The ageing extracellular matrix of the lung

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Illustration cover: aerial photograph of the Colorado Delta from *Wild West, America's Great Frontier*, BBC 2, 2016.

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Abstract

Ageing is characterized by a progressive decline of physiological integrity leading to impaired function and increased vulnerability for the development of diseases. Nowadays, ageing research emphasizes nine hallmarks that have been defined as common denominators in ageing, while less attention is paid to structural alterations of the extracellular matrix (ECM). In the ageing lung, substantial alterations of the ECM have been observed, as well as in respiratory diseases like chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). In this essay, we discuss whether the loss of proteostasis affects the integrity of the lung ECM in ageing and respiratory diseases. We have found that expression of mutant alpha-1 antitrypsin (A1AT) induced stress responses in endoplasmic reticulum via NF - κB and eLF2 in epithelial lungs cells. Further, we found that fibronectin EDA is necessary for TGF-β induced fibroblast differentiation. Further, we have discussed that fibronectin EDA induced ER stress responses, which is ameliorated by the introduction of chaperones. Therefore, it is reasonable to suggest that both A1AT and fibronectin EDA act separately in ER stress. We concluded that impaired proteostasis is accompanied by dysregulation of the ECM.

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Abbreviations

A1AT	Alpha-1-antitrypsin
BiP	Binding immunoglobin protein
cFN	Cellular fibronectin
COPD	Chronic obstructive pulmonary disease
ECM	Extracellular matrix
eLF2	Eukaryotic initiation factor 2
EOR	ER overload response
ER	Endoplasmic reticulum
hA1AT	Human alpha-1-antitrypsin
HSPs	Heat shock proteins
IL	Interleukin
IPF	Idiopathic pulmonary fibrosis
LAP	Latency-associated peptide
LTBP-1	Latent TGF-β-binding protein-1
MMPs	Matrix metalloproteases
mRNA	Messenger RNA
NF - κB	Nuclear factor - κB
PERK	Protein kinase R –like ER kinase
pFN	Plasma fibronectin
PTMs	Posttranslational modifications
UPR	Unfolded protein response
ZA1AT	Z alpha-1-antitrypsin

Introduction

Ageing is characterized by a progressive decline or loss of the physiological integrity necessary for survival and reproductivity that affects all individuals of a species. This natural phenomenon ultimately leads to an impaired cellular functionality in nearly all tissues and organs, and an increased risk for the development of diseases and vulnerability to death (Lopez-Otin et al. 2013). Ageing-associated diseases include among others cancer, atherosclerosis and cardiovascular diseases, diabetes, neurodegenerative diseases and respiratory diseases (Niccoli and Partridge 2012). In the last three decades, the intriguing field of ageing biology has experienced dynamic changes, which lead to a better understanding of the multifaceted process of ageing and the development of ageing-associated diseases.

Nowadays, ageing research primarily emphasizes nine tentative hallmarks (see Figure 1) that represent the common denominators in the process of ageing. These functional interconnected hallmarks of ageing are genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, altered intercellular communication, and stem cell exhaustion (Lopez-Otin et al. 2013).

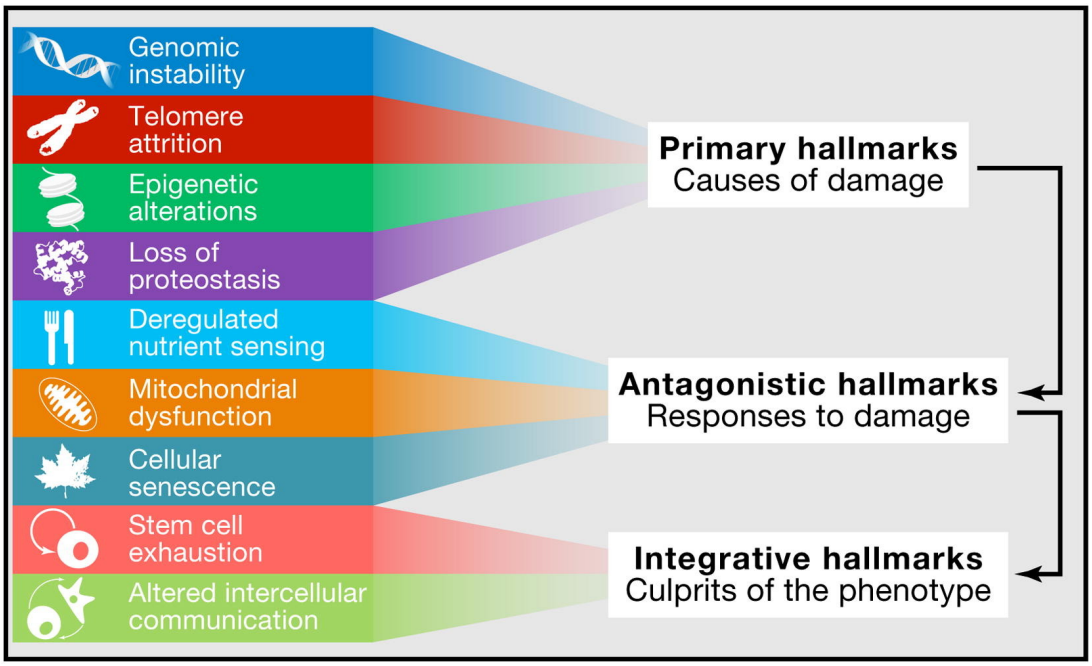

Figure 1: **The functional interconnected hallmarks of ageing.** The nine hallmarks of ageing are divided into three groups. The first group is proposed to be the primary causes of cellular damage. The antagonistic or compensatory hallmarks initially mitigate the damage, but gradually fail to compensate for the damage and they become deleterious themselves. The last group are integrative hallmarks, which are the consequences of the previous two groups, and are ultimately responsible for the functional impairment in ageing. The figure is adopted from Lopez-Otin *et al.*, 2013.

The extracellular matrix (ECM) was exclusively regarded to provide structure and stability to organs, but it is now widely acknowledged as a pivotal regulator in cellular responses (Karsdal et al. 2013). Normal ageing of the ECM is characterized by substantial alterations in the ECM composition, and are found in the whole human body. Moreover, alterations in the matrix composition are observed in several ageing-associated diseases for instance cancer and fibrosis (Minton 2014; Pickup, Mouw, and Weaver 2014). Hence, Meiners *et al.* have proposed dysregulation of the ECM as an additional hallmark of ageing (Meiners, Eickelberg, and Königshoff 2015).

The lungs are not spared from ageing as alterations in the matrix composition negatively affect the normal physiological integrity of the lungs with ageing. Further, two major lung diseases namely, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF) have an altered composition of the ECM. In addition, epidemiological studies have shown that the incidence of these two chronic respiratory diseases increase with ageing (Ley and Collard 2013; Raherison and Girodet 2009). It is plausible, therefore, that abnormalities in the ECM composition induced by ageing may contribute to or even may be a cause in the development of COPD and IPF.

In the development of chronic respiratory diseases, it is not completely understood how the hallmarks of ageing contribute to abnormalities in the composition of the ECM in the lung. Neither it is fully known whether COPD and IPF can be distinguished as accelerated ageing diseases. Since ageing is a complex process characterized by several hallmarks, we focus on one hallmark of ageing, namely loss of proteostasis. In this essay, we discuss whether alterations in the ECM are related to the loss of proteostasis or is a separate phenomenon in the process of normal lung ageing and respiratory diseases. To this end, PubMed was searched for the terms ECM, lung, plus ageing and combined with a separate search of these individual terms with the terms proteostasis, alpha-1 antitrypsin, fibronectin, and fibronectin EDA.

In the following paragraphs, we address the physiological ageing of the lung with a focus on alterations in the EMC homeostasis. Here, we discuss as well, the alterations in ECM composition in COPD and IPF. Further, we discuss the role of two proteins, namely, alpha-1 antitrypsin and fibronectin, in normal ageing and disease with a focus on dysregulation of the ECM. In the last paragraph, we combine the all the evidence, as we attempt to draw a conclusion.

The extracellular matrix in ageing and disease

The lung ECM includes a plethora of structural proteins that all contribute to maintain the structure, mechanical stability and elastic recoil. In addition, the ECM has a crucial function in the formation and maintenance of a tissue-specific microenvironment that instruct proper phenotypes and functionality of the cells in the lung (Kristensen et al. 2014). In the lungs, these structural proteins are mainly collagens, elastin, proteoglycans, fibronectin, and laminin. These structural components are believed to be produced by activated fibroblasts and myofibroblasts (Burgess et al. 2016). The pulmonary ECM is organized into two structural types with their own specific composition. The basement membrane is a thin sheet that consists mainly of type IV collagen and laminin, while the interstitial matrix constitutes a loose and fibril-like structure containing a myriad of extracellular proteins (Kruegel and Miosge 2010).

In the process of normal or physiological ageing, numerous alterations occur at the gross physio-anatomical level in the lungs (*e.g.* reduced total lung capacity, respiratory muscle strength, and lung elasticity). In the late eighties, ageing-associated changes in the composition of the ECM were already described by the group of Errico *et al.* The study reported a decline in elastic fibers along the alveolar walls without disruption when compared with that of non-elderly controls. Further, the levels of collagen type III were increased, as well as laminin and collagen type IV in the alveolar basement membrane (D'Errico et al. 1989). The turnover of other extracellular proteins is also affected with ageing. The collagen types I and II turnover is downregulated in old rodents, while type IV and V collagen are upregulated. The collagen types III and VI, and elastin turnover is not influenced by age (Karsdal et al. 2016). Studies in decellularized lungs from both human and ageing rodents confirm alterations in expression of collagens, fibronectin, and MMPs (Thannickal et al. 2015). Nowadays, ageing-associated alterations in the lung's ECM and extracellular proteins are widely acknowledged. However, several studies have presented sometimes contradictory results, as the ageing lung matrix is not yet extensively studied. It is reasonable to suggest that the ageing-associated alterations in the lung's matrix are highly heterogeneous in humans, as ageing results from both intrinsic and extrinsic factors. We do not address this issue further in the following paragraphs.

Chronic respiratory diseases, like COPD and IPF, are characterized by substantial alterations in expression, deposition, degradation, and turnover of the ECM. COPD is a common chronic inflammatory respiratory disease with a high global morbidity and mortality (Barnes et al. 2015). In the airways of patients who suffer from COPD, the alterations of the ECM are heterogeneous depending on severity of the disease, smoker or non-smoker, and location in the lung (Westergren-thorsson, Bjermer, and Hallgren 2014). Despite ECM's heterogeneous phenotypes in COPD, a well-established paradigm in COPD pathogenesis is the elastase and anti-elastase imbalance that leads to the destruction of elastic fibers. Recent findings extend ECM alterations to dysregulated matrix metalloproteases (MMPs) in human COPD (Meiners, Eickelberg, and Königshoff 2015). IPF is characterized by massive deposition of collagens (mainly type I and III) and fibronectin leading to structural alterations in the alveolar regions, which subsequently results in a progressive decline of the lung functionality. In IPF, several factors are involved in the imbalance of ECM formation, such as profibrotic growth factors that activate fibroblasts and myofibroblasts, and MMPs. The imbalance leads to structural alterations that results in distorted expression, turnover, or disposition of ECM components (Meiners, Eickelberg, and Königshoff 2015).

A hallmark of ageing: impaired proteostasis

Ageing and several ageing-related diseases (*e.g.* Alzheimer's disease and Parkinson's disease) are characterized by an altered protein homeostasis or more commonly referred to as proteostasis (Koga, Kaushik, and Cuervo 2011). Under normal conditions, proteostasis is maintained by the proteostasis network, which comprises pathways that control protein synthesis, folding, trafficking, aggregation, disaggregation, and degradation (Powers et al. 2009). In the process of ageing, the proteostasis network progressively deteriorates in the maintenance of these control pathways resulting in the loss of proteostasis. In particular, the protein folding machinery and proteolytic systems are impaired in ageing, which leads to unfolded or misfolded protein accumulation and aggregation, resulting in endoplasmic reticulum (ER) stress responses (Lopez-Otin et al. 2013).

The ER is an organelle that extends from the nuclear membrane, and has a crucial function in the folding of newly synthesized proteins. The correct folding of these nascent proteins is achieved by interactions with chaperones and enzymes, including members of the heat shock protein (HSP) family. After proper folding, the proteins are directed to the Golgi apparatus from where the proteins are sent to their destination. When a nascent protein misfolds or maintains in unfolded formation, the protein is retained within the ER lumen until it is properly folded. If the misfolded or unfolded protein in the ER is not properly folded, the protein is degraded via the proteasome or autophagy. However, when the protein-machinery is compromised, misfolded and unfolded proteins accumulate and aggregate in the ER inducing stress responses (Marciniak et al. 2016).

The accumulation and aggregation of proteins in the ER induces stress response signaling pathways. These stress pathways include the ER overload response (EOR) and unfolded protein response (UPR). In the EOR, nuclear factor (NF) - κB is rapidly induced via the activation of the IκB kinase (IKK) complex, which degrades inhibitors of NF - κB. The transcription factor NF - κB is localized to the nucleus, and subsequently promotes translation of inflammatory genes such as interleukins (ILs) and other cytoprotective genes (Catherine M Greene and McElvaney 2010). The UPR is another distinct ER stress pathway that is induced as a response to perturbations in the proteostasis network. Under normal conditions, the protein kinase R (PKR)-like ER kinase (PERK) is stabilized by HSP and a protein called binding immunoglobulin protein (BiP). In presence of misfolded proteins, BiP is removed enabling autophosphorylation of PERK, which then phosphorylates the eukaryotic initiation factor 2 (eLF2). This transcription factor attenuates gene translation in order to reduce the protein synthesis and subsequently reduces the protein accumulation in the ER (Manalo and Medina 2017).

In the following paragraphs, we discuss two proteins that are either indirectly or directly involved in the lung's matrix composition, and have a pivotal function in respiratory diseases, as well as in the process of ageing.

Alpha-1-antitrypsin isoforms in the lung

Alpha-1-antitrypsin (A1AT) is an anti-protease that maintains the integrity of the lung by preventing degradation of the ECM. This serine anti-protease is mainly produced in the liver from where it is transported to the lungs via the blood circulation (Catherine M. Greene et al. 2016). The deficiency of A1AT is an inherited genetic disorder that is most frequently caused by a point mutation in the *SERPINA1* gene. This point mutation ultimately leads to problems in both the liver and lungs. The amino acid substitution in A1AT deficiency causes misfolded A1AT proteins (see Figure 2A) during synthesis in the liver, which is most frequently the aberrant Z protein (*i.e.* ZA1AT). The mutant antitrypsin (AAT) variants polymerize and accumulate in the ER of hepatocytes (see Figure 2B). In the previous paragraph, we already discussed that ER stress responses are induced by protein accumulation. The same applies for the accumulation of mutant A1AT within the ER of hepatocytes, which can ultimately lead to fibrosis, cirrhosis or liver carcinomas (Edgar et al. 2017). An additional source of A1AT is the lung itself, where surface epithelial cells locally produce A1AT. In addition, unlike A1AT, mutant ZA1AT polymerizes and attracts neutrophils in the lung (Mulgrew et al. 2004). The article by Mulgrew *et al.* has not investigated the intracellular location of the ZA1AT polymers in lung epithelial cells. However, it is reasonable to suggest that the ZA1AT polymers may accumulate within the ER of lung epithelial cells, which induces ER stress responses and may ultimately lead to the development of fibrosis.

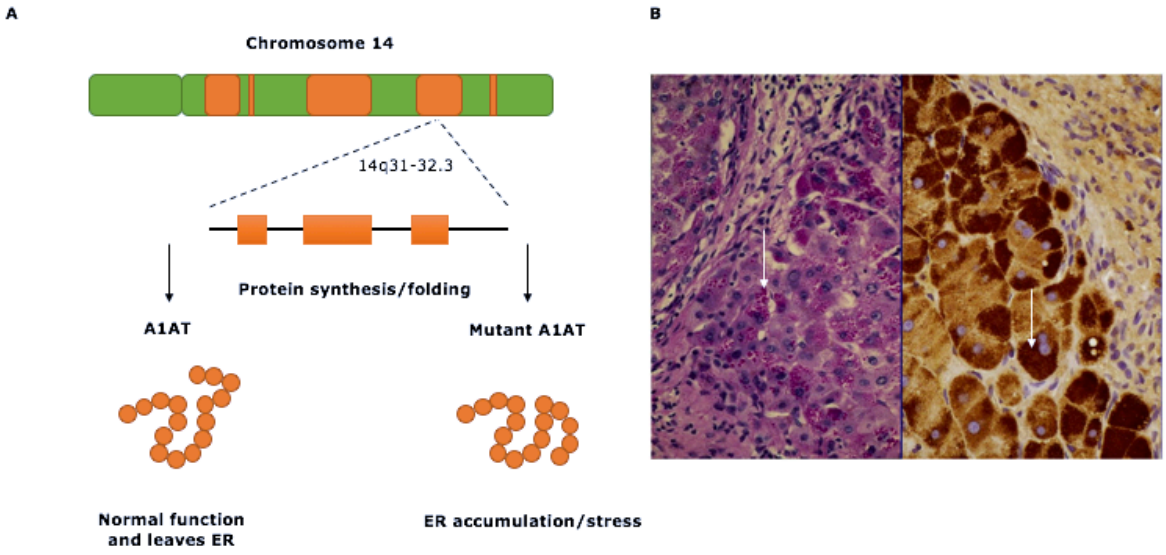

Figure 2: **A1AT protein folding after protein synthesis.** A) Under normal conditions, A1AT is transcribed from chromosome 14 (14q31 – 32.3) to mRNA. The A1AT protein is synthesized and folded to its functional formation, and ultimately leaves the ER. Mutant A1AT undergoes a similar process, but the protein is misfolded as results of the mutation. The mutant A1AT protein retains and accumulates within the ER leading to stress. B) Liver from a patient with defective A1AT protein that accumulates in globuli (white arrows) within hepatocytes visualized by periodic acid Schiff-positive diastase (left picture) and anti-A1AT antibodies (right picture). The microscopic pictures are adopted from (Nelson et al. 2012).

In the lungs, reduced A1AT levels allow proteolytic enzymes, primarily elastase secreted by neutrophils, to degrade the ECM in the pulmonary interstitium. This degradation subsequently triggers an inflammatory stress response similar to that involved in COPD and IPF (Bouchecareilh and Balch 2011). Indeed, patients who suffer from alpha-1 antitrypsin deficiency (A1ATD) are more likely to develop aggressive emphysema or COPD, and the disease usually manifests at a younger age (30 to 40 years old) when compared to persons who have a functional A1AT protein (Brode, Ling, and Chapman 2012). Hence, a paper by Bouchecareilh *et al*., suggests that airway stress diseases, like COPD and emphysema, have a common origin triggered by a challenge to the proteostasis machinery (Bouchecareilh and Balch 2011).

We already discussed that the ER stress response is mediated by the transcription factor NF - κB. In the liver, mutant ZA1AT elicits stress responses in the ER. Therefore, it is reasonable to suggest that similar responses occur in the lung, as surface epithelial cells in the lung produce A1AT (Mulgrew et al. 2004). The study by Lawless *et al*., showed an enhanced NF - κB nuclear localization in rodent ovary cells expressing mutant ZA1AT when compared with the control group. Moreover, mutant ZA1AT stimulated IL-6 and IL-8 protein expression (Lawless et al. 2004). Interestingly, a similar observation was established in human bronchial epithelial cells overexpressing mutant ZA1AT that displayed increased NF - κB gene expression and its upstream regulator the cellular inhibitor of apoptosis protein 1 (cAIP1) (Catherine M Greene and McElvaney 2010). More striking is a recently published study that showed that transgenic *Drosophila* expressing human A1AT (hA1AT) have a prolonged lifespan. The lifespan extension is a result of reduced inflammation by suppression of an ageing-induced NF - κB orthologue, which normally induces IL-6 and IL-8 expression (Yuan et al. 2017). These observations suggest that A1AT is an ageing suppressor, and might have potential therapeutic applications. In addition, it is reasonable to suggest that mutant A1AT induces ER stress, and subsequently inflammatory and cytoprotective genes by NK - κB – mediated pathways in the lung.

The UPR is another distinct ER-nuclear signaling pathway that is activated via the kinase PERK that phosphorylates eLF2. In human bronchial epithelial cells, the expression of mutant ZA1AT leads to PERK-independent phosphorylation of the transcription factor eIF2α that subsequently results in translational attenuations (C M Greene et al. 2010).

Altogether, these data suggest that misfolded A1AT consequences ECM dysregulation, and may be involved in the disposition of fibrosis development with ageing. However, an ageing-induced decline in functional A1AT proteins due to misfolding and accumulation in the lungs or other organs has, to the best of my knowledge, so far not been considered in the current literature. The ageing-induced decline in proper protein folding, and hence functionality, is feasible for A1AT as loss of proteostasis is a common phenomenon in the process of ageing.

The comeback of fibronectin in ageing

The second protein we discuss is the extracellular matrix protein fibronectin. This high molecular weight glycoprotein forms an interconnected network of fibrils in the lung's ECM. Fibronectin proteins are intimately involved in cell adhesion, attachment and migration, and cell proliferation. In humans, around 20 different fibronectin protein isoforms are observed, which are broadly divided in two variants: plasma fibronectin (pFN) and cellular fibronectin (cFN) (Eric S. White and Muro 2011). The fibronectin sequence and patterns of expression are highly conserved among vertebrates, suggestive of its biological importance. Indeed, transgenic mice without a functional fibronectin gene resulted in an early embryonic death displaying severe multiple cardiovascular defects (Astrof, Crowley, and Hynes 2007). The presence of fibronectin isoforms varies throughout life, as alternative spliced fibronectin isoforms are abundantly present during embryonic development, and decrease post-development (Früh et al. 2015)

The fibronectin protein isoforms are produced by alternative splicing of two exons within the fibronectin gene (see Figure 3). Alternative splicing is a process during gene expression that results in a single gene encoding for multiple proteins. The fibronectin gene has two exons that are called extra type III domain A (EDA) and extra type III domain B (EDB). These EDA and EDB domains can either be included or excluded from fibronectin messenger RNA (mRNA) (E. S. White, Baralle, and Muro 2008). As described previously, alternative spliced fibronectin isoforms are abundantly present during embryogenesis, and decreases substantially postpartum. However, EDA and EDB domain incorporation in fibronectin is temporally re-established under particular events, such as wound healing, angiogenesis, and fibrosis (Früh et al. 2015)

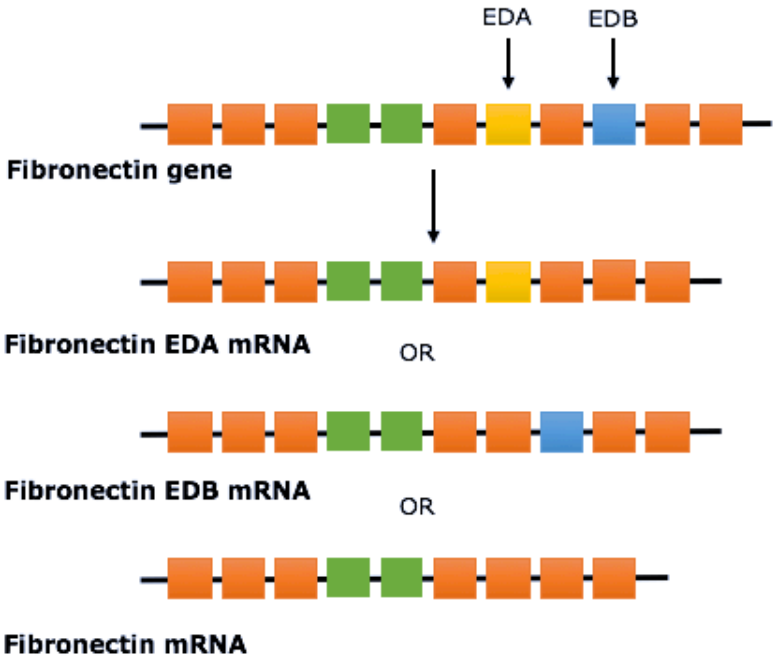

Figure 3: **Alternative splicing of the fibronectin gene.** The fibronectin EDA exon (yellow box) or exon EDB (blue box) mRNA is produced in response to tissue injury and during embryonic development, whereas these exons are mostly spliced out of the fibronectin mRNA.

There is growing evidence that fibronectin isoforms are involved in the process of ageing and disease. In both animal models and humans, fibronectin mRNA levels are increasing with ageing, as well as subsequent downstream fibronectin protein synthesis (Phillip et al. 2015). In ageing lungs of both mice and rats, alternative splicing of the fibronectin gene increases the EDA-containing fibronectin. In the lungs from patients who suffer from IPF there are higher levels of fibronectin EDA when compared to healthy individuals (Faner et al. 2012). On the other hand, fibronectin EDA protein expression is not changed in patients who suffer from COPD (Karvonen et al. 2013). Similarly, fibroblasts isolated from COPD patients showed no differences in fibronectin EDA secretion when compared with healthy individuals (Togo et al. 2008). In a study by Annoni *et al*., fibronectin protein levels are elevated in the inner layer, muscle layer and outer layer of small airways of COPD patients when compared with the control group. In the large airways and lung parenchyma there was no difference in fibronectin fractional area among groups (Annoni et al. 2012). These data suggest that EDA-containing fibronectin has a pivotal function in the development of fibrosis. Additionally, these data may explain the increased incidence of fibrosis during ageing as fibronectin EDA levels are increased with ageing. Speculatively, the alternative splicing of fibronectin may be a dominator in the development of either COPD or IPF.

The pivotal function of fibronectin EDA in lung fibrogenesis was established in transgenic mouse models by Muro *et al*. In their study, they revealed that fibronectin EDA null-mice fail to develop bleomycin-induced fibrosis, whereas the control wild-type mice have higher total lung collagen, which indicates fibrogenesis. This observation correlated with the diminished activation of the latent transforming growth factor (TGF) - β1, and a decreased lung fibroblast responsiveness to active TGF-β1, which is a potent profibrotic growth factor. Furthermore, *in vitro* studies revealed that lung fibroblast without functional fibronectin gene induce TGF-β1-mediated fibroblast differentiation, but only in presence of a matrix containing fibronectin EDA (Muro et al. 2008). Interestingly, the role of fibronectin EDA and TGF-β1 in fibrogenesis is not disease specific as similar results were observed in allergen-induced chronic asthma models, as well as in human renal epithelial cells. (Kohan et al. 2011; Phanish et al. 2015). These observations suggest that EDA-containing fibronectin and TGF-β contribute to the development of fibrosis. However, until now it is unclear how the exact mechanism works.

In all presented studies above, TGF-β1 and fibronectin EDA exhibit a mechanistic interplay in fibrogenesis. The mechanism that drives both contributors is currently unclear (Kohan et al. 2011; Muro et al. 2008; Phanish et al. 2015). In the matrix, TGF-β is captured in a latent state in a protein complex containing TGF-β, latency-associated peptide (LAP), and latent TGF-β-binding protein-1 (LTBP-1). In the presence of proteases like matrix metalloproteases (MMPs) and furin-like enzymes, TGF-β is cleaved and results in activation of latent TGF-β leading to myofibroblast activation, and subsequently fibrosis. In light of this mechanism, White *et al.* proposed that LTBP-1 requires a fibronectin EDA substrate for binding and localization of latent TGF-β1 (Eric S. White and Muro 2011). This proposed mechanism is plausible for lung fibrosis, as fibronectin EDA levels are increased in patients who suffer from lung fibrosis.

Fibronectin elicits stress response in the endoplasmic reticulum

In the previous paragraph, we discussed that ageing is accompanied by increased fibronectin levels, which isoforms differ between COPD and IPF patients. Further, we discussed that fibronectin EDA is considered necessary for TGF-β1-induced fibroblast differentiation. We argued that a similar mechanism is potentially involved in the development of lung fibrosis. Moreover, we already discussed that ageing is characterized by a progressive loss of proteostasis. Therefore, it is reasonable to suggest that aberrant fibronectin EDA expression as a result of ageing or impaired wound healing, elicits ER stress responses due to loss of the proteostasis machinery. Interestingly, studies in macrophages showed that fibronectin EDA isoform expression triggered ER stress, and this stress was inhibited by a chemical chaperone (4-phenyl butyric acid) or by overexpressing of the recombinant ER chaperone GRP78/BiP, which is a member of the HSP family (Du et al. 2015). The aberrant fibronectin EDA expression and impaired proteostasis in fibrosis may be an explanation for the predisposition for disrepair in aged lungs. In a study by Subeblinvong *et al.*, bleomycin-induced injury displayed worse lung fibrosis and higher mRNA expression of fibronectin EDA, MMP-2 and MMP-9 in old mice when compared with lungs from young mice. In addition, TGF-β receptor 1 and TGF-β expression and activity was increased in old mice (Sueblinvong et al. 2012). The apparent differences between young and aged ECM might explain the observation that anti-fibrotic treatments showed positive effects in young animal models, while the treatment later failed with older patients in clinical studies. These data suggest that aberrant fibronectin EDA expression causes ER stress, probably due to impaired proteostasis or protein overload, leading to fibrosis.

Conclusion and discussion

Ageing is characterized by a progressive decline in physiological integrity leading to an impaired cellular functionality, and an increased risk for the development of pathologies. One hallmark of ageing is the loss of proteostasis, which is mainly caused by an impaired protein folding machinery and proteolytic systems. The ageing lung exhibits progressive alterations in the ECM compositions that result in a functional decline of the lung. A similar process is reported in the development of two chronic respiratory diseases, namely, COPD and IPF. However, it is not completely understood whether the loss of proteostasis affects the integrity of the lung ECM in ageing and respiratory diseases. In this essay, we attempted to answer this question based on the current literature. Therefore, we discussed two proteins that are either directly or indirectly involved in the compositions of the lung's matrix.

The hepatic produced anti-proteolytic protein A1AT has a crucial function in the ECM maintenance of lung. However, misfolded A1AT proteins lead to aggregation and accumulation within the ER of hepatocytes, and subsequently to development of liver fibrosis as result of ER stress responses. It is plausible that a similar process occurs in the lungs as A1AT is also produced in the epithelial cells of the lung. There is *in vitro* evidence reporting that overexpression of mutant A1AT in human lung cells is accompanied by an increased gene expression of NF - κB, which is upregulated in response to protein overload responses in the ER. Further, increased eLF2 phosphorylation in ZA1AT expressing lung cells suggests the activation of unfolded protein pathway. These data suggest that aberrant A1AT proteins induce stress responses in the lung, and may contribute to ECM dysregulation. Further, in ageing lungs, it reasonable to suggest that altered gene expression (*i.e.* epigenetic alterations, histone modifications, and DNA methylation) and/or impaired proteostasis lead to the production of altered A1AT proteins. This combination may lead to ER stress, ECM dysregulation, and subsequently to development of lung fibrosis. The proposed mechanism is illustrated in Figure 4.

In the lungs, fibronectin and the alternative spliced variant fibronectin EDA increase with age. We further discussed that fibronectin EDA is considered necessary for TGF-β1-induced fibroblast differentiation. The fibronectin EDA protein is capable to elicit stress responses in the ER. We proposed that in the lungs, injury stimulates fibroblast differentiation via fibronectin EDA and TGF-β1. In the ageing lung, however, increased fibronectin EDA expression lead to an increased fibroblast and myofibroblast activation. In the ER stress responses stimulate downstream pathways in response to fibronectin EDA accumulation. The proteostasis network cannot overcome the stress. The proposed mechanism is illustrated in Figure 5.

In conclusion, dysregulated ECM is not a separate phenomenon in the process of ageing in the lungs, and is accompanied by loss of proteostasis. It is like that COPD and IPF are not accelerated ageing-diseases, but result from aberrant tissue repair responses.

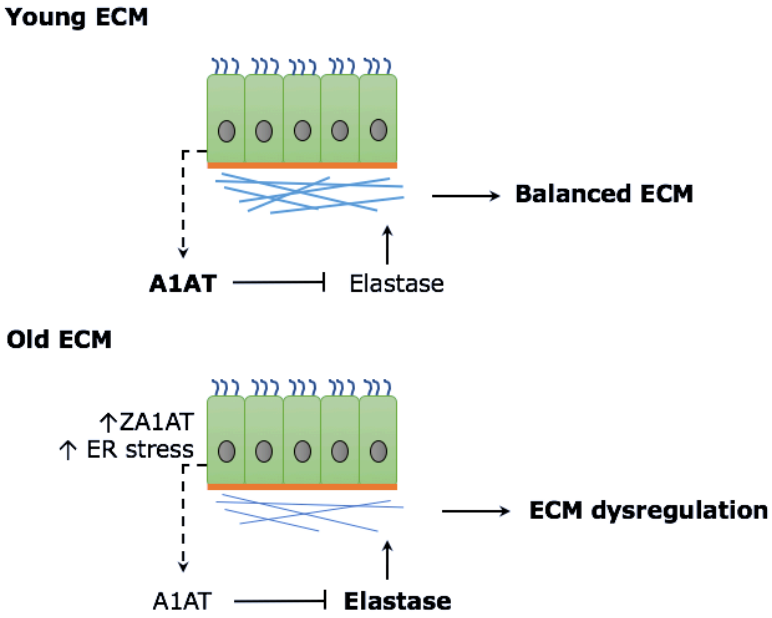

Figure 4: **Proposed mechanism of altered extracellular matrix proteostasis.** In the young ECM, turnover is balanced via the inhibition of elastase by local secreted A1AT. Altered gene expression and loss of proteostasis lead to increased mutant ZA1AT and decrease A1AT. The normal A1AT is not able to inhibit elastase resulting in an increased degradation of the ECM, and, hence, ECM dysregulation.

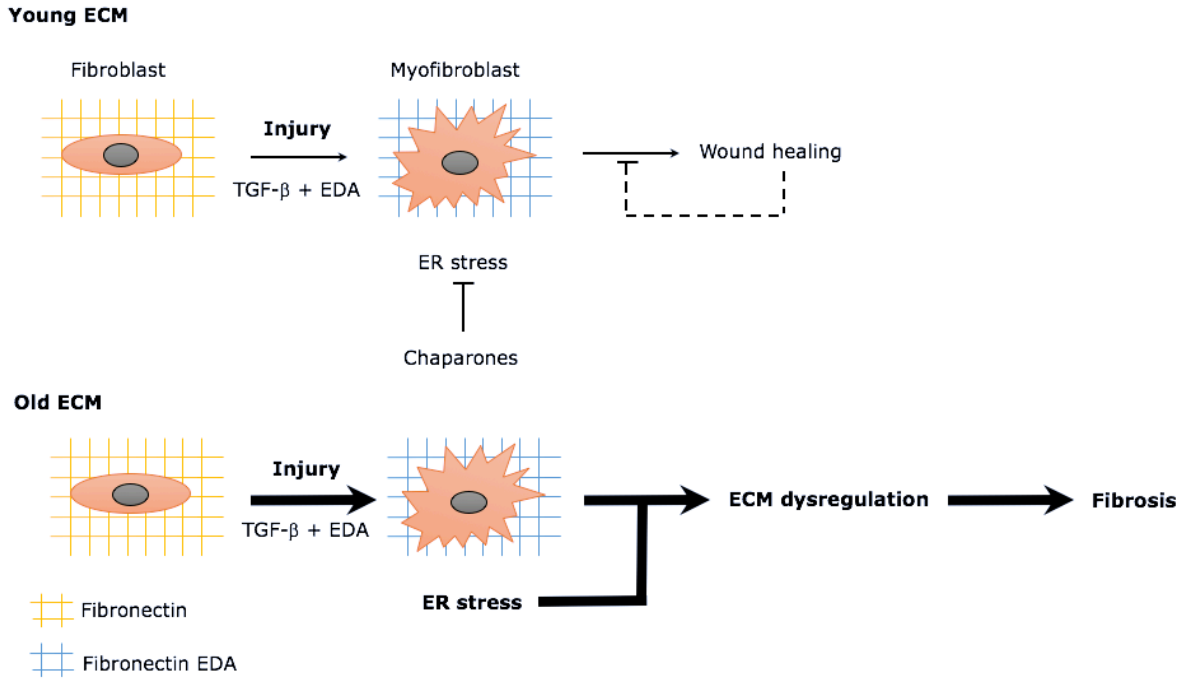

Figure 5: **Proposed mechanism in fibronectin EDA mediated wound healing.** In the young ECM, injury stimulates fibronectin EDA expression and induces fibroblast differentiation via a fibronectin EDA/ TGF-β1 – mediated pathway. The injury is repaired and fibronectin EDA induced stress is reduced via the proteostasis network. In the older ECM, increased fibronectin EDA and TGF-β1 extensively stimulate fibroblast differentiation in response to injury. The fibronectin EDA induced ER stress is not mitigated. The ECM is dysregulated and ultimately leads to fibrosis.

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