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

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CHAPTER 8

Summary, general discussion and future perspectives

SUMMARY

Since the pathogenesis of COPD is unclear and no treatment options are available to reduce disease progression, novel insights into the pathogenesis of COPD are urgently needed, such as elucidating the role of accelerated ageing in COPD. I hypothesized that accelerated ageing contributes to the pathogenesis of COPD, especially in the relatively young, severe, early-onset COPD (SEO-COPD) patients. These SEO-COPD patients represent a subgroup of COPD with a significant proportion of hospital admissions and healthcare costs. Recently, dysregulation of extracellular matrix (ECM) homeostasis has been described as an additional hallmark for lung ageing, although it is unknown whether accelerated ageing contributes to ECM dysregulation in COPD. Therefore, the overall aim of this thesis was to elucidate the role of accelerated ageing specifically in lung tissue repair and remodelling in COPD.

In **chapter 2**, the overlap between lung ageing and COPD was described and all data on ageing in COPD as available at the start of this thesis was reviewed. This comprehensive review demonstrates that most ageing hallmarks are observed in lung tissue from COPD patients, indicating that accelerated ageing may play a role in COPD. However, this overview also identified a lack of evidence as to whether accelerated ageing occurs in lung fibroblasts and airway smooth muscle cells from COPD patients and whether this impacts their functional roles in repair, remodelling and ECM regulation. Moreover, since no data were available including relatively young patients or SEO-COPD, this thesis is more focused on this group of patients with the hypothesis that accelerated ageing may especially play a role in SEO-COPD.

In **chapter 3**, potential gene-miRNA interactions were discovered that may play a role in normal ageing of the airways. Interestingly, genes that had a lower expression with age were part of pathways involved in three ageing hallmarks, including genomic instability, cellular senescence and altered intercellular communication, implicating a particular role for these ageing hallmarks in normal lung ageing.

In **chapter 4**, COPD-derived fibroblasts displayed features of accelerated ageing compared to non-COPD controls, with higher levels of cellular senescence, DNA damage and oxidative stress. Interestingly, some of these effects were most pronounced in fibroblasts from SEO-COPD patients. The observed increase in cellular senescence was correlated with lower gene expression of the ECM protein decorin (*DCN*) in COPD-derived fibroblasts. In addition, Paraquat-induced cellular senescence resulted in changes in ECM gene expression, including decreased *DCN* expression. Our study showed a clear link between cellular senescence and ECM dysregulation in COPD.

To give more insight into the potential consequences of accelerated ageing in lung fibroblasts, the senescence-associated secretory phenotype (SASP) of senescent COPD-derived lung fibroblasts was assessed in **chapter 5**. 124 SASP proteins from primary lung fibroblasts upon senescence induction were identified. 42 of these proteins were secreted

at higher levels by COPD-derived fibroblasts compared to non-COPD controls, and 35 were secreted at higher levels by SEO-COPD-derived fibroblasts compared to their matched non-COPD controls. Interestingly, multiple COPD-associated SASP proteins have been implicated in chronic inflammation, and as such might contribute to COPD pathogenesis.

In **chapter 6**, cellular senescence levels at baseline were higher in airway smooth muscle cells (ASMCs) compared to lung fibroblasts, but were not different between ASMCs from COPD patients compared to non-COPD controls. No link between cellular senescence and ECM gene expression in COPD-derived ASMCs was found. These results indicate that, in contrast to lung fibroblasts, higher levels of senescence in ASMCs do not appear to play a major role in COPD pathology.

Finally, in **chapter 7**, the potential of E-cigarettes, commonly used by COPD patients, to induce cellular senescence was assessed. E-vapour exposure induced cellular senescence in primary human lung fibroblasts. In addition, senescence induction by E-vapour exposure, similar to cigarette smoke exposure, and paraquat treatment resulted in an impaired wound healing capacity. Hence, E-cigarette vaping appears not a safe alternative for cigarette smoking and might even contribute to accelerated lung ageing and pathology.

A summary overview of all main findings in the studies of this thesis is depicted below in Figure 1.

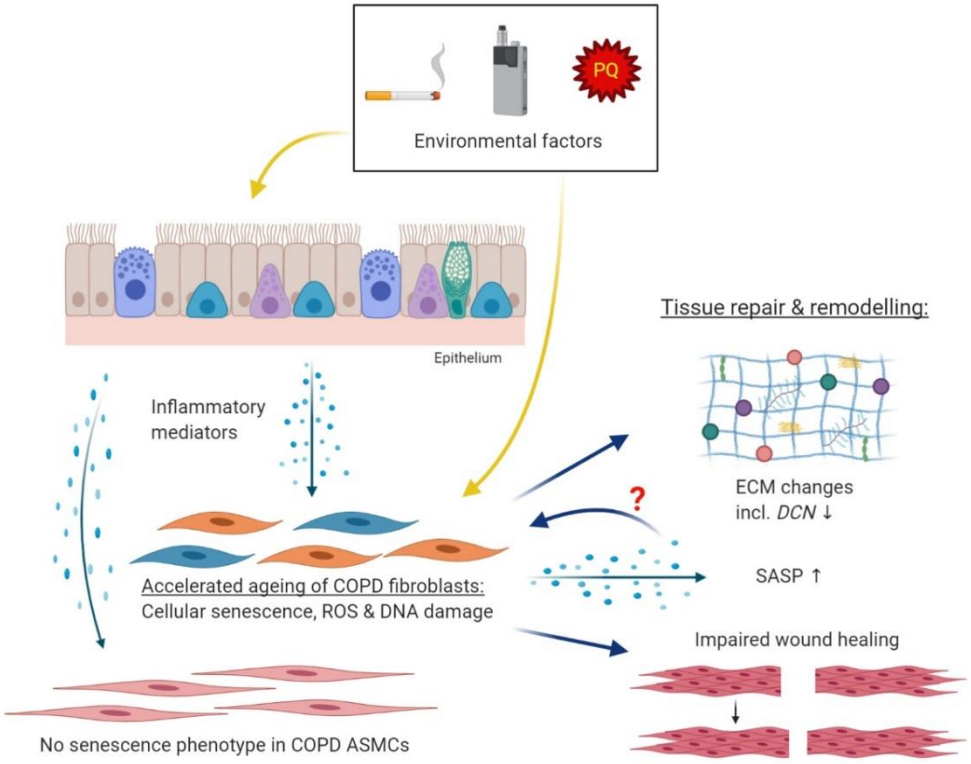

Figure 1: Overview of the role of accelerated ageing in lung tissue repair and remodelling in COPD. *Environmental factors, including cigarette smoke, E-cigarette vapour and Paraquat (PQ), can induce features of accelerated ageing (cellular senescence) in lung fibroblasts. COPD-derived fibroblasts have features of accelerated ageing with higher levels of cellular senescence, reactive oxygen species (ROS) and DNA damage. This accelerated ageing may be a direct result of environmental factors or indirect via induction of a pro-inflammatory environment in the lungs, for example, by secretion of inflammatory mediators by epithelial cells. Higher levels of cellular senescence resulted in extracellular matrix (ECM) changes, including lower DCN expression, higher SASP protein secretion and impaired wound healing capacity, and thus affect the tissue repair and remodelling functions of lung fibroblasts. These functional consequences have been implicated to play a role in COPD pathogenesis. Created with BioRender.com*

GENERAL DISCUSSION & FUTURE PERSPECTIVES

Cellular senescence in COPD

Cellular senescence can be induced by multiple factors, including environmental and lifestyle factors that are of relevance in COPD. Most of these factors induce cellular senescence via an increase in oxidative stress, mitochondrial dysfunction or DNA damage. Cigarette smoke exposure, the major risk factor of COPD, has been extensively described to induce oxidative stress, DNA damage, telomere dysfunction and cellular senescence *in vitro* in multiple structural lung cells (as reviewed in **chapter 2**) and also *in vivo* in mouse lungs (1-4). Both oxidative stress and DNA damage were higher in (SEO-) COPD-derived fibroblasts (**chapter 4**), which suggests that one or both of these stress factors may have caused the higher levels of cellular senescence observed in these cells. Future studies should assess whether one or both caused the induction in senescence by specifically inducing these stress factors separately, for example by radiation (DNA damage), and specifically reducing these stress factors separately, for example by anti-oxidants (oxidative stress), and assess the effects on senescence induction.

E-cigarettes are used as smoking cessation or replacement device and assumed to be a safer alternative than smoking cigarettes. Previous studies have already demonstrated that *in vitro* in multiple structural lung cells and *in vivo* in mouse lungs exposure to E-cigarette vapour can induce inflammation, oxidative stress and DNA damage (5, 6). In **chapter 7**, we showed that E-cigarette vapour exposure induces cellular senescence in primary human lung fibroblasts. The induction of senescence by E-cigarettes appeared to be nicotine-independent, but further studies should be done to elucidate the mechanisms that are involved in the induction of senescence, for example, oxidative stress and DNA damage. Moreover, future studies should assess the chronic effects of E-cigarettes as limited data on chronic use is available.

Only a few studies have been done to assess senescence induction by air pollution like particulate matter. Particulate matter has been demonstrated to induce inflammation and oxidative stress *in vivo* in mouse lungs (7-9). Till now, induction of cellular senescence has only been shown in fine particulate matter (PM2.5) treated corneal epithelial cells and keratinocytes (10, 11). Hence, future studies need to be done to assess the senescence inducing potential of air pollution in the lungs.

Finally, the inflammatory and fibrotic environment of COPD lungs can enhance the senescence phenotype as well, for example TGF-β, one of the best-known COPD-associated proteins (12, 13), has been demonstrated to be able to induce senescence (14, 15).

In summary, most of the environmental and lifestyle factors that are risk factors for COPD have the potential to induce cellular senescence in structural lung cells, which eventually may contribute to COPD pathogenesis. An important question that remains to be answered, is whether the induction of cellular senescence in COPD-derived lung

fibroblasts is caused directly by the environmental and lifestyle factors or by the pro-inflammatory environment in the lungs, for example, by inflammatory mediators secreted by epithelial cells. Therefore, experiments with co-cultures of exposed epithelial cells with lung fibroblasts or treatments of lung fibroblasts with conditioned media from exposed epithelial cells should be performed. It would also be of interest to assess whether exposed epithelial cells from COPD patients and non-COPD smokers provoke a different response in co-cultured or conditioned media treated lung fibroblasts.

Accumulation of senescent fibroblasts in lung tissue may be caused by an enhanced induction of cellular senescence and/or reduced clearance of senescent cells by immune cells and may have detrimental effects on the surrounding lung tissue. In **chapter 4**, higher levels of cellular senescence were observed in both lung tissue and lung fibroblasts from COPD patients, indicating that in COPD lung tissue senescent cells accumulate, including senescent fibroblasts. In this study, it could not be determined whether this accumulation is caused by an enhanced senescence induction or reduced senescence clearance or both. Till now, data assessing the capacity of senescence clearance by immune cells in COPD patients is very limited. Immune cells that can clear senescent cells by phagocytosis are macrophages, and cells that can induce apoptosis of senescent cells are NK cells and T cells, but the exact mechanisms are still not clear (16, 17). Numbers of macrophages are higher in lung tissue from COPD patients compared to smokers without COPD, which was associated with disease severity (18, 19), while levels of macrophage attractant MCP-1 were found to be higher in sputum (20) and BAL fluid (21) from COPD patients. Despite higher numbers of macrophages, the phagocytosis capacity of pathogens of COPD-derived alveolar macrophages was found to be reduced compared to non-COPD controls (22-25). Furthermore, levels of the senescence marker p21 are increased in macrophages from smokers (26), which indicates an impaired function of these cells upon smoking, but higher levels of senescence in COPD-derived macrophages have not been demonstrated yet. These studies suggest that clearance of senescent cells might be reduced in lung tissue from COPD patients as well, but future studies should assess the potential of senescent cell clearance of COPD-derived immune cells. These studies should include co-culture models of senescent cells (fibroblasts) with COPD-derived immune cells like macrophages to answer this question.

Functional consequences of senescence accumulation in COPD lung tissue

Cellular senescence is an important homeostatic mechanism, but accumulation of senescent cells can lead to pathology. Transient occurrence and clearance of senescent cells are part of normal physiology by contributing to embryogenesis, tissue development, and normal tissue repair and remodelling (27-30). The detrimental effects of cellular senescence are caused by an accumulation of senescent cells. These effects may be the result of altered

functions of the senescent cell itself and by the detrimental effects of the SASP. Cellular senescence in lung fibroblasts resulted in differential ECM expression (**chapter 4**) and an impaired wound healing capacity (**chapter 7**), which both are implicated in COPD pathogenesis. While little was known about ECM regulation in senescent cells in the lungs, several studies have found altered ECM and matrix metallopeptidase (MMP) regulation in senescent fibroblasts derived from other tissues like dermal and foreskin fibroblasts. Most of these studies have shown an increase in MMP secretion (31-33), which can degrade ECM proteins, and in general a decrease in expression of collagen genes (32-36). Therefore, this suggests that cellular senescence can directly affect the repair and remodelling function of fibroblasts. As cultures of senescent cells include non-senescent cells as well it cannot be excluded that the observed differences might be driven by the effect of senescent cells on neighbouring non-senescent cells. Sorting a pure population of senescent cells may answer this question. Since most of the current studies only describe an association between senescence and ECM changes or an effect of senescence on gene expression instead of ECM protein deposition and structure, future studies need to be done to elucidate the exact effect of cellular senescence in lung fibroblasts on the matrix biology in COPD lungs. It would be of interest to assess whether newly formed ECM from senescent lung fibroblasts is different in levels and biomechanical properties compared to non-senescent fibroblasts. In addition, whether these senescent fibroblasts change the existing ECM would be of interest as well.

The potential detrimental effects of SASP proteins on surrounding lung tissue include a wide range of processes; ECM interference, chronic inflammation, paracrine senescence, epithelial-mesenchymal transition (EMT) and tumorigenesis (27, 29, 37). In **chapter 5**, multiple SASP proteins were identified in senescent primary lung fibroblasts and COPD-associated SASP proteins that have been implicated in these detrimental processes. Firstly, multiple enzymes and proteases like MMPs that were identified as SASP proteins can have a direct effect on lung ECM, including MMP-2, -3, -9, -10. Proteases that were found to be secreted at higher levels by COPD-derived fibroblasts compared to non-COPD controls include MMP-9 and t-PA. Both have been implicated in ECM degradation, where MMP-9 can degrade collagen (38, 39) and decorin (40, 41), while tPA activates plasminogen and MMPs and thereby provokes ECM breakdown (42). Secondly, the SASP of senescent lung fibroblasts and the SASP of COPD-derived fibroblasts contained various cytokines and chemokines that have been implicated in inflammation. Several of our identified SASP proteins are COPD-associated inflammatory mediators, including the COPD-associated SASP proteins CCL15 and CXCL9 (43-45). Thirdly, single SASP proteins and also the full SASP profile can induce paracrine senescence, where the full SASP has been demonstrated to induce senescence in surrounding cells *in vitro* in multiple cell lines (46, 47) and even *in vivo* in mouse lungs (48, 49). Although paracrine senescence was observed in multiple tissues including liver, stroma, colon and muscle, paracrine senescence by the SASP has not been

demonstrated in lungs yet. Fourthly, multiple studies that co-cultured senescent cells with non-senescent cells or treated cells with conditioned media of senescent cells found that SASP can induce cell growth (27, 50-54) and characteristics of tumorigenesis, including morphogenesis, migration, invasion and angiogenesis (51, 53, 55-57). Whether the SASP can play a role in tumorigenesis in the lungs is still unknown and thus it remains to be elucidated whether the SASP may have a potential role in the COPD-lung cancer overlap. Finally, the SASP has also been demonstrated to cause EMT (58), which leads to a loss of epithelial function, where senescent fibroblasts and conditioned media from senescent fibroblasts caused EMT in human and mouse breast cancer cells (27, 51). Whether SASP protein secretion by COPD-derived lung fibroblasts can cause EMT of epithelial cells in COPD lungs has not been studied yet. In summary, the SASP has been demonstrated to induce detrimental effects on the surrounding tissue in multiple organs, but studies on the detrimental effects of the SASP (from senescent fibroblasts) on surrounding lung tissue are limited. Therefore, future studies should focus on the detrimental effects of the SASP proteins on the different structural lung cells and whether these effects are driven by a particular group of proteins or by the complete SASP composition.

In preliminary studies, I studied the potential autocrine effect of the SASP proteins secreted by senescence-induced primary lung fibroblasts. Conditioned media containing the SASP of senescent lung fibroblasts was used to treat naïve lung fibroblasts. Very low to no effects of senescent fibroblast-derived conditioned media were observed, with a small increase in cellular senescence in some donors and no effect on inflammation, ECM gene expression and wound healing capacity. Based on the preliminary findings, I expect that the SASP of senescent lung fibroblasts is more likely to have detrimental effects in a paracrine manner affecting its surrounding cells. For future studies, the effect of senescent fibroblasts on surrounding lung tissue needs to be addressed by co-culture models and treatment of different lung-derived cells with conditioned media from senescent fibroblasts. Furthermore, the effect of single COPD-associated SASP proteins, which are secreted at the highest levels, on lung-derived cells should be assessed to get more insight into the paracrine effect of senescent lung fibroblasts on COPD lung tissue.

Accelerated ageing in COPD

Although accelerated ageing has widely been described to contribute to COPD pathogenesis, causality has not been demonstrated and remains challenging. The fact that features of ageing were observed at a relatively young age (SEO-COPD) already, supports the hypothesis that accelerated ageing is involved in the disease. Since many ageing markers are increased upon cigarette smoking as well, like oxidative stress, DNA damage, and cellular senescence, proving that accelerated ageing contributes to COPD pathogenesis or is a result of the disease is difficult. To minimize the effect of smoking, ex-smoking COPD patients were compared to ex-smokers who did not develop COPD in the studies in this

thesis. However, this does not exclude that features of ageing may be a result of disease and not the cause of disease. To get more insight into this, our study and some other studies compared COPD patients with different Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages, showing that cellular senescence, telomere length, oxidative stress, mTOR activity and loss of proteostasis were associated with disease severity (59-65). In accordance, correlations between disease severity (FEV_1 % predicted) and cellular senescence, oxidative stress, and DNA damage were found in **chapter 4**. These results suggest that these ageing processes may contribute to disease progression.

An important question is whether accelerated ageing can be a driver of the development of COPD. Since in our studies samples from end-stage of disease were used, this question could not be answered. In mouse models, spontaneous emphysema occurs upon telomerase knockout and knockout of the anti-ageing gene Klotho (66-68). Genetic alterations of age-related repair mechanisms can enhance emphysema development in combination with cigarette smoke exposure, including a knockout of the anti-oxidant gene *NRF2* and knockdown of anti-ageing gene *SIRT1* (69, 70). Whether these age-related mechanisms can drive COPD development in humans is not known yet. So, while many studies suggest that accelerated ageing may contribute to the progression of the disease, it remains unclear whether accelerated ageing can be a driver of the development of COPD. Exposure to cigarette smoke and other noxious gases has been recognized to cause chronic inflammation and lung tissue damage in the development of COPD, but as not all smokers develop COPD it remains unclear which processes are involved in the onset of disease in combination with the exposures. Accelerated ageing might be one of these driving processes in COPD development. Confirming this hypothesis is difficult because most studies are done in patients that already developed symptoms. Therefore, longitudinal studies should be done in patients with no clinical disease yet who are at risk to develop COPD, because of the exposure to known COPD inducing stimuli, to assess ageing markers before the onset of disease. However, these studies also have their downsides in that they take a long time to finish, sample collection for example by bronchoscopy is invasive and large number of smoking individuals need to be included as not all smokers develop COPD and this is unpredictable. Studying the role of accelerated ageing in cohort studies including families with smokers who are diagnosed with COPD and families with smokers without COPD may help to reduce the study duration and to include relevant subjects. Examples of such studies are the COPDGene study (71), the ECLIPSE study (72), and a multicentre COPD susceptibility study (73, 74), which are studies of interest to assess the role of accelerated ageing in COPD development.

Why (SEO-) COPD-derived fibroblasts display more features of accelerated ageing remains an important unanswered question. On a cellular level, accelerated ageing is thought to be the result of environmental and inflammatory damages and impaired repair mechanisms (75, 76). Cigarette smoke causes cellular damages via oxidative stress,

mitochondrial dysfunction, DNA damage, and telomere dysfunction (2, 67, 77-80). In **chapter 4**, higher levels of oxidative stress and DNA damage in COPD-derived fibroblasts compared to non-COPD controls were observed. Since not all smokers develop COPD, an impairment in cellular repair and maintenance mechanisms has been hypothesized to result in a predisposition for accelerated ageing (81-83). The causes of these impaired mechanisms in COPD are largely unknown, but postulated to be influenced by genetic and epigenetic backgrounds (83, 84). Previous studies observed a reduction in protective repair mechanisms in lung tissue and epithelial cells from COPD patients, including reduced DNA damage repair, reduced telomerase activity, loss of proteostasis (autophagy and mitophagy), and reduced anti-oxidant as reviewed in **chapter 2**. In **chapter 4**, some markers of DNA damage repair and autophagy in COPD-derived fibroblasts were assessed, but no differences were observed between COPD and non-COPD at baseline. The limitations of this study were that only a few markers were selected to assess these repair mechanisms, measured at a single time-point, and that isolation and *in vitro* culture may have caused a loss of difference between COPD and non-COPD at baseline. Upon stimulation with Paraquat, which is a COPD risk factor by occupational exposure, COPD-derived fibroblasts were less capable to respond to the damage with lower up-regulation of the oxidative stress response genes *FOXO3* and *MGST1* compared to non-COPD derived fibroblasts. These results support the hypothesis that impaired repair mechanisms may cause a predisposition for accelerated ageing in COPD, but which exact mechanisms are involved needs to be elucidated. The genetic and epigenetic background of these patients may cause this predisposition. A chronic exposure *in vitro* model with cells derived from healthy smokers and COPD smokers may reveal repair and maintenance mechanisms that are involved in accelerated ageing, for example, autophagy, mitophagy or DNA damage repair. Upon unravelling these exact mechanisms that are involved in impaired repair in COPD-derived fibroblasts, therapeutic targets that restore these repair functions can be discovered.

Clinical implications and potential therapies

First of all, smoking cessation is at the moment the best option to prevent COPD development as cigarette smoke is a major source of oxidative stress and inducer of cellular senescence. E-cigarette vaping is becoming more popular as an alternative for cigarettes and as a cessation device, while younger individuals also start vaping without having smoked cigarettes before. In **chapter 7**, E-cigarette vapour appeared to be not harmless and might contribute to COPD pathology as it induces cellular senescence. Thus, E-cigarette use should be avoided as well. Since smoking cessation may be challenging for addicted smokers and 25-45% of COPD patients are non-smokers (85), therapeutics to stop the disease progression need to be developed as well. Therefore, interfering in the processes of accelerated ageing may be a promising approach for COPD patients. Currently, many studies are assessing the efficacy of anti-ageing drugs to improve healthy ageing and to prevent and

treat age-related diseases. Targeting senescent cells by senostatics that prevent induction of cellular senescence and senolytics that specifically kill senescent cells, seem promising approaches for multiple age-related diseases including COPD.

Senostatics are drugs that improve cellular repair mechanisms to prevent an accumulation of damage, which eventually prevents cellular senescence induction. The repair mechanisms that can or may be improved are DNA damage repair, telomerase activity, the proteostasis, mitochondrial function and normal nutrient sensing. Limited studies have been done to investigate the potential of improving DNA damage repair, telomerase activity and the proteostasis in COPD. Only one study showed that activation of an autophagy transcription factor reduced cigarette smoke-induced oxidative stress, cellular senescence and emphysema features in mice (86). Tested therapies that improve mitochondrial function are mainly anti-oxidants to reduce oxidative stress, but these are not clinically effective in COPD yet (87). Therapeutic approaches that are studied in more detail and seem to be beneficial are reduction of nutrient-sensing activity and activation of anti-ageing regulators. Inhibition of mTOR via rapamycin, AMPK activation via metformin and SIRT1 activation have all been found to reduce cellular senescence *in vitro* and *in vivo* and improve the lifespan of mice (88-92), but beneficial treatments in human clinical trials have not been demonstrated yet, while rapamycin has major side-effects (91). Regarding COPD, only AMPK activation reduced the mortality rate in patients with diabetes, but not in COPD patients without diabetes (93). So, senostatics may potentially be beneficial for COPD patients, but limited treatment options are currently available and only limited studies have been done in COPD. Furthermore, since the mechanisms that contribute to the predisposition of accelerated ageing in COPD are unclear yet, these need to be unravelled first to identify the specific mechanisms to target in COPD. More importantly, it is questionable whether severe COPD patients will benefit from these therapies as they already present features of accelerated ageing including senescence accumulation. Therefore, senostatics might be more beneficial for mild-moderate COPD patients to prevent disease progression. Hence, it is also important to know whether senescent cells continue to accumulate after smoking cessation, which still is critical to stop disease progression. For COPD patients that never smoked and thus are likely exposed to other environmental or occupational factors, senostatics may be especially beneficial to stop disease progression.

The other promising approach, senolytics, eliminate senescent cells by inducing apoptosis specifically in senescent cells and not in functional non-senescent cells. The first study to demonstrate the potential of senolytics found a delay in age-related disorders upon clearance of p16 positive senescent cells in mice (94). Since then, multiple senolytic treatments have been developed that eliminate senescent cells *in vitro* leading to reduced SASP protein secretion and reduced inflammation (95-97). Several mouse models in which treatment with senolytics was tested showed an extended lifespan and improved physical

activity and lung function (98-103). Interestingly, a recent study has demonstrated that senolytic CAR T cells that target uPAR, eliminated senescent cells *in vitro* and *in vivo* and extended the lifespan of mice (104), which is of particular interest as in **chapter 5** uPAR was found in the SASP of COPD-derived fibroblasts. The senolytic cocktail Dasatinib and Quercetin (D+Q) has been demonstrated to eliminate senescent foetal and senescent primary lung fibroblasts and thereby reducing fibrosis, improving pulmonary and physical health upon bleomycin-induced lung injury (105, 106). The D+Q cocktail has been used in a phase 1 clinical trial to treat idiopathic pulmonary fibrosis patients and did improve physical function without causing severe side-effects and thus may be a feasible treatment that needs further clinical trials (107). Currently, more clinical trials are being performed to assess the benefits of D+Q and other senolytics in various age-related diseases, but not yet including COPD. So, future studies should assess the potential benefits of senolytic treatments in COPD-derived structural cells, COPD mouse models and eventually in clinical trials in COPD patients.

Finally, another suggested anti-ageing therapeutic approach is targeting the pathways and release of SASP proteins, because SASP proteins can have multiple detrimental effects on lung tissue as discussed above. Since cellular senescence and the SASP have been recognized to be involved in COPD pathogenesis, targeting the SASP might be a potential therapeutic approach (108). However, activation pathways of SASP proteins are similar to the COPD-related inflammatory pathways, including NF-κB, p38 and JAK/STAT, and targeting these pathways have been demonstrated to be poorly effective and lead to major side-effects (44). Glucocorticoids have been demonstrated to suppress the SASP in irradiation-induced senescence (109). Although they reduce exacerbation risks and severity, they have proven to not reduce COPD progression (110). Therefore, targeting specific SASP proteins may be a more promising therapeutic approach, for example by targeting specific miRNAs (110). In **chapter 3**, 29 age-related miRNAs were identified, which might be involved in the regulation of ageing processes. The expression of these miRNAs was not assessed in the lungs of COPD patients yet. Hence, elucidating the role of miRNAs in accelerated ageing and COPD may reveal novel specific therapeutic targets. Ultimately, miRNA-based therapies with miRNA inhibition or miRNA activation might be a potential therapeutic approach to target accelerated ageing in COPD.

Since cellular senescence has multiple beneficial functions in healthy physiology, targeting cellular senescence may also lead to side-effects related to these normal functions. First of all, cellular senescence is a mechanism that prevents abnormal growth of a cell and thereby prevents tumour development. Thus, targeting cellular senescence may promote tumorigenesis. Furthermore, cellular senescence has also been demonstrated to play an important role in normal wound healing. Therefore, too low levels of cellular senescence can affect wound healing and thereby cause impaired tissue repair. Moreover, COPD is a heterogeneous disease, so a homogenous therapeutic approach might have more

detrimental side-effects than beneficial effects. Hence, more selective, site-targeted therapies should be developed. More studies to determine the healthy balance of cellular senescence levels need to be done to enable more site-targeted therapies, which may prevent these side-effects. Another opportunity to limit these side-effects and to help restore normal lung tissue would be combining senolytic treatments with lung tissue regeneration therapies, which recently are being studied more intensively and show promising benefits (111-113), but no clinical trials are done yet. So future *in vitro* and *in vivo* studies should assess the potential of such combination treatments.

Overall conclusion

The studies in this thesis support the hypothesis that accelerated ageing may play a role in aberrant tissue repair and remodelling in COPD and thereby contribute to disease pathology. Therefore, therapeutics that target the mechanisms of accelerated ageing may be a potential therapeutic approach in COPD. Future studies should unravel the exact mechanisms that lead to accelerated ageing in COPD to discover therapeutic targets and to develop therapies more specifically. These therapies can also be beneficial for other age-related diseases, which of the majority are comorbidities of COPD.

REFERENCES

(1) Tsuji T, Aoshiba K, Nagai A. Cigarette smoke induces senescence in alveolar epithelial cells. Am J Respir Cell Mol Biol 2004;31:643-649.
(2) Ahmad T, Sundar IK, Tormos AM, Lerner CA, Gerloff J, Yao H, Rahman I. Shelterin Telomere Protection Protein 1 Reduction Causes Telomere Attrition and Cellular Senescence via Sirtuin 1 Deacetylase in Chronic Obstructive Pulmonary Disease. Am J Respir Cell Mol Biol 2017;56:38-49.
(3) Vij N, Chandramani-Shivalingappa P, Van Westphal C, Hole R, Bodas M. Cigarette smoke-induced autophagy impairment accelerates lung aging, COPD-emphysema exacerbations and pathogenesis. Am J Physiol Cell Physiol 2018;314:C73-C87.
(4) Araya J, Tsubouchi K, Sato N, Ito S, Minagawa S, Hara H, Hosaka Y, Ichikawa A, Saito N, Kadota T, Yoshida M, Fujita Y, Utsumi H, Kobayashi K, Yanagisawa H, Hashimoto M, Wakui H, Ishikawa T, Numata T, Kaneko Y, Asano H, Yamashita M, Odaka M, Morikawa T, Nishimura SL, Nakayama K, Kuwano K. PRKN-regulated mitophagy and cellular senescence during COPD pathogenesis. Autophagy 2019;15:510-526.
(5) Bozier J, Chivers EK, Chapman DG, Larcombe AN, Bastian NA, Masso-Silva JA, Byun MK, McDonald CF, Crotty Alexander LE, Ween MP. The Evolving Landscape of e-Cigarettes: A Systematic Review of Recent Evidence. Chest 2020;157:1362-1390.
(6) Merecz-Sadowska A, Sitarek P, Zielinska-Blizniewska H, Malinowska K, Zajdel K, Zakonnik L, Zajdel R. A Summary of In Vitro and In Vivo Studies Evaluating the Impact of E-Cigarette Exposure on Living Organisms and the Environment. Int J Mol Sci 2020;21:10.3390/ijms21020652.
(7) Chan YL, Wang B, Chen H, Ho KF, Cao J, Hai G, Jalaludin B, Herbert C, Thomas PS, Saad S, Oliver BGG. Pulmonary inflammation induced by low-dose particulate matter exposure in mice. Am J Physiol Lung Cell Mol Physiol 2019;317:L424-L430.
(8) Cattani-Cavalieri I, Valenca SS, Lanzetti M, Carvalho GMC, Zin WA, Monte-Alto-Costa A, Porto LC, Romana-Souza B. Acute Exposure to Diesel-Biodiesel Particulate Matter Promotes Murine Lung Oxidative Stress by Nrf2/HO-1 and Inflammation Through the NF-kB/TNF-alpha Pathways. Inflammation 2019;42:526-537.
(9) Williams KM, Franzi LM, Last JA. Cell-specific oxidative stress and cytotoxicity after wildfire coarse particulate matter instillation into mouse lung. Toxicol Appl Pharmacol 2013;266:48-55.
(10) Gao ZX, Song XL, Li SS, Lai XR, Yang YL, Yang G, Li ZJ, Cui YH, Pan HW. Assessment of DNA Damage and Cell Senescence in Corneal Epithelial Cells Exposed to Airborne Particulate Matter (PM2.5) Collected in Guangzhou, China. Invest Ophthalmol Vis Sci 2016;57:3093-3102.
(11) Ryu YS, Kang KA, Piao MJ, Ahn MJ, Yi JM, Bossis G, Hyun YM, Park CO, Hyun JW. Particulate matter-induced senescence of skin keratinocytes involves oxidative stress-dependent epigenetic modifications. Exp Mol Med 2019;51:1-14.
(12) De Boer WI, van Schadewijk A, Sont JK, Sharma HS, Stolk J, Hiemstra PS, Van Krieken JH. Transforming growth factor beta1 and recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998;158:1951-1957.
(13) Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M, Okada Y, Yamasawa F, Nakahara K, Umeda A. Increased expression of transforming growth factor-beta1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). Am J Respir Crit Care Med 2001;163:1476-1483.
(14) Minagawa S, Araya J, Numata T, Nojiri S, Hara H, Yumino Y, Kawaishi M, Odaka M, Morikawa T, Nishimura SL, Nakayama K, Kuwano K. Accelerated epithelial cell senescence in IPF and the inhibitory role of SIRT6 in TGF-beta-induced senescence of human bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 2011;300:L391-401.
(15) Alvarez D, Cardenes N, Sellares J, Bueno M, Corey C, Hanumanthu VS, Peng Y, D'Cunha H, Sembrat J, Nouraie M, Shanker S, Caufield C, Shiva S, Armanios M, Mora AL, Rojas M. IPF lung fibroblasts have a senescent phenotype. Am J Physiol Lung Cell Mol Physiol 2017;313:L1164-L1173.
(16) Prata LGPL, Ovsyannikova IG, Tchkonia T, Kirkland JL. Senescent cell clearance by the immune system: Emerging therapeutic opportunities. Semin Immunol 2018;40:101275.
(17) Song P, An J, Zou MH. Immune Clearance of Senescent Cells to Combat Ageing and Chronic Diseases. Cells 2020;9:10.3390/cells9030671.

(18) Di Stefano A, Turato G, Maestrelli P, Mapp CE, Ruggieri MP, Roggeri A, Boschetto P, Fabbri LM, Saetta M. Airflow limitation in chronic bronchitis is associated with T-lymphocyte and macrophage infiltration of the bronchial mucosa. Am J Respir Crit Care Med 1996;153:629-632.
(19) Bazzan E, Turato G, Tine M, Radu CM, Balestro E, Rigobello C, Biondini D, Schiavon M, Lunardi F, Baraldo S, Rea F, Simioni P, Calabrese F, Saetta M, Cosio MG. Dual polarization of human alveolar macrophages progressively increases with smoking and COPD severity. Respir Res 2017;18:40-017-0522-0.
(20) Traves SL, Culpitt SV, Russell RE, Barnes PJ, Donnelly LE. Increased levels of the chemokines GROalpha and MCP-1 in sputum samples from patients with COPD. Thorax 2002;57:590-595.
(21) Capelli A, Di Stefano A, Gnemmi I, Balbo P, Cerutti CG, Balbi B, Lusuardi M, Donner CF. Increased MCP-1 and MIP-1beta in bronchoalveolar lavage fluid of chronic bronchitics. Eur Respir J 1999;14:160-165.
(22) Prieto A, Reyes E, Bernstein ED, Martinez B, Monserrat J, Izquierdo JL, Callol L, de LUCAS P, Alvarez-Sala R, Alvarez-Sala JL, Villarrubia VG, Alvarez-Mon M. Defective natural killer and phagocytic activities in chronic obstructive pulmonary disease are restored by glycophosphopeptical (inmunoferon). Am J Respir Crit Care Med 2001;163:1578-1583.
(23) Hodge S, Hodge G, Scicchitano R, Reynolds PN, Holmes M. Alveolar macrophages from subjects with chronic obstructive pulmonary disease are deficient in their ability to phagocytose apoptotic airway epithelial cells. Immunol Cell Biol 2003;81:289-296.
(24) Taylor AE, Finney-Hayward TK, Quint JK, Thomas CM, Tudhope SJ, Wedzicha JA, Barnes PJ, Donnelly LE. Defective macrophage phagocytosis of bacteria in COPD. Eur Respir J 2010;35:1039-1047.
(25) Berenson CS, Kruzel RL, Eberhardt E, Sethi S. Phagocytic dysfunction of human alveolar macrophages and severity of chronic obstructive pulmonary disease. J Infect Dis 2013;208:2036-2045.
(26) Tomita K, Caramori G, Lim S, Ito K, Hanazawa T, Oates T, Chiselita I, Jazrawi E, Chung KF, Barnes PJ, Adcock IM. Increased p21(CIP1/WAF1) and B cell lymphoma leukemia-x(L) expression and reduced apoptosis in alveolar macrophages from smokers. Am J Respir Crit Care Med 2002;166:724-731.
(27) Coppe JP, Patil CK, Rodier F, Sun Y, Munoz DP, Goldstein J, Nelson PS, Desprez PY, Campisi J. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 2008;6:2853-2868.
(28) Demaria M, Ohtani N, Youssef SA, Rodier F, Toussaint W, Mitchell JR, Laberge RM, Vijg J, Van Steeg H, Dolle ME, Hoeijmakers JH, de Bruin A, Hara E, Campisi J. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev Cell 2014;31:722-733.
(29) Munoz-Espin D, Serrano M. Cellular senescence: from physiology to pathology. Nat Rev Mol Cell Biol 2014;15:482-496.
(30) He S, Sharpless NE. Senescence in Health and Disease. Cell 2017;169:1000-1011.
(31) Millis AJ, Hoyle M, McCue HM, Martini H. Differential expression of metalloproteinase and tissue inhibitor of metalloproteinase genes in aged human fibroblasts. Exp Cell Res 1992;201:373-379.
(32) Jun JI, Lau LF. The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. Nat Cell Biol 2010;12:676-685.
(33) Ezure T, Sugahara M, Amano S. Senescent dermal fibroblasts negatively influence fibroblast extracellular matrix-related gene expression partly via secretion of complement factor D. Biofactors 2019;45:556-562.
(34) Mancini M, Saintigny G, Mahe C, Annicchiarico-Petruzzelli M, Melino G, Candi E. MicroRNA-152 and -181a participate in human dermal fibroblasts senescence acting on cell adhesion and remodeling of the extra-cellular matrix. Aging (Albany NY) 2012;4:843-853.
(35) Mellone M, Hanley CJ, Thirdborough S, Mellows T, Garcia E, Woo J, Tod J, Frampton S, Jenei V, Moutasim KA, Kabir TD, Brennan PA, Venturi G, Ford K, Herranz N, Lim KP, Clarke J, Lambert DW, Prime SS, Underwood TJ, Vijayanand P, Eliceiri KW, Woelk C, King EV, Gil J, Ottensmeier CH, Thomas GJ. Induction of fibroblast senescence generates a non-fibrogenic myofibroblast phenotype that differentially impacts on cancer prognosis. Aging (Albany NY) 2016;9:114-132.
(36) Lago JC, Puzzi MB. The effect of aging in primary human dermal fibroblasts. PLoS One 2019;14:e0219165.
(37) Basisty N, Kale A, Jeon OH, Kuehnemann C, Payne T, Rao C, Holtz A, Shah S, Sharma V, Ferrucci L, Campisi J, Schilling B. A proteomic atlas of senescence-associated secretomes for aging biomarker development. PLoS Biol 2020;18:e3000599.

(38) Yasumitsu H, Miyazaki K, Umenishi F, Koshikawa N, Umeda M. Comparison of extracellular matrix-degrading activities between 64-kDa and 90-kDa gelatinases purified in inhibitor-free forms from human schwannoma cells. J Biochem 1992;111:74-80.
(39) Van den Steen PE, Proost P, Brand DD, Kang AH, Van Damme J, Opdenakker G. Generation of glycosylated remnant epitopes from human collagen type II by gelatinase B. Biochemistry 2004;43:10809-10816.
(40) Imai K, Hiramatsu A, Fukushima D, Pierschbacher MD, Okada Y. Degradation of decorin by matrix metalloproteinases: identification of the cleavage sites, kinetic analyses and transforming growth factor-beta1 release. Biochem J 1997;322 (Pt 3):809-814.
(41) Tanaka T, Terai Y, Ohmichi M. Association of matrix metalloproteinase-9 and decorin expression with the infiltration of cervical cancer. Oncol Lett 2019;17:1306-1312.
(42) Adibhatla RM, Hatcher JF. Tissue plasminogen activator (tPA) and matrix metalloproteinases in the pathogenesis of stroke: therapeutic strategies. CNS Neurol Disord Drug Targets 2008;7:243-253.
(43) Chung KF. Cytokines in chronic obstructive pulmonary disease. Eur Respir J Suppl 2001;34:50s-59s.
(44) Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 2016;138:16-27.
(45) Henrot P, Prevel R, Berger P, Dupin I. Chemokines in COPD: From Implication to Therapeutic Use. Int J Mol Sci 2019;20:10.3390/ijms20112785.
(46) Hubackova S, Krejcikova K, Bartek J, Hodny Z. IL1- and TGFbeta-Nox4 signaling, oxidative stress and DNA damage response are shared features of replicative, oncogene-induced, and drug-induced paracrine 'bystander senescence'. Aging (Albany NY) 2012;4:932-951.
(47) Alessio N, Aprile D, Squillaro T, Di Bernardo G, Finicelli M, Melone MA, Peluso G, Galderisi U. The senescence-associated secretory phenotype (SASP) from mesenchymal stromal cells impairs growth of immortalized prostate cells but has no effect on metastatic prostatic cancer cells. Aging (Albany NY) 2019;11:5817-5828.
(48) Acosta JC, Banito A, Wuestefeld T, Georgilis A, Janich P, Morton JP, Athineos D, Kang TW, Lasitschka F, Andrulis M, Pascual G, Morris KJ, Khan S, Jin H, Dharmalingam G, Snijders AP, Carroll T, Capper D, Pritchard C, Inman GJ, Longerich T, Sansom OJ, Benitah SA, Zender L, Gil J. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. Nat Cell Biol 2013;15:978-990.
(49) da Silva PFL, Ogrodnik M, Kucheryavenko O, Glibert J, Miwa S, Cameron K, Ishaq A, Saretzki G, Nagaraja-Grellscheid S, Nelson G, von Zglinicki T. The bystander effect contributes to the accumulation of senescent cells in vivo. Aging Cell 2019;18:e12848.
(50) Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proc Natl Acad Sci U S A 2001;98:12072-12077.
(51) Parrinello S, Coppe JP, Krtolica A, Campisi J. Stromal-epithelial interactions in aging and cancer: senescent fibroblasts alter epithelial cell differentiation. J Cell Sci 2005;118:485-496.
(52) Bavik C, Coleman I, Dean JP, Knudsen B, Plymate S, Nelson PS. The gene expression program of prostate fibroblast senescence modulates neoplastic epithelial cell proliferation through paracrine mechanisms. Cancer Res 2006;66:794-802.
(53) Tsai KK, Chuang EY, Little JB, Yuan ZM. Cellular mechanisms for low-dose ionizing radiation-induced perturbation of the breast tissue microenvironment. Cancer Res 2005;65:6734-6744.
(54) Liu D, Hornsby PJ. Senescent human fibroblasts increase the early growth of xenograft tumors via matrix metalloproteinase secretion. Cancer Res 2007;67:3117-3126.
(55) Farsam V, Basu A, Gatzka M, Treiber N, Schneider LA, Mulaw MA, Lucas T, Kochanek S, Dummer R, Levesque MP, Wlaschek M, Scharffetter-Kochanek K. Senescent fibroblast-derived Chemerin promotes squamous cell carcinoma migration. Oncotarget 2016;7:83554-83569.
(56) Coppe JP, Kauser K, Campisi J, Beausejour CM. Secretion of vascular endothelial growth factor by primary human fibroblasts at senescence. J Biol Chem 2006;281:29568-29574.
(57) Gonzalez-Meljem JM, Martinez-Barbera JP. Senescence drives non-cell autonomous tumorigenesis in the pituitary gland. Mol Cell Oncol 2018;5:e1435180.
(58) Laberge RM, Awad P, Campisi J, Desprez PY. Epithelial-mesenchymal transition induced by senescent fibroblasts. Cancer Microenviron 2012;5:39-44.
(59) Tsuji T, Aoshiba K, Nagai A. Alveolar cell senescence in patients with pulmonary emphysema. Am J Respir Crit Care Med 2006;174:886-893.

(60) Zhang J, Wu L, Qu JM, Bai CX, Merrilees MJ, Black PN. Pro-inflammatory phenotype of COPD fibroblasts not compatible with repair in COPD lung. J Cell Mol Med 2012;16:1522-1532.
(61) Ishii T, Gemma A, Kida K. Senescence is involved in the pathogenesis of chronic obstructive pulmonary disease through effects on telomeres and the anti-aging molecule fibroblast growth factor 23. Geriatr Gerontol Int 2015;15:827-833.
(62) Rutten EP, Gopal P, Wouters EF, Franssen FM, Hageman GJ, Vanfleteren LE, Spruit MA, Reynaert NL. Various Mechanistic Pathways Representing the Aging Process Are Altered in COPD. Chest 2016;149:53-61.
(63) Rahman I, van Schadewijk AA, Crowther AJ, Hiemstra PS, Stolk J, MacNee W, De Boer WI. 4-Hydroxy-2-nonenal, a specific lipid peroxidation product, is elevated in lungs of patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2002;166:490-495.
(64) Mitani A, Ito K, Vuppusetty C, Barnes PJ, Mercado N. Restoration of Corticosteroid Sensitivity in Chronic Obstructive Pulmonary Disease by Inhibition of Mammalian Target of Rapamycin. Am J Respir Crit Care Med 2016;193:143-153.
(65) Min T, Bodas M, Mazur S, Vij N. Critical role of proteostasis-imbalance in pathogenesis of COPD and severe emphysema. J Mol Med (Berl) 2011;89:577-593.
(66) Lee J, Reddy R, Barsky L, Scholes J, Chen H, Shi W, Driscoll B. Lung alveolar integrity is compromised by telomere shortening in telomerase-null mice. Am J Physiol Lung Cell Mol Physiol 2009;296:L57-70.
(67) Birch J, Anderson RK, Correia-Melo C, Jurk D, Hewitt G, Marques FM, Green NJ, Moisey E, Birrell MA, Belvisi MG, Black F, Taylor JJ, Fisher AJ, De Soyza A, Passos JF. DNA damage response at telomeres contributes to lung aging and chronic obstructive pulmonary disease. Am J Physiol Lung Cell Mol Physiol 2015;309:L1124-37.
(68) Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R, Nabeshima YI. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 1997;390:45-51.
(69) Iizuka T, Ishii Y, Itoh K, Kiwamoto T, Kimura T, Matsuno Y, Morishima Y, Hegab AE, Homma S, Nomura A, Sakamoto T, Shimura M, Yoshida A, Yamamoto M, Sekizawa K. Nrf2-deficient mice are highly susceptible to cigarette smoke-induced emphysema. Genes Cells 2005;10:1113-1125.
(70) Yao H, Chung S, Hwang JW, Rajendrasozhan S, Sundar IK, Dean DA, McBurney MW, Guarente L, Gu W, Ronty M, Kinnula VL, Rahman I. SIRT1 protects against emphysema via FOXO3-mediated reduction of premature senescence in mice. J Clin Invest 2012;122:2032-2045.
(71) Maselli DJ, Bhatt SP, Anzueto A, Bowler RP, DeMeo DL, Diaz AA, Dransfield MT, Fawzy A, Foreman MG, Hanania NA, Hersh CP, Kim V, Kinney GL, Putcha N, Wan ES, Wells JM, Westney GE, Young KA, Silverman EK, Han MK, Make BJ. Clinical Epidemiology of COPD: Insights From 10 Years of the COPDGene Study. Chest 2019;156:228-238.
(72) Siedlinski M, Tingley D, Lipman PJ, Cho MH, Litonjua AA, Sparrow D, Bakke P, Gulsvik A, Lomas DA, Anderson W, Kong X, Rennard SI, Beaty TH, Hokanson JE, Crapo JD, Lange C, Silverman EK, COPDGene and ECLIPSE Investigators. Dissecting direct and indirect genetic effects on chronic obstructive pulmonary disease (COPD) susceptibility. Hum Genet 2013;132:431-441.
(73) Lo Tam Loi AT, Hoonhorst SJ, Franciosi L, Bischoff R, Hoffmann RF, Heijink I, van Oosterhout AJ, Boezen HM, Timens W, Postma DS, Lammers JW, Koenderman L, Ten Hacken NH. Acute and chronic inflammatory responses induced by smoking in individuals susceptible and non-susceptible to development of COPD: from specific disease phenotyping towards novel therapy. Protocol of a cross-sectional study. BMJ Open 2013;3:10.1136/bmjopen-2012-002178. Print 2013.
(74) Hoonhorst SJ, Timens W, Koenderman L, Lo Tam Loi AT, Lammers JW, Boezen HM, van Oosterhout AJ, Postma DS, Ten Hacken NH. Increased activation of blood neutrophils after cigarette smoking in young individuals susceptible to COPD. Respir Res 2014;15:121-014-0121-2.
(75) Hogg JC, Timens W. The pathology of chronic obstructive pulmonary disease. Annu Rev Pathol 2009;4:435-459.
(76) Ogrodnik M, Salmonowicz H, Gladyshev VN. Integrating cellular senescence with the concept of damage accumulation in aging: Relevance for clearance of senescent cells. Aging Cell 2019;18:e12841.
(77) Nyunoya T, Monick MM, Klingelhutz A, Yarovinsky TO, Cagley JR, Hunninghake GW. Cigarette smoke induces cellular senescence. Am J Respir Cell Mol Biol 2006;35:681-688.

(78) Fujii S, Hara H, Araya J, Takasaka N, Kojima J, Ito S, Minagawa S, Yumino Y, Ishikawa T, Numata T, Kawaishi M, Hirano J, Odaka M, Morikawa T, Nishimura S, Nakayama K, Kuwano K. Insufficient autophagy promotes bronchial epithelial cell senescence in chronic obstructive pulmonary disease. Oncoimmunology 2012;1:630-641.
(79) Hara H, Araya J, Ito S, Kobayashi K, Takasaka N, Yoshii Y, Wakui H, Kojima J, Shimizu K, Numata T, Kawaishi M, Kamiya N, Odaka M, Morikawa T, Kaneko Y, Nakayama K, Kuwano K. Mitochondrial fragmentation in cigarette smoke-induced bronchial epithelial cell senescence. Am J Physiol Lung Cell Mol Physiol 2013;305:L737-46.
(80) Takasaka N, Araya J, Hara H, Ito S, Kobayashi K, Kurita Y, Wakui H, Yoshii Y, Yumino Y, Fujii S, Minagawa S, Tsurushige C, Kojima J, Numata T, Shimizu K, Kawaishi M, Kaneko Y, Kamiya N, Hirano J, Odaka M, Morikawa T, Nishimura SL, Nakayama K, Kuwano K. Autophagy induction by SIRT6 through attenuation of insulin-like growth factor signaling is involved in the regulation of human bronchial epithelial cell senescence. J Immunol 2014;192:958-968.
(81) Tuder RM, Yoshida T, Fijalkowka I, Biswal S, Petrache I. Role of lung maintenance program in the heterogeneity of lung destruction in emphysema. Proc Am Thorac Soc 2006;3:673-679.
(82) Barbera JA, Peinado VI. Disruption of the lung structure maintenance programme: a comprehensive view of emphysema development. Eur Respir J 2011;37:752-754.
(83) Agusti A, Faner R. Chronic Obstructive Pulmonary Disease Pathogenesis. Clin Chest Med 2020;41:307-314.
(84) Agusti A, Hogg JC. Update on the Pathogenesis of Chronic Obstructive Pulmonary Disease. N Engl J Med 2019;381:1248-1256.
(85) Salvi SS, Barnes PJ. Chronic obstructive pulmonary disease in non-smokers. Lancet 2009;374:733-743.
(86) Bodas M, Patel N, Silverberg D, Walworth K, Vij N. Master Autophagy Regulator Transcription Factor EB Regulates Cigarette Smoke-Induced Autophagy Impairment and Chronic Obstructive Pulmonary Disease-Emphysema Pathogenesis. Antioxid Redox Signal 2017;27:150-167.
(87) Barnes PJ. Oxidative stress-based therapeutics in COPD. Redox Biol 2020;33:101544.
(88) Mercken EM, Mitchell SJ, Martin-Montalvo A, Minor RK, Almeida M, Gomes AP, Scheibye-Knudsen M, Palacios HH, Licata JJ, Zhang Y, Becker KG, Khraiwesh H, Gonzalez-Reyes JA, Villalba JM, Baur JA, Elliott P, Westphal C, Vlasuk GP, Ellis JL, Sinclair DA, Bernier M, de Cabo R. SRT2104 extends survival of male mice on a standard diet and preserves bone and muscle mass. Aging Cell 2014;13:787-796.
(89) Mitchell SJ, Martin-Montalvo A, Mercken EM, Palacios HH, Ward TM, Abulwerdi G, Minor RK, Vlasuk GP, Ellis JL, Sinclair DA, Dawson J, Allison DB, Zhang Y, Becker KG, Bernier M, de Cabo R. The SIRT1 activator SRT1720 extends lifespan and improves health of mice fed a standard diet. Cell Rep 2014;6:836-843.
(90) Gu C, Li Y, Xu WL, Yan JP, Xia YJ, Ma YY, Chen C, Wang HJ, Tao HQ. Sirtuin 1 Activator SRT1720 Protects Against Lung Injury via Reduction of Type II Alveolar Epithelial Cells Apoptosis in Emphysema. COPD 2015;12:444-452.
(91) Lamming DW, Ye L, Sabatini DM, Baur JA. Rapalogs and mTOR inhibitors as anti-aging therapeutics. J Clin Invest 2013;123:980-989.
(92) Houssaini A, Breau M, Kebe K, Abid S, Marcos E, Lipskaia L, Rideau D, Parpaleix A, Huang J, Amsellem V, Vienney N, Validire P, Maitre B, Attwe A, Lukas C, Vindrieux D, Boczkowski J, Derumeaux G, Pende M, Bernard D, Meiners S, Adnot S. mTOR pathway activation drives lung cell senescence and emphysema. JCI Insight 2018;3:10.1172/jci.insight.93203. eCollection 2018 Feb 8.
(93) Yen FS, Chen W, Wei JC, Hsu CC, Hwu CM. Effects of metformin use on total mortality in patients with type 2 diabetes and chronic obstructive pulmonary disease: A matched-subject design. PLoS One 2018;13:e0204859.
(94) Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL, van Deursen JM. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 2011;479:232-236.
(95) Zhu Y, Tchkonia T, Fuhrmann-Stroissnigg H, Dai HM, Ling YY, Stout MB, Pirtskhalava T, Giorgadze N, Johnson KO, Giles CB, Wren JD, Niedernhofer LJ, Robbins PD, Kirkland JL. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. Aging Cell 2016;15:428-435.
(96) Kirkland JL, Tchkonia T. Cellular Senescence: A Translational Perspective. EBioMedicine 2017;21:21-28.
(97) Kirkland JL, Tchkonia T, Zhu Y, Niedernhofer LJ, Robbins PD. The Clinical Potential of Senolytic Drugs. J Am Geriatr Soc 2017;65:2297-2301.
(98) Hashimoto M, Asai A, Kawagishi H, Mikawa R, Iwashita Y, Kanayama K, Sugimoto K, Sato T, Maruyama M, Sugimoto M. Elimination of p19(ARF)-expressing cells enhances pulmonary function in mice. JCI Insight 2016;1:e87732.

(99) Baar MP, Brandt RMC, Putavet DA, Klein JDD, Derks KWJ, Bourgeois BRM, Stryeck S, Rijksen Y, van Willigenburg H, Feijtel DA, van der Pluijm I, Essers J, van Cappellen WA, van IJcken WF, Houtsmuller AB, Pothof J, de Bruin RWF, Madl T, Hoeijmakers JHJ, Campisi J, de Keizer PLJ. Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging. Cell 2017;169:132-147.e16.
(100) Fuhrmann-Stroissnigg H, Ling YY, Zhao J, McGowan SJ, Zhu Y, Brooks RW, Grassi D, Gregg SQ, Stripay JL, Dorronsoro A, Corbo L, Tang P, Bukata C, Ring N, Giacca M, Li X, Tchkonia T, Kirkland JL, Niedernhofer LJ, Robbins PD. Identification of HSP90 inhibitors as a novel class of senolytics. Nat Commun 2017;8:422-017-00314-z.
(101) Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weivoda MM, Inman CL, Ogrodnik MB, Hachfeld CM, Fraser DG, Onken JL, Johnson KO, Verzosa GC, Langhi LGP, Weigl M, Giorgadze N, LeBrasseur NK, Miller JD, Jurk D, Singh RJ, Allison DB, Ejima K, Hubbard GB, Ikeno Y, Cubro H, Garovic VD, Hou X, Weroha SJ, Robbins PD, Niedernhofer LJ, Khosla S, Tchkonia T, Kirkland JL. Senolytics improve physical function and increase lifespan in old age. Nat Med 2018;24:1246-1256.
(102) Mikawa R, Suzuki Y, Baskoro H, Kanayama K, Sugimoto K, Sato T, Sugimoto M. Elimination of p19(ARF) - expressing cells protects against pulmonary emphysema in mice. Aging Cell 2018;17:e12827.
(103) Barnes PJ, Baker J, Donnelly LE. Cellular Senescence as a Mechanism and Target in Chronic Lung Diseases. Am J Respir Crit Care Med 2019;200:556-564.
(104) Amor C, Feucht J, Leibold J, Ho YJ, Zhu C, Alonso-Curbelo D, Mansilla-Soto J, Boyer JA, Li X, Giavridis T, Kulick A, Houlihan S, Peerschke E, Friedman SL, Ponomarev V, Piersigilli A, Sadelain M, Lowe SW. Senolytic CAR T cells reverse senescence-associated pathologies. Nature 2020;583:127-132.
(105) Schafer MJ, White TA, Iijima K, Haak AJ, Ligresti G, Atkinson EJ, Oberg AL, Birch J, Salmonowicz H, Zhu Y, Mazula DL, Brooks RW, Fuhrmann-Stroissnigg H, Pirtskhalava T, Prakash YS, Tchkonia T, Robbins PD, Aubry MC, Passos JF, Kirkland JL, Tschumperlin DJ, Kita H, LeBrasseur NK. Cellular senescence mediates fibrotic pulmonary disease. Nat Commun 2017;8:14532.
(106) Lehmann M, Korfei M, Mutze K, Klee S, Skronska-Wasek W, Alsafadi HN, Ota C, Costa R, Schiller HB, Lindner M, Wagner DE, Gunther A, Konigshoff M. Senolytic drugs target alveolar epithelial cell function and attenuate experimental lung fibrosis ex vivo. Eur Respir J 2017;50:10.1183/13993003.02367-2016. Print 2017 Aug.
(107) Justice JN, Nambiar AM, Tchkonia T, LeBrasseur NK, Pascual R, Hashmi SK, Prata L, Masternak MM, Kritchevsky SB, Musi N, Kirkland JL. Senolytics in idiopathic pulmonary fibrosis: Results from a first-in-human, open-label, pilot study. EBioMedicine 2019;40:554-563.
(108) Birch J, Barnes PJ, Passos JF. Mitochondria, telomeres and cell senescence: Implications for lung ageing and disease. Pharmacol Ther 2018;183:34-49.
(109) Laberge RM, Zhou L, Sarantos MR, Rodier F, Freund A, de Keizer PL, Liu S, Demaria M, Cong YS, Kapahi P, Desprez PY, Hughes RE, Campisi J. Glucocorticoids suppress selected components of the senescence-associated secretory phenotype. Aging Cell 2012;11:569-578.
(110) Baker JR, Donnelly LE, Barnes PJ. Senotherapy: A New Horizon for COPD Therapy. Chest 2020;158:562-570.
(111) Navarro S, Driscoll B. Regeneration of the Aging Lung: A Mini-Review. Gerontology 2017;63:270-280.
(112) Kruk DMLW, Heijink IH, Slebos DJ, Timens W, Ten Hacken NH. Mesenchymal Stromal Cells to Regenerate Emphysema: On the Horizon? Respiration 2018;96:148-158.
(113) Behnke J, Kremer S, Shahzad T, Chao CM, Bottcher-Friebertshauser E, Morty RE, Bellusci S, Ehrhardt H. MSC Based Therapies-New Perspectives for the Injured Lung. J Clin Med 2020;9:10.3390/jcm9030682.