliance of the chest wall decreases progressively, which can be explained by several, synergistically acting, age-related processes. Firstly, the shape of the thorax may change with age due to reduced thickness of intervertebral discs, leading to reduced intra thoracic volume. Secondly, age-associated osteoporosis may cause vertebral fractures resulting in changes in the shape of the thorax. Thirdly, the stiffness of the ribs increases with age, thereby enhancing the forces needed for movement of the chest (5,9). In general, muscle

strength diminishes with age. This loss in muscle strength is also reflected in the diaphragm, the most important respiratory muscle, and thus affects the breathing pattern (5). These structural changes of the ageing lung have a clear effect on the overall lung function and several physiologically parameters are altered upon ageing. Both the forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) are decreasing with age and the rate of decline has shown to be higher for males than for females. As a consequence of the reduced elastic recoil and compliance of the chest wall, the residual volume (RV) increases, while the vital capacity (VC) decreases (9). Of interest, the total lung capacity (TLC) does not change with increasing age, since the reduction in elastic recoil observed upon ageing is counterbalanced by the decrease in chest wall compliance and muscle strength of the chest (5,9). Although the distribution of alveolar ventilation and perfusion across the lungs is very heterogeneous as a consequence of the decline in alveolar surface area, density of lung capillaries and pulmonary capillary blood volume, the overall transfer capacity of the lung for carbon monoxide (TLCO) is reduced with increasing age. Clinically, this might influence the physical activity and the development of sleep-discorded breathing (5,9).

Next to changes in lung function with increasing age, the natural defence mechanisms of the lungs are also gradually less functional, leading to increased infection risk (4). For example, the antioxidant response to prohibit the accumulation of reactive oxygen species (ROS) is deteriorated in the ageing lung, consistent with an increase in ROS levels upon ageing (10). Furthermore, intercellular communications become less effective with ageing (11) contributing to two phenomena known as immunosenescence and inflammageing. The first relates to dampened immune responses following an infection or injury, and the second term relates to the chronic activation of immune responses in aged subjects in the absence of a real immunologic challenge (12). As a result of immunosenescence, innate and adaptive immune responses decrease with age, which is characterized by an increase in memory and effector cells at the expense of naïve T cells and the overall T cell repertoire (13,14). Of interest, several of the pro-inflammatory mediators associated with inflammageing, like tumour necrosis factor (TN)-α, interleukin (IL)-1β and IL-6, are present as pro-inflammatory mediators in the senescence-associated secretory phenotype (SASP). Another factor contributing to increased inflammation in aged lungs is poor airway clearance of particles. Over time, muscles become atrophic, resulting in less strength for effective cough (15). Also, mucociliary clearance is known to be compromised with age (16), which might in particular contribute to viral and bacterial inflammation and thus acute exacerbations of lung diseases like COPD.

Pathology of COPD

COPD is a heterogeneous disease involving both the alveolar and airway compartment resulting in (small) airways disease and emphysema (Figure 1). The extent of pathologic changes in these different lung compartments is however variable in individual patients (17). The aetiology involves in general exposure to external noxious particles or gases. In the Western world this is in particular by (cigarette) smoking, and in the non-Western world mainly by indoor cooking. COPD pathology is driven by chronic inflammation (18-20), which

is still observed after stopping smoking (21) even after one or more years (22,23). The combination of the exposure and inflammation leads to lung tissue damage resulting in remodelling of the lung. This remodelling shows remarkable features: a common main aetiology, smoke exposure, leads to fibrosis (extracellular matrix increase) with thickening of (large and in a particular small) airway walls with lumen reduction and concurrently to emphysema with ECM destruction in the lung parenchyma. (19,24). Another main histopathologic feature of COPD is seen in the vasculature with in particular increased thickness of the arterioles, resulting in pulmonary hypertension as an important complication of COPD (25).

The chronic inflammation is mainly characterized by macrophages and ($CD8^+$) T cells and can also show increased plasma cells, neutrophilic granulocytes and sometimes eosinophilic granulocytes (26-28). B-cells are also found, often in aggregates or small primary or secondary follicles (29-31). These have been described in association with airways as tertiary follicles, or reactive bronchus associated lymphoid tissue (BALT), but also have been observed scattered in the parenchyma (31,32). The presence of such follicles is variable, most pronounced in patients with severe COPD (30,32), and not only seen in COPD patients but also, to a lesser extent, in heavy smokers without COPD (31). As oligoclonality of these follicles has been shown (31). It is most likely that these are induced by local antigen stimulation (33-35). Cigarette smoke components, micro-organisms and matrix components (36) have been considered as etiologic factors for this antigenic stimulation, but none have been convincingly shown yet (34,37).

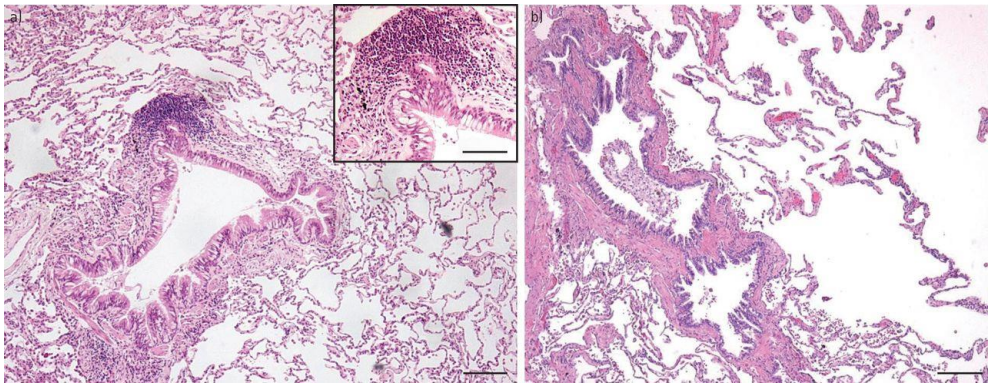

Figure 1: Pathologic changes in COPD. A) *Characteristic picture of airways changes, with increase of goblet cells, a thickened airway wall with some adventitial inflammation and a small lymphoid follicle at the left upper side. Emphysema is hardly present here (haematoxylin and eosin, x200).Insert shows the magnification of part of the airway with a lymphoid follicle.* ***B)*** *At the left almost longitudinal cross-section of a small airway, while at the right side severe parenchymal destruction by emphysema (haematoxylin and eosin, x200).*

In the large and small airways, epithelial changes are observed with increase of goblet cells, basal cell hyperplasia and squamous metaplasia, sometimes with dysplasia (20,38-40). Overall, these changes are in general more pronounced in the large airways, where the primary and most intensive exposure takes place. In the airway lumen increased and more sticky mucus can be present, which is produced by the increased number of goblet cells in combination with the enlarged mucus glands. The airway walls are thickened, caused by increased inflammation, increase of smooth muscle mass, increase in size of mucus glands, and, in due time, further changes with increase of extracellular matrix (ECM). The matrix changes in the airways are in general of a fibrotic nature with increase of collagens in the submucosa but also in the adventitia of smaller airways (41-44). Similar to asthma, a thickening of the basement membrane is seen, although more irregular and of different composition (43). An interesting finding was that in the peribronchial area of small airways in severe COPD an impressive reduction was found for proteoglycans, most prominent for decorin and to a lesser extent biglycan (45,46). A main characteristic of decorin is that it can bind TGF-β, one of the main cytokines regulating matrix production, which is consistently upregulated in COPD (47,48). Another important feature is that decorin is the main proteoglycan connecting collagen fibrils, in this way regulating rigidness of the collagen (48,49). As decorin is reduced in COPD, this will result in a very loose type of fibrosis, contributing to increased airway collapsibility and reduced peribronchial tensile strength of the parenchymal attachments (19,24). An interesting observation by Hogg *et al.* in a limited number of patients was a reduction in the number of small airways with increasing emphysema severity within COPD lungs suggesting that part of the airflow limitation within COPD is primarily caused by destruction of small airways (50,51).

Emphysema is the characteristic pathology of COPD that occurs in the lung parenchyma. This is characterized by net destruction of alveolar walls, as a result from increased destruction in combination with failing tissue repair (19). The destructive part is caused by an unbalance between exposure to oxygen radicals from cigarette smoke and neutrophils and proteases from macrophages and neutrophils, and their counterparts, oxygen scavengers and anti-proteases (52). Also in the parenchyma inflammation is present, but far less research has been published with respect to this compartment (53,54). Similar to the airways, cytotoxic (CD8+) T cells are important infiltrating cells in alveolar septa and arterioles in COPD when compared to non-COPD controls (53). Whereas neutrophils were and are considered as a main inflammatory cell contributing to emphysema, already in early studies in smokers no association was found between parenchymal neutrophils and the severity of the destructive index (55). In addition to possible direct effects of both smoke components and proteases released from inflammatory cells, indirect effects have been shown to destroy alveolar walls in COPD by inducing apoptosis of endothelial and alveolar type 2 epithelial cells, likely contributing to emphysema (56,57).

The important vascular changes in COPD are mainly seen in the arterioles. Here, intimal thickening with smooth muscle proliferation and increase of collagen and elastin, together with hyperplastic increase of the media have been observed (25,58-61). Initially

this was thought to be the result of hypoxia, but these events are also present in mild and early cases (60). More recently, the vascular changes have been attributed to "endothelial dysfunction" i.e. pathophysiological changes in the normal biochemical function of the endothelium (25,61,62). The end result are arterioles with a thickened wall, increased contraction and reduced lumen, but also with the reduced ability to vasodilate. Functionally, this leads to pulmonary hypertension which is a major cause of morbidity in COPD and a predictor of mortality (25,61).

Similarities and differences between lung ageing and COPD

One of the first reports on senile lungs compared with normal and emphysematous lungs by Verbeken *et al.* (63,64) demonstrated that the airspace enlargement in ageing, although comparable with smoking induced emphysema, differed in the fact that it was more regular in distribution without clear-cut destruction. Furthermore, increased thickening of alveolar septa was observed without inflammation or fibrosis with reduced density of the membranous bronchioles. They proposed the term senile lung for this condition. So, although similar in enlargement of airspaces at least part of the pathogenesis appears to be different. For loss of elasticity it is less clear whether this is a destructive effect in COPD or whether in both conditions there is an underlying defect in elastin fibrillogenesis. The functional effects on small airways in milder forms of emphysema are however comparable with the senile lung with loss of elastic recoil (64).

In the ageing process, Ito and Barnes (1) proposed that with increasing age the lung is less able to maintain organ integrity and protect itself against oxidative injury. Also, Kirkwood (65,66) indicated that cellular defects often cause inflammatory reactions contributing to damage, thereby causing a vicious circle of ongoing microscopic damage in due time with ageing. As yet, it is not readily clear to what extent these events are present during the total life course and when effects on tissue homeostasis become effective. In addition, it is not clear what the variation in the natural course of these events is with regard to their contribution to deterioration of the normal ageing lung. Taking the above mechanisms into account, several components observed in COPD, like the ongoing inflammation, unbalanced oxidative stress, and changes in the ECM are quite comparable as observed in the normal ageing lung. However in COPD, these changes will occur in general at an earlier age and to a larger extent compared to normal lung ageing. In the paragraphs below we will discuss in more detail whether premature or abnormal (lung) ageing aspects may or may not play a role in pathogenesis and natural course of COPD.

Lung ageing and COPD phenotypes

As described above, COPD is a very heterogeneous lung disease presented by different (mixed) phenotypes. Well-known phenotypes in COPD are chronic bronchitis with predominant airway related changes (inflammation and airway wall thickening) and increased mucus production, and emphysema with (severe) alveolar wall destruction, hyperinflation and impaired gas exchange. Other phenotypes of COPD are related to the number of exacerbations (i.e. the frequent exacerbator) (67,68) or the age of onset of the

disease, i.e. severe early onset COPD (SEO-COPD) (69). Given the difference in underlying pathology of these phenotypes, it can be envisaged that lung ageing is more or differently involved in some of these phenotypes than others. As discussed above, senile emphysema is an important hallmark of lung ageing and together with the structural changes, the ageing lung is in particular inclined to develop an emphysema-like phenotype. This is different from the bronchitis phenotype, where, apart from increased inflammation, there are very little similarities with the ageing lung and there is no indication of increased mucus production or airway wall thickening in the ageing lung, although decreased ciliary function with ageing likely contributes to increased coughing and decreased mucociliary clearance (15). The frequent exacerbator is an interesting phenotype as exacerbations are in general linked to infections and the susceptibility for infections increases with age (70,71). Moreover, age is a risk factor for COPD exacerbations (72) and hospital admissions for acute exacerbations of COPD (73).

Severe early-onset COPD is an interesting COPD phenotype with respect to ageing. Patients with this phenotype develop very severe COPD at a relatively young age, i.e. <53 according to Silverman *et al.* (69) and often with a relatively low number of pack years of smoking. This severe early-onset COPD (SEO-COPD) leads to a high personal burden and huge societal costs due to loss in working days and frequent hospitalizations. As these patients progress so quickly, we propose that, if accelerated ageing is an important contributor to COPD pathology, it should be most clear in these SEO-COPD patients. With respect to the pathology these patients are characterized by severe emphysema (69).

Ageing hallmarks in COPD

The main hallmarks of ageing were recently summarized in a review by Lopez-Orin (11) and this was followed by an overview of these hallmarks in lung ageing and lung disease (74). Broadly, the ageing hallmarks can be divided in processes affecting transcription (genomic instability, telomere attrition and epigenetic alterations), processes affecting the metabolism (loss of proteostasis, deregulated nutrient sensing and mitochondrial dysfunction) and cellular processes (cellular senescence, stem cell exhaustion and altered intracellular communication). We will now discuss the current knowledge about the possible role for these ageing hallmarks in COPD and mainly focus on findings in structural cells (alveolar and bronchial epithelial cells, smooth muscle cells and fibroblasts) and lung tissue. Subsequently, we will summarize the main evidence regarding the role of ageing hallmarks in disturbed repair and remodelling in COPD. All findings discussed in the paragraphs below are summarized in Table 1.

Transcription

Genomic instability

Ageing leads to increased DNA damage and to impaired ability to prevent and repair DNA damage. Several markers related to these features have also been demonstrated in COPD lungs and may contribute to the pathologic processes.

The DNA damage marker gamma- H2A histone family member X (ɣ-H2A.X) was increased in alveolar walls, including type I and type II epithelial cells and endothelial cells (75), as well as in small airways of COPD patients compared to controls (75,76). Another study, however, showed no differences in small airways in COPD versus control (77).

Smoke exposure increases ɣ-H2A.X levels in experimental animal models and cigarette smoke extract (CSE) treatment increases ɣ-H2A.X levels in bronchial epithelial cells and fibroblasts *in vitro* (76,78,79), suggesting an important role for oxidative stress. In addition, the anti-ageing protein sirtuin 6 (SIRT6) is considered to be protective against DNA damage and senescence. SIRT6 levels are decreased in lung tissue homogenates from COPD patients and overexpression and knockdown of SIRT6 in bronchial epithelial cells resulted in a decrease and an increase in ɣH2A.X levels, respectively (79).

The DNA repair marker Ku86 was decreased in parenchymal lung tissue of COPD patients, including small airways (77,80), while no differences were observed in Ku70 expression in these samples. Ku70 is another DNA repair marker which was decreased in leukocytes derived from COPD patients and its expression was negatively correlated with age (80).

Table 1: Evidence for ageing hallmarks in COPD

Hallmark	Marker	COPD vs non-COPD	COPD cell origin	CSE treated cells	References
Genomic instability	γ-H2A.X	↑	Lung tissue sections, AT1, AT2, HBEC & PV-EC	HBEC, HFL1 & MRC-5	(75,76,78,79)
	Ku70	↓	Peripheral leukocytes		(80)
	Ku80	↓	Lung homogenates		(77)
Telomere shortening	length	↓	Lung homogenates AT2, PA-SMC, PV-EC & peripheral leukocytes	SAEC (COPD) & HFL1	(78,81-86)
	telomerase	↓	PV-EC		(84)
	TPP1	↓	Lung homogenates	SAEC & HFL1	(78)
Epigenetic changes	HDAC activity	↓	Lung homogenates and bronchial biopsies		(92)
	SIRT-1 & -6	↓	Lung homogenates		(94,95)
Loss of proteostasis	Autophagy	↑	HBEC	SAEC	(100)
	Autophagy	↓		HBEC (COPD)	(100)
	Autophagosomes	↑	Lung homogenates	HBEC & BEAS-2B	(79,99,100)
	Ubiquitin	↑	Lung homogenates		(98,100)
	p62	↑	Lung homogenates		(79,100)
Deregulated nutrient sensing	S6K (mTOR)	↑*	Lung homogenates & peripheral leukocytes	HBEC	(79,103)
	IGF1	↑	SAEC		(104)
Mitochondrial dysfunction	ROS	↑		HBEC, BEAS-2B & MRC-5	(76,108,109)
	Ox-DNA	↑	Lung homogenates		(77)
	lipid peroxidation	↑	Lung homogenates		(105,106)
	NO	↑	Lung homogenates		(106)
	mitophagy	↑	Lung homogenates	HBEC & BEAS-2B	(108,109)
	Antioxidant	↓	Lung homogenates & HBEC		(107)
	Mitochondrial membrane potential	↓		BEAS-2B	(108)
Immune dysregulation	Klotho	↓	Lung homogenates	HBEC	(112)
	NF-κB	↑	Lung homogenates		(113)
	pro-inflammatory cytokines	↑	Lung homogenates		(113)
Senescence	SA-β-gal	↑	SAEC, PA-SMC, PV-EC & fibroblasts	SAEC (COPD), HBEC, A549, HFL1 & MRC-5	(76,78,79,84-86,115-117)
	p16	↑	Lung tissue sections, AT1, AT2, PA-SMC, PV-EC & fibroblasts	HFL1	(75,76,78,84-86,116,117)
	p21	↑	Lung homogenates, AT2, PA-SMC, PV-EC & peripheral leukocytes	HBEC, A549 & HFL1	(78-80,84-86,117)
	IL-6 & IL-8	↑	Lung tissue sections, AT1, AT2, PA-SMC & PV-EC	MRC-5	(75,76,84,85)
ECM dysregulation	ECM proteins	↑	Lung homogenates		(42)
	Elastogenesis genes	↑	Lung homogenates		(125)
	MMP/TIMP dysregulation	↑	Lung homogenates		(123)
Stem cell exhaustion	Circulating progenitor cells	↓*	Endothelial and haemopoietic progenitor cells		(137,138)
	Regenerative capacity	↓	Basal progenitor cells		(135)
	Stem cell function	↓	HBEC		(142)
	WNT signalling	↓*	Lung homogenates, AT2 & SAEC		(144,147)
	Notch pathway	↓*	SAEC		(143)

CSE: cigarette smoke extract; AT1: type I alveolar cells; AT2: type II alveolar cells; HBEC: human bronchial epithelial cells; PV-EC: pulmonary vascular endothelial cells; HFL1: foetal lung fibroblasts; MRC-5: foetal lung fibroblasts; TPP1: telomere protection protein 1; PA-SMC: pulmonary artery smooth muscle cells; SAEC: small airway epithelial cells; HDAC: histone deacetylase; SIRT: sirtuin; BEAS-2B: bronchial epithelial cell line (virus); mTOR: mechanistic target of rapamycin; IGF1: insulin-like growth factor 1; ROS: reactive oxygen species; SA-β-gal: senescence-associated-β-galactosidase; IL: interleukin; A549: alveolar basal epithelial cell line (carcinoma); ECM: extracellular matrix; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of metalloproteinase. #: mean age was significantly different between COPD and control group; for the other studies, information was not available or mean age was not different between groups.

Telomere shortening

Telomere shortening is an important inducer of senescence and a well-known phenomenon in ageing. Reduced telomere length in circulating leukocytes in COPD has been demonstrated in several studies (80-83), while data regarding telomere shortening in structural cells is still scarce.

Reduced telomere length was demonstrated in pulmonary vascular endothelial cells and pulmonary artery smooth muscle cells that were derived from COPD patients when compared to cells derived from smoking controls (84,85). Tsjui *et al.* used fluorescent in situ hybridization (FISH) to assess telomere length in alveolar type II and endothelial cells and demonstrated decreased telomere length in COPD patients when compared to non-smoking controls, but not compared to smoking controls (86). A recent study from Ahmad *et al.* assessed telomere length in lung tissue and reported an association with levels of telomere protection protein 1 (TPP1) (78). Both telomere length and TTP1 levels were reduced in lung homogenates from COPD patients compared to non-smoking controls, but not compared to smoking controls. This was further supported by decreased TTP1 levels and telomere length in CSE treated airway epithelial cells and lung fibroblasts (78).

The above findings of Tsuji *et al.* and Ahmad *et al.* (78,86) suggest an association with smoking rather than being COPD specific, although given that most COPD-patients are (ex-) smokers, this might be a contributing factor to disease risk and development.

Epigenetic changes

Epigenetic alterations caused by DNA methylation, histone modifications and noncoding RNA's are highly dynamic and influenced by ageing (87). It has even been postulated that the DNA methylation status of particular CpG-sites, also known as the 'epigenetic clock', can be used in an algorithm to predict the biological age (88,89). However, as far as we know, this algorithm has not been applied yet to COPD patients to test if the biological age of COPD patients determined by their methylation status is indeed increased compared to controls as would be expected. The majority of epigenome-wide methylation studies have been performed in whole blood and not much data is available on DNA methylation in whole lung tissue and lung tissue-specific cell types. While it has been widely established that cigarette smoke affects DNA methylation (90) and that COPD is highly associated with cigarette smoke exposure, in a recent systematic review by *Machin et al.*, no consistent differences were found in DNA methylation in peripheral blood in association with COPD or lung function (91). Therefore, the role of DNA methylation in COPD and thereby the role of age-associated differences in DNA methylation in COPD remains unclear.

Histone deacetylase (HDAC) enzymes can reduce the acetylation of histones, leading to enhanced expression of inflammatory genes involved in the disease pathogenesis of COPD. It has been shown that the HDAC activity is reduced in peripheral lung tissue, alveolar macrophages and bronchial biopsies of COPD patients compared to controls and this activity is further associated with the disease severity of COPD in peripheral lung tissue (92). The NAD^+-dependent Class III protein deacetylases known as the Sirtuin family are frequently described as anti-ageing enzymes (93). The fact that SIRT1 and 6 have been

shown to be decreased in peripheral lung tissue, and SIRT1 also in serum, of COPD patients compared to controls and, suggests age-associated acetylation differences in COPD (94,95). *Baker et al.* postulate that the reduced expression of both of the Sirtuins is regulated by the micro-RNA MiR-34a, a small endogenous non-coding RNA, which appears to be increased in COPD patients compared to controls. While the role of micro-RNAs in COPD has been extensively reviewed (96) the role of micro-RNAs in accelerated lung ageing is not extensively investigated and remains rather elusive (97).

Metabolism

Loss of proteostasis

Ageing cells are less able to maintain the homeostasis of proteins and contain more damaged proteins. In COPD lungs proteostasis of cells is decreased as well, which results in accumulation of damaged proteins. Accumulation of ubiquitinated proteins and the de-ubiquitinating enzyme and aggregation marker, ubiquitin C-terminal hydrolase L1 (UCH-L1), is increased in lung tissue of patients with severe COPD, and these levels negatively correlate with FEV_1 % predicted (98). Furthermore, several autophagy markers are increased in COPD lung tissue including p62, microtubule-associated proteins 1A/1B light chain 3B (LC3-II), autophagy related 4 (Atg4), Atg5-Atg12 and Atg7 (79,99).

Functional studies showed that autophagy activity (LC3-II flux) is increased in bronchial epithelial cells of COPD patients, without further increase upon CSE treatment (100). However, CSE treatment does increase the amount of autophagosomes in airway and bronchial epithelial cells (79,99). Inhibition of autophagy in bronchial epithelial cells results in accumulation of ubiquitinated protein and p62 (100). Again, the anti-ageing molecule SIRT6 may also regulate autophagy, as SIRT6 overexpression and knockdown resulted in an increase and a decrease of autophagosomes respectively (79).

Deregulated nutrient sensing

Nutrient sensing is a cell's ability to recognize and respond to fuel substrates such as glucose and recent findings suggest that nutrient sensing is increased in COPD lungs. Caloric restriction is strongly associated with longevity, and this is possibly mediated via two main pathways involved in nutrient sensing: mechanistic target of rapamycin (mTOR) and insulin like growth factor (IGF1)-signalling (101,102). The activity of mTOR, an important protein kinase in cell metabolism and nutrient sensing, is increased in total lung tissue and leukocytes of COPD patients (103) as well as in CSE-treated bronchial epithelial cells (79). In addition, SIRT6 is considered to attenuate the IGF1-mTOR pathway. The IGF1 pathway is important in cell growth and interacts with mTOR in the regulation of energy metabolism. Here, SIRT6 may play a role, as overexpression and knockdown of *SIRT6* resulted in a decrease in mTOR activity and increase in IGF1 signalling, respectively (79). Of interest, IGF1 protein levels were found to be increased in airway epithelial cells of patients with chronic bronchitis (104).

Mitochondrial dysfunction

With ageing, the function of the mitochondria decreases, which can lead to oxidative stress. Of interest, increased levels of oxidative stress are observed in COPD lungs as well. In whole lung tissue of COPD patients increased oxidative stress was found, as determined by reactive oxygen species (ROS) levels, oxidized-DNA, lipid peroxidation and nitric oxide (NO) levels (77,105,106). Moreover, lipid peroxidation correlated negatively with FEV_1 % predicted (105). In addition, gene expression and protein levels of the anti-oxidant nuclear factor, erythroid 2 like 2 (NRF2) were decreased in total lung tissue and bronchial epithelial cells of COPD patients (107) and *NRF2* expression was positively correlated with airway obstruction (FEV_1/FVC). Mitophagy, the degradation of mitochondria by autophagy, was increased in total lung tissue of COPD patients (108).

Furthermore, CSE treatment of bronchial epithelial cells resulted in increased ROS levels and mitophagy and decreased mitochondrial potential (108,109). Also in fibroblasts CSE treatment resulted in higher ROS levels (76).

Cellular processes

Immunosenescence and inflammageing

As described above, ageing is associated with immunosenescence and inflammageing. These two definitions underlie most of age-associated diseases and are important during COPD development in aged individuals. Several recent studies have investigated how ageing might affect immune dysregulation in COPD. In a study from 2016, John-Schuster *et al.* (110) demonstrated that aged mice exposed to cigarette-smoke are more susceptible to develop emphysema than younger mice. Aged animals had increased lung inflammation, with higher levels of inflammatory cells and mediators associated with lower repair. Two studies, also from 2015, observed that Klotho, an anti-ageing protein with anti-inflammatory properties, is reduced in alveolar macrophages (111) and airway epithelial cells of patients with COPD (112). The reduction was associated with high levels of oxidative stress, inflammation and apoptosis (111,112). Furthermore, decreased expression of miR-125a and b levels in COPD have been linked to inflammation and an impaired immune response. MiR-125a reduction resulted in NF-κB activation with a classical induction of pro-inflammatory cytokines, while in parallel, low levels of miR-125a and b suppress viral clearance (113). These data underscore the potential of targeting inflammation and at the same time increasing resistance to infections in the aged individual with COPD.

Cellular senescence

Cellular senescence is a cell state in which normal cells stop to divide as a mechanism to prevent tumorigenesis and tissue damage. Senescent cells can be cleared by the immune system, however upon ageing the number of senescent cells is accumulating in tissues. In here, these cells can have detrimental effects as they secrete several inflammatory factors and may disturb normal tissue homeostasis and repair due to the loss of their proliferative

capacity and normal physiologic function (114). Evidence is accumulating for increased cellular senescence in COPD lungs.

The percentage of senescent-associated β-galactosidase positive cells was increased in multiple cell types in COPD patients, including airway epithelial cells, smooth muscle cells, endothelial cells and fibroblasts as well as in CSE treated alveolar and bronchial epithelial cells (76,78,79,84,85,115,116). Another senescence marker, the cell cycle inhibitor p16, was found to be increased in total lung tissue, alveolar cells, airway epithelial cells, smooth muscle cells, endothelial cells and fibroblasts of COPD patients (75,76,84-86,116). Similarly, the presence of p21, another cell cycle inhibitor, was increased in total lung tissue, alveolar cells, smooth muscle cells, endothelial cells and leukocytes of COPD patients (79,80,84-86). P21 was also increased in CSE treated bronchial epithelial cells and fibroblasts (78,79,117). Moreover, the percentages of p16 and p21 positive cells were negatively correlated with FEV_1% predicted in alveolar type II and endothelial cells (86). The levels of IL-6 and IL-8, two important cytokines that are secreted by senescent cells as part of the senescence-associated secretory phenotype, were increased in total lung tissue, alveolar cells, smooth muscle cells and endothelial cells of COPD patients as well as in CSE treated fibroblasts (75,76,84,85). Though these cytokines can also be the result of ongoing inflammation in COPD, these observations cannot directly be related to an increase in cellular senescence.

ECM dysregulation

Age-related changes in the lung can also be observed at the extracellular levels. Comparable to the ageing lung (118,119) the extracellular matrix (ECM) is altered in COPD (120). The main alterations in COPD include increases of several ECM proteins such as collagens, fibronectin and laminin (42), changes in the structural organization of collagen with more disorganized collagen fibres (121), and also a reduction in elastic fibres (122). An important contributing factor to these ECM changes is the imbalance between proteases, such as matrix metalloproteinase 12 (MMP12) and neutrophil elastase, and anti-proteases, like α-1 antitrypsin and tissue inhibitor of metalloproteinase (TIMP) 1-4, as reviewed by Navratilova *et al.* (123).

Elastin degradation plays an important role in the pathogenesis of COPD. Elastin fragments alone are known to induce inflammation, leading to destruction of lung tissue (124). It was previously thought that in COPD the lung loses its ability to repair, however, it has become increasingly more evident that there may be aberrant attempts at repair. A number of genes encoding for elastogenesis components, such as fibulin-5 (*FBLN5*), microfibril associated protein 4 (*MFAP4*), latent transforming growth factor binding protein 2 (*LTBP2*) and elastin (*ELN*) itself were identified in a large COPD patient cohort to be higher expressed (125). Whether these components are beneficial or further drive disease pathogenesis remains unclear, as extracellular proteins have the potential to interfere with different cellular pathways (126,127).

Another interesting observation in COPD is the change in lung fibroblast responses *in vitro*. Lung fibroblasts are the main cells involved in ECM homeostasis and repair in the

lung and several studies have shown differences in terms of ECM production when comparing COPD fibroblasts to those derived from non-COPD controls (128-131), suggesting a disturbed or abnormal repair capacity of these cells.

Stem cell exhaustion

Adult lung tissue is thought to reside in a quiescent state. Upon injury, (stem) cells can get activated and are able to proliferate and (trans) differentiate into other cell types, according to their plasticity (132). Indeed, the lung harbours different cell populations including stem cells, responsible for its unique homeostatic capacity to ensure gas exchange (133,134). Airway basal cells represent a well-characterized stem cell population located in the trachea and bronchi. These cells have the ability to self-renew and give rise to secretory, ciliated and neuroendocrine cells (135). In the distal lung, alveolar type II cells (ATII) (136) have been shown to be able to replenish lost ATII and trans differentiate to alveolar type I cells (ATI), thus ensuring proper gas exchange (132,133). It is most likely that other progenitor or stem cell subpopulations exist, which is indicated by several studies in mouse tissue over the past years, however, the existence of these cell in the human tissue and the relevance for tissue injury and potentially impaired repair, remains elusive.

Moreover, COPD has been associated with reduced numbers and dysfunction of circulating progenitor cells (137,138). Cigarette-smoke, a major risk factor for COPD, was shown to reduce the repair potential of endothelial progenitor cells (139), and bone marrow mesenchymal stem cells by interfering with cell homing and proliferation capacities (140). Thus, stem cell exhaustion might contribute to COPD pathogenesis by reducing the endogenous renewal and repair capacity of the lung by local as well as recruited cells. Stem cell niches fail to respond effectively to additional demands for cell-turnover, moreover, deranged metabolic signalling and premature senescence might occur (141). In line with this, reduced regenerative capacity of basal progenitor cells has been reported in COPD (135). In addition, in a different study, an abnormal population of $TRP63^+$ $KRT5^+$ $KRT14^+$ basal cells was identified in regions of hyperplasia from sections of COPD human airways (142), suggesting abnormal stem cell function. Developmental pathways, such as WNT, Sonic Hedgehog and Notch, are important susceptibility factors for COPD (143-145) and are associated with the regulation of different stem cell functions (146). Canonical WNT signalling, which relies on stabilization of β-catenin for transcriptional activation, is decreased in COPD (144,147,148). Notably, pharmaceutical activation of the pathway led to an increase in surfactant protein C production and secretion along with increased alveolar type I cell marker expression in COPD lung tissue *ex vivo*, thus suggesting that the initiation of stem cell mediated repair in the COPD lung is possible (149).

<u>Evidence for ageing hallmarks and abnormal tissue repair in COPD</u>

Increased levels of DNA damage and decreased levels of DNA repair markers have been demonstrated in COPD, in particular in the alveolar compartment. Although the data is derived from a limited number of studies, it does indicate a role for these ageing markers

in COPD, in particular in relation to emphysema development with increased alveolar wall destruction and lack of repair.

Another key hallmark in ageing is telomere shortening. Since information on telomere length in structural cells is mostly lacking it is difficult to speculate on a role of telomere shorting in relation to tissue repair and remodelling in COPD. Reduced TPP1 levels in relation to smoking in lung tissue and structural cells however does suggest an effect of smoking. Whether this also relates to smoking-induced COPD remains to be elucidated.

Regarding epigenetic changes in COPD, solid data on DNA methylation is lacking, and thus it is yet not possible to infer a role for DNA methylation in abnormal tissue repair in COPD. Several studies however have indicated involvement of histone modification (HDAC and Sirtuins) and miRNAs in COPD and it is of great interest to further evaluate if and how these changes contribute to accelerated lung ageing and abnormal tissue repair in COPD.

With respect to the metabolic changes in COPD, increased autophagy and accumulation of damaged proteins reflects ongoing tissue damage and high protein turnover in COPD. Whether this is cause or consequence (or both) of the abnormal repair response is currently unclear. Similarly, disturbed nutrient sensing (IGF-1-mTOR) and the oxidant anti-oxidant imbalance, indicate that, as with normal ageing, cell homeostasis is disturbed, which makes the cells vulnerable to disease. However, whether and how this contributes to abnormal repair should be evaluated by further studies.

Of all ageing hallmarks in COPD, the changes in cellular processes are probably the best studied. Multiple studies have shown increased cellular senescence and changes in ECM regulation in COPD, in particular in structural cells, including epithelial cells, smooth muscle cells and fibroblasts. The latter are of particular interest, as fibroblasts are the main regulators of tissue repair in the lung and changes in these cells possibly underlie the abnormal tissue repair responses in COPD. Together with the reduced numbers, dysfunction and regenerative capacity of progenitor cells in COPD these age-related cellular changes may very well explain the disturbed repair and remodelling capacity of COPD lungs, both in the alveolar and airway compartment.

Implications for treatment

COPD exacerbations are of major concerns in elderly, as they are highly susceptible to infections. Due to a dampened immune system, vaccination is not considered a successful preventive measure (150). Having this in mind, strategies which boost the immune system have been proposed for lung disease treatment. One of the strategies is the interference with gut microbiota (151). Local microbiota influences immunity at distal sites and organs. *Bifidobacterium breve* and *Lactobacillus rhamnosus* have been shown to reduce inflammatory responses in macrophages that were exposed to cigarette smoke extract *in vitro* (152). Another potential future therapy is based on the application and usage of stem cells. In 2013, bone marrow-derived mesenchymal stem cells (MSCs) were first transplanted to patients with no adverse effects observed in older patients (153). More recently, an immunomodulatory mechanism has been associated with MSCs treatment which

decreased lung inflammation and improved lung function (154). Furthermore, as mentioned above, pharmaceutical activation of the WNT pathway showed promising effects *ex vivo* and indicates the opportunities to induce endogenous stem cell mediated repair in COPD (149).

Although stem cell therapies hold promise as future therapeutic options, more regulations and clinical trials on the matter are needed to optimize therapeutic schemes, dosages, infusion rates and further identify possible risk groups and specific adverse effects (155). Ultimately, understanding the molecular biology of ageing in the lung is crucial for finding new ways of manageing COPD in older but also younger (SEO-COPD) patients.

Summary and conclusions

As summarized in this review, main ageing hallmarks are present in COPD and this supports the hypothesis that (abnormal) ageing contributes to COPD development. With respect to the role of abnormal ageing in tissue repair in COPD, the strongest indications come from cellular changes, i.e. increased cellular senescence, ECM dysregulation and stem cell exhaustion. Yet, to be able to answer our question whether accelerated or abnormal ageing is causally contributing to COPD pathogenesis and in particular impaired tissue repair, we need to integrate all findings and assess how age-related changes affect ECM homeostasis and tissue repair in the lung. Ideally this should not be restricted to single-cell culture models with primary lung cells, but also involve more complex co-culture and organoid models, lung tissue slices and/or lab-on-a-chip approaches. An important aspect that needs to be taken into account is the age-matching between the control and COPD groups. Indeed for some studies discussed in this review, the mean age was significantly different between the control and COPD group (indicated in Table 1). This may come as a challenge to distinguish the effects which are related to age and which are related to COPD. Finally, translation and comparison to *in vivo* models and to what happens in the lungs of the actual COPD patients is important for the identification of potential new therapeutic approaches. Evaluation in well-defined clinical samples is crucial to understand the clinical implications and potential benefit for COPD patients. This information may also guide us towards novel approaches aiming to stop or at least slow down accelerated lung ageing in COPD. Providing a future perspective for the most vulnerable group of COPD patients that suffers from the highest disease burden and lacks adequate treatment; severe early onset COPD.

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