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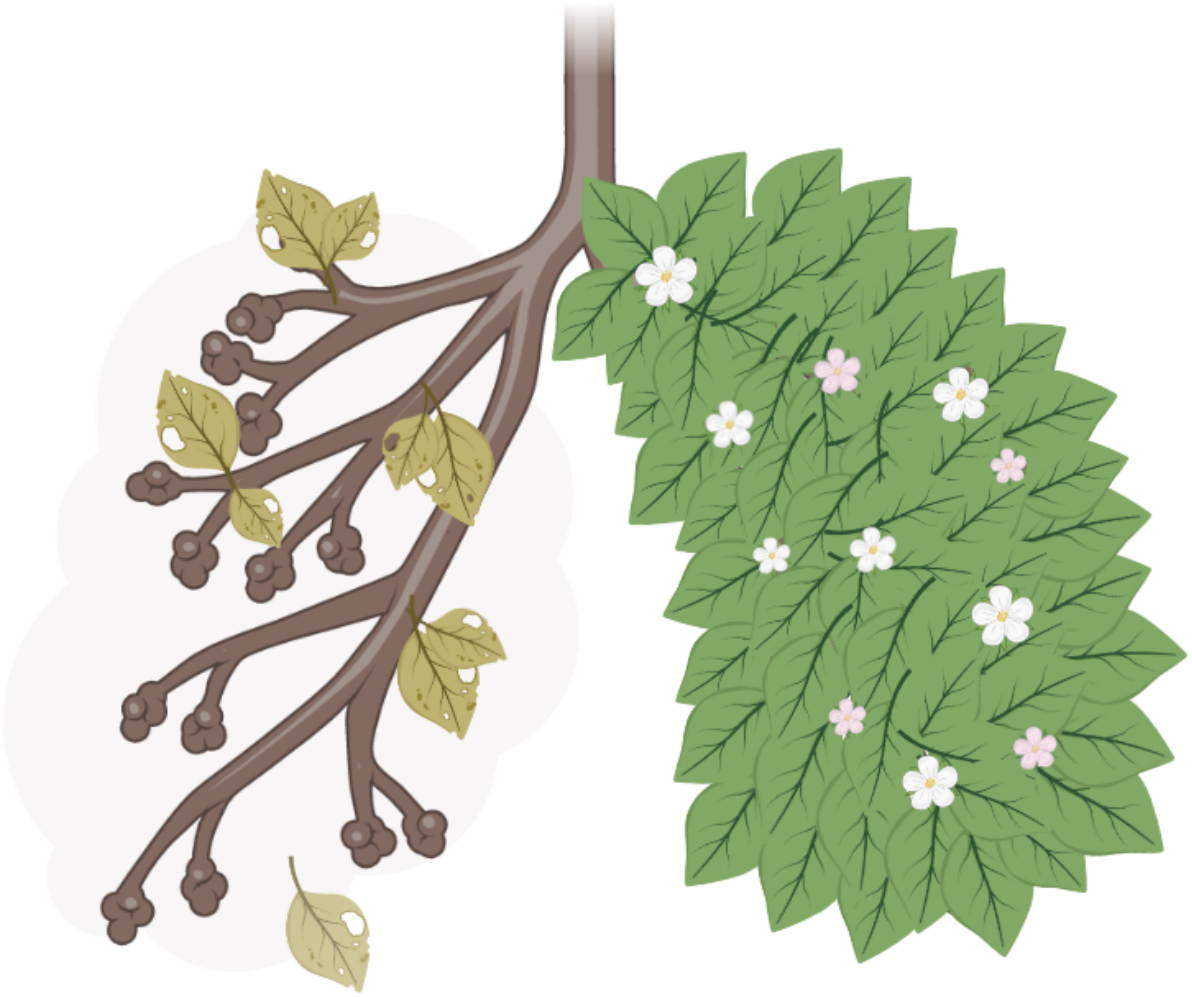
Accelerated lung aging in COPD: finding potential anti-aging therapies



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Abstract

As of this moment, the human society has reached an age that is higher than has ever been shown in history. Age-related diseases are increasing and so is the evidence for the relationship between chronic inflammatory diseases and aging. One of the most common causes of mortality and morbidity worldwide is the lung disease Chronic Obstructive Pulmonary Disease (COPD). COPD is an irreversible and progressive chronic inflammatory lung disease which affects millions of people worldwide. The disease is characterized by chronic bronchitis and emphysema and major risk factors for the progression and initiation of COPD are cigarette smoke and aging. Several studies showed that the pathogenesis of COPD is associated with an accelerated form of aging in the lung.

Importantly, current therapy options are not sufficient since they mostly focus on treating the symptoms of COPD rather than preventing the onset or progression of the disease. Thus, understanding the pathomechanisms related to aging and its association to COPD is critical for discovering novel treatment strategies. There are several hallmarks of aging which are associated with the accelerated lung aging shown in COPD. Targeting these hallmarks by finding anti-aging therapies is hypothesized to reduce or even prevent the development and progression of COPD. Therefore, the aim of this thesis is targeting hallmarks of accelerated lung aging in COPD: finding potential anti-aging therapies. Functional and cellular changes in the normal aging lung showed a lot of similarity with the COPD lung. Additionally, hallmarks of accelerated lung aging were shown in the COPD lungs, whereas the most important ones were oxidative stress, telomere attrition, stem cell exhaustion and cellular senescence.

Three anti-aging molecules were found to be reduced in COPD; klotho, SMP-30 and SIRT. Klotho and SMP-30 knockout mice showed these proteins play an important role in the pathogenesis of COPD and are therefore considered to become promising therapeutic targets for preventing and/or reducing COPD pathogenesis. Moreover, reduced levels of SIRT1 and SIRT6 were also associated with several hallmarks of accelerated lung aging in COPD. Activating SIRT1 via pharmacologic agents' resveratrol and sRT1720 in preclinical studies showed potential for becoming a promising anti-aging treatment option in COPD. Additionally, current anti-aging treatments; antioxidant therapy, stem cell regenerative therapy and senolytics showed promising outcomes in pre-clinical studies for targeting hallmarks of accelerated lung aging in COPD. However, almost no clinical studies investigated the effects of these anti-aging therapies in COPD patients and little clinical studies that did investigate the effects showed no beneficial effects for the pathogenesis of COPD. In addition, some therapies were associated with serious side effects.

In conclusion, despite the many advances shown in pre-clinical studies on the anti-aging molecules and treatments discussed in this thesis, more research is required to draw a conclusion on which anti-aging therapy is the most promising for reducing or preventing the development of COPD in the aging society. Moreover, current pharmacological targets need modifications and extensive examination in pre-clinical and clinical studies to be used safely and protect the health of the aging COPD lung.

Nomenclature

ASL	=	Airway Surface Liquid
COPD	=	Chronic Obstructive Pulmonary Disease
FEV1	=	Forced Expiratory Volume in one second
FOXO3	=	Transcription Factor Forkhead Box O3
GSH	=	Glutathione
IPF	=	Idiopathic Pulmonary Fibrosis
$KL^{-/-}$	=	Homozygous mutant klotho mice
$KL^{-/+}$	=	Heterozygous mutant klotho mice
MAPK	=	Mitogen-Activated Protein Kinase
MSCs	=	Mesenchymal Stem Cells
NAD^{+}	=	Nicotinamide Adenine Dinucleotide
NF-κB	=	Nuclear Factor-κB
Nrf2	=	Nuclear Factor Erytroid-2-Related Factor 2
ROS	=	Reactive Oxygen Species
SASP	=	Senescence-Associated Secretory Phenotype
SIRT	=	Sirtuin
SMP-30	=	Senescence Marker Protein-30
SMP30Y/-	=	Knock-out SMP-30 mouse model
SMP30Y/+	=	Wild-type SMP-30 mouse model
SOD	=	Superoxide Dismutase

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1. Introduction

Healthy aging is a very popular subject discussed in the recent years. As of this moment, the human society has reached an age that is higher than has ever been shown in history (Schneider et al., 2021). When the reproductive phase of human life is completed, homeostasis in the human body undergoes a progressive decline which is defined as aging. Subsequently, this decline provides susceptibility to diseases or even death. One of the causes of disease and death are the lack of success of organs to repair shortening of telomeres upon constant cell division, which is a programmed form of aging, and by failure to repair damage in DNA caused by oxidative stress, which is a nonprogrammed form of aging (Ito & Barnes, 2009). The aging society is growing, and so are aging-related diseases. By understanding the biological sequences of events in age-related diseases, this might provide targets to manipulate biological processes which delay, or even cure these diseases (Schneider et al., 2021). Overall, evidence for the relationship between chronic inflammatory diseases and aging is increasing (Ito & Barnes, 2009).

One of the most common causes of mortality and morbidity worldwide is the lung disease Chronic Obstructive Pulmonary Disease (COPD). COPD is an irreversible and progressive chronic inflammatory lung disease which affects millions of people worldwide (Varmaghani et al., 2019). Major risk factors for the progression and initiation of COPD are cigarette smoke and aging (Moriyama et al., 2010). The disease is characterized by emphysema, defined as the enlargement of air spaces in the lung with destruction of alveolar walls, and by chronic bronchitis which is defined as chronic inflammation of the larger airways accompanied by overproduction of mucus. (Goldklang & Stockley, 2016; Kim & Criner, 2013). The progression of the disease is very slowly and therefore mostly elderly are affected by the disease (Ito & Barnes, 2009). Moreover, remodeling of the small airways, enlargement of the alveolar spaces, hypersecretion of mucus, chronic airway inflammation and accelerated aging of the lung are important characteristics shown to contribute to the pathology and clinical symptoms of COPD. Interestingly, these characteristics and pathological changes show similarities with pathogenesis of the normal aging lung (John-Schuster et al., 2016; Mercado et al., 2015). Besides, there is growing evidence that the susceptibility for the pathogenesis of COPD is enhanced by age-associated functional and structural changes in the lungs (Fukuchi, 2009) (John-Schuster et al., 2016).

Several studies showed that the pathogenesis of COPD is associated with an accelerated form of aging in the lung. (Mercado et al., 2015). Moreover, it is suggested that cigarette smoke worsens structural and functional changes in the lung, by reducing molecules involved in anti-aging enhancing the accelerated development of COPD even more (Ito & Barnes, 2009; Mercado et al., 2015). However, it should be noted that not all smokers develop COPD, indicating other factors might also contribute to the development of COPD such as genetics, air pollution and more (Terzikhan et al., 2016). The study of *Ito et al* showed a very clear hypothesis indicating how the development of COPD is provided by accelerated lung aging (figure 1).

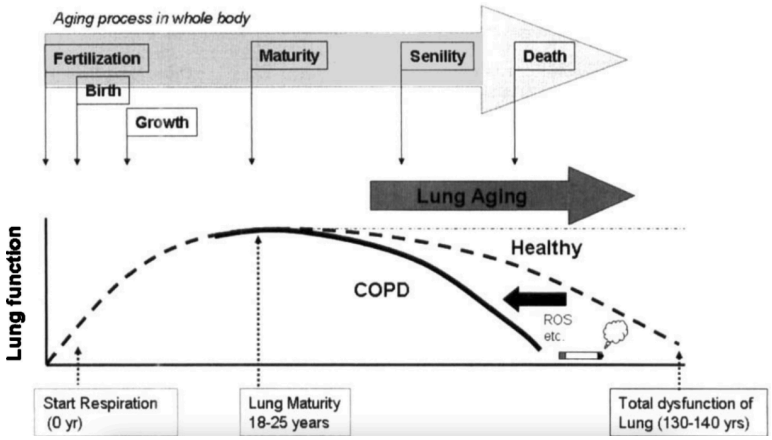

Figure 1: Hypothesis of *Ito et al* for the development of COPD as a consequence of accelerated aging of the lung. Progressive decline of homeostasis is defined as aging as a consequence of organ failure to maintain

oxidative stress, telomere shortening and DNA damage. Pulmonary function progressively declines during aging and parenchymal and small airway lung structures change and subsequently increase pulmonary inflammation. Exposure of the lungs to environmental cigarette smoke is indicated to possibly accelerate lung function which is defective upon aging. Retrieved from (Ito & Barnes, 2009; figure 1).

The hypothesis in figure 1 shows that lung function decreases in both the normal aging lung and in COPD. Interestingly, lung function decreases faster in COPD patients leading to earlier death. This indicates accelerated aging resulting from failure of maintenance and repair by the lungs due to exposure of the lungs to cigarette smoke (Ito & Barnes, 2009). The molecular sequence of events and pathways contributing to the phenotype of accelerated aging of COPD is still widely investigated (Navarro & Driscoll, 2017). Moreover, the question whether accelerated lung aging plays a role in the onset of COPD or can be seen as a consequence of the disease remains to be elucidated.

As of this moment, there is no cure for COPD and it should also be considered that in elderly, treatment of COPD is not so easy due to its high association with comorbidities such as cardiovascular diseases and many more. These comorbidities also have their own pharmacological treatments making therapies for COPD patients even more challenging and complex. Importantly, with the lifespan that is increasing of the world population, the prevalence of the age-related disease COPD will be enhanced further. Eventually, a burden will be put on the healthcare systems and the quality of life will be affected of these patients (Easter et al., 2020). Moreover, current therapies are not sufficient since they mostly focus on treating the symptoms of COPD rather than preventing the progression or mortality of the disease. Thus, understanding the pathomechanisms related to aging and its association to COPD is critical for discovering novel treatment strategies (Easter et al., 2020; Ito & Mercado, 2014). There are several hallmarks of aging which are associated with the accelerated lung aging shown in COPD such as oxidative stress, telomere attrition, stem cell exhaustion and cellular senescence (Meiners et al., 2015). Targeting these hallmarks of aging by finding anti-aging therapies is hypothesized to reduce or even prevent the development and progression of COPD (MacNee, 2016). Therefore, the aim of this thesis is targeting hallmarks of accelerated lung aging in COPD: finding potential anti-aging therapies. In order to address this aim, the functional and cellular changes in the normal lungs and their relevance to COPD are discussed in order to understand the relationship between the normal aging lungs and the COPD lung. Secondly, the hallmarks of aging which are involved in accelerated lung aging in COPD will be discussed in order to understand how the aging lung contributes to the development and progression of COPD. Next, several anti-aging molecules will be discussed which are downregulated in the pathogenesis of COPD, and their relevance as a target for anti-aging therapies. Lastly, current potential therapies will be discussed which target hallmarks of accelerated lung aging in COPD.

2. Functional and cellular changes in the aging lung and their relevance in COPD

In chronic lung diseases, one of the biggest risk factors is aging, however the underlying mechanisms that drive aging in the lung remain largely to be elucidated (Schneider et al., 2021). It is hypothesized that changes in function and structure in the lung related to age may enhance the susceptibility of developing COPD (Fukuchi, 2009). In order to understand the relationship between aging and COPD it is important to highlight the processes occurring in the normal aging lung. In this chapter, the known functional, structural, and cellular changes in the aging lung, and their relevance for COPD, will be discussed.

2.1 Functional and structural changes

Functional and structural changes are both characteristics of the aging lung. A structural and morphological change in the aging lung is the enlargement of the alveoli. This change is similar to emphysema, however in the aging lung there is no alveolar wall destruction. Therefore, in the aging lung this phenomenon is called senile emphysema. Destruction of the alveolar wall is a pathologic characteristic of pulmonary emphysema. Senile emphysema is however suggested to be susceptible to persuade into pulmonary emphysema (Fukuchi, 2009). Other structural changes in the aging lung are decrease of elastic recoil, loss of strength of respiratory muscles and an increase in stiffness of the chest wall. These three structural changes lead to a decrease in Forced Expiratory Volume in one second (FEV1) (Fukuchi, 2009). FEV1 is the volume of breath exhaled in one second (David & Edwards W., 2020). Overall, functional changes in the aging lung are associated with structural integrity and elasticity loss, increased inflammation and decreased pulmonary function. In COPD, these functional changes are key characteristics of the disease indicating there is a relationship between the normal aging lung and COPD (Rashid et al., 2018).

2.1.1 Aging reduces mucociliary clearance

Another functional change occurring in the aging lung is the change in mucociliary clearance. The respiratory lung epithelium is the major barrier between the outside environment and the inside tissues of humans. When foreign material such as pathogens and particles are inhaled, this barrier functions as a defense mechanism by trapping the foreign material in the mucus layer on the epithelium and removing the foreign material through movement of the cilia (Schneider et al., 2021). Therefore, major characteristics of mucociliary clearance are transport and production of mucus. When mucociliary clearance becomes dysregulated, the susceptibility of airway epithelial infections increases (Garth et al., 2020). For example, failed transport of mucus leads to obstructed airways due to mucus accumulation and leads to increased inflammatory changes which are also shown in COPD (Ramos et al., 2014). The study of *Svartengren et al*, showed that in the small and large airways, mucociliary clearance was decreased as the age increased in the healthy aging lung. These results implicate that age-related mucociliary clearance impairment might be associated to the prevalence and development of chronic bronchitis, which is also shown to have decreased mucociliary clearance (Svartengren et al., 2005).

2.2 Cellular changes

Besides functional and structural changes there are also cellular changes that contribute to the pathogenesis of the aging lung. At cellular level, inability to maintain homeostasis at baseline, adult stem cell depletion, reduced stress response, mitochondrial dysfunction and increased damaged DNA accumulation (leading to shortening of telomeres) are markers of aging (MacNee, 2016).
One of the cellular changes is the depletion of adult lung stem cells which is related to aging. Adult stem cells are important in the lung for the ability to remodel, repair and regenerate the respiratory system.

These processes are highly involved in disease and injury (Schneider et al., 2021). When lung resident stem cells are depleted, the lung loses its capability to repair injury and loses its regenerative capacity which contribute to emphysema. In the next chapter age-related stem cell depletion in the aging lung and the relevance in COPD will be further described since it is an important hallmark of accelerated lung aging (Kotton & Morrisey, 2014).

3. Hallmarks of accelerated lung aging in COPD

As shown in the previous chapter, there are several features of COPD which overlap with healthy lung aging. Several hallmarks of accelerated lung aging are associated with COPD (Ito & Barnes, 2009). In this chapter, the hallmarks of accelerated lung aging which are also involved in the pathogenesis of COPD will be discussed in order to obtain more insights in how lung aging is involved in the development or progression of COPD.

3.1 Oxidative stress

Oxidative stress is one of the most important hallmarks of accelerated lung aging that is also associated with the progression and pathogenesis of COPD. When the age of the lung is increasing, the ability of the lung to maintain its integrity is reduced and the lung is less able to protect itself from oxidative injuries (Ito & Barnes, 2009). Mitochondrial functioning is decreased in the aging lung which can lead to oxidative stress by for example mitochondrial derived Reactive Oxygen Species (ROS). Besides, mitochondrial dysfunction is also shown to cause oxidative stress in patients with COPD (Brandsma et al., 2017; Easter et al., 2020). There are also environmental sources which contribute to oxidative stress such as airborne pollution, car exhaust fumes and indoor cooking fires. However, the most important environmental ROS in the onset of COPD is cigarette smoke (Kirkham & Barnes, 2013). Several studies showed that oxidative stress accelerates aging-associated changes, while antioxidants showed to slow aging (Ito & Barnes, 2009). In the healthy lung, antioxidant mechanisms neutralize ROS in the lung to prevent ROS induced damage upon oxidative stress (Kirkham & Barnes, 2013). Antioxidant enzymes are shown to be decreased in the aging lung and the COPD lung by genetically impairment of antioxidant defense mechanisms leading to oxidative stress upon the inability to neutralize increased ROS levels (Ito & Barnes, 2009; Kirkham & Barnes, 2013).

Thus, an important indication of oxidative stress is the accumulation of ROS (Ito & Barnes, 2009). During normal oxygen metabolism, ROS is formed. When free radicals derived from oxygen are increased, they are able to activate several transcriptional factors. These transcriptional factors are subsequently able to activate the transcription of genes which encode for proinflammatory molecules (Rahman & Adcock, 2006). One of these transcription factors is nuclear factor-κB (NF-κB). NF-κB is known to regulate processes of inflammation in the lung and therefore maintains inflammatory homeostasis. During aging this homeostasis is disturbed and inhibitory κBα is hyperphosphorylated due to accumulation of increased ROS levels. Subsequently NF-κB activity is increased leading to downstream signaling of pro-inflammatory molecules such as IL-6 (Fulop et al., 2006). Furthermore, accumulation of ROS shown in the aging lung and in the COPD lung leads to reduction of anti-aging protein Sirtuin, DNA damage, inhibition of the proteasome and activation of stress responsible kinases such as PI3K and mitogen-activated protein kinase (MAPK). Additionally, these factors lead to amplified inflammation, instability of chromatin structure, hyperplasia, and structural changes due to apoptosis. Cigarette smoke, an environmental ROS, is able to enhance these mechanisms resulting in more inflammation, accumulation of oxidated proteins and decreased antioxidants. The study of *Ito & Barnes* suggests these enhanced mechanisms contribute to the accelerated aged COPD lung which is indicated in figure 2 (Ito & Barnes, 2009).

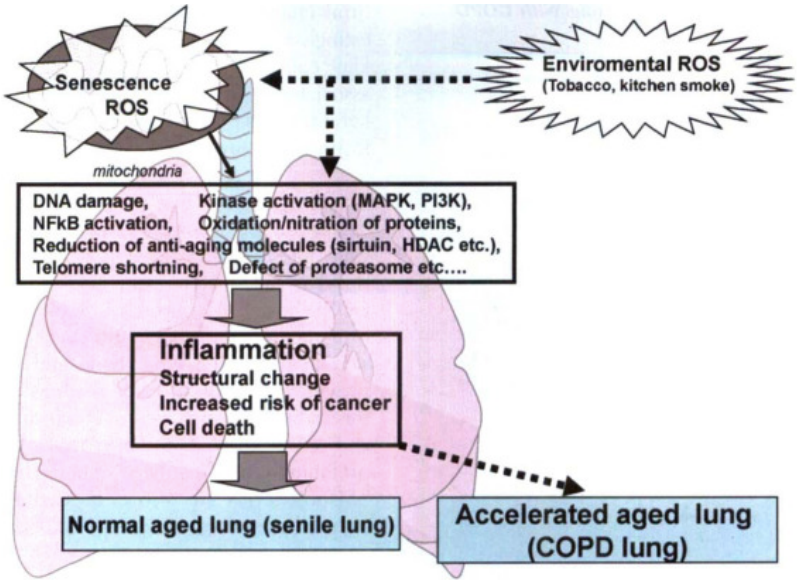

Figure 2: Molecular mechanisms upon oxidative stress during aging and development of COPD according to *Ito & Barnes.* When endogenous ROS is accumulated this induces DNA damage, activation of oxidative stress responsible kinases such as MAPK and PI3K, inhibition of proteasomes, activation of NF-κB due to hyperphosphorylation of κBα, and reduction of sirtuin activity. These processes lead to instability of the chromatin structure, structural changes in the lungs due to hyperplasia and apoptosis and amplified inflammation. All these mechanisms are enhanced by cigarette smoke, also leading to the reduction of HDAC expression and antioxidant activity resulting in enhanced inflammation and accumulation of oxidated proteins. Retrieved from (Ito & Barnes, 2009; Figure 2).

3.2 Telomere attrition

Telomere attrition is another important hallmark of accelerated lung aging and COPD. According to the study of *Houben et al*, it is suggested that one of the most important contributors to telomere attrition is oxidative stress. Oxidative stress is even shown to accelerate the shortening process (Houben et al., 2008). This is due to the fact that upon ROS accumulation in the aging lungs, DNA damage is induced as a consequence of oxidative stress (Ito & Barnes, 2009). The ends of chromosomes are protected by telomeres, which are structures of DNA and protein. Upon normal cell division and during aging, telomeres shorten. When telomeres are shortened and dysfunction, they send a signal of DNA damage which elicit apoptosis and cellular senescence. Telomeres also have a telomerase enzyme which is a polymerase that is involved in the synthesis of new repeats in the telomere (Stanley et al., 2015). Moreover, chronic exposure of cigarette smoke is associated with accelerated telomere breakdown in COPD patients. Besides, several studies have suggested that telomere length in circulating leukocytes is a biomarker for the progression of COPD (Schneider et al., 2021).

The study of *Stanley et al*, showed that a mouse model with short telomeres is susceptible to develop emphysema after chronic exposure of cigarette smoke. Subsequently, early onset emphysema was shown to be associated with deletion of a specific RNA component of the telomerase enzyme in a familial germline. These results suggest that telomeric length in COPD is an important genetic susceptibility factor (Stanley et al., 2015). Furthermore, the study of *Córdoba-Lanús et al,* showed that COPD patients have shorter telomeres than age-matched smoker controls upon investigating telomere shortening over time. At baseline and after 3 years they measured the telomere lengths in DNA isolated from leukocytes in venous blood. Interestingly, they observed shortening of telomeres to be accelerated in COPD patients (70 patients) compared to age-matched smoker controls (73 patients) after 3 years of follow up. The amount of telomere attrition over 3 years was shown to be dependent on the length of telomeres at baseline (Córdoba-Lanús et al., 2017).

3.3 Stem cell exhaustion

In the previous chapter, it was described that stem cell exhaustion is found to play an important role in the aging lung. Stem cell exhaustion is another hallmark of accelerated lung aging, and its mechanism is associated with the pathogenesis of COPD (Schneider et al., 2021). Proliferation of stem cells in the lung is infrequent and therefore the lung is considered to be an organ which renews slowly (Stripp & Reynolds, 2008). As a consequence, injury in the lung results in depletion and restricted capacity of proliferative stem cells (Ghosh et al., 2018). During injury one of the cells that participates in the lung's response are the basal progenitor cells in the airway. Basal progenitor cells are multipotent, which means they are able to differentiate into ciliated, basal, and secretory cells. Besides, alveolar type 2 cells are stem cells in the distal lung. Alveolar type 2 cells are able to replace lost alveolar type 2 cells and are able to differentiate into type 1 alveolar cells. Type 1 alveolar cells are important for gas exchange in the alveoli and account for 95% of the epithelial cells in the alveoli (Barkauskas et al., 2013).

Regenerative capacity and function of stem cells are decreasing with age. A decline of capacity of stem cells with regenerative potential in the tissue is shown to contribute to diseases related to aging and in particularly leads to lung failure (Signer & Morrison, 2013) (Navarro & Driscoll, 2017). Exhaustion of age-associated stem cells arises from mechanisms such as mitochondrial dysfunction, telomere shortening, epigenetic changes and oxidative stress responses (Navarro & Driscoll, 2017). In COPD, it is shown that airway basal cells have a decrease in differentiation ability followed by early exhaustion (Staudt et al., 2014). Furthermore, during aging the amount of alveolar type 2 cells remains unchanged but the differentiation capacity and self-renewal is exhibited leading to less alveolar type 1 cells and therefore impaired gas exchange (Watson et al., 2020). Moreover, the study of *Paschalaki et al* showed that the major risk factor for COPD cigarette smoke, reduced the repair ability of progenitor endothelial cells, suggesting stem cell exhaustion contributes to the pathogenesis of COPD (Paschalaki et al., 2013) Overall, when the lung is depleted of stem cells, regenerative and repair mechanisms are impaired and unable to repair tissue damage upon lung failure leading to pulmonary diseases such as emphysema (Kotton & Morrisey, 2014).

3.4 Cellular senescence

Cellular senescence is an important aging process and mechanism for the suppression of tumors which is characterized by resistance to apoptosis, mitochondrial dysfunction, irreversible growth arrest, production of senescence-associated secretory phenotype (SASP) and alterations in chromatin and DNA (Pignolo et al., 2020). Cellular senescence is one of the most widely described mechanisms contributing to accelerated lung aging events in COPD (Chilosi et al., 2013). The irreversible arrest in growth in cellular senescence is caused by stressors such as oxidative stress, DNA damage, shortening of telomeres and depletion of stem cells which are also all described in the other hallmarks above (Meiners et al., 2015). One of the inducers of cellular senescence in COPD is cigarette smoke. Cigarette smoke causes DNA damage by oxidative stress in the lungs and triggers cellular senescence (Rashid et al., 2018). Subsequently, cigarette smoke contributes to the expression of p21 which is a marker of senescence induced in fibroblasts and epithelial cells. Moreover, cellular senescence markers p21, p19 and p16 are increased in expression in emphysematous lungs and function as inhibitors of cyclin kinases (Tuder et al., 2012).

Senescent cells accumulate in the tissues of the lungs while aging (Antony & Thannickal, 2018). Preventing this accumulation of senescent cells is shown to delay diseases related to aging. However, the mechanism of how senescent cells accumulate is not completely understood. It might be that increased production of senescent cells lead to accumulation or that the removal of senescent cells is decreased with aging, or both (Karin & Alon, 2021). When senescent cells accumulate in tissues during aging, tissue function and repair are impaired (Rashid et al., 2018). Moreover, in the COPD lung, senescent cells prevent repair of cells, and lose their regenerative capacity leading to worsening of lung function and progression of emphysema (Easter et al., 2020).

Senescent cells go into growth arrest while remaining metabolically active. Several factors are still being released called SASP (Schneider et al., 2021). SASP proteins are pro-inflammatory mediators. This phenotype causes an increase in damage of neighboring cells when secreted leading to tissue dysfunction and chronic inflammation (Muñoz-Espín & Serrano, 2014) (Rashid et al., 2018). The SASP phenotype is specific for each cell type and the impact is dependent on the level of protein secretion and composition. SASP proteins are for example chemokines, interleukins, proteases, and growth factors (Woldhuis et al., 2021). The recent study of *Woldhuis et al,* investigated the SASP profile of primary fibroblasts derived from COPD patients. The study found several SASP proteins secreted by COPD derived fibroblasts such as important chemokines and cytokines involved in inflammatory responses. These findings indicate a possible relationship to chronic inflammatory responses found in COPD which subsequently contribute to the pathogenesis of the disease. Next to higher SASP levels, cellular senescence was also upregulated in COPD-derived fibroblasts compared to the control groups. Both upregulated SASP levels and cellular senescence indicate involvement of accumulated senescence with the disease pathology of COPD (Woldhuis et al., 2021).

4. Anti-aging molecules in the pathogenesis of COPD and their relevance as a therapeutic target

Animal models are widely used to study diseases. In COPD, animal models are a valuable manner to understand molecular mechanisms underlying the disease due to the ability to modulate pharmacological pathways and genes (Vandivier & Ghosh, 2017). Anti-aging molecules protect against age-related diseases. In the pathogenesis of COPD, it is shown that various anti-aging molecules are downregulated, suggesting targeting the deficiency of these molecules is a promising therapeutic option to prevent of delay age-related diseases. In this chapter several anti-aging molecules will be discussed which are studied in mouse models and their relevance as a target for the treatment of COPD.

4.1 Klotho

One of the most extensively studied anti-aging proteins in the pathogenesis of COPD is klotho. Klotho exists in a soluble circulating form and a membrane form. These forms arise from alternative splicing or proteolytic cleavage (Y. Wang & Sun, 2009; Mencke et al., 2017). As an anti-aging molecule, klotho exerts anti-inflammatory, anti-oxidative and anti-proliferative functions in organs such as the kidney, heart, and lung (Garth et al., 2020). Interestingly, absence of expression of klotho in the lung is described by other reports. These reports suggest that the kidney synthesizes klotho and cleaves it at the level of the transmembrane domain. After cleavage, it is secreted into the blood circulation and absorbed in the lungs (Ravikumar et al., 2014; Gazdhar et al., 2018). In COPD, klotho protein levels are downregulated in the human airway epithelium (Gao et al., 2015). Next to klotho's anti-aging properties, it also implicates longevity. The protein showed to prevent apoptosis and cellular senescence, decreases oxidative stress, maintains endothelial function and integrity, and lastly preserves stem cells (Pako et al., 2017).

One of the first findings of klotho and its association to aging was found in klotho knock-out mice. The study of *Suga et al* showed that lung pathology of homozygous mutant klotho ($KL^{-/-}$) mice resembled pulmonary emphysema as is shown in humans both functional and histological. When mutant mice were 4 weeks old, they showed air space enlargements and destruction of the walls of alveoli which progressed as the mice got older. Heterozygous mutant klotho ($KL^{-/+}$) mice showed pulmonary emphysema when they were 120 weeks old (late in life) indicating maintenance of pulmonary integrity upon klotho protein expression during postnatal life is essential. $KL^{-/-}$ mice died around week 8/10 whereas $KL^{-/+}$ mice survived longer than 120 weeks while also developing emphysema, suggesting the klotho gene has a gene dose effect. These results implicate that expression of the klotho gene is essential in adulthood to maintain normal architecture of alveoli (Suga et al., 2000).

As was previous described, lung aging is associated with reduced mucociliary clearance which might subsequently be associated with the development of chronic bronchitis. *Garth et al*, showed that deficiency in the klotho gene led to mucociliary clearance impairment and reduction of airway surface liquid (ASL) volume. The study showed that when klotho is overexpressed, ASL volumes were increased subsequently leading to increased activation of BK channels (Ca^{2+}-activated, voltage-dependent potassium channel). Together with CFTR (cystic fibrosis transmembrane conductance regulator) channel, BK channels maintain ion flux in airway epithelial membranes which additionally help regulating ASL volume and mucociliary clearance (Garth et al., 2020). In COPD, dysregulation of BK and CFTR channels were shown in the pathogenesis (Dransfield et al., 2013; Raju et al., 2016). Furthermore, IL-8 levels were also downregulated upon overexpression of klotho which is an important finding since ASL volume is negatively regulated by IL-8 (Garth et al., 2020). Together, these findings provide new insights for discovering anti-aging and anti-inflammatory therapies in order to enlarge signaling of klotho in the airway and restore levels of klotho by improving mucociliary clearance and ASL volume. Finding these therapies would benefit airway inflammation diseases (Garth et al., 2020).

4.2 SMP-30

Besides klotho there is another anti-aging molecule which is studied in knock-out mice models called Senescence Marker Protein-30 (SMP-30). The molecular weight of SMP-30 is 30 kilo Dalton. Furthermore, the amino acid sequence of the protein is highly evolutionary preserved in rats, mice, and human. During aging, the protein decreases independent of androgen (Fukuchi, 2009). Similarly, to klotho, SMP-30 protein is decreased in the lung tissue of COPD patients (Ito & Barnes, 2009; Fan et al., 2012). A knock-out SMP-30 (SMP30Y/-) mouse model was developed for studying the role of SMP-30 in organ disorders related to aging. Similar to klotho knock-out mice, SMP-30 knock-out (SMP30Y/-) mice have a shorter life span indicating SMP-30 plays an important role in aging (Ishigami et al., 2004). Besides, knock-out SMP-30 (SMP30Y/-) mice showed to have enlargement of peripheral airspace. However, no alveolar destruction was shown, indicating SMP30Y/- mice are a model for the senile lung which is related to lung aging (Mori et al., 2004).

The study of *Sato et al,* investigated whether, during aging, SMP30Y/- mice would be susceptible to oxidative stress and whether the mouse model would generate pulmonary emphysema upon exposure to cigarette smoke (Sato et al., 2006). The investigators looked at protein carbonyl levels since these are biomarkers of oxidative stress shown to be increased in the lungs of COPD patients (Rahman et al., 2002; Dalle-Donne et al., 2003). Results showed that protein carbonyl levels were significantly increased in SMP30Y/- mice compared to wild-type SMP30Y/+ mice after cigarette exposure. Subsequently, after chronic cigarette exposure of 8 weeks, SMP30Y/- mice generated pulmonary emphysema whereas SMP30Y/+ mice did not. In addition, apoptosis was induced in lung cells of SMP30Y/- mice after cigarette smoke exposure but not in wild-type mice. Altogether, this study showed an increase in oxidative stress in SMP30Y/- mice indicating a lack of the SMP-30 protein causes higher susceptibility to oxidative stress upon cigarette smoke exposure. In conclusion, SMP-30 is shown to protect mice lungs from oxidative stress related to aging and smoking (Sato et al., 2006).

4.3 Sirtuin

Another promising anti-aging molecule is sirtuin (SIRT). SIRT controls inflammation, resistance to oxidative stress and DNA repair (Ito & Barnes, 2009). Besides, SIRT showed to have positive effects on diseases related to aging (Chun, 2015). Sirtuins are histone/protein deacetylases that are dependent of nicotinamide adenine dinucleotide (NAD^+). As of this moment, there are seven sirtuins identified in humans (SIRT1-SIRT7). These proteins show specificity against acetylated substrates leading to different physiological functions; regulation of the cell cycle, apoptosis, metabolism, gene expression and aging (Grubisha et al., 2005). Previously it was described that in the aging lung, SIRT is decreased as a consequence of oxidative stress. Of the seven sirtuins, there are two sirtuins (SIRT1 and SIRT6) which are implicated to have protective functions against COPD.

SIRT1 might be able to slow down or improve COPD since it is implicated to protect against inflammation responses in cells of the lung upon oxidative stress induced by cigarette smoke (Yao et al., 2012). Furthermore, SIRT1 was shown to be decreased in lungs of COPD patients after cigarette smoke exposure which indicates the protein plays a role in the disease pathogenesis (Rajendrasozhan et al., 2008). In a previous chapter it was described that NF-κB plays a role in the transcription of pro-inflammatory cytokines in the lungs upon oxidative stress. Studies showed that reduced SIRT1 levels upregulate NF-κB by acetylation which subsequently leads to increased transcription of inflammatory molecules and therefore inflammatory responses. In contrast, when SIRT1 is overexpressed, NF-κB will be deacetylated resulting in a decreased transcription of inflammatory molecules (Schug et al., 2010; Yoshizaki et al., 2010). Besides protection against inflammation, SIRT1 is also shown to protect against cellular senescence. When p53 is acetylated, the protein will bind to DNA leading to the transcription of genes involved in apoptosis, cell cycle arrest and senescence (Schlereth et al., 2010). The study of *Langley et al* showed that SIRT1 represses and deacetylates p53 leading to prevention of cellular senescence (Langley et al., 2002). In COPD patients, SIRT1 levels are reduced in blood outgrowth

endothelial cells. When SIRT1 levels are inhibited in these cells, this results in an increase of p53 acetylation indicating protection of SIRT1 against cellular senescence by p53 deacetylation (Chun, 2015).

Autophagy is shown to be regulated by SIRT6 expression in the lungs of COPD patients (Chun, 2015). In the COPD lung, SIRT6 expression is reduced suggesting prevention of cellular senescence induced by cigarette smoke in the development of the disease (Rajendrasozhan et al., 2008). *Takasaka et al*, showed an inhibition of senescence in bronchial epithelial cells of humans after SIRT6 was overexpressed. Additionally, the study showed induction of autophagy by overexpression of SIRT6 via weakening of insulin-like growth factor-Akt-mammalian target of rapamycin (IGF-Akt-mTOR) signaling. The insufficient elimination of cellular damaged components by autophagy upon IGF-Akt-mTOR activation is suggested to be involved in developing COPD. This implicates that deficiency of SRT6 might provide to developing COPD (Takasaka et al., 2014).
SRT6 is also shown to be related to cellular senescence. *Minagawa et al* showed inhibition of the induction of cellular senescence by TGF-β after breakdown of p21 due to overexpression of SIRT6 in bronchial epithelial cells. In contrast, when SIRT6 was knocked down by short interfering RNA, cellular senescence was increased (Minagawa et al., 2011). Therefore, it is suggested SRT6 plays an important role in cellular senescence inhibition.

4.4 Treatment strategies for klotho, SMP-30 and sirtuin

In the pathogenesis of COPD, anti-aging molecules klotho and SMP-30 were shown to be decreased. Both anti-aging molecules showed pre-mature aging and development of emphysema in knock-out mice models, indicating these models are of great interest for studying accelerated lung aging. Besides, klotho deficiency reduced mucociliary clearance which might be associated with the prevalence of chronic bronchitis (Svartengren et al., 2005). Moreover, a lack of SMP-30 was shown to increase oxidative stress in knock-out mice after cigarette smoke exposure. Since oxidative stress is one of the hallmarks of accelerated lung aging in COPD, SMP-30 might be an interesting anti-aging target contributing to protecting smokers from developing pulmonary diseases (Fan et al., 2012). Using klotho and SMP-30 as a therapeutic target might prevent or reduce the development of emphysema, chronic bronchitis, and pre-mature aging. Unfortunately, as of this moment there are no clinical studies showing supplementation or targeting pathways of klotho and SMP-30 as a therapeutic treatment for COPD.

Another promising anti-aging molecule shown was SIRT. SIRT1 protects against inflammatory responses via NF-κB and protects against cellular senescence via p53. Moreover, SIRT6 increases autophagy via IGF-Akt-mTOR and inhibits cellular senescence via TGF-β. Since SIRT is decreased in the lungs of COPD patients upon oxidative stress, activation of SIRT1 and SRT6 via pharmacologic agents might be a promising treatment option for targeting hallmarks of accelerated lung aging in COPD (Chun, 2015). One of the most widely investigated activators of SIRT1 is resveratrol. Resveratrol is a natural compound found in plants such as berries, peanuts and grapes. Moreover, it is found in wines (mostly red wines) (Alarcón De La Lastra & Villegas, 2007). Resveratrol was even shown to activate SIRT1 in 8-fold (Borra et al., 2005). Resveratrol exerts antioxidant and anti-inflammatory properties making it a potential treatment for COPD (Beijers et al., 2018). *X. L. Wang et al* studied resveratrol treatments in rat COPD models and found increased levels of SIRT1 upon resveratrol administration compared to control COPD rats. Furthermore, pro-inflammatory cytokines were downregulated and anti-oxidant levels were upregulated upon resveratrol administration in COPD rats. These findings suggest an important role of resveratrol in oxidant and inflammatory regulation by SIRT1 in COPD making it a potential treatment option in the future (X. L. Wang et al., 2017). Next to the natural compound resveratrol, there is also a synthetic SIRT1 activating compound investigated in COPD named SRT1720. *Yao et al,* showed sRT1720 administration in mice protected against emphysema after cigarette smoke induction by reducing senescent cells. This reduction of senescent cells was due to the deacetylation of stress response transcription factor forkhead box O3 (FOXO3) (Yao et al., 2012). Moreover, the study of *Gu et al* showed administration of sRT1720 in cigarette-smoke induced

emphysematous rats improved lung function, upregulated FOXO3 protein and SIRT1 activity, downregulated p53 and inhibited apoptosis of type 2 alveolar epithelial cell. Type 2 alveolar epithelial cells are important for tissue repair in the lung and alveolar homeostasis. Upon inhibition of type 2 alveolar epithelial cells after SRT1720 administration, lung injury as a consequence of emphysema was alleviated (Gu et al., 2015). Together, animal studies using administration of SIRT1 activating compounds resveratrol and SRT170 showed promising outcomes for becoming a therapeutic future therapy for COPD. Unfortunately, there are currently no activating compounds of SIRT6 studied in COPD.

5. Treatments targeting hallmarks of accelerated lung aging in COPD

Previous chapters showed there are many resemblances between aging processes in the normal aging lung and the COPD lung contributing further to the evidence that accelerated aging plays an important role in the pathogenesis of COPD. Understanding these mechanisms provides novel targets for these age-related conditions associated with COPD (MacNee, 2016). This is important since current used therapies mainly focus on treating symptoms of the disease (Easter et al., 2020). In this chapter, anti-aging treatments will be discussed which are aimed to target hallmarks of accelerated lung aging in COPD and therefore are suggested to contribute to prevent or reduce the development of the disease.

5.1 Antioxidant treatments

Reducing oxidative stress, one of the most important hallmarks of accelerated lung aging in COPD is suggested by using antioxidant treatment (Easter et al., 2020). Previously it was described that antioxidants slow aging but are also decreased upon cigarette smoke exposure (Ito & Barnes, 2009). Some examples of antioxidants which are decreased and play an important role in the lungs are glutathione (GSH), nuclear factor erythroid-2-related factor 2 (Nrf2), thioredoxin and superoxide dismutase (SOD) (Taniguchi et al., 2021). There are many antioxidants investigated in the recent years. According to the recent study of *Taniguchi et al*, approaches which are most encouraging for antioxidant therapy are activators of Nrf2. Nrf2 is a transcription factor which is involved in oxidative damage protection. Activators of Nrf2 activate various antioxidant genes and target the dysfunction of Nrf2 as a response to oxidative stress found in COPD patients (Bellezza et al., 2018; Taniguchi et al., 2021). With the progression of COPD, Nrf2 decreases in the lungs and emphysema incidence was shown to be increased in Nrf2-deficient mice (Rangasamy et al., 2004). Therefore, activators of Nrf2 might provide reduction of oxidative stress and work protectively against ROS found in cigarette smoke (Taniguchi et al., 2021).

Currently investigated activators of Nrf2 as therapeutic agents are bardoxolone methyl, dimethyl fumarate and sulforaphane. Sulforaphane, found in various vegetables, promotes the expression of various antioxidant genes mediated by Nrf2 and showed to improve the susceptibility for corticosteroid in COPD patients (Yagishita et al., 2020). However, sulforaphane did not show an increase in the expression of antioxidant genes in clinical trials with COPD patients and therefore, no suppression of oxidative stress was shown (Wise et al., 2016). Bardoxolone methyl showed to decrease the susceptibility for smoke-exposed mouse models to develop emphysema and therefore having beneficial effects for preventing COPD progression (Sussan et al., 2009). Unfortunately, no clinical studies have investigated the effects of bardoxolone in patients. In the study of *Cattani-Cavalieri et al*, dimethyl fumarate showed to reduce oxidative stress, lung injury and inflammation in a mouse model which was exposed to diesel exhaust particles (Cattani-Cavalieri et al., 2020). Interestingly, exacerbations of COPD can also be triggered by exposure to air pollution (Shin et al., 2021). *Taniguchi et al* states that it is crucial to develop specific activators of Nrf2 and therefore, it is implicated as an important area for future research (Taniguchi et al., 2021). Yet again, there are no clinical studies showing the effects of dimethyl fumarate in COPD patients. However dimethyl fumarate did show beneficial effects in clinical studies for relapses of multiple sclerosis. Unfortunately, in this study dimethyl fumarate supplementation was associated with serious side effects such as nausea, flushing and diarrhea (Gold et al., 2012). Research in developing potential antioxidants is still ongoing and according to the study of *Taniguchi et al*, it is expected that in future studies bioactive and pharmaceutical compounds will be revealed which are safe in use and protect the health of COPD lung (Taniguchi et al., 2021).

5.2 Stem cell regenerative therapy

As was previously described, airway basal cells have a decrease in differentiation ability followed by early exhaustion. Therefore, a potential benefit for COPD might be stem cell regenerative therapy (Easter et al., 2020). As a potential treatment for COPD, mesenchymal stem cells (MSCs) were studied in the recent years. These stem cells are multipotent with a morphology similar to fibroblasts and have the ability to differentiate into osteoblasts, muscle cells, adipocytes, and chondrocytes. MSCs are shown to have clinical potential for regeneration and repair of the lung. Besides, MSCs secrete tissue repair growth factors and anti-inflammatory molecules making them a potential treatment for the inflammation shown in the airways of COPD patients (Liu et al., 2016). In preclinical studies with COPD/emphysema animal models, MSCs showed to have potential beneficial effects after administration (Antunes et al., 2014; Zhao et al., 2014). Besides, these studies showed evidence for MSCs therapeutic efficacy, safety, mechanism of action, and toxicity which hold promise for application and administration of MSCs in the future in COPD patients (Liu et al., 2016). Unfortunately, MSCs therapy is followed by rates of low differentiation and engraftment in lung injury and is associated with detrimental fibrosis (Zhang et al., 2014). Moreover, clinical studies showed disappointing results and lack of efficacy after administration of MSCs in COPD-patients (Weiss et al., 2013). Nevertheless, the potential findings shown in pre-clinical studies upon MSCs administration hold promise for MSCs to become a useful treatment option for COPD patients in the future.

5.3 Senolytics

For targeting senescent cells, senolytics are explored a promising therapeutic therapy in the recent years. Senolytics are found as a therapeutic strategy to remove senescent cells by targeting apoptotic pathways and driving them into apoptosis. Removing senescent cells and thereby reducing them, is suggested to decrease inflammation in the airways of COPD patients and increase tissue repair by progenitor and stem cells (Kirkland et al., 2017). This investigation is important since it was described previously that when senescent cells accumulate in tissues during aging, tissue function and repair are impaired leading to the progression of emphysema (Rashid et al., 2018). In animal studies, it was shown that using pharmacological or genetic approaches to eliminate senescent cells prevented or delayed chronic diseases, various age-related diseases, and geriatric syndromes (Kirkland et al., 2017). As of this moment, there are various senolytics drugs under investigation such as dasatinib and quercetin, which are tyrosine kinases that target anti-apoptotic pathways, and navitovlax and cardiag glycosides which target the Bcl-2 family. Dasatinib and quercetin are in phase 2 of the clinical trials and navitovlax and cardiag glycosides are as of this moment only studied in animal models (table 1) (Easter et al., 2020).

Table 1: Overview of current investigated senolytic drugs; molecular drug targets and therapy progress for eliminating senescent cells. Retrieved and modified from (Easter et al., 2020; Table 1).

Senolytics	Target	Model
Dasatinib	Tyrosine Kinases	Phase 2 Clincal Trial
Quercetin	Tyrosine Kinases	Phase 2 Clincal Trial
Navitovlax	BCL–2 Family	Animal Models
Cardiag Glycosides	BCL–2 Family	Animal Models

Navitoclax, targets Bcl-2, Bcl-x and Bcl-w and showed to effectively remove some types of senescent cells in animal models (Zhu et al., 2016). However, navitoclax was shown to cause blood cell dyscrasias upon high toxicity (Justice et al., 2019). This is due to the fact that inhibitors of Bcl-xL are able to interfere with platelet and neutrophil viability leading to thrombocytopenia (Wilson et al., 2010). Additionally, ouabain and digoxin (cardiac glycosides) inhibited the Bcl-2 family by inhibiting Na+/K+

ATPase and thereby showing senolytic effects. Besides, ouabain and digoxin showed senolytic effects on epithelial cells which express p16 (Guerrero et al., 2019; Easter et al., 2020). Moreover, studies showed that treatment of aged hypercholesterolemic mice with dasatinib and quercetin reduced senescent cells (Roos et al., 2016). Recently, dasatinib and quercetin were shown as a treatment therapy in clinical studies for Idiopathic Pulmonary Fibrosis (IPF), a lung disease which is also associated to cellular senescence. Dasatinib and Quercetin showed reduction of senescent cells in humans and SASP proteins MMP-9, IL-1 and IL-6 in the blood. The clinical trial showed significant promising physical improvements in 13 of the 14 IPF patients (Justice et al., 2019). However, the clinical study of IPF used a small patient cohort and did not compare their findings to control groups suggesting larger controlled trials are necessary to demonstrate dasatinib and quercetins efficacy and safety (Justice et al., 2019). Senolytics navitoclax, dasatinib, and quercetin have a downside in the fact that they are limited in the types of senescent cells they can target. Navitoclax is even more limited than dasatinib and quercetin further emphasizing chemical optimization of the medicines is needed to target particular diseases (Zhu et al., 2016). As of this moment, there are no clinical trials investigating the effects of senolytics on COPD patients. Altogether, findings on senolytics showed senescent cells can be removed by targeting them in various ways in preclinical animal models and in clinical trials of patients with diseases related to aging. Therefore, targeting senescent cells with senolytics in COPD is implicated to become a potential therapy option (Easter et al., 2020).

6. Conclusion/discussion

The focus of this thesis was targeting hallmarks of accelerated lung aging in COPD: finding potential anti-aging therapies. It was hypothesized that by finding anti-aging therapies that address the hallmarks of accelerated lung aging in COPD, the development and progression of the disease will be reduced or even prevented. Functional and cellular changes in the normal aging lung showed a lot of similarity with the COPD lung. Additionally, hallmarks of accelerated lung aging were shown in the COPD lungs, whereas the most important ones were oxidative stress, telomere attrition, stem cell exhaustion and cellular senescence. *Ito & Mercado*, states that anti-aging therapies should not focus on immortalization or extending life span but on prevention pre-mature aging (Ito & Mercado, 2014). Therefore, targeting hallmarks of accelerated lung aging is considered an even more promising treatment strategy to prevent or reduce the development of COPD.

Three anti-aging molecules were found to be reduced in COPD; klotho, SMP-30 and SIRT. Both klotho and SMP-30 knockout mice showed these proteins play an important role in the pathogenesis of COPD and deficiency was associated with several hallmarks of accelerated lung aging in COPD. However, clinical studies are lacking using supplementation or targeting pathways of klotho and SMP-30 as a therapeutic target for COPD. Therefore, a recommendation for further research would be to discover anti-aging and anti-inflammatory therapies that focus on enlarging the signaling of klotho and SMP-30 in the airway and restore the levels of these proteins subsequently leading to preventing the onset or progression of the disease pathology in COPD.
Reduced levels of SIRT1 and SIRT6 were also associated with several hallmarks of accelerated lung aging in COPD. Activating SIRT1 via pharmacologic agents' resveratrol and sRT1720 in preclinical studies showed opportunities for becoming a promising treatment option for targeting several hallmarks of accelerated lung aging in COPD. It is therefore recommended to extensively examine the safety of these SIRT activating molecules in pre-clinical studies and subsequently study their benefits for the pathogenesis of COPD in clinical trials.

Various anti-aging treatments currently studied showed potential for targeting hallmarks of accelerated lung aging in COPD. Antioxidant therapies showed promising outcomes to reduce oxidative stress upon activation of Nrf2 by sulforaphane, bardoxolone methyl and dimethyl fumarate in preclinical models. However, promising outcomes of clinical trials in COPD are lacking and outcomes of a clinical trial using dimethyl fumarate in multiple sclerosis showed serious side effects. Therefore, it is suggested that these antioxidant therapies need chemical modifications and wide-scale examination in pre-clinical studies to be used safely and beneficial in clinical COPD trials.
Unfortunately, there are no specific treatment options shown yet for targeting the hallmark of accelerated telomere attrition in COPD patients. However, oxidative stress is a major cause of the shortening and loss of telomeres (Houben et al., 2008). Targeting oxidative stress may therefore provide less susceptibility for the development of telomere attrition shown in COPD patients.
Stem cell regenerative therapies showed potential pre-clinical outcomes and safe, efficient and non-toxic usage of MSCs for regeneration and repair of COPD lungs depleted of stem cells. Additionally, clinical studies also showed safe usage of MSCs administration. Unfortunately, the clinical outcomes showed a lack of efficacy of MSCs administration in COPD patients. A recommendation for further research would be to use larger-scale clinical trials to be able to fully examine the promising effects of MSCs in COPD patients.
Lastly, senolytics navitovlax and cardiag glycosides ouabain and digoxin showed efficient removal of senescent cells in animal models. Moreover, dasatinib and quercetin showed promising senolytic outcomes in clinical trials for IPF. Unfortunately, navitoclax is associated with high toxicity and dasatinib and quercetin are not studied yet in clinical trials for COPD patients. Besides, current senolytics are limited in the types of senescent cells they can target. Overall, it is requested that senolytics need chemical optimization to target specific diseases and need extensive preclinical investigations to be used safely before studies in clinical trials with COPD patients are possible.

An important remark associated with the findings in this thesis is that one anti-aging treatment might not be effective in reducing the whole spectrum of pathophysiology contributing to the disease manifestations in COPD. Therefore, combining several treatments might be necessary. However, large-scale studies on the safety of anti-aging treatments in COPD patients is required to draw conclusions on safely combining treatments. Moreover, it should be noted that finding anti-aging therapies that show reduction or prevention of the pathogenesis of COPD in clinical studies, also contributes to the remaining question whether accelerated lung aging plays an active role in the onset of COPD or can be seen as a consequence of the disease. Therefore, investigating anti-aging therapies for COPD is valuable research providing many future potentials.

Altogether, anti-aging molecules klotho, SMP-30 and SIRT show potential for becoming therapeutic targets in COPD. Moreover, antioxidant treatments, stem cell regenerative therapy and senolytics show promising features as therapeutic anti-aging therapies for targeting the hallmarks of accelerated lung aging in COPD and eventually they might reduce or prevent the development and progression of the disease pathogenesis.
To conclude, despite the many advances shown in pre-clinical studies on the anti-aging molecules and treatments discussed in this thesis, more research is required to draw a conclusion on which anti-aging therapy is the most promising for reducing or preventing the development of COPD in the aging society. Moreover, current pharmacological targets need modifications and extensive examination in pre-clinical and clinical studies to be used safely and protect the health of the aging COPD lung.

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