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Martina Korfei*, Clemens Ruppert, Benjamin Loeh, Poornima Mahavadi#, Andreas Guenther#

The role of Endoplasmic Reticulum (ER) stress in pulmonary fibrosis

DOI 10.1515/ersc-2016-0002
Received December 8, 2015; accepted April 19, 2016

Abstract: The activation of Endoplasmic Reticulum (ER) stress and Unfolded Protein Response (UPR) was first observed in patients with familial interstitial pneumonia (FIP) carrying mutations in the C-terminal BRICHOS domain of surfactant protein C (*SFTPC*). Here, aggresome formation and severe ER stress was demonstrated in type-II alveolar epithelial cells (AECII), which specifically express this very hydrophobic surfactant protein. In subsequent studies, FIP-patients with mutations in the gene encoding surfactant protein A2 (*SFTPA2*) were discovered, whose overexpression in epithelial cells *in vitro* also resulted in significant induction of ER stress. Moreover, prominent ER stress in AECII was also observed in FIP-patients not carrying the *SFTPC*/*SFTPA2* mutations, as well as in patients with the more common sporadic forms of IP. Additionally, cases of adult-onset FIP with mutations in *Telomerase* genes and other telomere-associated components were reported. These mutations were associated with telomere shortening, which is a potential cause for triggering a persistent DNA damage response and replicative senescence in affected cells. Moreover, shortened telomeres were observed directly in the AECII of FIP-patients, and even sporadic IP cases, in the absence of any gene mutations. Here, we try to figure out the possible origins of ER stress in sporadic IP cases and non-*SFTPC*/*SFTPA2*-associated FIP.

Keywords: Idiopathic Interstitial Pneumonia; endoplasmic reticulum stress; type-II alveolar epithelial cell; surfactant processing

***Corresponding author: Martina Korfei,** Clinical Research Group Lung Fibrosis, Biomedical Research Center Seltersberg, Justus-Liebig-University Giessen, Schubertstrasse 81, D-35392 Giessen, Germany, E-mail: Martina.Korfei@innere.med.uni-giessen.de
Martina Korfei, Clemens Ruppert, Benjamin Loeh, Poornima Mahavadi, Andreas Guenther, Dept. of Internal Medicine, Clinical Research Group Lung Fibrosis, Justus-Liebig-University Giessen, D-35392 Giessen, Germany.
Martina Korfei, Clemens Ruppert, Benjamin Loeh, Poornima Mahavadi, Andreas Guenther, Universities of Giessen and Marburg Lung Center (UGMLC), Member of the German Center for Lung Research (DZL).
Clemens Ruppert, Andreas Guenther, Excellence Cluster Cardio-Pulmonary System (ECCPS), D-35392 Giessen, Germany.
Andreas Guenther, Agaplesion Lung Clinic Waldhof Elgershausen, D-35753 Greifenstein, Germany.
Andreas Guenther, European IPF Network and European IPF Registry.
# both authors contributed equally.

1 The principles of ER stress signaling

1.1 Adaptive and maladaptive UPR pathways

The endoplasmic reticulum (ER) is the main subcellular compartment involved in protein folding and secretion. A complex network of chaperones, foldases, co-factors, quality control mechanisms and processing enzymes assist the correct folding of proteins at the ER, in order to ensure an efficient production of proteins, and thus protein homeostasis ('proteostasis'). Multiple physiologic or pathologic conditions that affect ER homeostasis and ER capacity can trigger ER stress, including glucose starvation, impaired glycosylation, changes in the redox or ionic conditions of the ER lumen, elevated protein synthesis (e. g. due to viral infection), insufficient lysosomal degradation, gene mutations causing malfolding, and oxidative stress, which all consecutively lead to the accumulation of unfolded and misfolded proteins in the ER due to the failure of protein folding and processing under these conditions. ER stress triggers an adaptive mechanism to cope with protein misfolding known as the unfolded protein response (UPR), which aims to restore ER homeostasis by increasing the capacity of the ER to fold and process client proteins and to reduce the protein load in the ER [1-3].

Three highly conserved stress sensors at the ER membrane, known as PKR-like ER kinase (PERK), activating transcription factor 6 alpha (ATF6α) and

inositol-requiring enzyme 1 alpha (IRE1α), initiate the cellular answer to ER stress, the UPR. Immunoglobulin heavy-chain-binding protein (Bip), also referred to as glucose regulated protein 78 (GRP78), is bound to these transmembrane stress sensor-proteins, keeping them in an inactive state. As accumulation of (misfolded, unfolded) proteins occur in the ER, GRP78 dissociates from PERK, ATF6α and IRE1α in order to bind and fold these client proteins, thereby activating the UPR signaling pathways governed by these three ER stress-sensor proteins [3]. Upon activation by autophosphorylation and dimerization, PERK signaling leads to global translational repression, via direct phosphorylation of its target eukaryotic translation initiation factor alpha (eIF2α), which leads to a reduced protein burden in the ER and in turn allows the selective translation of activating transcription factor 4 (ATF4) [1-3,4]. ATF4 has been shown to increase the expression of genes involved in amino acid metabolism, antioxidant responses and autophagy [4]. ATF4 is also essential for the transition from adaptive to proapoptotic ER stess-programs when cells are suffering from excessive or long-term accumulation of unfolding proteins. The major mechanism driving apoptosis by ATF4 is the induction of the pro-apoptotic transcription factor CCAAT/enhancer binding protein (EBP) homologous protein (CHOP) [5, 6] which downstream induces the mitochondrial apoptosis pathway [7].

When activated by ER stress, 90kDa ATF6α is transported to the Golgi apparatus, where it is processed by specific site1 and site2 proteases, thereby releasing the NH_2-terminal cytosolic fragment p50ATF6(N), which migrates to the nucleus to serve as a potent transcription factor for ER chaperones (GRP78, GRP94, calreticulin, calnexin), thus increasing the folding capacity of the ER [1-3].

IRE1α is a kinase and endoribonuclease which is acticated upon ER stress by dimerization and autophosphorylation. Active IRE1α catalyzes the unconventional splicing of the mRNA encoding X-box binding protein 1 (XBP1), resulting in a spliced variant encoding for an active transcription factor (XBP1s), which, in turn, further upregulates chaperone production and proteins involved in ERAD (ER associated degradation) function, the pathway involved in disposal of terminally misfolded proteins by the ubiquitin-proteasome pathway [1-3,8]. In addition, p50ATF6(N) is also indirectly involved in induction of ERAD through upregulating the *XBP1* mRNA [9].

If ER stress is not resolved by adaptive UPR signaling, several ER stress-mediated apoptosis pathways are induced to enforce the death of the concerned cell, such as activation of c-Jun amino-terminal kinase (JNK) via a complex of (dimerized) phosphorylated (p)IRE1α, TRAF2 (TNF receptor-associated factor 2) and ASK1 (apoptosis signal-regulating kinase 1) [10], activation of ER stress-specific caspases 4 (human) and 12 (mouse) [11,12], and induction of the pro-apoptotic ER-stress associated transcription factor CHOP [5,6]. CHOP is a member of the C/EBP transcription factor family that heterodimerizes with other C/EBPs and mediates induction of apoptosis through the mitochondrial pathway. These heterodimers control the expression of Bcl-2 family members and enforce dimerization of Bax proteins which triggers cytochrome c-release and formation of the apoptosome [7,13]. According to the current paradigm, the ATF4 - , ATF6α - and XBP1-pathways coordinate to activate the CHOP gene to the optimal level under severe ER stress conditions [2,3,14,15].

Additionally, and more recently, new molecular mechanisms of apoptosis triggered by hyperactivated IRE1α have been identified: i) The Regulated IRE1 Dependent Decay (RIDD) dependent degradation of mRNAs by IRE1α has been proposed to perform the role of a cell death-executioner by degrading mRNAs encoding pro-survival proteins (i. e. chaperones) during prolonged ER stress [16,17]. ii) IRE1α also degrades selected microRNAs (miRs), such as *miR-17*, *miR34a*, *miR-96*, and *miR-125b* to promote translation of caspase-2 (which is normally repressed by these miRs). Increased expression of *Casp2* mRNA then contributes to apoptotic cell death through proteolytic cleavage of Bid, which then causes cytochrome c release from mitochondria [18]. iii) IRE1α and PERK signaling were shown to induce pro-oxidant TXNIP (Thioredoxin-interacting protein), leading to activation of the NLRP3 inflammasome and IL-1β expression, and consecutively, cell death [19,20]. Whether hyperactivation of IRE1α occurs independently of CHOP induction or after CHOP induction, has not yet been clarified.

1.2 Induction and role of CHOP in ER stress-induced apoptosis

Induction of the transcription factor CHOP, also known as growth arrest- and DNA damage-inducible gene 153 [GADD153]), is a critical cellular response for the transcriptional control of ER stress-induced apoptosis. CHOP is expressed at very low levels in non-stress conditions, but is robustly upregulated in chronic ER stress [3,6]. *In vitro* overexpression of CHOP results in apoptosis [21-23], while deficiency of CHOP protects cells from apoptosis [24,25].

To date, it is believed that the transcriptional induction of CHOP during ER stress is regulated by four *cis*-acting

elements, namely by two amino-acid-response elements AARE1 and AARE2 (also designated as C/EBP-ATF binding sites), and by two ER stress-response elements ERSE1 and ERSE2 [1-3]. It has been documented that PERK/eIF2α signaling is an absolute requirement for induction of the *CHOP* gene during ER stress and that ATF4 may activate *CHOP* transcription during the early stress response by binding to both AARE´s [4,15,26], whereas the IRE1α/XBP1- and ATF6α pathways have been implicated in induction of CHOP expression in late ER stress through binding to the ERSE elements [14,27]. Of note, induction of CHOP *in vitro* could be achieved in HeLa cells with single overexpression of nuclear p50ATF6(N) as ER stress trigger [14].

Upon induction and nuclear translocation, CHOP upregulates the transcription of proapoptotic factors such as BH-3 only (Bim) [28] and death receptor 5 (DR5) [3], and down-regulates anti-apoptotic genes such as B-cell leukemia/lymphoma 2 protein (Bcl-2) [13]. Moreover, CHOP has been implicated in exaggerated ROS production by upregulation of the UPR-regulated oxidative protein folding machinery (represented by ER oxidoreductin 1α, ERO1α) in the ER, which is directly contributing to ROS generation through the oxidation of disulfide bonds [29]. Furthermore, CHOP induces expression of GADD34 (growth arrest and DNA damage 34), a subunit of type 1 protein phosphatase that directs elongation factor eIF2α-dephosphorylation to increase mRNA translation, thereby enhancing protein synthesis in the stressed ER [29]. Taken together, persistent ER stress, through induction of CHOP, may lead to an "overactive" UPR resulting in excessive protein synthesis and ROS accumulation, and, finally, cell death [29].

CHOP belongs to the 'basic leucine zipper domain' (bZIP) family, and it is suggested that the majority of the proapoptotic function of CHOP resides in the carboxy-terminal bZIP region. In order to function as an apoptotic promotor, CHOP heterodimerizes with other members of the bZIP family such as ATF4 [30], and most notably with C/EBPβ (isoforms C/EBPβ-LIP and C/EBPβ-LAP [31-33]). Importantly, *Chop*$^{(-/-)}$ mouse embryonic fibroblasts (MEFs) are more resistant to ER stress-induced apoptosis (tunicamycin treatment) than their wild-type counterparts [6]. In addition, the same was observed for *Cebpb*$^{(-/-)}$ MEFs [6], thus underscoring the relevance of this transcription factor in regulation of genes downstream of CHOP during severe ER stress. Moreover, CHOP has been shown to be involved in IL6 induction through trapping of inhibitory C/EBPβ-LIP and promoting of C/EBPβ-LAP-mediated transcription of the *IL6* gene [34], thus being a potent inducer of inflammatory events in response to ER stress. In line with this, the ER stress induced CHOP pathway has been reported to be involved in interleukin-1β (IL1β) production via the induction of caspase-11, and which occurs also in response to non-inflammatory stimuli such as tunicamycin [25,35].

Other identified target genes of *CHOP* involve close homologues of the actin-binding proteins villin and gelsolin [32]. The latter has been found to undergo cleavage by caspase 3 during programmed cell death, and the N-terminal fragment generated by this cleavage causes cytoskeletal collapse resulting in apoptotic cell death [36].

Tribbles homolog 3 (*TRB3*) is a proapoptotic gene induced by cooperation of ATF4 and CHOP [30]. It has been reported to dephosphorylate the survival-related protein AKT, whose inactivation is essential for the conformational change and translocation of Bax-proteins to mitochondria [37,38]. Thus, TRB3 represents a link between ER stress-induced CHOP and AKT dephosphorylation in inducing Bax-translocation to induce apoptosome formation [30].

More recently, *CHAC1*, encoding the glutathione-specific gamma-glutamylcyclo-transferase 1-enzyme, has been reported as a proapoptotic target gene of the ATF4-ATF3-CHOP pathway, but inhibition of CHOP by RNAi mediated gene silencing was sufficient to prevent UPR-induced *CHAC1* expression in response to tunicamycin in several tumor cell lines [39]. CHAC1 is reported to lead to depletion of intracellular glutathione levels through degradation, which is an important factor for apoptosis initiation and execution [40]. Moreover, CHAC1 overexpression *in vitro* resulted in augmentation of TUNEL staining, cleavage of PARP, and nuclear translocation of AIF, but not in caspase-3 cleavage (but which is induced by CHOP through the mitochondrial apoptosis pathway), thus underscoring the ability of CHOP to induce various apoptotic pathways in response to severe ER stress [39].

2 The Idiopathic Interstitial Pneumonias – an introduction

Idiopathic interstitial pneumonias (IIPs) are a heterogenous subgroup of the family of diffuse parenchymal lung diseases (DPLDs), the latter of which also includes DPLD of known causes such as drugs (i. e. amiodarone), association with collagen vascular disease, or granulomatous DPLD such as sarcoidosis [41,42]. According to the revised ATS/ERS classification of the IIPs, the major IIPs comprise the following entities: (1) idiopathic pulmonary fibrosis (IPF), (2) idiopathic non-specific interstitial pneumonia (NSIP), (3) respiratory bronchiolitis-interstitial lung disease (RB-ILD), (4)

desquamative interstitial pneumonia (DIP), (5) cryptogenic organising pneumonia (COP) and (6) acute interstitial pneumonia [41,42]. Although all IIPs are characterized by their unknown aetiology, the common histological feature is a distortion of the lung interstitium by highly variable combinations of inflammation and fibrosis - "interstitial pneumonia" [41,42]. The "fibrotic change" in the IIPs has been commonly suggested as thickening of alveolar walls, resulting from deposition of fibrotic tissue between the alveolar epithelial and capillary endothelial basement membranes. The process of alveolar wall thickening is thought to start with repetitive injuries to alveolar epithelial cells (AEC), resulting in collapse of alveoli, and alveolar exudates promote consecutive proliferation and migration of fibroblasts from the interstitium. A subgroup of fibroblasts may differentiate into contractile protein-expressing myofibroblasts, which produce and secrete large amounts of ECM components, thereby resulting in the formation of fibroblastic tissue and scars. The outcome of fibroblastic tissue and scar formation is variable among the IIPs and seems to be determined primarily by alveolar epithelial cell integrity after injury [41,42].

IPF is the most common form of the IIPs, and has a poor prognosis. It is histologically defined by the usual interstitial pneumonia (UIP) pattern, and typically affects individuals aged 50 to 75 years and occurs somewhat more frequently in men [41,43,44]. UIP is characterized by a dense fibrosis that causes destruction of the alveolar architecture, with frequent honeycombing and bronchiolization of the alveoli, scattered fibroblast foci, and patchy lung involvement. At low magnification, the lung characteristically has a heterogenous appearance with alternating areas of normal parenchyma, fibrosis, and (bronchiolized) honeycomb cysts [41,43,44]. Up to one in five patients with IPF have a family history of interstitial lung disease, comprising the syndrome of familial interstitial pneumonia (FIP) [45]. Familial and sporadic IP diseases are clinically and histologically indistinguishable, but FIP tends to present at an earlier age [44,46]. FIP has been linked to rare heterozygous mutations in genes encoding for components of the pulmonary surfactant system [surfactant protein A2 (*SFTPA2*), surfactant protein C (*SFTPC)*] or proteins involved in telomere maintenance [telomerase reverse transcriptase (*TERT*), telomerase RNA component (*TERC*), dyskerin (*DKC1*) and some other telomere-associated genes], that account for 10-15% of FIP cases [46].

The molecular pathomechanisms underlying IPF and other IIP in general are incompletely understood. According to the current paradigm, IPF is thought to be the result of chronic injury to the alveolar epithelium that activates aberrant repair processes resulting in replacement of alveolar architecture with fibrotic scars and cysts lined by abnormal bronchiolar epithelium [43,47,48]. Consistent with a central role for the alveolar epithelium in disease pathogenesis, apoptosis of type-II alveolar epithelial cells (AECII) is a prominent finding in IPF [49-52], which has been also observed in patients with chronic extrinsic allergic alveolitis (EAA), NSIP and other ILD´s [23,53,54]. Multiple lines of evidence underscore a pivotal role of alveolar epithelial ER stress leading to AECII apoptosis in the pathogenesis of IPF. Mutations in the gene for surfactant protein (SP)-C, an AECII-specific protein, were associated with ER stress in AECII and familial interstitial pneumonia (FIP) in both children and adults (55-58). Moreover, the finding of proapoptotic ER stress in AECII is also prominent in patients with sporadic IPF (and other IIP) in the absence of any gene mutation (23,58,62) – and does not seem to differ in extent from familial IPF cases [58]. Besides, induction and nuclear translocation of CHOP in AECII is an eminent observation in UIP lungs [59,63], but also in patients with Hermansky-Pudlak Syndrome 1 (HPS1)-associated IP (HPS-IP) [64], as well as in individuals with systemic sclerosis (*Ssc*)-associated ILD [23]. In both of these latter diseases, pulmonary fibrosis is the leading cause of mortality and morbidity and frequently imposes with an UIP pattern [64,65]. Additionally, increased protein contents of p-IRE1α have been encountered in the AECII of IPF patients [61]. It can thus be hypothesized that pro-apoptotic signaling in IPF-AECII largely involves CHOP and p-IRE1α. Moreover, increased inflammation, which has been reported as a secondary and end-stage effect in IPF, may underly increased proapoptotic signaling through p-IRE1α as well as CHOP, which both are able to produce IL1β [19,20,25,35].

The evidence for such prominent role of ER stress in IPF (and other IIP, such as fibrotic NSIP) is detailed in the following chapters.

3 ER stress in genetically defined forms of pulmonary fibrosis

3.1 ER stress in Familial Interstitial Pneumonia (FIP) due to SFTPC mutations

FIP is most commonly associated with UIP on lung biopsy [44], and according to recent reports it has been suggested that up to 20% of cases of IPF might be familial [44,46]. Initial insights into the role of ER stress in IPF came from the observation that *SFTPC* mutations were associated

with FIP in both adults (mostly IPF) and pediatric patients (mostly NSIP, DIP). Meanwhile, over 60 different mutations in the *SFTPC* gene have been described in FIP [66].

SP-C is a very hydrophobic peptide which is synthesized exclusively by AECII as a 21 kDa precursor (proSP-C) and is further processed into its 4.2 kDa mature form through more than four sequential proteolytical cleavages. Only then it will be secreted together with surfactant protein B (SP-B) and phospholipids and other surfactant components into the alveolar space [67]. The enzymes involved in SP-C processing are the aspartyl proteases napsin A [68] and pepsinogen C [69] and the cysteine protease cathepsin H [70]. Importantly, SP-C processing is a sensitive multistep-enzymatic process and is dependent on the successful transport through the regulated secretory pathway and the correct lysosomal environment [67].

Many of the known SP-C mutations reside within the preprotein´s BRICHOS domain, a ~100 amino acid region in the COOH-terminal area. This region bears homology to a number of proteins linked to familial neurodegenerative disease and amyloid formation [71]. The mutations result in misfolding of the proprotein and subsequent intracellular aggregate formation [56,72]. In detail, Nogee *et al*. first described in 2001 a *SFTPC* gene mutation in the BRICHOS domain (c.460 + 1 G → A) on one allele, which resulted in alternative splicing of the SP-C transcript and deletion of exon 4 including its 37 amino acids (Δexon4) [55]. Due to elimination of crucial disulfide bridge-forming cysteines (C120/C121), the mutated proprotein cannot be properly folded for proteolytical cleavages, and thus cannot be processed into its mature form. As consequence of misfolding and complete abrogation of post-translational processing, the abnormal SP-C-proprotein is retained inside the ER, followed by cytosolic accumulation and aggregation of that unprocessed 21 kDa proSP-C and induction of severe ER stress and caspase-3-activation (shown *in vitro* by overexpression in alveolar epithelial tumor cell lines) [57,72,73]. Moreover, due to aggresome formation, clearance of misfolded proSP-C by ER associated degradation (ERAD) pathways is inhibited [57,73]. Likewise, a different reported BRICHOS *SFTPC* mutation leads to substitution of glutamine for leucine at amino acid position 188 of proSP-C, which is very close to another crucial disulfide bridge-forming cysteine (C189), and which, *in vitro*, resulted in aggresome formation, proteasome inhibition and apoptosis [56,73]. In patients, these two heterozygous mutations were associated with NSIP, DIP and PAP in pediatric cases and NSIP or (mostly) UIP in adults, with prominent ER stress in AECII and no detectable mature SP-C in patients´ lungs, suggesting a dominant-negative effect of the mutant allele [55,56]. Indeed, it could be shown *in vitro* that SP-C$^{\Delta Exon4}$ and SP-C^{L188Q} bind to SP-C^{wt} via heteromeric association during dimeric sorting in the Golgi-apparatus, thereby inhibiting the trafficking and further processing of SP-C^{wt} in distal vesicles through misleadingly guiding it to aggresome formation together with the mutant SP-C proteins [72,74].

Interestingly, overexpression of both BRICHOS domain mutants SP-C$^{\Delta Exon4}$ and SP-C^{L188Q} in lung epithelial cells *in vitro* did not result in induction of the ER stress-associated proapoptotic transcription factor CHOP, but in increased c-jun kinase (JNK) signaling as well as activation of the ER-specific caspase-4, which both lead to caspase-3 mediated cell death in the setting of overwhelming ER stress [75]. In contrast to these reports, two independent research groups observed that alveolar epithelial A549 cell lines underwent epithelial-to-mesenchymal transition (EMT) in response to ER stress induced by *in vitro*-overexpression of SP-C$^{\Delta Exon4}$ and SP-C^{L188Q}, as indicated by induced expression of myofibroblastic cell markers α-SMA and S100A4 in transfected cells, and without any signs of apoptosis [76,77]. In line with these observations, conditional overexpression of human SP-C^{L188Q} in AECII of transgenic mice *in vivo* revealed pronounced induction of ER stress, but without apoptotic signaling in alveolar epithelium [78]. Consequently, AECII death and the development of lung fibrosis were not observed in these mice, not even with the transgene being shut-on for six months. However, elevated development of lung fibrosis was observed in SP-C^{L188Q} overexpressing mice in response to bleomycin treatment in comparison to *wt* (background) mice, suggesting the requirement of a "second hit" on top of alveolar epithelial ER stress [78]. In line with this notion, Bridges *et al*.. (2006) described that HEK293 cells with stable overexpression of SP-C$^{\Delta Exon4}$ adapted to chronic ER stress imposed by the mutant SP-C proprotein, presumably due to activation of cytoprotective UPR pathways, but revealed an increased susceptibility to virus infection in comparison to empty-vector transfected cells. Moreover, virus-infected HEK293 cells stably expressing the SP-C$^{\Delta Exon4}$-mutant indicated an aggravated ER stress-response with induction of cell death when compared to non-infected SP-C$^{\Delta Exon4}$ expressing cells, presumably as a result of overwhelmed ERAD and cellular quality control-apparatus due to accumulation of both the virus-envelope proteins and the misfolded SP-C mutant [79]. Indeed, herpesvirus DNA and proteins (EBV, CMV, HHV-8) have been detected in the lungs of patients with IPF, with early studies showing herpesvirus infection (EBV) localized to AECII [80]. In addition, two studies from the Vanderbuilt group (Lawson *et al*., 2008; Kropski *et al*., 2015) have

reported that various herpesvirus (envelope) proteins were localized in AECIIs of patients with sporadic and familial IPF in IHC-studies and colocalized with XBP1 and GRP78, suggesting that herpesviruses may indeed play a role in disease progression of IPF [58,81]. On the other hand, ER stress is also commonly detected in lungs of IPF patients without herpesvirus infection [81].

These observations nevertheless suggest that the low penetrance of these mutations in FIP, with great variability in the severity and the age of onset of lung fibrosis, may be related to environmental insults [82]. It is also an astonishing fact that genetically related individuals with *SFTPC* associated-FIP raised in different environments developed either IPF as adults or the milder entities DIP, PAP and NSIP in childhood [56,83].

In addition to these both clinically characterized BRICHOS domain-SP-C mutations, over 60 additional *SFTPC* mutations associated with interstitial lung disease have been reported, with various locations within the SP-C propeptide [66]. A heterozygous non-BRICHOS missense mutation, leading to a substitution of threonine for isoleucine at position 73 of proSP-C (SP-C^{I73T}), has been identified in multiple unrelated families and is the most frequent *SFTPC* mutation observed to date [84,85]. Importantly, this gene mutation occurs also in sporadic cases of IP and demonstrates that even sporadic IP may have a genetic basis due to spontaneous mutation [86]. Similarly to SP-C$^{\Delta Exon4}$ and SP-C^{L188Q}, there is a strong variability in the "ILD-outcome" and onset of disease amongst mutation carriers, ranging from early fatal disease in children and UIP/IPF in adults [84,85]. Again, this example further illustrates that childhood ILD and adult IPF represent the two faces of the very same, genetically based pathomechanism, which presumably requires environmental modifiers for ultimative disease-development.

The cellular consequences of mutant SP-C^{I73T} expression *in vitro* and *in vivo* were mistrafficking of the proprotein to the plasma membrane and marked processing defects of both proSP-C as well as proSP-B, resulting in consecutive aberrant secretion and exaggerated accumulation of unprocessed hydrophobic surfactant proteins together with SP-A, phospholipids, and periodic acid-Schiff (PAS)-positive macromolecules into the alveolar space [87-89]. Moreover, Stewart *et al.* observed that SDS-insoluble SP-C proprotein-aggregates were much more abundant in SP-C^{I73T} expressing HEK293 cells as compared to cells expressing SP-C^{wt}, SP-C^{L188Q} and SP-C$^{\Delta Exon4}$, thereby confirming the cell-surface localization of the accumulated SP-C^{I73T} mutant [87,90]. In accordance with these observations, Hawkins and co-workers recently observed that the overexpressed SP-C^{I73T} mutant is re-internalized through the endosomal compartment and led to an aberrant protein turnover within the lysosomal/autophagic machinery, resulting in marked disruption of autophagy-dependent proteostasis [91]. As a consequence, accumulation of large vacuoles containing both unprocessed proteinacous debris and elevated autophagy markers (LC3, p62, parkin) occured, which is indicative of an impaired cellular degradative capacity in SP-C^{I73T} overexpressing cells [91] and which might also involve development of ER stress in affected cells. In line with this suggestion, Woischnik *et al.* showed that disturbed cellular function in epithelial MLE12 cells overexpressing mutant SP-C^{I73T} could be alleviated by exposure of cells to pharmaceutical drugs used for ILD therapy, such as cyclophosphamide, hydroxychloroquine and methylprednisolon, which led to an induction of chaperones Hsp70/Hsp90, and the ER quality control-apparatus component calreticulin [92]. In another study the same group could show that stable overexpression of mutant SP-C^{I73T} in A549 cells led to marked intracellular accumulation of misprocessed proSP-C-intermediates in the molecular weight-range 16-25 kDa, that were also seen in cells expressing the BRICHOS mutants SP-C^{A116D} and SP-C^{L188Q}, when compared to SP-C^{wt} expressing cells. Despite these pronounced cellular alterations, A549 cells overexpressing mutant SP-C^{I73T} did neither show intracellular accumulation of Congo red-positive aggregates nor indicated any signs of increased ER stress [93]. Moreover, the authors did not observe the development of significant ER stress in A549 cells in response to stable overexpression of SP-C-BRICHOS mutants SP-C^{L110R}, SP-C^{A116D}, SP-C^{L188Q} and SP-C^{P115L}, although all these SP-C mutants led to intracellular accumulation of Congo red-positive aggregates, and were associated with generation of aberrant, misprocessed proSP-C intermediates (except SP-C^{P115L}) in transfected cells [93]. However, it should be mentioned that the authors analyzed only the expression of GRP78 as ER stress-readout-parameter in their *in vitro*-models, whereas direct activation of the three UPR sensors ATF6α, IRE1α and PERK was not assessed [93]. Under conditions of ER stress when unfolded or misfolded proteins accumulate in the lumen of the ER, (constitutively expressed) bound GRP78 dissociates from ATF6α, IRE1α and PERK to chaperone the misfolded proteins thereby permitting the activation of one or more of these ER stress transducers, which may include the upregulation of GRP78 and/or other ER stress-response-element (ERSE) genes by the ATF6α-pathway [1-3]. However, whether there is induction of GRP78 in response to misfolded proteins, might depend on the nature of the malfolded

protein. Several abnormalities, including exposure of hydrophobic regions, unpaired cysteines and the tendency to form aggregates, might also result either in immediate activation of proapoptotic ER stress signaling pathways or in induction of ER stress-mediated autophagy. The latter has been shown to be linked to PERK/ATF4-activation [4]. ER stress has also recently been reported to upregulate the E3 ubiquitin ligase Nedd4-2 through spliced XBP1 to induce autophagy in the livers of high-fructose and high-fat diet-fed mice [94]. Vice versa, various reports exist that impaired autophagy can induce proapoptotic ER stress [95].

Taken together, both the *in vitro* and *in vivo* studies about the cellular consequences of mutant SP-C^{I73T} overexpression should include a more comprehensive analysis for ER stress markers; such studies are also awaited for other disease-associated *SFTPC* mutations.

Despite the reported finding of insufficient processing of SP-C^{I73T} in A549 cells *in vitro*, completely processed mature SP-C could be found in BALF of mutation carriers *in vivo* [89]. Importantly, and in line with this observation, the majority of the non-BRICHOS domain *SFTPC* mutations (e. g. I73T, E66K) do not seem to exert a dominant-negative effect [66,89]. As an exception, the non-BRICHOS domain mutant SP-C$^{\Delta 91\text{-}93}$, which has been linked to severe ILD in infants, has been shown to result in a substantial loss of both mature SP-C and SP-B in BALF of mutation carriers, and the minimum surface tension of the patient´s surfactant was increased (20 mN/m, normal < 5 mN/m). Moreover, some AECII were found to appear with disorganized appearing lamellar bodies, and aggregation of proSP-C was observed in a compartment separate from SP-B [96]. These results add to the concept that aggregation of misfolded SP-C also exerts a processing- and maturation defect of SP-B [66,96]. Furthermore, the missense mutation P30L is the only *SFTPC* mutation described to date in the NH_2-terminal region of proSPC, and has been associated with unspecified ILD [67,93,97]. Overexpression of SP-C^{P30L} in A549 *in vitro* resulted in complete retention of the proprotein in the ER, formation of Congo red-positive aggregates, and processing of the mutant proprotein arrested in the 16 kDa-stage [93]. More recently, the novel missense SP-C mutation L55F has been identified in a pediatric patient with ILD from Japan, and was associated with the genesis of abnormally small and dense lamellar bodies in the AECII of patients´ lung [98]. Congo red-positive aggregates were not detected in explanted lung tissue. Moreover, A549 cells stably overexpressing SP-C^{L55F} displayed numerous abnormal lysosomal-like organelles and increased dense core bodies of lysosomal-like compartments. In contrast to *wt*-proSP-C, mutant proSP-C^{L55F} was only partially trafficked to (normal) CD63-positive cytoplasmic vesicles [98]. However, analyses of markers for ER stress and ERAD- or other degradation pathways such as autophagy were not addressed in this study [98].

Of note, there has never been a report on gene mutations in *SFTPC* in COPD patients. This strengthens the evidence that these dominantly expressed mutations are disease-causing for IP [86]. Although more than 60 *SFTPC* gene mutations have been detected and associated with specified or unspecified ILD, a comprehensive analysis of the lung phenotypes including surfactant-BAL profiles from affected patients as well as of the detailed molecular pathomechanisms resulting from these mutated SP-C proteins remain still elusive for many BRICHOS domain- (e. g. L110R, P115L, A116D, A112T) and non-BRICHOS domain-*SFTPC* mutations. We hypothesize that alveolar epithelial ER stress is present in all of these familial ILD patients.

3.2 ER stress in Familial Interstitial Pneumonia due to SFTPA2 mutations

The identification of *SFTPC* mutations raised speculations that gene mutations in the other surfactant proteins might be found in FIP as well. Such was the case with *SFTPA2* in 2009, when the first family with adult-onset IPF, but also, interestingly, with adenocarcinoma in some individuals of the same familiy, was linked to *SFTPA2* mutations [99].

Surfactant protein A (SP-A) is a major protein of pulmonary surfactant, is mainly hydrophilic, and is found in the airways and alveoli. SP-A comprises two lung-specific isoforms, SP-A1 and SP-A2, and belongs to the family of collectins that are innate-immune defense proteins, characterized by a NH_2-terminal collagen-like region and COOH-terminal carbohydrate recognition (CRD) lectin domain [100]. SP-A is a large multimeric protein, because it assembles into a 18-subunit oligomer which is composed of six disulfide-bridged trimers (each consists of two SP-A1- and one SP-A2 monomer/s), forming a "flower bouquet" conformation [101]. Only a few years ago, the first two rare heterozygous missense mutations in exon 6 of the gene encoding SP-A2 (*SFTPA2*) have been identified in two families with adult-onset pulmonary fibrosis [99,102]. Both dominant mutations (G231V and F198S) are located in the CRD domain and affect the highly conserved residues F198 and G231 which are conserved in all SP-A proteins of vertebrate air-breathing species [99]. In detail, Maitra and coworkers transiently overexpressed the SP-A2^{G231V} and SP-A2^{F198S} mutants in A549 cells and observed, that both mutant proteins were not secreted into the medium, whereas another rare SP-A2^{Q223K} variant

(with a mutation in the non-conserved residue Q223 in the CRD region), which has not been linked to development of lung fibrosis in affected kindreds, was detected in the same amounts like *wt*SP-A1 and *wt*SP-A2 in the medium of transiently transfected cells [102]. Furthermore, SP-A2^{G231V} and SP-A2^{F198S} mutants, but not the SP-A2^{Q223K} variant, were not able to associate with both co-expressed *wt*SP-A isoforms and to form the typical trimer oligomers, presumably as a result of disruption of the tertiary structure of the CRD domain due to missense mutations of conserved crucial residues phenylalanine and glycine at positions 198 and 231 in SP-A2, respectively [102]. Instead, both mutants SP-A2^{G231V} and SP-A2^{F198S} retained in the ER and induced ER stress as shown by significant Grp78 upregulation and activation of the IRE1α/XBP1 pathway, and were rapidly degraded by the ERAD pathway. Despite observed proteasomal degradation, nonidet P-40-insoluble fractions were encountered in A549 cells overexpressing SP-A2^{G231V} and SP-A2^{F198S} mutants. Moreover, the SP-A2^{G231V} mutant sequestered coexpressed *wt*SP-A1 and *wt*SP-A2 into the detergent-insoluble fraction in A549 cells, thereby exerting a dominant-negative effect with similarities to the SP-C-BRICHOS domain mutants [102]. Importantly, apoptosis could not be detected in A549 cell lines transiently overexpressing the SP-A2^{G231V} and SP-A2^{F198S} mutants, thus indicating a milder cellular consequence of *SFTPA2* gene mutations in comparison to *SFTPC* mutations which can cause ILD in children and younger adults [102]. Another reason why *SFTPA2* mutations have been found associated exclusively with adult-onset disease might be the fact that the toxicity/ability of the mutated hydrophilic SP-A2 to induce severe ER stress is more reduced when compared to the very hydrophobic SP-C proteins, which can form insoluble, non-degradable amyloid fibril deposits (*wt*- and mutated SP-C) [103]. Furthermore, translated SP-A proteins are not subjected to an intensive, multi-step proteolytic processing through the secretory pathway in multiple subcellular compartments/vesicles as observed for proSP-C and proSP-B [67,104], and which may thus intrinsically contribute to the higher susceptibility to ER stress of the AECII in case of failure due to *SFTPC*/*SFTPB* gene mutations. On the other side, ER stress may be easily elevated in patients with *SFTPA2* mutations, maybe due to increased protein synthesis of SP-A in the stressed ER as possible cellular response to lack of secreted SP-A2 in the alveolar space [102].

It was speculated that the occurence of pulmonary fibrosis and adenocarcinoma in *SFTPA2* mutation carriers of the same family may be caused by a modulated ER stress-response [102]. Many ER resident proteins and markers of "adaptive ER stress" implicated in quality control/ERAD are upregulated in cancers in order to promote oncogenic transformation and tumor growth, and presumably to maintain the production of growth factors and 'cancerous' survival proteins, thereby increasing aggressiveness and resistance to certain chemotherapeutic agents [105,106]. Some scientists describe this phenomenon as the 'ER proteostasis addiction in cancer' [106]. Though, protective ER stress in normal cells can be quickly converted into maladaptive, severe ER stress involving death signals by second hits such as environmental factors or by critically overwhelming protein synthesis in the ER [5,79,107]. Thus, it can be presumed that ER stress underlies both lung fibrosis and concomitant adenocarcinoma in patients with *SFTPA2* mutations [102]. In line with this suggestion, many different epidemiology studies have noted an association between pulmonary fibrosis and lung cancer, especially in sporadic forms of IPF without any gene mutations [108].

Very recently, three new *SFTPA2* mutations were found in FIP, again with localization in exon 6: N210T, G231R and N171Y [109]. All three missense mutations were predicted to have deleterious consequences by *in silico* studies, and caused UIP (with features of DIP) and concomitant adenocarcinoma in heterozygous mutation carriers. However, the new mutations have yet not been experimentally tested [109].

In general, future research should comprise a detailed *in vivo*-analysis of ER stress-protein markers in AECII and adenocarcinoma cells, as well as of accumulated SP-A2 in non-native aggregates in lung tissue of patients with *SFTPA2* mutations by means of immunohistochemistry or immuno-EM, and should also include the quantitative analysis of SP-A in BALF of affected patients – because such studies have not been performed so far [99,102,109].

3.3 ER stress in Familial Interstitial Pneumonia due to ABCA3 mutations

Gene mutations in *ABCA3* (adenosine triphosphate-binding cassette transporter A3) have been shown to cause surfactant deficiency and fatal respiratory distress syndrome in full-term neonates [110-112] and chronic ILD including IPF in children and young adults [113], and have also been associated with the induction of ER stress [114]. The *ABCA3* gene encodes a 1704-amino acid lipid transporter that is highly expressed in the lung and is predominantly expressed at the limiting membrane of lamellar bodies in AECIIs [115]. The lamellar bodies are lysosome-related organelles and are critical storage organelles for pulmonary surfactant within the AECII; and ABCA3 transports surfactant phospholipids into lamellar

bodies and is thus crucially involved in the proper biogenesis of these 'secretory lysosomes', including the distal steps of hydrophobic surfactant protein processing, which occur in lamellar bodies necessarily for their maturation and secretion [110,116-118]. Of note, more than 150 distinct mutations have been identified in the *ABCA3* gene, making this the largest class of mutations that i) cause abnormalities in surfactant (protein) processing and metabolism due to loss or reduction of ABCA3 protein function and ii) result in genetic ILD (FIP) [111,113,119]. Most *ABCA3* mutations are "private" and are restricted to families [119]. Interestingly, *ABCA3* gene mutations are distributed throughout the whole gene and include nonsense-, frameshift-, missense-, splice site-, insertion- and deletion mutations. Lung disease resulting from *ABCA3* mutations is expressed in an autosomal-recessive manner, thus requiring mutations on both alleles [119]. Importantly, among patients with homozygous *ABCA3* mutations or with two different (compound heterozygous) *ABCA3* mutations, the pulmonary phenotype varies in terms of age and presentation and disease severity [111,119,120]. It is suggested that the phenotypic heterogeneity of lung disease associated with *ABCA3* mutations may be presumably related to the amount of residual protein function (decreased protein function versus no functional protein), and the type of mutation (impaired trafficking/processing of ABCA3 versus defective lipid transport/ATP hydrolysis mediated lipid transport) [119,121,122]. In accordance with this notion, patients with the homozygous nonsense mutation in codon 1142 (W1142X), which results in no residual ABCA3 protein due to nonsense-mediated degradation of mRNA [123], as well as patients with compound heterozygous mutations such as L982P/G1221S, which lead to retention of mutant $ABCA3^{L982P}$ in the ER and significant impairment of ATP hydrolysis activity of the (correctly localized) $ABCA3^{G1221S}$ mutant [122], usually die because of surfactant deficiency within the neonatal period [123]. Other identified *ABCA3* gene mutations associated with fatal lung disease and respiratory distress in full-term newborns (occuring homozygously or compound heterozygously) are the missense mutations R43L, R280C and L101P. Histologically, affected newborn-patients indicate either a PAP or DIP pattern, and, on ultrastructural level, reveal abnormal small lamellar bodies with more densely packed membranes (known as 'electron dense bodies') [118,123]. The fact that homozygous $Abca3^{(-/-)}$ knockout mice die soon after birth, with no lamellar bodies and mature forms of SP-B/C in their AECII, underscores the relevance of this lipid transporter for proper surfactant processing and functional surfactant metabolism and thus life [117].

As pathogenetic principle, two independent groups observed that some mutant ABCA3 proteins were retained completely ($ABCA3^{L982P}$ and $ABCA3^{L101P}$) or partially ($ABCA3^{R280C}$) in the ER, presumably as a result of misfolding, and were consequently not (or only partially) post-translationally processed through the secretory pathway [114,122]. In addition, Weichert and co-workers showed that A549 cells overexpressing the $ABCA3^{L101P}$ mutant developed significant ER stress and apoptosis, as shown by increases in GRP78 expression, IRE1α-mediated *XBP1*-splicing, Annexin V surface staining, caspase-3 activation and decreases of intracellular levels of the antioxidant glutathione. These nocent effects were also encountered in $ABCA3^{R280C}$ overexpressing cell lines, but to lesser extent. In contrast, the $ABCA3^{R43L}$ mutant, which is successfully processed through the secretory pathway and found in LAMP3 positive vesicles, was not shown to induce ER stress and apoptosis, despite severe ILD in affected newborns and a marked functional defect of this mutant in lipid uptake and transport [114]. Similarly, the recently identified homozygous missense mutation G964D, which has been associated with a 'milder' outcome and found in a teenage patient with IPF and an adult relative with NSIP, resulted in reduced ABCA3-mediated surfactant phospholipid accumulation with consequent defective lamellar body genesis. Again, the authors did not observe induction of ER stress in A549 cells overexpressing the $ABCA3^{G964D}$ mutant, which revealed complete maturation and localization in lysosomal vesicles [121]. In addition, the most common ABCA3 disease-causing variant, the missense mutation E292V, which has been discovered in many unrelated families, has been recently shown to occur in adult IPF homozygous or in pediatric ILD compound heterozygous [86,111,124]. The cellular consequences of overexpression of mutant $ABCA3^{E292V}$ in HEK293 cells were impaired lipid transport due to reduction of ATP hydrolysis of the completely processed, mutant transporter. Still, lipid transport in the mutant was decreased to 37% only of that of the wild-type protein, thus allowing a moderately preserved transport function of the mutant $ABCA3^{E292V}$ protein [125]. The missense mutations E690K and T1114M, which were shown to occur only compound heterozygously with the E292V mutation in pediatric ILD, also revealed normal intracellular processing and localization but indicated much more severe defects in ATP-dependent catalysis of lipid transport than the E292V mutation [125]. These results suggest that the E292V mutation is responsible for the development of pediatric ILD in these patients, the phenotype of which is milder than that of fatal surfactant deficiency in neonates [125].

In summary, *ABCA3* mutations affecting the lipid metabolism have yet not been disclosed to induce ER stress *in vitro* and *in vivo*, but they may interact with regular processing of SP-B and SP-C. Similar to SP-C, SP-B is translated as a large 42 kDa precursor and processed by more than six proteolytical steps into its 8 kDa-mature form which is secreted as a dimer within the lamellar body into the alveolar space [104,116]. Importantly, the final steps of proSP-B and proSP-C processing occur in multivesicular bodies and lamellar bodies [104,110,116-118]. Because lipid-transport affecting *ABCA3* mutations have been predicted or in part even shown to cause abnormal lamellar body formation [111,118], proSP-B and proSP-C may be at risk of not being fully processed into their mature forms, thereby leading to intracellular accumulation of unprocessed proSP-B/-C intermediates. Indeed, infants with E292V mutation indicated accumulation of proSP-B, and lack of completely processed, mature SP-B in their AECII [111]. Moreover, accumulation of proSP-B occured also in the alveolar space, presumably as a result of aberrant secretion of unprocessed proSP-B [111]. This is in line with the study of Brasch *et al*. demonstrating unprocessed ~23 and ~17 kDa SP-B intermediates by immunoblotting in BALF of full-term newborns with various homozygous *ABCA3* mutations affecting either processing or (ATP-mediated) lipid transport of this transporter [118]. Again, mature forms of SP-B and SP-C were absent or barely detectable in BALF of affected patients [118].

Due to predicted intracellular accumulation of hydrophobic proSP-B (and also proSP-C) within the AECII as a result of defective lamellar body genesis in almost all *ABCA3* mutations (including lipid transport-affecting mutations), it is intriguing to speculate that *ABCA3* mutations are in principle associated with induction of ER stress, despite these two previous mentioned reports describing no ER stress induction in A549 cells overexpressing the $ABCA3^{R43L}$ - or the $ABCA3^{G964D}$ mutant [114,121]. However, the research for ER stress was only restricted to markers GRP78, calnexin and XBP1, thereby ignoring the ATF6α- and PERK/ATF4 pathways [114,121]. Furthermore, for the vast majority of *ABCA3* mutations the cellular consequences of the altered proteins have not been disclosed which are presumably related to defective hydrophobic surfactant processing, abnormal intracellular protein accumulation, defective surfactant lipid composition, and lysosomal- and ER stress. We believe, that there is an urgent need to analyse the *in vivo* expression patterns of a complete set of ER stress-markers in lung biopsies of pediatric and adult ILD patients with *ABCA3* gene mutations.

3.4 ER stress in Familial Interstitial Pneumonia: Mutations in Telomerase genes and other telomere-associated genes

Pulmonary fibrosis is observed in 20% of individuals with the genetic disorder dyskeratosis congenita (DC) [126]. DC is a disease of telomere shortening originally described as a rare inherited syndrome characterized by mucocutaneous findings that includes skin hyperpigmentation, nail dystrophy and oral leukoplakia, along with aplastic anemia [126,127]. DC is most commonly caused by gene mutations in dyskerin (*DKC1*), which is a component of the telomerase ribonucleoprotein-complex. In addition, mutations in *Telomerase* genes *TERT* (Telomerase-Reverse Transcriptase) and *TERC* (Telomerase RNA component), the latter of which is a specialized RNA that forms the template for the addition of $(TTAGGG)_n$ -repeats by the TERT enzyme, have also been reported as being responsible for DC [127]. *Telomerase* is responsible for elongation of telomeres in germ cells, stem cells and progenitor cells (but also cancer cells) during cell division. *Telomerase* activity in these cells provides the possibility for these cells to divide an unlimited number of times, whereas division number of somatic cells is limited, because somatic cells lack *TERT* expression [128].

In 2007, two groups reported cases of adult-onset FIP (mostly IPF), who had no manifestations of DC, with heterozygous mutations in *TERT* or *TERC*. Both groups demonstrated that these mutations were associated with telomere shortening [129,130], which is a potential cause for triggering activation of a persistent DNA damage response at telomeres and the subsequent induction of cellular senescence or apoptosis in affected cells. Moreover, shortened telomeres were observed directly in the AECII of familial IPF patients, and were even commonly detected in IPF-AECII of sporadic cases, in the absence of any gene mutations in the Telomerase complex [131].

In the case of adult stem cells, criticial shortening of telomeres impairs the stem cells capacity to regenerate tissues both in mice and humans, leading to many different age-related pathologies [132]. Conversely, critical short telomeres can also trigger re-activation and abnormal overexpression of *TERT* resulting in cancerous transformation of cells: this is the reason why telomere-shortening syndromes are also associated with cancer [128].

Importantly, in contrast to the rare *SFTPC* and *SFTPA2* gene mutations which account only for 1-2% of all adult cases of FIP [133], *TERT* and *TERC* mutations are the most common identified genetic cause of FIP, representing

15% of FIP kindreds [46]. Recently, two independent US groups identified missense mutations in *Dyskerin*/*DKC1* in two kindreds with FIP [134,135]. These mutations were demonstrated to be functionally deleterious, as they ultimately led to reduced expression of the *TERC* subunit and consequent telomere shortening [135], with undetectably short telomeres in AECII in one patient who had died from IPF [134]. Dyskerin functions to stabilize *TERC* in the telomerase complex, by interacting with *TERC* through an essential RNA binding domain, which is affected by gene mutations [134]. Furthermore, gene mutations in components of the 'telomere-protecting' *Shelterin*-telosome have been identified in adult FIP patients with concomitant other telomere syndromes such as idiopathic infertility and DC [136,137]. The *Telomerase*-holoenzyme builts up a complex with the *Shelterin* proteins (POT1 = protection of telomeres 1, PTOP = POT1 and TINF2-interacting protein, TERF1/2 = telomeric repeat-binding factor 1/2, TINF2 = TERF1 interacting nuclear factor 2, TERF2IP = Telomeric repeat-binding factor 2-interacting protein 1), from which many bind and cap directly telomeric DNA, thereby sheltering (protecting) the telomeres from being recognized as dsDNA breaks or ssDNA, and from ATR/ATM-mediated DNA damage response-pathways [138]. Without the protective activity of the *Shelterin*-telosome, telomeres are no longer hidden from DNA repair mechanisms and chromosome ends are therefore incorrectly processed by these pathways [138,139].

In detail, the *Shelterin* component *TINF2* has been found to be mutated by two independent researchers in a small subset of families with DC showing pulmonary fibrosis and marked telomere shortening thereby demonstrating that severe telomere uncapping contributes to dysfunction of telomere-metabolism [136,137]. Further support for the impact of telomere-related pathways in pulmonary fibrosis stems from the very recent discovery of rare loss-of-function mutations in the gene encoding for regulator of telomere elongation helicase 1 (*RTEL1*) in many (independent) families with FIP from France and US [140-142]. RTEL1 is an ATP-dependent DNA helicase involved in DNA replication, and is crucial for unwinding the T-loop structure at the telomeric ends [143]. Structural analyses of mutated RTEL1 proteins predict a deleterious function of mutants [141], which is supported by recent findings from other scientists reporting aberrant cleavages and releases of circular, telomeric DNA fragments in genomic PBMC-DNA fractions of patients carrying *RTEL* mutations [140]. Consistent with this, nearly all affected patients indicated sigificantly reduced telomere length in comparison to age-matched healthy volunteers [140-142].

However, 50% of FIP cases with shortened telomeres still lacks a reasonable explanation on genetic basis, and the significant telomere shortening in sporadic IIP (mostly IPF) remains unexplained [141]. Moreover, telomere shortening syndromes are malicious, because telomere length is a heritable trait [144]; and several FIP-offsprings of heterozygous *TERT* or *RTEL1* mutation carriers who did not inherit their parents´s mutation have short telomeres [140,145]. Also, the precise mechanisms by which reduced telomere length leads to pulmonary fibrosis is yet unresolved. A more recent study by Povedano and co-workers documents that homozygous *Tert*$^{(-/-)}$ ko mice from the second (G2) and fourth (G4) generations with progressively shorter telomeres develop pulmonary fibrosis in response to low-dose bleomycin which was well-tolerated in wild-typ mice and thus not sufficent to induce lung fibrosis in wild-typ animals [146]. However, these results stand in contrast with the work of Degryse *et al.* describing that telomere shortening due to telomerase deficiency alone did not accelerate or augment bleomycin-induced lung fibrosis in early- and late-generation *Tert*$^{(-/-)}$ ko mice [147]. Moreover, these both papers are also conflicting with the studies from Liu and coworkers suggesting that telomerase activity is required for bleomycin-induced pulmonary fibrosis in mice [148]. Early research by this group indicated that telomerase activity is highly induced in lung fibroblasts and collagen-I expressing bone marrow progenitor cells from rodent models of lung fibrosis induced by bleomycin [149,150]. In subsequent studies, the authors showed that homozygous *Tert*$^{(-/-)}$ ko mice from third (G3) generations revealed significantly reduced lung fibrosis following bleomycin-administration, when compared to treated wild-typ mice. Lung fibroblasts isolated from bleomycin-challenged *Tert*$^{(-/-)}$ ko mice showed significantly decreased proliferation and increased apoptosis rates compared with fibroblasts isolated from treated wild-type mice and sham-treated controls [148]. Because transplantation of bone marrow (BM) from wild-type mice to *Tert*$^{(-/-)}$ ko mice reconstituted the 'wild-type-response' to bleomycin-induced lung fibrosis, the authors concluded that lung telomerase induction in BM-derived fibroblast-like cells in response to bleomycin-treatment is important for the development of pulmonary fibrosis [148]. However, Liu *et al.* never addressed the role of AECII or Clara cells in *Tert*$^{(-/-)}$ ko mice in response to bleomycin, although lung alveolar integrity is compromised in telomerase-null mice due to increased apoptosis in AECII [151]. In addition, many reports exist which describe robust induction of telomerase expression in wild-type murine AECII or AECII-like subpopulations after a single dose of intratracheal bleomycin [152-

154], suggesting that AECII may be an important stem/progenitor cell population in the lung which is activated in response to lung injury [152,153].

Taken together, the reasons for the discrepancy between the results from Povedano JM/Degryse AL and those from Liu *et al.* remain unclear and are difficult to interpret. Based on the outcome of *TERT* mutations in FIP we and others hypothesize that these families developed lung fibrosis because of progressive telomere shortening in the alveolar epithelium [130,131].

In line with these thoughts, Povedano *et al.* showed that conditional, AECII-specific deletion of *Trf1* (Terf1, telomeric repeat-binding factor 1) in mice led to persistent telomeric damage in alveolar epithelium through *shelterin* disruption and consequent severe telomere uncapping, and was able to trigger alone pulmonary fibrosis in mice [146]. Taken together, it is apparent from the data in humans and mice that there is an important relationship between defective telomere maintenance, genomic instability and lung fibrosis [129-131,134,137,140-142,146].

Until now, ER stress-/UPR signaling in response to genomic instability involving concomitant DNA damage-response pathways and (aberrant) DNA repair has only been poorly explored. Failure to correct genomic alterations has a variety of negative consequences, ranging from cell death on the one side, and to uncontrolled oncogenic transformation on the other side, with genomic instability being a prominent feature in most cancers [155]. The development of an apoptotic ER stress-reponse may be a cellular consequence to genomic instability, DNA damage and consequent aberrant DNA repair. It could represent a translation of genomic alterations into 'proteotoxicity', such as abundant protein overexpression due to chromosome imbalance [156], or the expression and translation of nonsense- or truncated proteins (due to premature stop-codons), which may misfold, accumulate and be retained in the ER. In addition, genomic instability can be associated with epigenomic imbalance, and may result in global dysregulation of gene- and protein expression profiles, leading to the development of ER stress. It is also intriguing to speculate that defective telomere maintenance in the IPF-AECII affects surfactant production and processing in these cells.

The UPR and DNA damage responses are simultaneously activated in cells suffering from genomic stress. It has been shown that ER stress enhances proteasomal degradation of Rad51, a key protein involved in the homologous recombination pathway, and which was associated with decreased DNA repair and cell death in irradiated cells [157]. In line with such reasoning, it has been more recently reported that ER stress is dominant following DNA damage in cultured tumor cell lines, causing an increase in p53-mediated, genotoxic-induced apoptosis [158].

On the other side, spliced XBP1 has been shown to upregulate various DNA damage response and repair genes suggesting that ER stress- and DNA repair mechanism might cooperate in genetically unstable cells to generate cancerous cells [159], by accumulating genetic defects through chromosomal rearrangements (causing loss-of-function of tumor suppressors or conferring additional proliferative advantages) that promote a state of increased aggressiveness [155].

However, *in vivo*-expression of ER stress-pathway molecules has not yet been investigated in lung tissues of patients with *Telomerase*/*Shelterin* pathway mutations or other telomere-processing pathway mutations. In 2007, Lawson and colleagues reported ER stress in non-*SFTPC* familial forms of IPF which was indistinguishable from sporadic IPF and familial IPF associated with *SFTPC* mutation. However, the underlying genetic basis of these FIP cases has not been disclosed to the readers [58].

Similarly, it is yet not known whether protein products of the various identified *TERT*-, *DKC1*-, *TINF2*-, *RTEL1* mutations per se lead to misfolding and accumulation in the ER and development of ER stress, as structural analyses of some of the above mentioned mutations predict beside impaired function a defective protein structure or reduced stability of the mutant proteins [141].

3.5 ER stress in Hermansky-Pudlak Syndrome associated Interstitial Pneumonia (HPS-IP)

Numerous lysosomal storage diseases, although primarily affecting the vesicle trafficking and transport machinery for lysosomes, are associated with the induction of a severe ER stress response [160-162]. Importantly, multiple genetic lysosomal storage disorders have been described in which pulmonary fibrosis may be a manifestation of disease, including mucolipidosis II [163], Niemann-Pick disease [164], Gaucher disease [165], and Hermansky-Pudlak syndrome (HPS) [166]. HPS represents a group of inherited autosomal recessive disorders that occur worldwide. In humans, nine genetic subtypes, known as *HPS1* through *HPS9*, have been described, with each mutation affecting the function of lysosome-related organelles (LROs), such as platelets, melanosomes, and the lung lamellar bodies [167]. The dysfunction of melanosomes accounts for the oculocutaneous albinism found in all HPS patients. The bleeding disorder that is often the presenting complaint of the disease, is caused by the dysfunction of platelets [167].

In addition, pathological accumulation and deposition of lipofuscin-like material also occurs in multiple organs, especially in the lung [167,168]. More importantly, the development of pulmonary fibrosis in some HPS patients (HPS-IP) has been linked to the dysfunction of lamellar bodies. Adults with HPS1 or HPS4, whose genetic defects lead to a loss of function of the ″biogenesis of lysosome-related organelle complex 3″ (BLOC3) develop lung fibrosis which reveals exactly the usual interstitial pneumonia (UIP) histology found in IPF [166-170], whereas children and young adults with the rare HPS2, who have a defect in the β3a subunit of the adaptor protein 3 (AP3) complex, seem to develop predominantly other histopathological patterns of pulmonary fibrosis [171,172]. The AP3 complex regulates the biogenesis of lysosome-related organelles and is crucially implicated in intracellular trafficking of proteins to late endosomes, lysosomes and lysosome-related organelles [173]. Of note, pulmonary fibrosis has not been reported in patients with BLOC2-defects (i.e., HPS3, HPS5, or HPS6).

Importantly, patients with *HPS1* gene mutation and UIP pattern have been reported to reveal histopathologically enlarged AECIIs with ‘‘giant’’ lamellar bodies due to intracellular over-accumulation of surfactant [170]. We could show that hyperplastic AECII of patients with HPS1 indicate pro-apoptotic ER stress, as shown by immunohistochemistry for the ER stress markers ATF4 and CHOP and the apoptosis-marker cleaved caspase-3 [64]. In contrast, ultrastructural findings in lung tissue of HPS2-patients with ILD demonstrated prominent AECII cell hyperplasia, but no lamellar body enlargement, thus underscoring the significant difference between *HPS1* and *HPS2* (or *AP3B1*) gene mutations [171]. However, the expression of ER stress markers has not yet been addressed in lungs of HPS2-patients with concomitant ILD.

Moreover, the development of pulmonary fibrosis does not occur in naturally occurring HPS mouse models, although they share many features of the human disease [174]. Nevertheless, *Hps1* mutant mice have an exaggerated fibrotic response to silica [175], and both the *Hps1*- and *Hps2* mutant mice reveal remarkably a higher susceptibility to bleomycin-induced lung fibrosis, thereby presumably indicating the need of a ’second hit’ for development of pulmonary fibrosis [176]. Our group previously reported the development of spontaneous lung fibrosis involving severe alveolar epithelial ER stress in aged *Hps1*/*Hps2* double-homozygous-mutant mice, which were generated by mating the *Hps1*- and *Hps2* mutant mice [64]. Additionally, these HPS double-mutant mice indicated severe defects in processing and secretion of hydrophobic surfactant proteins SP-B and SP-C, because both mature forms as well as the unprocessed intermediates and precursors of SP-B & SP-C were found to be accumulated in lung tissue, whereas BALF of these mice lacked mature surfactant proteins. In addition, lung tissues of *Hps1*/*Hps2* double-mutant mice revealed marked accumulation of (surfactant) phospholipids and proapoptotic glucosylceramides. Of note, these mutant deleterious effects were not pronounced in the individual mutant *Hps1* and *Hps2* strains [64]. Taken together, these results indicated that the processing and secretion of surfactant was severely impaired/blocked due to transport- and trafficking defects in the secretory pathway as cause of ’additive’ *Hps1*/*Hps2* gene mutations. Importantly, lysosomal stress, as proven by increased expression of cathepsin D and of its apoptotic pro-form, seemed to precede the induction of severe alveolar epithelial ER stress, which was mainly dominated by the pro-apoptotic ATF4/CHOP pathway in hyperplastic, ″giant″ AECII of *Hps1*/*Hps2* double-mutant mice [64]. In line with such notion, numerous lysosomal storage diseases end in the induction of a severe ER stress response, due to the consequent accumulation of unprocessed proteins and lipids in distal lysosomes, vesicles, Golgi and (finally) also in the proximal ER, as a result of defective trafficking, targeting and secretion of glycoproteins and -lipids [160].

Finally, Young and colleagues could demonstrate that susceptibility to bleomycin-induced fibrosis occurred only in those HPS single-mutant mouse models with HPS gene defects corresponding to human genotypes associated with ILD development (*Hps1*-, *Hps2*- and *Hps2*$^{(-/-)}$-knockout mice), whereas mice with HPS3 or Chediak-Higashi syndrome (CHS), a lysosomal disorder with features overlapping with HPS [174], revealed a susceptibility to bleomycin-induced fibrosis comparable to wild-typ background mice [176]. Moreover, targeted re-expression of the missing Hps2-subunit (β3a subunit/*AP3b1*) of the AP3 complex in lung epithelium of *Hps2* mutant mice resulted in restoration of functional AECII homeostasis and significantly reduced susceptibility of AECII death in response to low-dose bleomycin, thus underscoring the relevance of the alveolar epithelium in the fibrotic susceptibility of HPS mice. Interestingly, despite the key role of AECII apoptosis in the fibrotic susceptibility of bleomycin-treated *Hps1*-, *Hps2*- and *Hps2*$^{(-/-)}$-ko mice, no evidence of increased ER stress could be observed in isolated AECII and whole lung tissue of *Hps1*- and *Hps2* mice in comparison to wild-typ mice at baseline and after bleomycin treatment [176]. Thus, the observed AECII apoptosis may be also a result of ’lysosomal stress’ which is directly connected to cell death pathways through cathepsins, which are released into the cytoplasm to

initiate the 'lysosomal pathways of apoptosis' through the cleavage of Bid and the degradation of the anti-apoptotic BCL2 protein family [177]. However, lysosomal stress pathways leading to apoptosis were not addressed in this study which would clarify if defective intracellular trafficking and transport in murine HPS1 and HPS2 lead to AECII dysfunction and lung fibrosis through mechanisms other than ER stress. Furthermore, expression analysis of ER stress markers Grp78/Bip, Atf6α, Xbp1, Atf4 and Chop was mainly assessed on the transcriptomic level, and lacked protein expression studies by immunoblotting or IHC for Xbp1, Atf6 and Chop [176]. Because development of ER stress involving Chop induction has been observed in general in bleomycin treated mice and rats (presumably as cause of increased oxidative stress in this model which results in protein misfolding through oxidation of proteins) [178,179], it can be suggested that some ER stress markers may have accidentally escaped detection in this great study, also due to the relatively low number of samples examined, and the nature of the ER stress-response which is often not reflected at the transcriptomic level [60].

Taken together, HPS-IP underscores the link between defective AECII homeostasis and lysosomal stress/ER stress in the development of pulmonary fibrosis, presumably caused by impaired lamellar body genesis and disturbed surfactant processing [64,170,176].

4 ER stress in sporadic Interstitial Pneumonia

In non-familial, sporadic forms of IPF (and other IIP) without any gene mutation, chronic ER stress is also a prominent finding in the AECII of these patients. In detail, Lawson *et al.* reported that AECIIs of patients with sporadic IPF indicate upregulation of GRP78, EDEM and XBP1 to same extent as observed for familial cases associated with *SFTPC* mutations as well as without any gene mutation in *SFTPC* (thereby being genetically unexplained in this report) [58]. Our group observed significant ATF6α-upregulation and -cleavage, and activation of the IRE1α/XBP1-pathway, as well as significant increases in ATF4- and CHOP expression in AECII of sporadic IPF patients, but not in normal AECIIs of organ donors. Of note, fibroblast foci in IPF were negative for ATF6α, ATF4 and CHOP. In line with induction of the pro-apoptotic CHOP protein in IPF-AECIIs, caspase-3 cleavage and thus execution of apoptosis was observed in these cells [59]. Similarly, AECII near areas of dense uniform fibrosis in fibrotic NSIP (fNSIP) lungs indicated significantly induced protein expression of various ER stress markers, including XBP1, whereas immunoreactivity for these proteins was negative in the interstitium in fNSIP [62].

Interestingly, Oikonomou *et al.* observed significant overexpression of the CHOP-target gene gelsolin in AECII, but not in the fibrotic interstitium in IPF and fNSIP patients, whereas AECII of patients with cellular NSIP (cNSIP) as well as of normal control lungs indicated no expression of gelsolin [180]. Increased gelsolin (*Gsn*) expression was also noted in alveolar epithelium of bleomycin treated C57BL/6 *wt*-mice, whereas $Gsn^{(-/-)}$ mice were found to be protected from fibrosis development. Moreover, caspase-3-mediated gelsolin fragmentation was shown to be an apoptotic effector mechanism in epithelial apoptosis in bleomycin-induced lung fibrosis, and disease protection in $Gsn^{(-/-)}$ mice was largely correlated with reduced epithelial apoptosis [180].

Lastly, and in full accordance with our results, Cha and co-workers detected by IHC significantly induced caspase-cleaved cytokeratin-18 (cCK-18) in AECIIs of IPF patients in comparison to normal lungs. Cell lysates of isolated AECIIs from patients with IPF revealed increased *XBP1*-splicing and concomitantly elevated levels of activated, phosphorylated IRE1α (p-IRE1α) [61], which has been reported to induce directly various pro-apoptotic pathways independent of those activated by XBP1 [16-20]. In full agreement with this notion, incubation of A549 cells with thapsigargin resulted in increased IRE1α-phosphorylation and formation of cCK-18 [61].

However, the origin of ER stress in sporadic cases of IPF and fNSIP remain still elusive. Similar to FIP, altered molecular pathways such as disturbed surfactant processing seem to be present also in sporadic IPF and fNSIP. Qualitative changes in the alveolar surfactant pool have been observed in sporadic IPF and may contribute to alveolar collapse [181,182]. As has been shown previously, such cyclic alveolar collapse was caused by a significant increase in the alveolar surface tension to approximately 17mN/m as compared to healthy controls (< 1mN/Mm) [181]. Moreover, altered lung function in IPF was shown to correlate to significant qualitative changes in the alveolar surfactant pool, including a decline in lamellar bodies and tubular myelin (comprising the so-called *large aggregate surfactant*) and a substantial loss of dipalmitoylated phosphatidylcholine (DPPC) species [182]. Additionally, it could be observed that IPF- and fNSIP lungs revealed depletion of mature forms of SP-B and SP-C, with concomitant abnormal intracellular accumulation of unprocessed SP-B proproteins and induction of ER stress markers [personal communications, see ref. 183]. These processing defects resembled in part to those observed in murine HPS-IP [64] or in response to hereditary ABCA3

deficiency [118]. Indeed, *ABCA3* gene expression has been observed to be downregulated in sporadic IPF and NSIP [184].

In addition, documented evidence indicates that alveolar oxidative stress plays a role in the pathogenesis of IPF and other IIP´s, and it is caused by a disequilibrium of the alveolar oxidant-antioxidant balance due to an increased production of oxidants and depletion of antioxidants [185]. Alveolar macrophages (AMs) and neutrophils, which dominate in the lower respiratory tract of patients with IPF, are capable of inducing oxidant-mediated injury to lung parenchymal cells [186]. This is supported by experimental studies from Ye and coworkers indicating markedly reduced protein levels of the antioxidant acting enzyme haem oxygenase-1 (HO-1) in AMs [187]. In addition, superoxide radicals and hydrogen peroxide are released spontaneously by bronchoalveolar lavage (BAL) cells of IPF patients, which consecutively may result in injury to the alveolar epithelium [186]. In line with this, oxidative damage of mitochondrial DNA has been observed in lung epithelial cells of IPF patients [176]. Other biomarkers of oxidative stress, such as 8-isoprostane, a lipid peroxidation product, are elevated in BALF of patients with IPF [189]. Conversely, there is a deficiency of cellular antioxidants in IPF patients. Glutathione, a major component of the lung antioxidant defence system, is decreased in the epithelial lining fluid (ELF), both in the bleomycin model of lung fibrosis and in IPF patients [190]. Additionally, low expression of enzymatic antioxidants, including extracellular superoxide dismutase (*Ec*SOD), manganese superoxide dismutase (*Mn*SOD) and catalase have been observed in the fibrotic areas [185,191].

Of note, oxidative stress is known to disrupt protein folding through formation of protein carbonyls and other oxidative modifications, which consequently results in induction of ER stress [1-3]. Indeed, BALF samples of patients with IPF reveal an increase in oxidized proteins [192]. In addition, oxidative stress and ER stress-response are tightly interconnected through the PERK/NRF2 pathway, which induces cytoprotective and antioxidant acting genes such as peroxiredoxins in response to increased ROS levels and oxidant stress [193]. Furthermore, it has already been shown that NRF2 is induced and upregulated in AECIIs of both fNSIP- and IPF lungs, whereas “normal” AECIIs in control donor lungs did not indicate notable protein levels of this transcription factor [191,194]. This finding points to the possibility that an upregulation of the antioxidant defense system occurs under conditions of severe oxidative- and ER stress in IPF, but seems to be unable to counterbalance the oxidant burden. As described before, CHOP is upregulated in IPF-AECIIs [59,63] and has been reported to further increase ROS levels through dephosphylation of eIF2α and consequent upregulation of the oxidative folding machinery, and through depletion of intracellular glutathione which is mediated by its target gene CHAC1 [39,40].

Interestingly, the NRF2-target genes peroxiredoxin 1 (PRDX1) and proteasome activator complex subunit 1 (PSME1), the latter which is implicated in the degradation of oxidized, misfolded proteins, have both been observed to be exclusively induced in hyperplastic AECIIs in areas of thickened alveolar septae in patients with fNSIP, but never in AECIIs near areas of dense uniform fibrosis in IPF- as well as fNSIP-lungs itself [62]. We therefore concluded that the impressive finding of an AECII-localized upregulation of antioxidant acting enzymes as well as of factors involved in the removal of oxidized proteins in fNSIP may represent an attempt of these AECIIs to survive under conditions of persistent ER- and oxidative stress, thereby resulting in the maintenance of septated alveolar structure in this distinct histologic subtype [62]. In accordance with this notion, antioxidants have been reported to reduce ER stress and improve protein secretion in an *in vitro* model of protein misfolding [195].

Importantly, oxidative stress is also a leading cause of telomere shortening [196], and cigarette smoke, which releases a myriad of oxidants, free radicals and chemical compounds, including hydroxyl radicals and hydrogene peroxide, has been shown to be a risk factor for sporadic and familial IPF [82] and to cause telomere shortening in a dose-dependent manner [197]. Due to the end replication problem, telomere shortening inevitably occurs in proliferating cells over time (during aging) and is accelerated in telomere shortening syndomes. Critically short telomeres can activate a DNA damage response that manifests in cellular senescence, with permanent cell cycle arrest or cell death [198]. Because telomere shortening specifically occurs in AECII of patients with familial and sporadic IIP [131,134], it can be suggested that this progenitor cell population suffers from premature senescence and loss of replicative potential, and the inability to respond to alveolar cell injury that leads to failure of appropriate epithelial repair mechanisms and irreversible tissue renewal by proliferating fibroblasts. Given the frequency of telomere shortening in FIP and sporadic IP, in can be suggested that i) mutations in other telomere-related genes will be identified in the future, and that ii) till yet unidentifed defects ″in the upstream″ of telomerase- and telomere-related genes associated with deficiency of their protein products will be identified as underlying reason. In line with the latter notion, lung

tissues of patients with sporadic IPF reveal decreased mRNA transcripts of *TERT* and *TERC* [199]. In addition, Yang *et al.* have shown by comparative transcriptome profiling that gene expression levels for the *TERC*-interacting protein TEP1 (*TEP1*, Telomerase protein component 1) were reduced in IPF- and NSIP lungs in comparison to non-diseased normal controls, and were even 1.8-fold downregulated in the IPF- versus NSIP transcriptome [184]. In addition to familial genetic disease, these data in lungs from sporadic IP patients highlight the putative role of impaired expression and function of telomerase pathways as general pathomechanisms in IP [184,199].

As already outlined, ER stress-pathways are activated during severe DNA damage and genomic instability [156-158], presumbly mainly triggered by proteotoxicity due to translation of genomic alterations [156], and alveolar epithelial ER stress and shortened telomeres are unequivocally common in familial and sporadic cases of IPF. However, the assessment of the detailed mechansims leading to ER stress in response to severe telomere dysfunction is still lacking.

Another common phenomenon in IPF are respiratory infections, which not only seem to frequently antecede the clinical appearance of the disease, but also seem to accelerate the clinical course [200]. IPF is characterized by an increased bacterial burden in BAL that predicts decline in lung function and death [201]. Consistent with these observations, bacterial infections can induce severe ER-stress [35,202]. Endo and coworkers showed that the ER stress-mediated apoptosis pathway involving Chop was induced in alveolar epithelium of lipopolysaccharide (LPS)-treated mice, and alveolar epithelial apoptosis induced by LPS treatment was suppressed in lungs of homozygous *Chop*$^{(-/-)}$-knockout mice [202]. Viral infection/replication may - even more compelling - induce and/or aggravate ER stress and repetitive injury to the alveolar epithelium in IPF. DNA and envelope proteins of various herpesviruses have been detected in AECII from a number of patients with IPF, and co-localized with ER stress-marker proteins [58]. In line with this, herpesviruses have been demonstrated to lead to ER stress and pulmonary fibrosis in aged, but not young mice, with localization of ER stress and apoptosis in AECIIs of infected, aged mice [203]. Based on the observation that IPF increases with age this great study also highlighted that the aging lung is more susceptible to injury and lung fibrosis associated with alveolar epithelial ER stress (in response to herpesvirus infection) [203].

Finally, an interesting study from Atanelishvili and coworkers suggests a link between the increase of coagulation proteases and CHOP-mediated apoptosis in fibrotic lung disease [23]. Enhanced activation of the coagulation cascade including the significant overexpression of coagulation proteases, such as thrombin, has been demonstrated in the setting of pulmonary fibrosis [204]. Atanelishvili *et al.* [23] showed that incubation of primary AECII or A549 with thrombin resulted in induction of CHOP expression through a novel, yet not described Ets1-dependent pathway, and not via ATF4, and was associated with consequent apoptosis in both epithelial cell lines. In contrast, thrombin decreased tunicamycin-induced CHOP expression in lung fibroblasts through Myc-dependent mechanisms, and protected these cells from apoptosis. Importantly, overexpression of CHOP in lung fibroblasts led to apoptosis of cells, even in the presence of thrombin, thus clearly indicating that CHOP-mediated pathways are downstream of thrombin [23]. Additionally, the authors showed for the first time that induced CHOP overexpression was localized in AECIIs of patients with (*Ssc*)-associated pulmonary fibrosis, and never in myofibroblastic cell populations or in any cells of normal lung tissue. As expected from *in vitro* data, CHOP overexpression in AECIIs co-localized with apoptotic TUNEL staining and with the expression of the thrombin receptor protease-activated receptor 1 (PAR1). The authors then concluded, that regulation of CHOP expression by thrombin may contribute to the persistent fibroproliferation in (*Ssc*)-associated lung fibrosis and other IIP, in which thrombin directs AECIIs through induction of CHOP towards apoptosis while promoting survival of lung fibroblasts [23].

5 ER stress in drug-induced interstitial lung disease (ILD)

The development of alveolar epithelial ER stress plays also a major role in the pathogenesis of drug-induced ILD (DILD). DILD can be caused by chemotherapeutic agents, antibiotics, antiarrhythmic drugs, and immunosuppressive agents [205]. The chemotherapeutic bleomycin is used to treat different types of neoplasms and is the drug most commonly studied as a cause of DILD [205]. Lung toxicity/ILD occurs in up to 46% of the patients under treatment; for this reason the clinical use of bleomycin as anti-cancer agent in humans is limited [206]. At present bleomycin administration is the most important and widely used method for inducing lung fibrosis in rodent models. In C57Bl/6 mice, lung fibrosis appears between 14 and 28 days after a single-dose (intratracheal) administration of bleomycin; and within 6 weeks, the lung repairs itself and minimal or no fibrosis remains. The histologic pattern

is characterized by patchy parenchymal inflammation, basement membrane and alveolar epithelial cell injury with reactive hyperplasia, and differentiation of fibroblasts into myofibroblasts [207]. Bleomycin exerts its cytotoxic effect by induction of free radicals and reactive oxygene species (ROS) through reaction with endogenous metal ions and oxygene, with concomitant single- and double-strand DNA breaks through oxidative damage mechanisms, and causes a consecutive, pronounced inflammatory response [208,209]. Production of non-functional, oxidatively modified proteins that are prone to misfolding, has been shown to occur under these conditions [210]. Moreover, activated inflammatory cells contribute to increased ROS- and reactive nitrogen species [RNS] production [209]. In accordance with increased oxidative- and DNA damage stress in response to bleomycin, proapoptotic ER stress mediated by Chop has been recently shown by Tanaka *et al.* to play a role in the development of bleomycin-induced pulmonary fibrosis in mice [179]. In this study, nuclear Chop induction in AECII and airway epithelial cells was present already at day 1 and 3 after bleomycin instillation, and AECII were observed to undergo apoptosis at day 3 as shown by TUNEL-assay, thereby indicating that epithelial cell injury is beside inflammation a primary event in bleomycin-induced cell injury. In addition, gene expression of target genes of Chop such as *Il1b* and *Casp11* was upregulated in bleomycin-treated mice, indicating that alveolar epithelial-derived Chop is also significantly involved in the inflammatory phase of bleomycin-induced lung fibrosis. In addition, the administration of tauroursodeoxycholic acid (TUDCA), a pharmacological agent that inhibit ER stress, resulted in a significant attenuation of pulmonary fibrosis and inflammation in bleomycin-treated mice, and *Chop* expression on mRNA level in a dose-dependent manner. Moreover, AECII apoptosis and pulmonary fibrosis was remarkably attenuated in bleomycin-treated heterozygous *Chop*$^{(+/-)}$-ko mice [179]. In contrast, a report in 2014 on the International Colloquium on Lung and Airway Fibrosis (ICLAF)-congress by Ask and coworkers revealed that full blockade of Chop by use of homozygous *Chop*$^{(-/-)}$ knockout mice did not protect from bleomycin-induced fibrosis, and even wild-typ mice were better protected from bleomycin-induced fibrosis than the homozygous Chop$^{(-/-)}$ ko mice [Ask K, personal communications, see ref. 211]. As pathomechanism, the authors discovered that Chop-mediated macrophage apoptosis was found to be protective towards bleomycin-induced fibrosis in wild-type mice. In contrast, Chop$^{(-/-)}$ knockout mice were unable to mediate ER stress-induced apoptosis in macrophages in response to bleomycin-treatment, which led to increased inflammation, interstitial fibrosis and abnormal high levels of macrophages both in the BALF and in the lung parenchyma of affected knockout-mice. These data indicated that proapoptotic UPR and ER stress pathways are also activated in macrophages of lungs from bleomycin-treated wild-typ mice [211]. However, all these results stand in marked contrast with the very recent work of Yao and coworkers describing that full Chop deficiency in homozygous *Chop*$^{(-/-)}$ ko mice protected against bleomycin-induced lung injury and fibrosis, paradoxically by attenuating M2 macrophage production [212].

In general, many publications document full-blown activation of the UPR in the bleomycin-induced lung fibrosis mouse model, as shown by various protein expression studies of whole lung tissue-lysates for UPR markers indicating upregulation of Grp78, p50Atf6, p-eIF2α, p-Ire1α, Xbp1 and Chop at early and late fibrotic phases [179,213,214]. Some of these studies demonstrate the involvement of adaptive UPR-signaling in epithelial-mesenchymal transition (EMT) during bleomycin-induced pulmonary fibrosis [213,214]. It can therefore be suggested, that alveolar epithelial pro-apoptotic ER stress is paralleled by adaptive UPR mechanisms in mesenchymal cells and in 'non-apoptotic epithelial cells during EMT' in fibrotic lungs of bleomycin-treated mice [179,213,214]. It has been shown, that daily administration of 4-phenylbutyrate (4-PBA) to bleomycin-treated mice resulted in marked downregulation of above described ER stress markers, with concomitant reduction of α-SMA and type I-collagen expression, and significant alleviation of fibrosis [213]. Similarly, melatonin, a hormone with antioxidant properties, has also been reported to protect from bleomycin-induced fibrosis by reducing ER stress [214].

Despite some limitations, rodent models of bleomycin-induced pulmonary fibrosis are widely used in the assessment of potential antifibrotic agents for treatment of human IPF [206,213]. In addition to a large number of compounds, ER stress inhibitors such as TUDCA or 4-PBA have been more recently shown to attenuate fibrosis and to prevent disease progression in this model [179,213], and might thus be suggested as attractive candidates for therapy of human IPF.

The pathogenic and molecular events underlying amiodarone (AD)-induced pulmonary fibrosis are also associated with alveolar epithelial ER stress, but the signatures triggering ER stress and AECII apoptosis differ somewhat from bleomycin. Amiodarone (AD) is a highly efficient antiarrhythmic drug with potentially serious side effects, including severe pulmonary toxicity [215]. Several forms of ILD occur among patients treated with AD, such as ARDS, chronic interstitial pneumonia, organizing

pneumonia and pulmonary fibrosis. It affects as many as 6% of individuals receiving the drug and, amongst these cases, fatality rates range from 10-20% [215]. Because molecular pathways underlying AD-induced ILD were still elusive, our group has recently established a mouse model for AD-induced lung fibrosis, in which C57Bl/6 mice received intratracheally 0.8 mg/kg body weight amiodarone every 5th day [216]. Extensive lung fibrosis and AECII hyperplasia were observed in AD-treated mice already at day 7, and remained until day 28. Interestingly, a pronounced intracellular accumulation of pro- and mature forms of SP-B & SP-C could be encountered in lung tissue of AD-treated mice at d7, d14, d21 and d28, with concomitant abnormally increased tissue-phospholipids [216], thereby indicating high similarities to observed surfactant processing disturbances in *Hps1*/*Hps2* double-mutant mice [64]. In full agreement with hyperplastic AECIIs near areas of dense fibrosis, electron microscopy indicated an increase in number and size of lamellar bodies, and also intra-alveolar surfactant appeared more abundant after AD-treatment. As anticipitated from our results in murine and human HPS-IP, AECIIs of AD-treated mice indicated increased lysosomal- and ER stress with parallel induction of apoptosis as determined by immunoblotting and IHC for respective marker proteins cathepsin D, Atf6α, Chop and cleaved caspase-3. Additional *in vitro*-studies with use of RNAi-mediated *Ctsd* silencing revealed increased caspase-3-activation in AD-treated MLE12 cells, despite full blockade of cathepsin D, and thus execution of apoptosis independent of the lysosomal apoptosis pathway [216]. These results suggested at first glance that alveolar epithelial apoptosis in AD-induced pulmonary fibrosis might be mainly due to ER stress involving the Chop protein which was significantly increased in MLE12 cells in response to AD [216]. However, we speculated also on the involvement of other lysosomal- and ER stress-pathways in driving AECII apoptosis in this model, due to severe intracellular accumulation of surfactant in subcellular compartments of the secretory pathway. In addition to ERAD, cells can also eliminate proteins that accumulate in the ER through activation of autophagy, which is a catabolic process mediated through the lysosomal pathway [217-219]. Although extremely important in maintaining cellular homeostasis [220], ER-/lysosomal stress mediated autophagy has also been reported to be able to induce cell death, especially in non-transformed cells, because its degradative capability might turn out to be too extensive and lead to excessive consumption of normal cellular constituents and therefore contribute negatively to cell survival [221].

In addition to ER stress-induction, primary murine AECII and MLE12 cells revealed increased (macro) autophagy and autophagy flux in response to AD, as shown by ten-fold induction of the autophagy marker microtubule-associated protein 1 light chain-3B (LC3B), and by induced autophagosome maturation and fusion with lysosomal compartments in AD-treated cells [222]. Moreover, induction of apoptosis seemed to be dependent on LC3B in alveolar epithelial cells *in vitro*, as RNAi-mediated knockdown of *Map1lc3b* resulted in reduced caspase-3-cleavage in AD-treated cells. *In vivo*, in non-treated and AD-treated C57Bl/6 mice, LC3B was localized at the limiting membrane of lamellar bodies, which were closely connected to the autophagosomal structures in AECII, thus underscoring the reasoning of induction of macroautophagy processes in response to AD-induced defective surfactant storage and consequent lysosomal and ER stress [222].

Taken together, AD exerts inhibitory effects on the trafficking- and transport mechanisms in vesicles and lysosomal compartments of the secretory pathway [216,222], which is in line with previous observations that AD induces excessive intracellular phospholipidosis in lungs of treated patients [223] and gets itself enriched within the lysosomes of various cell types, also due to its uncommon long half-life [224]. However, *in vivo*-expression patterns of ER stress and autophagy markers are still not researched in lungs of patients treated with AD.

ER stress in asbestos-induced lung fibrosis

A prototypcial type of pulmonary fibrosis is caused by asbestos exposure, which is prevalent worldwide as at least 125 million are exposed to harzardous levels [225]. Recent evidence suggests that ER stress plays a major role in the pathogenesis of asbestos-induced pulmonary fibrosis. Kamp and coworkers observed a significant induction of transcript levels for *IRE1*, *XBP1*, *GRP78* and *CHOP* in A549 cells or primary rat AECII already after 30 min exposure to asbestos, and *XBP1*-activation and *CHOP* induction persisted over 24 hours in treated cells [226]. Moreover, IRE1α protein overexpression and *XBP1*-splicing in response to amosite asbestos was comparable to that induced by the ER stress-inducer thapsigargin. Additionally, exposure of primary AECII to asbestos induced Ca^{2+} release from the ER and thus a depletion of ER Ca^{2+} stores, resulting in activation of the intrinsic apoptosis pathway. Importantly, asbestos has also been reported to trigger AECII apoptosis by both the mitochondria- and p53-regulated death pathways [226,227]. Because overexpression of a SOD/catalase mimetic reduced asbestos-induced ER-Ca^{2+} release and concomitant IRE1α-activation and apoptosis in AECII, the

authors concluded that asbestos-induced ER stress due to Ca^{2+} release into cytosol is mainly caused by oxidant-induced mitochondrial dysfunction and consecutive mitochondrial ROS production. Asbestos-induced ER-Ca^{2+} release and proapoptotic ER stress in AECII could also be prevented by overexpression of the antiapoptotic protein Bcl-X_L, but not with the chemical chaperone 4-PBA [226].

Similarly, Ryan and coworkers observed rapid and sustained increases in cytosolic Ca^{2+}-levels in macrophage-like THP-1 cell lines in response to crysotile-exposure, with concomitant activation of ATF6α- and XBP1-pathways [228]. Anisomycin, an inhibitor of translocon Ca^{2+} leak, inhibited induction of ER stress markers suggesting that ER Ca^{2+} depletion may be the major cause of ER stress-activation in macrophages exposed to chrysotile [228]. Moreover, BALF cells of asbestosis patients indicated significantly increased expression of ER stress-markers *GRP78*, *ATF6*, *ERO1A* and *CHOP*. This result was supported by observations in lungs of chrysotile-exposed C57Bl/6 mice which revealed destruction of alveolar architecture by collagen deposition, and exhibited significant Xbp1-activation in their BALF cells but not in whole fibrotic lung tissue, with macrophages being the predominant cells in BALF. Of note, no significant apoptosis was observed in macrophages of chrysotile-exposed C57Bl/6 mice, and active TGF-β levels were increased in BALF of such mice. The authors then concluded, that bronchoalveolar macrophages undergo adaptive/protective ER stress for cell survival in response to chrysotile exposure, and that this is an important contributory factor for development of asbestos-induced lung fibrosis [228]. However, the authors did not analyse by means of immunohistochemistry the expression patterns of ER stress-markers in fibrotic lungs of chrysotile exposured mice, and the putative involvement of AECII in disease pathogenesis was also not addressed in this study [228].

Additionally, it has been reported that airborne particulate matter (PM) selectively activates ER stress in the lung (and also liver tissues) [229]. Air PM pollution is a complex mixture of chemical and/or biological elements, consisting of salts, carbonaceous material, volatile organic compounds, polycyclic aromatic hydrocarbons, and endotoxins [229,230]. Laing and coworkers demonstrated that C57Bl/6J mice exposed to fine particulate matter pollution with an aerodynamic diameter < 2.5 μm ($PM_{2.5}$) indicated significant Perk activation, eIF2α-phosphorylation, and Atf4- and Chop induction in their lungs [229]. Furthermore, lungs of mice exposed to $PM_{2.5}$ displayed an increased number of mitochondria and expansion of ER compartment when compared to lungs from control mice exposed to filtered air (FA). The redox state in lung tissues of $PM_{2.5}$-exposed mice indicated stimulation of oxidative stress, as shown by increases of mRNAs for *Gpx1* (glutathione peroxidase), *Hmox1* (heme oxygenase), *Sod1* and *Sod2* (superoxide dismutases 1 and 2). In addition, apoptotic cells as determined by DNA fragmentation staining were sevenfold higher increased in lungs of $PM_{2.5}$-exposed mice in comparison to FA-exposed animals. Moreover, Chop-knockout mice were protected from increased apoptosis induced by $PM_{2.5}$ particles in comparison to exposed wild-type control mice, thus underscoring the relevance of ER stress-induced Chop for $PM_{2.5}$-induced apoptosis [229]. Further studies indicated that elevated ROS generated through mitochondrial NADPH oxidase pathways or electron transport was responsible for the induction of that $PM_{2.5}$-induced pro-apoptotic ER stress-pathway via Perk/Atf4 activation with consecutive Chop induction. Interestingly, $PM_{2.5}$ exposure appeared to suppress the activity of Ire1α in splicing the *Xbp1*-mRNA, involving the suppression of typical *Xbp1*-target genes such as Edem and ERdj4 [229]. In addition to this report, various *in vitro*-studies with use of lung epithelial cell lines exist that document the induction of ER stress in response to coarse (PM_{10}) and fine ($PM_{2.5}$) exposure [231].

Taken together, ER stress may be a mechanism by which PM exerts toxicity, that may be related to air pollution-associated pathogenesis [229,231], or may represent a 'second hit' on top of a genetically susceptible individual prone to develop chronic lung disease, such as pulmonary fibrosis [82].

6 Adaptive ER stress in mesenchymal cells of IPF lungs

Various studies document that selected (prosurvival-acting) ER stress pathways may contribute directly to fibrogenesis and ECM production in IPF [232,233]. The activation and differentiation of fibroblasts into apoptosis-resistant, ECM-producing myofibroblasts is a critical factor towards the development of IPF. The accumulation of myofibroblasts within pathologic lesions called fibroblast foci (FF) is a hallmark of IPF lungs, and their fatal apoptosis-resistance promotes their expansion and the consecutive exaggerated ECM deposition in IPF lungs [43,234,235]. Transforming growth factor-beta (TGF-β) is a major stimulus of ECM production in fibroproliferative diseases [236]. With regard to this, adaptive (protective) ER stress has been shown to play a crucial role in TGF-β-mediated myogenic differentiation of lung fibroblasts into ECM-secreting myofibroblasts [232]. In detail, Baek *et al.*

showed that mouse and human lung fibroblasts indicated beside overexpression of α-SMA and type-I collagen a significant induction of adaptive ER stress-markers GRP78, ATF6α and XBP1 in response to TGF-β, but not of the severe ER stress-indicators p-eIF2a or CHOP. Conversely, the depletion of GRP78 by RNAi resulted in reduction of α-SMA expression and collagen-I-biosynthesis in TGF-β-treated fibroblast populations. Moreover, GRP78 was found to be significantly overexpressed in myofibroblasts of FF in IPF lungs *in vivo* [232]. In line with these results, a more recent study by Murphy *et al.* reports the significant upregulation of the IRE1α/XBP1 pathway in human lung fibroblasts in response to TGF-β, and specific inhibition of this UPR branch by the IRE1α endonuclease inhibitor STF-083010 resulted in reduced fibroblast proliferation and impaired myofibroblast differentiation in TGF-β stimulated fibroblasts [personal communications, see ref. 237]. These results lead to the suggestion, that profibrotic signaling and production of large amounts of ECM components in myofibroblasts require protective ER stress in order to deal with the protein turnover in the ER. In line with this notion, a study from Semren *et al.* demonstrated increased 26S proteasome activity in primary human lung fibroblasts in response to TGF-β due to augmented expression of the regulatory 19S subunit *PSMD11*/RPN6, which is known to function as a molecular clamp stabilizing the interaction between the 19S regulatory particle and the 20S catalytic core [238]. Moreover, RPN6 expression and protein levels of K48-polyubiquitinated substrates were upregulated in IPF lungs *in vivo*, and overexpressed exclusively in myofibroblasts of fibroblast foci and overlying (hyperplastic) bronchiolar basal cells at sites of aberrant re-epithelialization in IPF, presumably as the result of an enhanced turnover rate of proteins in these metabolically active cells. Importantly, *PSMD11* silencing prevented TGF-β mediated formation of 26S proteasomes and ubiquitin-dependent protein degradation in primary human lung fibroblasts, with consequent inhibition of myofibroblast differentiation and collagen production. Taken together, the results by Semren *et al.* suggest that myofibroblasts use increased ERAD function as shown by elevated 26S proteasomal activity in order to maintain ER homeostasis and cell survival in response to TGF-β-mediated elevation of ECM protein-production [238].

The direct profibrotic role of UPR mediators in activation of TGF-β signaling in lung fibroblasts could also be proven by a study from Zimmerman and coworkers who showed that the ER chaperone calreticulin is required for TGF-β induced expression of *Col1a1* and *Fn*, whereas knockout of *Calr* resulted in abrogation of cellular responsiveness to TGF-β even in the presence of active Smad 2/3 signaling [233]. Importantly, calreticulin was required for TGF-β induced ECM synthesis under conditions of ER stress, as tunicamycin-induced ER stress was insufficient to induce ECM production in TGF-β stimulated *Calr*$^{(-/-)}$ MEFs (but induced ECM production in TGF-β stimulated *Calr*$^{(+/+)}$ *wt*-MEFs, due to upregulation of *Calr* in response to ER stress) [233]. Taken together, all these results point out that selected protective UPR pathways are involved in fibroproliferative remodeling that are independent of severe ER stress and apoptosis [232,233,238].

Moreover, TGF-β has been shown itself to increase expression of calreticulin in human fibroblasts [239], and calreticulin is upregulated in numerous different fibrotic tissues, including rodent models for bleomycin-induced lung fibrosis and streptozotocin-induced diabetic nephropathy [239-241]. Calreticulin is a ATF6- and XBP1-target gene [1,242] and acts in ECM producing cells as a collagen chaperone to mediate ER-to-Golgi trafficking, processing, and incorporation into the ECM [243]. In addition to its chaperone function, calreticulin has a role as a crucial regulator of ER-Ca^{2+} signaling [233]. Regarding this, Zimmerman *et al.* also showed that calreticulin mediates Ca^{2+} release into the cytosol in response to TGF-β in MEF cells *in vitro*, thereby inducing calcineurin activation and consequent nuclear translocation of factor of activated T cells (NFAT) which directly stimulates transcription of ECM components *COL1A1* and *FN* together with other known matrix-inducing transcription factors such as AP-1 and SP-1. Taken together, the data by Zimmerman *et al.* identified calreticulin-regulated functions as a critical molecular link between ER stress and TGF-β fibrotic signaling [233].

Interestingly, the chemical chaperone 4-phenylbutyrate (4-PBA) has been shown to inhibit TGF-β-induced α-SMA expression and type-I collagen synthesis in primary human and murine lung fibroblasts *in vitro* [232,244], paradoxically despite its ability to enhance the folding capacity of the ER [245]. Because 4-PBA has been reported to significantly reduce protein expression of adaptive ER stress-markers (ATF6α, XBP1, GRP78) in response to TGF-β [232], and even the expression of GRP78 on mRNA level [246], it can be suggested that epigenetic silencing mechanisms may play a major role in this process as 4-PBA is a HDAC6- and a weak class I-HDAC-inhibitor (HDACi) [247]. In line with this notion, we observed also a downregulation of type-I collagen on gene and protein expression level in response to the weak class-I-HDACi valproic acid in primary IPF fibroblasts *in vitro*, concomitantly with slight reduction of mRNA level for adaptive - (*ATF6*) and proapoptotic

ER stress-markers (*CHOP*), whereas the strong pan-HDACi panobinostat/LBH589 strongly reduced *COL1A1* production by concomitantly inducing a pro-apoptotic ER stress-response [248].

In conclusion, a cytoprotective (adaptive) ER stress-response takes places in contractile protein-expressing myofibroblasts, presumably in order to restore and maintain ER proteostasis under conditions of elevated ECM protein production [232,233,238], and maybe also to allow the continuous production of various survival-related proteins, such as survivin, PI3Kγ, and members of the inhibitor of apoptosis (IAP) protein family which maintain the apoptosis-resistance of myofibroblasts and which all have been reported to be upregulated in myofibroblastic cell populations in experimental lung fibrosis and IPF *in vivo* [234,235,249].

7 Possible therapeutic approaches for pulmonary fibrosis targeting ER stress.

We hypothesize that interfering with apoptotic ER stress in human IPF may increase the survival of AECII and thus prevent the subsequent aberrant fibrogenesis process. As outlined in this review, it is obvious that proapoptotic ER stress-signaling in AECII of patients with various IIP largely involves CHOP which may serve as novel, AECII-specific target for therapeutic intervention. In line with this, targeted disruption of the *Chop* gene has been shown to attenuate pulmonary fibrosis and inflammation in bleomycin-treated mice [179]. However, no small molecule inhibitors for CHOP activity have been identified to date; and the lack of specific CHOP inhibitors impedes current efforts in this field. Therefore, future research should aim to screen effective ER stress modulators inhibiting CHOP-mediated cell death. This could be achieved by fusing the *CHOP* promotor with a fluorescence gene (or luciferase reporter), followed by generation of a stable cell line with this construct, which could then be used to select 'small molecule'-ER stress modulators by measuring suppression of the ER stress-induced fluorescence signal (or -luciferase activity).

Chemical chaperones, such as 4-PBA and TUDCA, are low-molecular-weight compounds reported to mimic endogenous chaperones, and improve ER folding capacity and promote trafficking of misfolded (mutant) proteins in a nonselective manner [245]. Due to their ability to reduce apoptotic ER stress in thapsigargin-treated hepatocytes *in vitro* [250] or in mouse models for ER stress-mediated liver disease *in vivo* [251], 4-PBA and TUDCA have been regarded as promising novel therapeutic molecules for intervention with alveolar apoptotic ER stress and thus treatment of pulmonary fibrosis. Although administration of TUDCA and 4-PBA to bleomycin-treated mice was proven to result in reduction of severe ER stress (including CHOP downregulation) and significant attenuation of pulmonary fibrosis [179,213], however, 4-PBA-treatment of HEK293 cells with stable overexpression of mutant SP-C proproteins (SP-C^{I73T}, SP-C^{L188Q} and SP-C$^{\Delta Exon4}$) *in vitro* was shown to increase the amount of all SP-C mutants and to promote the accumulation of SDS-insoluble SP-C aggregates in a dose-dependent manner, despite restoration of trafficking and processing of the proprotein to mature peptide in case of the SP-C^{L188Q} mutant [90]. Moreover, abnormal SDS-insoluble SP-C-proprotein aggregation in response to 4-PBA was also apparent in cells expressing SP-C^{wt}, and did not differ much from aforementioned SP-C mutants [90]. Due to the high susceptibility of all mutant SP-C proproteins as well as SP-C^{wt} to form detergent-insoluble aggregates [90,103], which may have cytotoxic outcome, we carefully suggest that the use of chemical chaperones especially for treatment of alveolar epithelial ER stress in IIP should be rather avoided due to concomitant unwanted (hydrophobic) SP-C proprotein stabilization in the AECII.

There is documented evidence that ER stress-induced autophagy may play a crucial role in removal of misfolded protein aggregates, which cannot be cleared by the ERAD pathway [252,253], whereas prolonged ER stress may impair autophagy with consequent induction of cell death [254]. Recently, the well-known mTOR (mammalian target of rapamycin) inhibitor, rapamycin, has been reported to suppress ER stress through activation of autophagy in various cell types *in vivo*. Wang *et al.* demonstrated that restoration of autophagy by rapamycin attenuated hepatic ER stress and insulin resistance in high fructose-fed mice [255]. Similarly, treatment of diabetic Akita mice with rapamycin prevented pancreatic β-cell dysfunction and apoptosis induced by ER stress, and improved diabetes [256]. Moreover, pretreatment of wild-type C57Bl/6J mice with rapamycin attenuated bleomycin-induced fibrosis in two independent studies [257,258]. These results suggest that activation of autophagy by rapamycin via mTOR inhibition may be an effective pharmacological modulator of ER stress, and that ER stress-induced cellular damage by aggregates of misfolded proteins can be overcome by induction of autophagy [255-258].

More recently and surprisingly, Hidvegi *et al.* have been discovered that transgenic PiZ mice with constitutive, tissue-unspecific overexpression of the common misfolded (human) variant α1-antitrypsin Z (Z-A1AT) develop

spontaneously lung fibrosis based on respiratory epithelial cell 'proteinopathy', with similarities to the pathogenesis of disease-associated mutant SP-A/C in human IPF [259]. The PiZ mouse was previously reported by the same group and other researchers to robustly model the hepatic disease of Alpha-1 antitrypsin (A1AT) deficiency, as the result of gain-of-function proteotoxicity with the activation of the autophagic response in hepatocytes to mitigate the sequelae of cellular dysfunction by degrading misfolded, aggregated Z-A1AT mutants [260,261]. The sudden observation of 'proteinopathy' in respiratory epithelium in PiZ mice due to overexpression and accumulation of misfolded Z-A1AT might be also due to the fact, that AEC and bronchial epithelial cells are generally known to be capable of producing significant levels of A1AT in the lung [259,262].

The authors then suggested that the endogenous autophagic response and presumably other proteostasis mechanisms (such as ERAD) are insufficient to counteract the proteinopathy in transgenic PiZ mice, thereby consecutively resulting in fibrosis in the liver and the lung as the pathological outcome [259]. In line with such suggestions, the same researchers found out that therapy with autophagy enhancer drugs carbamazepine (CBZ) and fluphenazine (Flu) reversed hepatic fibrosis in PiZ mice [263,264]. Moreover, they have very recently shown that the same drugs or lung-directed gene transfer of TFEB (transcription factor EB), a master activator of the autophagolysosomal system, abrogated pulmonary fibrosis in the same mice [259].

Taken together, ER stress-induced cellular damage by aggregates of misfolded proteins can be overcome by induction of autophagy [259,263,264], and autophagy enhancing therapies should be evaluated not only in SFTPA/C-associated IPF, but also in sporadic forms of IPF. Of note, CBZ and Flu are approved by the US Food and Drug Administration (FDA) which additionally makes these drugs very attractive for immediate assessment in clinical trials [259]. Though, enhanced autophagy has also been suggested to lead to cell death associated with excessive cytoplasmic vacuolization [265], and the autophagic protein LC3B was shown to be capable to activate extrinsic and intrinsic apoptosis in response to various stress stimuli (i. e. amiodarone, cigarette smoke) [222,266,267]. Thus, careful consideration and further research are needed for the design of autophagy enhancing therapies.

As illustrated in our review, IPF and other forms of pulmonary fibrosis are characterized by apoptotic alveolar epithelial ER stress on the one side, and by exaggerated adaptive, survival-associated UPR signaling in fibroblast populations on the other side. Thus, it is intriguing to speculate that the outputs of UPR deregulation in IPF could be modulated in both distinct cell types to control cell fate under ER stress. It has been recently reported that the master UPR regulator IRE1α is capable of being drugged with small molecules [268,269]. Murphy and coworkers showed that inhibition of IRE1α-mediated *XBP1*-splicing by the salicylaldehyde-based endonuclease inhibitor STF-083010 (which specifically inhibits the RNase activity of IRE1α without affecting IRE1α-kinase activity) abrogated fibroblast proliferation, myofibroblast differentiation and the consequent ECM-protein production in response to TGF-β in human lung fibroblasts *in vitro* [personal communications, see ref. 237]. Because spliced XBP1 is reported as a potent inducer of the pro-apoptotic acting CHOP protein under maladaptive, severe ER stress conditions [2,3], and since IRE1α itself becomes hyperactivated and degrades a large number of ER-localized mRNAs (encoding prosurvival proteins) under irremediable ER stress [16,18,19], such strategy could also be suitable for preventing ER stress-mediated apoptosis in alveolar epithelial cells. In line with this notion, a study from Ghosh and coworkers reported the successful attenuation of ER stress-induced apoptosis in epithelial MLE12 cells by using a similar, direct IRE1α-RNase inhibitor (DRI). Moreover, they could show that DRI administration into C57Bl/6 mice evidently reduced lung fibrosis in the bleomycin-tunicamycin model which the authors had established before as a model of ER stress-induced pulmonary fibrosis [personal communications, see ref. 270].

Recently, the 'more advanced' ATP-competitive IRE1α Kinase-Inhibiting RNase Attenuators (KIRAs) have been developed that allosterically inhibit the RNase activity of IRE1α through its kinase domain by preventing autophosphorylation and breaking the homo-oligomerization of IRE1α through its cytosolic kinase/endoribonuclease (RNAse) domains [19,269,271]. The present best advanced KIRA, 'KIRA6', has been shown to be efficient in two different animal models of ER stress-induced cell degeneration. It preserved photoreceptor functional viability in rat models of ER stress-induced retinal degeneration when injected into the vitreous [271]. Moreover, it prevented β-cell apoptosis and reduced hyperglycemia in a mouse model of diabetes (Akita mice) [19,271]. However, the therapeutic potential of KIRAs in mouse models for pulmonary fibrosis *in vivo* has not yet been addressed. Taken together, therapeutic intervention of adaptive as well as pro-apoptotic IRE1α-signaling by 'KIRA6' in fibrosis mouse models *in vivo* appears possible and efficient and should thus be further investigated in

the future.

In conclusion, therapeutic strategies to modulate and control ER stress may be essential for the treatment of pulmonary fibrosis. But care should be taken to evaluate the question whether systemic suppression of ER stress is good for health.

8 Summary/Conclusion

Taken together, all the independent observations summarized in this review suggest that ER stress-induced apoptosis of AECII represents a common pathomechanistic principle in IPF, as well as in other forms of lung fibrosis, and that various mechanisms such as defective protein folding and processing, or DNA damage stress and genomic instability due to short dysfunctional telomeres, or viral/bacterial infection, or oxidative stress, can induce or aggravate ER stress and thereby contribute to the loss of AECII. On the other side, adaptive antiapoptotic ER stress mechanisms maintain fibroblast survival and abnormal ECM production by these cells in IPF, thereby contributing to "endless healing" and irreversible scar formation in this fatal disease. Since ER stress mediated-apoptosis of alveolar epithelium is the triggering event for IPF, therapeutic strategies should primarily aim to interfere with the AECII-localized proapoptotic ER stress-response.

Figure 1 summarizes the origins of alveolar epithelial ER stress pathomechanisms in familial and sporadic idiopathic interstitial pneumonia.

This work has been funded by grants from the German Center for Lung Research (DZL) and from the Bundesministerium für Bildung und Forschung ("German

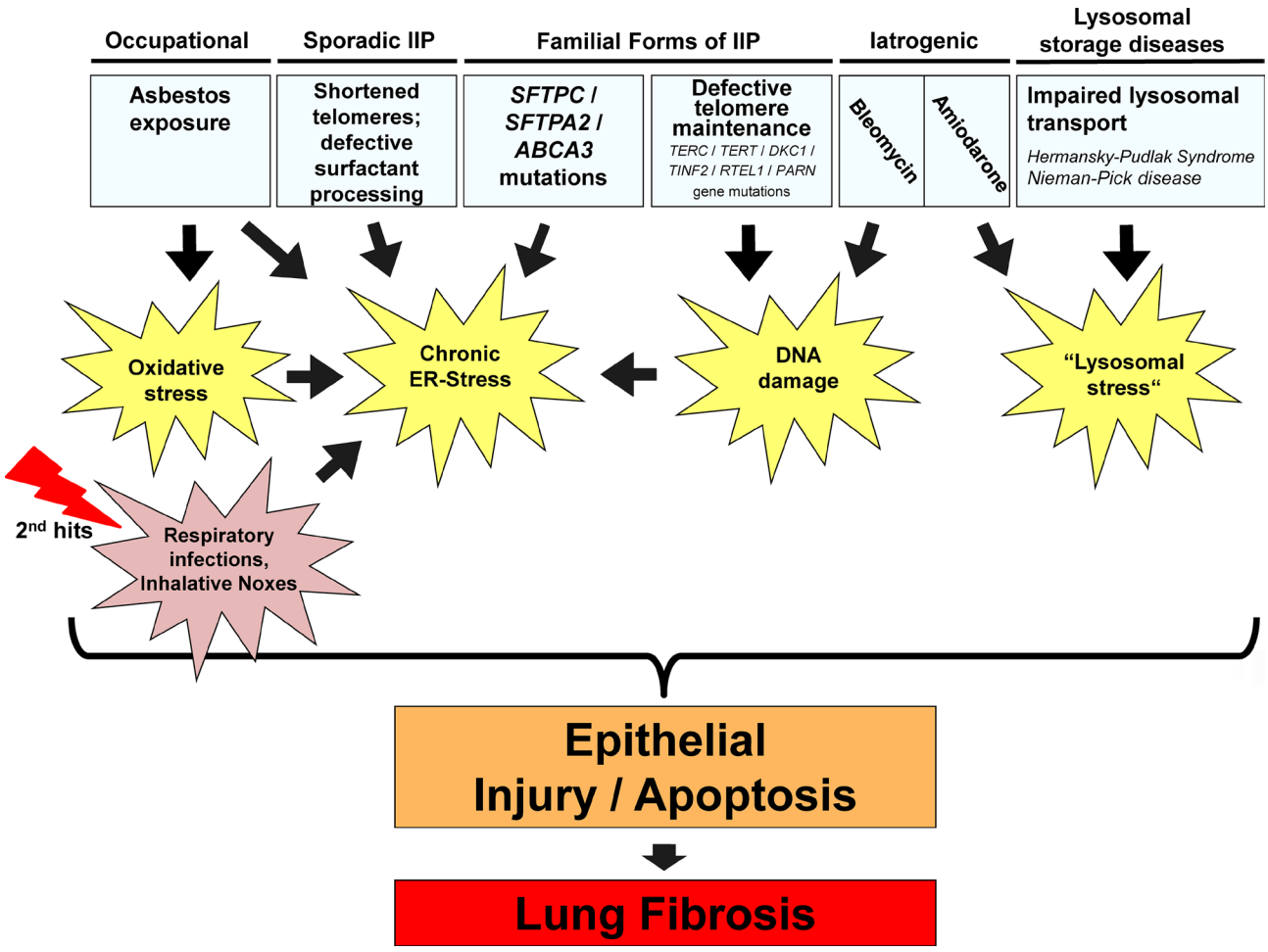

Figure 1. Proposed pathomechanisms in familial and sporadic idiopathic interstitial pneumonia (IIP).

Network for Diffuse Parenchymal Lung Diseases", GOLDnet), from the German Research Council (Clinical Research Group 118 "Lung Fibrosis" and Excellence Cluster "Cardiopulmonary System" [www.eccps.de]), as well as from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. HEALTH-F2-2007-202224 eurIPFnet.

Conflict of interest statement: Authors state no conflict of interest

References

[1] Wang S, Kaufman RJ: The impact of the unfolded protein response on human disease, The Journal of cell biology 2012, 197:857-867

[2] Kitamura M: Endoplasmic reticulum stress in the kidney, Clinical and experimental nephrology 2008, 12:317-325

[3] Yoshida H: ER stress and diseases, The FEBS journal 2007, 274:630-658

[4] Harding HP, Zhang Y, Bertolotti A, Zeng H, Ron D: Perk is essential for translational regulation and cell survival during the unfolded protein response, Molecular cell 2000, 5:897-904

[5] Ma Y, Brewer JW, Diehl JA, Hendershot LM: Two distinct stress signaling pathways converge upon the CHOP promoter during the mammalian unfolded protein response, Journal of molecular biology 2002, 318:1351-1365

[6] Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, Stevens JL, Ron D: CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum, Genes & development 1998, 12:982-995

[7] Gotoh T, Terada K, Oyadomari S, Mori M: hsp70-DnaJ chaperone pair prevents nitric oxide- and CHOP-induced apoptosis by inhibiting translocation of Bax to mitochondria, Cell death and differentiation 2004, 11:390-402

[8] Lee K, Tirasophon W, Shen X, Michalak M, Prywes R, Okada T, Yoshida H, Mori K, Kaufman RJ: IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response, Genes & development 2002, 16:452-466

[9] Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K: XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor, Cell 2001, 107:881-891

[10] Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, Ron D: Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1, Science (New York, NY 2000, 287:664-666

[11] Hitomi J, Katayama T, Eguchi Y, Kudo T, Taniguchi M, Koyama Y, Manabe T, Yamagishi S, Bando Y, Imaizumi K, Tsujimoto Y, Tohyama M: Involvement of caspase-4 in endoplasmic reticulum stress-induced apoptosis and Abeta-induced cell death, The Journal of cell biology 2004, 165:347-356

[12] Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, Yuan J: Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta, Nature 2000, 403:98-103

[13] McCullough KD, Martindale JL, Klotz LO, Aw TY, Holbrook NJ: Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state, Molecular and cellular biology 2001, 21:1249-1259

[14] Yoshida H, Okada T, Haze K, Yanagi H, Yura T, Negishi M, Mori K: ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response, Molecular and cellular biology 2000, 20:6755-6767

[15] Averous J, Bruhat A, Jousse C, Carraro V, Thiel G, Fafournoux P: Induction of CHOP expression by amino acid limitation requires both ATF4 expression and ATF2 phosphorylation, The Journal of biological chemistry 2004, 279:5288-5297

[16] Han D, Lerner AG, Vande Walle L, Upton JP, Xu W, Hagen A, Backes BJ, Oakes SA, Papa FR: IRE1alpha kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates, Cell 2009, 138:562-575

[17] Tabas I, Ron D: Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress, Nature cell biology 2011, 13:184-190

[18] Upton JP, Wang L, Han D, Wang ES, Huskey NE, Lim L, Truitt M, McManus MT, Ruggero D, Goga A, Papa FR, Oakes SA: IRE1alpha cleaves select microRNAs during ER stress to derepress translation of proapoptotic Caspase-2, Science 2012, 338:818-822

[19] Lerner AG, Upton JP, Praveen PV, Ghosh R, Nakagawa Y, Igbaria A, Shen S, Nguyen V, Backes BJ, Heiman M, Heintz N, Greengard P, Hui S, Tang Q, Trusina A, Oakes SA, Papa FR: IRE1alpha induces thioredoxin-interacting protein to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress, Cell metabolism 2012, 16:250-264

[20] Oslowski CM, Hara T, O'Sullivan-Murphy B, Kanekura K, Lu S, Hara M, Ishigaki S, Zhu LJ, Hayashi E, Hui ST, Greiner D, Kaufman RJ, Bortell R, Urano F: Thioredoxin-interacting protein mediates ER stress-induced beta cell death through initiation of the inflammasome, Cell metabolism 2012, 16:265-273

[21] Matsumoto M, Minami M, Takeda K, Sakao Y, Akira S: Ectopic expression of CHOP (GADD153) induces apoptosis in M1 myeloblastic leukemia cells, FEBS letters 1996, 395:143-147

[22] Maytin EV, Ubeda M, Lin JC, Habener JF: Stress-inducible transcription factor CHOP/gadd153 induces apoptosis in mammalian cells via p38 kinase-dependent and -independent mechanisms, Experimental cell research 2001, 267:193-204

[23] Atanelishvili I, Liang J, Akter T, Spyropoulos DD, Silver RM, Bogatkevich GS: Thrombin increases lung fibroblast survival while promoting alveolar epithelial cell apoptosis via the endoplasmic reticulum stress marker, CCAAT enhancer-binding homologous protein, American journal of respiratory cell and molecular biology 2014, 50:893-902

[24] Oyadomari S, Koizumi A, Takeda K, Gotoh T, Akira S, Araki E, Mori M: Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes, The Journal of clinical investigation 2002, 109:525-532

[25] Namba T, Tanaka K, Ito Y, Ishihara T, Hoshino T, Gotoh T, Endo M, Sato K, Mizushima T: Positive role of CCAAT/enhancer-binding protein homologous protein, a transcription factor involved in the endoplasmic reticulum stress response in the

development of colitis, The American journal of pathology 2009, 174:1786-1798
[26] Chen H, Pan YX, Dudenhausen EE, Kilberg MS: Amino acid deprivation induces the transcription rate of the human asparagine synthetase gene through a timed program of expression and promoter binding of nutrient-responsive basic region/leucine zipper transcription factors as well as localized histone acetylation, The Journal of biological chemistry 2004, 279:50829-50839
[27] Donati G, Imbriano C, Mantovani R: Dynamic recruitment of transcription factors and epigenetic changes on the ER stress response gene promoters, Nucleic acids research 2006, 34:3116-3127
[28] Puthalakath H, O'Reilly LA, Gunn P, Lee L, Kelly PN, Huntington ND, Hughes PD, Michalak EM, McKimm-Breschkin J, Motoyama N, Gotoh T, Akira S, Bouillet P, Strasser A: ER stress triggers apoptosis by activating BH3-only protein Bim, Cell 2007, 129:1337-1349
[29] Marciniak SJ, Yun CY, Oyadomari S, Novoa I, Zhang Y, Jungreis R, Nagata K, Harding HP, Ron D: CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum, Genes & development 2004, 18:3066-3077
[30] Ohoka N, Yoshii S, Hattori T, Onozaki K, Hayashi H: TRB3, a novel ER stress-inducible gene, is induced via ATF4-CHOP pathway and is involved in cell death, The EMBO journal 2005, 24:1243-1255
[31] Wang XZ, Kuroda M, Sok J, Batchvarova N, Kimmel R, Chung P, Zinszner H, Ron D: Identification of novel stress-induced genes downstream of chop, The EMBO journal 1998, 17:3619-3630
[32] Ron D, Habener JF: CHOP, a novel developmentally regulated nuclear protein that dimerizes with transcription factors C/EBP and LAP and functions as a dominant-negative inhibitor of gene transcription, Genes & development 1992, 6:439-453
[33] Ubeda M, Wang XZ, Zinszner H, Wu I, Habener JF, Ron D: Stress-induced binding of the transcriptional factor CHOP to a novel DNA control element, Molecular and cellular biology 1996, 16:1479-1489
[34] Akira S, Isshiki H, Sugita T, Tanabe O, Kinoshita S, Nishio Y, Nakajima T, Hirano T, Kishimoto T: A nuclear factor for IL-6 expression (NF-IL6) is a member of a C/EBP family, The EMBO journal 1990, 9:1897-1906
[35] Endo M, Mori M, Akira S, Gotoh T: C/EBP homologous protein (CHOP) is crucial for the induction of caspase-11 and the pathogenesis of lipopolysaccharide-induced inflammation, J Immunol 2006, 176:6245-6253
[36] Geng YJ, Azuma T, Tang JX, Hartwig JH, Muszynski M, Wu Q, Libby P, Kwiatkowski DJ: Caspase-3-induced gelsolin fragmentation contributes to actin cytoskeletal collapse, nucleolysis, and apoptosis of vascular smooth muscle cells exposed to proinflammatory cytokines, European journal of cell biology 1998, 77:294-302
[37] Du K, Herzig S, Kulkarni RN, Montminy M: TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver, Science 2003, 300:1574-1577
[38] Rathmell JC, Fox CJ, Plas DR, Hammerman PS, Cinalli RM, Thompson CB: Akt-directed glucose metabolism can prevent Bax conformation change and promote growth factor-independent survival, Molecular and cellular biology 2003, 23:7315-7328
[39] Mungrue IN, Pagnon J, Kohannim O, Gargalovic PS, Lusis AJ: CHAC1/MGC4504 is a novel proapoptotic component of the unfolded protein response, downstream of the ATF4-ATF3-CHOP cascade, J Immunol 2009, 182:466-476
[40] Kumar A, Tikoo S, Maity S, Sengupta S, Kaur A, Bachhawat AK: Mammalian proapoptotic factor ChaC1 and its homologues function as gamma-glutamyl cyclotransferases acting specifically on glutathione, EMBO reports 2012, 13:1095-1101
[41] American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001, American journal of respiratory and critical care medicine 2002, 165:277-304
[42] Strollo DC, Franks TJ, Galvin JR: The idiopathic interstitial pneumonias: histology and imaging, Seminars in roentgenology 2015, 50:8-15
[43] American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS), American journal of respiratory and critical care medicine 2000, 161:646-664
[44] Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby TV, Cordier JF, Flaherty KR, Lasky JA, Lynch DA, Ryu JH, Swigris JJ, Wells AU, Ancochea J, Bouros D, Carvalho C, Costabel U, Ebina M, Hansell DM, Johkoh T, Kim DS, King TE, Jr., Kondoh Y, Myers J, Muller NL, Nicholson AG, Richeldi L, Selman M, Dudden RF, Griss BS, Protzko SL, Schunemann HJ: An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management, American journal of respiratory and critical care medicine 2011, 183:788-824
[45] Garcia-Sancho C, Buendia-Roldan I, Fernandez-Plata MR, Navarro C, Perez-Padilla R, Vargas MH, Loyd JE, Selman M: Familial pulmonary fibrosis is the strongest risk factor for idiopathic pulmonary fibrosis, Respiratory medicine 2011, 105:1902-1907
[46] Kropski JA, Lawson WE, Young LR, Blackwell TS: Genetic studies provide clues on the pathogenesis of idiopathic pulmonary fibrosis, Disease models & mechanisms 2013, 6:9-17
[47] Selman M, Pardo A: Idiopathic pulmonary fibrosis: misunderstandings between epithelial cells and fibroblasts?, Sarcoidosis Vasc Diffuse Lung Dis 2004, 21:165-172
[48] King TE, Jr., Pardo A, Selman M: Idiopathic pulmonary fibrosis, Lancet 2011, 378:1949-1961
[49] Myers JL, Katzenstein AL: Epithelial necrosis and alveolar collapse in the pathogenesis of usual interstitial pneumonia, Chest 1988, 94:1309-1311
[50] Uhal BD, Joshi I, Hughes WF, Ramos C, Pardo A, Selman M: Alveolar epithelial cell death adjacent to underlying myofibroblasts in advanced fibrotic human lung, The American journal of physiology 1998, 275:L1192-1199
[51] Barbas-Filho JV, Ferreira MA, Sesso A, Kairalla RA, Carvalho CR, Capelozzi VL: Evidence of type II pneumocyte apoptosis in the pathogenesis of idiopathic pulmonary fibrosis (IFP)/usual interstitial pneumonia (UIP), Journal of clinical pathology 2001, 54:132-138

[52] Kuwano K, Kunitake R, Kawasaki M, Nomoto Y, Hagimoto N, Nakanishi Y, Hara N:P21Waf1/Cip1/Sdi1 and p53 expression in association with DNA strand breaks in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 1996, 154:477-483
[53] Nakashima N, Kuwano K, Maeyama T, Hagimoto N, Yoshimi M, Hamada N, Yamada M, Nakanishi Y: The p53-Mdm2 association in epithelial cells in idiopathic pulmonary fibrosis and non-specific interstitial pneumonia. J Clin Pathol 2005, 58:583-589
[54] Jinta T, Miyazaki Y, Kishi M, Akashi T, Takemura T, Inase N, Yoshizawa Y: The pathogenesis of chronic hypersensitivity pneumonitis in common with idiopathic pulmonary fibrosis: expression of apoptotic markers. Am J Clin Pathol 2010, 134:613-620
[55] Nogee LM, Dunbar AE, 3rd, Wert SE, Askin F, Hamvas A, Whitsett JA: A mutation in the surfactant protein C gene associated with familial interstitial lung disease, The New England journal of medicine 2001, 344:573-579
[56] Thomas AQ, Lane K, Phillips J, 3rd, Prince M, Markin C, Speer M, Schwartz DA, Gaddipati R, Marney A, Johnson J, Roberts R, Haines J, Stahlman M, Loyd JE: Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred, American journal of respiratory and critical care medicine 2002, 165:1322-1328
[57] Mulugeta S, Nguyen V, Russo SJ, Muniswamy M, Beers MF: A surfactant protein C precursor protein BRICHOS domain mutation causes endoplasmic reticulum stress, proteasome dysfunction, and caspase 3 activation, American journal of respiratory cell and molecular biology 2005, 32:521-530
[58] Lawson WE, Crossno PF, Polosukhin VV, Roldan J, Cheng DS, Lane KB, Blackwell TR, Xu C, Markin C, Ware LB, Miller GG, Loyd JE, Blackwell TS: Endoplasmic reticulum stress in alveolar epithelial cells is prominent in IPF: association with altered surfactant protein processing and herpesvirus infection, American journal of physiology 2008, 294:L1119-1126
[59] Korfei M, Ruppert C, Mahavadi P, Henneke I, Markart P, Koch M, Lang G, Fink L, Bohle RM, Seeger W, Weaver TE, Guenther A: Epithelial endoplasmic reticulum stress and apoptosis in sporadic idiopathic pulmonary fibrosis, American journal of respiratory and critical care medicine 2008, 178:838-846
[60] Korfei M, Schmitt S, Ruppert C, Henneke I, Markart P, Loeh B, Mahavadi P, Wygrecka M, Klepetko W, Fink L, Bonniaud P, Preissner KT, Lochnit G, Schaefer L, Seeger W, Guenther A: Comparative proteomic analysis of lung tissue from patients with idiopathic pulmonary fibrosis (IPF) and lung transplant donor lungs, Journal of proteome research 2011, 10:2185-2205
[61] Cha SI, Ryerson CJ, Lee JS, Kukreja J, Barry SS, Jones KD, Elicker BM, Kim DS, Papa FR, Collard HR, Wolters PJ: Cleaved cytokeratin-18 is a mechanistically informative biomarker in idiopathic pulmonary fibrosis, Respiratory research 2012, 13:105
[62] Korfei M, von der Beck D, Henneke I, Markart P, Ruppert C, Mahavadi P, Ghanim B, Klepetko W, Fink L, Meiners S, Kramer OH, Seeger W, Vancheri C, Guenther A: Comparative proteome analysis of lung tissue from patients with idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonia (NSIP) and organ donors, Journal of proteomics 2013, 85:109-128
[63] Chia, J. Y.; Jung Y.; Liang J.; Jiang D.; Brown K. K.; du Bois R. M.; Homer R. J.; Noble P. W., Gene expression profiles and immunohistochemical analysis highlight role of endoplasmic reticulum stress in pulmonary fibrosis. *Am J Respir Crit Care Med* 179; 2009:A2175
[64] Mahavadi P, Korfei M, Henneke I, Liebisch G, Schmitz G, Gochuico BR, Markart P, Bellusci S, Seeger W, Ruppert C, Guenther A: Epithelial stress and apoptosis underlie Hermansky-Pudlak syndrome-associated interstitial pneumonia, American journal of respiratory and critical care medicine 2010, 182:207-219
[65] Park JH, Kim DS, Park IN, Jang SJ, Kitaichi M, Nicholson AG, Colby TV: Prognosis of fibrotic interstitial pneumonia: idiopathic versus collagen vascular disease-related subtypes, American journal of respiratory and critical care medicine 2007, 175:705-711
[66] Mulugeta S, Nureki S, Beers MF: Lost after translation: insights from pulmonary surfactant for understanding the role of alveolar epithelial dysfunction and cellular quality control in fibrotic lung disease, American journal of physiology 2015, 309:L507-525
[67] Beers MF, Mulugeta S: Surfactant protein C biosynthesis and its emerging role in conformational lung disease, Annual review of physiology 2005, 67:663-696
[68] Brasch F, Johnen G, Schauer-Vukasinovic V, Ten Brinke A, Kapp N. Napsin A is involved in the processing of proSP-C. American journal of respiratory and critical care medicine 2001, 163:A560
[69] Kotorashvili A, Mulugeta S, Guttentag S, Beers MF. Pepsinogen C is a candidate protease for post-translational processing of surfactant protein C. American journal of respiratory and critical care medicine 2009, 179:A6265
[70] Brasch F, Ten Brinke A, Johnen G, Ochs M, Kapp N, Muller KM, Beers MF, Fehrenbach H, Richter J, Batenburg JJ, Buhling F: Involvement of cathepsin H in the processing of the hydrophobic surfactant-associated protein C in type II pneumocytes, American journal of respiratory cell and molecular biology 2002, 26:659-670
[71] Sanchez-Pulido L, Devos D, Valencia A: BRICHOS: a conserved domain in proteins associated with dementia, respiratory distress and cancer, Trends in biochemical sciences 2002, 27:329-332
[72] Wang WJ, Mulugeta S, Russo SJ, Beers MF: Deletion of exon 4 from human surfactant protein C results in aggresome formation and generation of a dominant negative, Journal of cell science 2003, 116:683-692
[73] Mulugeta S, Maguire JA, Newitt JL, Russo SJ, Kotorashvili A, Beers MF: Misfolded BRICHOS SP-C mutant proteins induce apoptosis via caspase-4- and cytochrome c-related mechanisms, American journal of physiology 2007, 293:L720-729
[74] Wang WJ, Russo SJ, Mulugeta S, Beers MF: Biosynthesis of surfactant protein C (SP-C). Sorting of SP-C proprotein involves homomeric association via a signal anchor domain, The Journal of biological chemistry 2002, 277:19929-19937
[75] Maguire JA, Mulugeta S, Beers MF: Multiple ways to die: delineation of the unfolded protein response and apoptosis induced by Surfactant Protein C BRICHOS mutants, The international journal of biochemistry & cell biology 2012, 44:101-112
[76] Zhong Q, Zhou B, Ann DK, Minoo P, Liu Y, Banfalvi A, Krishnaveni MS, Dubourd M, Demaio L, Willis BC, Kim KJ, duBois RM, Crandall ED, Beers MF, Borok Z: Role of

endoplasmic reticulum stress in epithelial-mesenchymal transition of alveolar epithelial cells: effects of misfolded surfactant protein, American journal of respiratory cell and molecular biology 2011, 45:498-509

[77] Tanjore H, Cheng DS, Degryse AL, Zoz DF, Abdolrasulnia R, Lawson WE, Blackwell TS: Alveolar epithelial cells undergo epithelial-to-mesenchymal transition in response to endoplasmic reticulum stress, The Journal of biological chemistry 2011, 286:30972-30980

[78] Lawson WE, Cheng DS, Degryse AL, Tanjore H, Polosukhin VV, Xu XC, Newcomb DC, Jones BR, Roldan J, Lane KB, Morrisey EE, Beers MF, Yull FE, Blackwell TS: Endoplasmic reticulum stress enhances fibrotic remodeling in the lungs, Proceedings of the National Academy of Sciences of the United States of America 2011, 108:10562-10567

[79] Bridges JP, Xu Y, Na CL, Wong HR, Weaver TE: Adaptation and increased susceptibility to infection associated with constitutive expression of misfolded SP-C, The Journal of cell biology 2006, 172:395-407

[80] Egan JJ, Stewart JP, Hasleton PS, Arrand JR, Carroll KB, Woodcock AA: Epstein-Barr virus replication within pulmonary epithelial cells in cryptogenic fibrosing alveolitis, Thorax 1995, 50:1234-1239

[81] Kropski JA, Pritchett JM, Zoz DF, Crossno PF, Markin C, Garnett ET, Degryse AL, Mitchell DB, Polosukhin VV, Rickman OB, Choi L, Cheng DS, McConaha ME, Jones BR, Gleaves LA, McMahon FB, Worrell JA, Solus JF, Ware LB, Lee JW, Massion PP, Zaynagetdinov R, White ES, Kurtis JD, Johnson JE, Groshong SD, Lancaster LH, Young LR, Steele MP, Phillips Iii JA, Cogan JD, Loyd JE, Lawson WE, Blackwell TS: Extensive phenotyping of individuals at risk for familial interstitial pneumonia reveals clues to the pathogenesis of interstitial lung disease, American journal of respiratory and critical care medicine 2015, 191:417-426

[82] Steele MP, Speer MC, Loyd JE, Brown KK, Herron A, Slifer SH, Burch LH, Wahidi MM, Phillips JA, 3rd, Sporn TA, McAdams HP, Schwarz MI, Schwartz DA: Clinical and pathologic features of familial interstitial pneumonia, American journal of respiratory and critical care medicine 2005, 172:1146-1152

[83] Chibbar R, Shih F, Baga M, Torlakovic E, Ramlall K, Skomro R, Cockcroft DW, Lemire EG: Nonspecific interstitial pneumonia and usual interstitial pneumonia with mutation in surfactant protein C in familial pulmonary fibrosis, Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc 2004, 17:973-980

[84] Cameron HS, Somaschini M, Carrera P, Hamvas A, Whitsett JA, Wert SE, Deutsch G, Nogee LM: A common mutation in the surfactant protein C gene associated with lung disease, The Journal of pediatrics 2005, 146:370-375

[85] Guillot L, Epaud R, Thouvenin G, Jonard L, Mohsni A, Couderc R, Counil F, de Blic J, Taam RA, Le Bourgeois M, Reix P, Flamein F, Clement A, Feldmann D: New surfactant protein C gene mutations associated with diffuse lung disease, Journal of medical genetics 2009, 46:490-494

[86] Coghlan MA, Shifren A, Huang HJ, Russell TD, Mitra RD, Zhang Q, Wegner DJ, Cole FS, Hamvas A: Sequencing of idiopathic pulmonary fibrosis-related genes reveals independent single gene associations, BMJ open respiratory research 2014, 1:e000057

[87] Beers MF, Hawkins A, Maguire JA, Kotorashvili A, Zhao M, Newitt JL, Ding W, Russo S, Guttentag S, Gonzales L, Mulugeta S: A nonaggregating surfactant protein C mutant is misdirected to early endosomes and disrupts phospholipid recycling, Traffic 2011, 12:1196-1210

[88] Brasch F, Griese M, Tredano M, Johnen G, Ochs M, Rieger C, Mulugeta S, Muller KM, Bahuau M, Beers MF: Interstitial lung disease in a baby with a de novo mutation in the SFTPC gene, The European respiratory journal 2004, 24:30-39

[89] Tredano M, Griese M, Brasch F, Schumacher S, de Blic J, Marque S, Houdayer C, Elion J, Couderc R, Bahuau M: Mutation of SFTPC in infantile pulmonary alveolar proteinosis with or without fibrosing lung disease, American journal of medical genetics Part A 2004, 126A:18-26

[90] Stewart GA, Ridsdale R, Martin EP, Na CL, Xu Y, Mandapaka K, Weaver TE: 4-Phenylbutyric acid treatment rescues trafficking and processing of a mutant surfactant protein-C, American journal of respiratory cell and molecular biology 2012, 47:324-331

[91] Hawkins A, Guttentag SH, Deterding R, Funkhouser WK, Goralski JL, Chatterjee S, Mulugeta S, Beers MF: A non-BRICHOS SFTPC mutant (SP-CI73T) linked to interstitial lung disease promotes a late block in macroautophagy disrupting cellular proteostasis and mitophagy, American journal of physiology 2015, 308:L33-47

[92] Woischnik M, Sparr C, Kern S, Thurm T, Hector A, Hartl D, Liebisch G, Mulugeta S, Beers MF, Schmitz G, Griese M: A non-BRICHOS surfactant protein c mutation disrupts epithelial cell function and intercellular signaling, BMC cell biology 2010, 11:88

[93] Thurm T, Kaltenborn E, Kern S, Griese M, Zarbock R: SFTPC mutations cause SP-C degradation and aggregate formation without increasing ER stress, European journal of clinical investigation 2013, 43:791-800

[94] Wang H, Sun RQ, Camera D, Zeng XY, Jo E, Chan SM, Herbert TP, Molero JC, Ye JM: Endoplasmic reticulum stress up-regulates Nedd4-2 to induce autophagy, FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2016 [Epub ahead of print]

[95] Wang Z, Du T, Dong X, Li Z, Wu G, Zhang R: Autophagy inhibition facilitates erlotinib cytotoxicity in lung cancer cells through modulation of endoplasmic reticulum stress, International journal of oncology 2016 [Epub ahead of print].

[96] Hamvas A, Nogee LM, White FV, Schuler P, Hackett BP, Huddleston CB, Mendeloff EN, Hsu FF, Wert SE, Gonzales LW, Beers MF, Ballard PL: Progressive lung disease and surfactant dysfunction with a deletion in surfactant protein C gene, American journal of respiratory cell and molecular biology 2004, 30:771-776

[97] Nogee LM, Dunbar AE, 3rd, Wert S, Askin F, Hamvas A, Whitsett JA: Mutations in the surfactant protein C gene associated with interstitial lung disease, Chest 2002, 121:20S-21S

[98] Liu T, Sano K, Ogiwara N, Kobayashi N: A novel surfactant protein C L55F mutation associated with interstitial lung disease alters subcellular localization of proSP-C in A549 cells, Pediatric research 2015 [Epub ahead of print].

[99] Wang Y, Kuan PJ, Xing C, Cronkhite JT, Torres F, Rosenblatt RL, DiMaio JM, Kinch LN, Grishin NV, Garcia CK: Genetic defects in surfactant protein A2 are associated with pulmonary fibrosis

and lung cancer, American journal of human genetics 2009, 84:52-59
[100] Gupta G, Surolia A: Collectins: sentinels of innate immunity, BioEssays : news and reviews in molecular, cellular and developmental biology 2007, 29:452-464
[101] Korfhagen TR, LeVine AM, Whitsett JA: Surfactant protein A (SP-A) gene targeted mice, Biochimica et biophysica acta 1998, 1408:296-302
[102] Maitra M, Wang Y, Gerard RD, Mendelson CR, Garcia CK: Surfactant protein A2 mutations associated with pulmonary fibrosis lead to protein instability and endoplasmic reticulum stress, The Journal of biological chemistry 2010, 285:22103-22113
[103] Gustafsson M, Thyberg J, Naslund J, Eliasson E, Johansson J: Amyloid fibril formation by pulmonary surfactant protein C, FEBS letters 1999, 464:138-142
[104] Whitsett JA, Weaver TE: Hydrophobic surfactant proteins in lung function and disease, The New England journal of medicine 2002, 347:2141-2148
[105] Wang M, Kaufman RJ: The impact of the endoplasmic reticulum protein-folding environment on cancer development, Nature reviews Cancer 2014, 14:581-597
[106] Dejeans N, Manie S, Hetz C, Bard F, Hupp T, Agostinis P, Samali A, Chevet E: Addicted to secrete - novel concepts and targets in cancer therapy, Trends in molecular medicine 2014, 20:242-250
[107] Xu C, Bailly-Maitre B, Reed JC: Endoplasmic reticulum stress: cell life and death decisions, The Journal of clinical investigation 2005, 115:2656-2664
[108] Antoniou KM, Tomassetti S, Tsitoura E, Vancheri C: Idiopathic pulmonary fibrosis and lung cancer: a clinical and pathogenesis update, Current opinion in pulmonary medicine 2015, 21:626-633
[109] van Moorsel CH, Ten Klooster L, van Oosterhout MF, de Jong PA, Adams H, Wouter van Es H, Ruven HJ, van der Vis JJ, Grutters JC: SFTPA2 Mutations in Familial and Sporadic Idiopathic Interstitial Pneumonia, American journal of respiratory and critical care medicine 2015, 192:1249-1252
[110] Bullard JE, Wert SE, Nogee LM: ABCA3 deficiency: neonatal respiratory failure and interstitial lung disease, Seminars in perinatology 2006, 30:327-334
[111] Bullard JE, Wert SE, Whitsett JA, Dean M, Nogee LM: ABCA3 mutations associated with pediatric interstitial lung disease, American journal of respiratory and critical care medicine 2005, 172:1026-1031
[112] Garmany TH, Moxley MA, White FV, Dean M, Hull WM, Whitsett JA, Nogee LM, Hamvas A: Surfactant composition and function in patients with ABCA3 mutations, Pediatric research 2006, 59:801-805
[113] Young LR, Nogee LM, Barnett B, Panos RJ, Colby TV, Deutsch GH: Usual interstitial pneumonia in an adolescent with ABCA3 mutations, Chest 2008, 134:192-195
[114] Weichert N, Kaltenborn E, Hector A, Woischnik M, Schams A, Holzinger A, Kern S, Griese M: Some ABCA3 mutations elevate ER stress and initiate apoptosis of lung epithelial cells, Respiratory research 2011, 12:4
[115] Yamano G, Funahashi H, Kawanami O, Zhao LX, Ban N, Uchida Y, Morohoshi T, Ogawa J, Shioda S, Inagaki N: ABCA3 is a lamellar body membrane protein in human lung alveolar type II cells, FEBS letters 2001, 508:221-225
[116] Weaver TE, Na CL, Stahlman M: Biogenesis of lamellar bodies, lysosome-related organelles involved in storage and secretion of pulmonary surfactant, Seminars in cell & developmental biology 2002, 13:263-270
[117] Cheong N, Zhang H, Madesh M, Zhao M, Yu K, Dodia C, Fisher AB, Savani RC, Shuman H: ABCA3 is critical for lamellar body biogenesis in vivo, The Journal of biological chemistry 2007, 282:23811-23817
[118] Brasch F, Schimanski S, Muhlfeld C, Barlage S, Langmann T, Aslanidis C, Boettcher A, Dada A, Schroten H, Mildenberger E, Prueter E, Ballmann M, Ochs M, Johnen G, Griese M, Schmitz G: Alteration of the pulmonary surfactant system in full-term infants with hereditary ABCA3 deficiency, American journal of respiratory and critical care medicine 2006, 174:571-580
[119] Wambach JA, Casey AM, Fishman MP, Wegner DJ, Wert SE, Cole FS, Hamvas A, Nogee LM: Genotype-phenotype correlations for infants and children with ABCA3 deficiency, American journal of respiratory and critical care medicine 2014, 189:1538-1543
[120] Hartl D, Griese M: Interstitial lung disease in children -- genetic background and associated phenotypes, Respiratory research 2005, 6:32
[121] Campo I, Zorzetto M, Mariani F, Kadija Z, Morbini P, Dore R, Kaltenborn E, Frixel S, Zarbock R, Liebisch G, Hegermann J, Wrede C, Griese M, Luisetti M: A large kindred of pulmonary fibrosis associated with a novel ABCA3 gene variant, Respiratory research 2014, 15:43
[122] Matsumura Y, Ban N, Ueda K, Inagaki N: Characterization and classification of ATP-binding cassette transporter ABCA3 mutants in fatal surfactant deficiency, The Journal of biological chemistry 2006, 281:34503-34514
[123] Shulenin S, Nogee LM, Annilo T, Wert SE, Whitsett JA, Dean M: ABCA3 gene mutations in newborns with fatal surfactant deficiency, The New England journal of medicine 2004, 350:1296-1303
[124] Nogee LM: Genetics of pediatric interstitial lung disease, Current opinion in pediatrics 2006, 18:287-292
[125] Matsumura Y, Ban N, Inagaki N: Aberrant catalytic cycle and impaired lipid transport into intracellular vesicles in ABCA3 mutants associated with nonfatal pediatric interstitial lung disease, American journal of physiology 2008, 295:L698-707
[126] Dokal I: Dyskeratosis congenita in all its forms, British journal of haematology 2000, 110:768-779
[127] Armanios M, Blackburn EH: The telomere syndromes, Nature reviews Genetics 2012, 13:693-704
[128] Cong YS, Wright WE, Shay JW: Human telomerase and its regulation, Microbiology and molecular biology reviews : MMBR 2002, 66:407-425, table of contents
[129] Armanios MY, Chen JJ, Cogan JD, Alder JK, Ingersoll RG, Markin C, Lawson WE, Xie M, Vulto I, Phillips JA, 3rd, Lansdorp PM, Greider CW, Loyd JE: Telomerase mutations in families with idiopathic pulmonary fibrosis, The New England journal of medicine 2007, 356:1317-1326
[130] Tsakiri KD, Cronkhite JT, Kuan PJ, Xing C, Raghu G, Weissler JC, Rosenblatt RL, Shay JW, Garcia CK: Adult-onset pulmonary fibrosis caused by mutations in telomerase, Proceedings of the National Academy of Sciences of the United States of America 2007, 104:7552-7557
[131] Alder JK, Chen JJ, Lancaster L, Danoff S, Su SC, Cogan JD, Vulto I, Xie M, Qi X, Tuder RM, Phillips JA, 3rd, Lansdorp PM,

Loyd JE, Armanios MY: Short telomeres are a risk factor for idiopathic pulmonary fibrosis, Proceedings of the National Academy of Sciences of the United States of America 2008, 105:13051-13056

[132] Armanios M, Alder JK, Parry EM, Karim B, Strong MA, Greider CW: Short telomeres are sufficient to cause the degenerative defects associated with aging, American journal of human genetics 2009, 85:823-832

[133] Garcia CK: Idiopathic pulmonary fibrosis: update on genetic discoveries, Proceedings of the American Thoracic Society 2011, 8:158-162

[134] Kropski JA, Mitchell DB, Markin C, Polosukhin VV, Choi L, Johnson JE, Lawson WE, Phillips JA, Cogan JD, Blackwell TS, Loyd JE: A novel dyskerin (DKC1) mutation is associated with familial interstitial pneumonia, Chest 2014, 146:e1-7

[135] Alder JK, Parry EM, Yegnasubramanian S, Wagner CL, Lieblich LM, Auerbach R, Auerbach AD, Wheelan SJ, Armanios M: Telomere phenotypes in females with heterozygous mutations in the dyskeratosis congenita 1 (DKC1) gene, Human mutation 2013, 34:1481-1485

[136] Fukuhara A, Tanino Y, Ishii T, Inokoshi Y, Saito K, Fukuhara N, Sato S, Saito J, Ishida T, Yamaguchi H, Munakata M: Pulmonary fibrosis in dyskeratosis congenita with TINF2 gene mutation, Eur Respir J 2013, 42:1757-1759

[137] Alder JK, Stanley SE, Wagner CL, Hamilton M, Hanumanthu VS, Armanios M: Exome sequencing identifies mutant TINF2 in a family with pulmonary fibrosis, Chest 2015, 147:1361-1368

[138] Palm W, de Lange T: How shelterin protects mammalian telomeres, Annual review of genetics 2008, 42:301-334

[139] Sfeir A, de Lange T: Removal of shelterin reveals the telomere end-protection problem, Science (New York, NY 2012, 336:593-597

[140] Cogan JD, Kropski JA, Zhao M, Mitchell DB, Rives L, Markin C, Garnett ET, Montgomery KH, Mason WR, McKean DF, Powers J, Murphy E, Olson LM, Choi L, Cheng DS, Blue EM, Young LR, Lancaster LH, Steele MP, Brown KK, Schwarz MI, Fingerlin TE, Schwartz DA, Lawson WE, Loyd JE, Zhao Z, Phillips JA, 3rd, Blackwell TS: Rare variants in RTEL1 are associated with familial interstitial pneumonia, American journal of respiratory and critical care medicine 2015, 191:646-655

[141] Kannengiesser C, Borie R, Menard C, Reocreux M, Nitschke P, Gazal S, Mal H, Taille C, Cadranel J, Nunes H, Valeyre D, Cordier JF, Callebaut I, Boileau C, Cottin V, Grandchamp B, Revy P, Crestani B: Heterozygous RTEL1 mutations are associated with familial pulmonary fibrosis, Eur Respir J 2015, 46:474-485

[142] Stuart BD, Choi J, Zaidi S, Xing C, Holohan B, Chen R, Choi M, Dharwadkar P, Torres F, Girod CE, Weissler J, Fitzgerald J, Kershaw C, Klesney-Tait J, Mageto Y, Shay JW, Ji W, Bilguvar K, Mane S, Lifton RP, Garcia CK: Exome sequencing links mutations in PARN and RTEL1 with familial pulmonary fibrosis and telomere shortening, Nature genetics 2015, 47:512-517

[143] Vannier JB, Sarek G, Boulton SJ: RTEL1: functions of a disease-associated helicase, Trends in cell biology 2014, 24:416-425

[144] Hao LY, Armanios M, Strong MA, Karim B, Feldser DM, Huso D, Greider CW: Short telomeres, even in the presence of telomerase, limit tissue renewal capacity, Cell 2005, 123:1121-1131

[145] Diaz de Leon A, Cronkhite JT, Katzenstein AL, Godwin JD, Raghu G, Glazer CS, Rosenblatt RL, Girod CE, Garrity ER, Xing C, Garcia CK: Telomere lengths, pulmonary fibrosis and telomerase (TERT) mutations, PloS one 2010, 5:e10680

[146] Povedano JM, Martinez P, Flores JM, Mulero F, Blasco MA: Mice with Pulmonary Fibrosis Driven by Telomere Dysfunction, Cell reports 2015, 12:286-299

[147] Degryse AL, Xu XC, Newman JL, Mitchell DB, Tanjore H, Polosukhin VV, Jones BR, McMahon FB, Gleaves LA, Phillips JA, 3rd, Cogan JD, Blackwell TS, Lawson WE: Telomerase deficiency does not alter bleomycin-induced fibrosis in mice, Experimental lung research 2012, 38:124-134

[148] Liu T, Chung MJ, Ullenbruch M, Yu H, Jin H, Hu B, Choi YY, Ishikawa F, Phan SH: Telomerase activity is required for bleomycin-induced pulmonary fibrosis in mice, The Journal of clinical investigation 2007, 117:3800-3809

[149] Nozaki Y, Liu T, Hatano K, Gharaee-Kermani M, Phan SH: Induction of telomerase activity in fibroblasts from bleomycin-injured lungs, American journal of respiratory cell and molecular biology 2000, 23:460-465

[150] Hashimoto N, Jin H, Liu T, Chensue SW, Phan SH: Bone marrow-derived progenitor cells in pulmonary fibrosis, The Journal of clinical investigation 2004, 113:243-252

[151] Lee J, Reddy R, Barsky L, Scholes J, Chen H, Shi W, Driscoll B: Lung alveolar integrity is compromised by telomere shortening in telomerase-null mice, American journal of physiology Lung cellular and molecular physiology 2009, 296:L57-70

[152] Driscoll B, Buckley S, Bui KC, Anderson KD, Warburton D: Telomerase in alveolar epithelial development and repair, American journal of physiology Lung cellular and molecular physiology 2000, 279:L1191-1198

[153] Reddy R, Buckley S, Doerken M, Barsky L, Weinberg K, Anderson KD, Warburton D, Driscoll B: Isolation of a putative progenitor subpopulation of alveolar epithelial type 2 cells, American journal of physiology Lung cellular and molecular physiology 2004, 286:L658-667

[154] Fridlender ZG, Cohen PY, Golan O, Arish N, Wallach-Dayan S, Breuer R: Telomerase activity in bleomycin-induced epithelial cell apoptosis and lung fibrosis, The European respiratory journal 2007, 30:205-213

[155] Lord CJ, Ashworth A: The DNA damage response and cancer therapy, Nature 2012, 481:287-294

[156] Oromendia AB, Dodgson SE, Amon A: Aneuploidy causes proteotoxic stress in yeast, Genes & development 2012, 26:2696-2708

[157] Yamamori T, Meike S, Nagane M, Yasui H, Inanami O: ER stress suppresses DNA double-strand break repair and sensitizes tumor cells to ionizing radiation by stimulating proteasomal degradation of Rad51, FEBS letters 2013, 587:3348-3353

[158] Mlynarczyk C, Fahraeus R: Endoplasmic reticulum stress sensitizes cells to DNA damage-induced apoptosis through p53-dependent suppression of p21(CDKN1A), Nature communications 2014, 5:5067

[159] Acosta-Alvear D, Zhou Y, Blais A, Tsikitis M, Lents NH, Arias C, Lennon CJ, Kluger Y, Dynlacht BD: XBP1 controls diverse cell type- and condition-specific transcriptional regulatory networks, Molecular cell 2007, 27:53-66

[160] Tessitore A, del PMM, Sano R, Ma Y, Mann L, Ingrassia A, Laywell ED, Steindler DA, Hendershot LM, d'Azzo A: GM1-ganglioside-mediated activation of the unfolded protein

response causes neuronal death in a neurodegenerative gangliosidosis, Molecular cell 2004, 15:753-766

[161] Maor G, Rencus-Lazar S, Filocamo M, Steller H, Segal D, Horowitz M: Unfolded protein response in Gaucher disease: from human to Drosophila, Orphanet journal of rare diseases 2013, 8:140

[162] Sano R, Annunziata I, Patterson A, Moshiach S, Gomero E, Opferman J, Forte M, d'Azzo A: GM1-ganglioside accumulation at the mitochondria-associated ER membranes links ER stress to Ca(2+)-dependent mitochondrial apoptosis, Molecular cell 2009, 36:500-511

[163] Ishak M, Zambrano EV, Bazzy-Asaad A, Esquibies AE: Unusual pulmonary findings in mucolipidosis II, Pediatric pulmonology 2012, 47:719-721

[164] Terry RD, Sperry WM, Brodoff B: Adult lipidosis resembling Niemann-Pick's disease, The American journal of pathology 1954, 30:263-285

[165] Schneider EL, Epstein CJ, Kaback MJ, Brandes D: Severe pulmonary involvement in adult Gaucher's disease. Report of three cases and review of the literature, The American journal of medicine 1977, 63:475-480

[166] Brantly M, Avila NA, Shotelersuk V, Lucero C, Huizing M, Gahl WA: Pulmonary function and high-resolution CT findings in patients with an inherited form of pulmonary fibrosis, Hermansky-Pudlak syndrome, due to mutations in HPS-1, Chest 2000, 117:129-136

[167] Gahl WA, Brantly M, Kaiser-Kupfer MI, Iwata F, Hazelwood S, Shotelersuk V, Duffy LF, Kuehl EM, Troendle J, Bernardini I: Genetic defects and clinical characteristics of patients with a form of oculocutaneous albinism (Hermansky-Pudlak syndrome), The New England journal of medicine 1998, 338:1258-1264

[168] Pierson DM, Ionescu D, Qing G, Yonan AM, Parkinson K, Colby TC, Leslie K: Pulmonary fibrosis in hermansky-pudlak syndrome. a case report and review, Respiration; international review of thoracic diseases 2006, 73:382-395

[169] Anderson PD, Huizing M, Claassen DA, White J, Gahl WA: Hermansky-Pudlak syndrome type 4 (HPS-4): clinical and molecular characteristics, Human genetics 2003, 113:10-17

[170] Nakatani Y, Nakamura N, Sano J, Inayama Y, Kawano N, Yamanaka S, Miyagi Y, Nagashima Y, Ohbayashi C, Mizushima M, Manabe T, Kuroda M, Yokoi T, Matsubara O: Interstitial pneumonia in Hermansky-Pudlak syndrome: significance of florid foamy swelling/degeneration (giant lamellar body degeneration) of type-2 pneumocytes, Virchows Arch 2000, 437:304-313

[171] Gochuico BR, Huizing M, Golas GA, Scher CD, Tsokos M, Denver SD, Frei-Jones MJ, Gahl WA: Interstitial lung disease and pulmonary fibrosis in Hermansky-Pudlak syndrome type 2, an adaptor protein-3 complex disease, Mol Med 2012, 18:56-64

[172] Wenham M, Grieve S, Cummins M, Jones ML, Booth S, Kilner R, Ancliff PJ, Griffiths GM, Mumford AD: Two patients with Hermansky Pudlak syndrome type 2 and novel mutations in AP3B1, Haematologica 2010, 95:333-337

[173] Feng L, Seymour AB, Jiang S, To A, Peden AA, Novak EK, Zhen L, Rusiniak ME, Eicher EM, Robinson MS, Gorin MB, Swank RT: The beta3A subunit gene (Ap3b1) of the AP-3 adaptor complex is altered in the mouse hypopigmentation mutant pearl, a model for Hermansky-Pudlak syndrome and night blindness, Human molecular genetics 1999, 8:323-330

[174] McGarry MP, Reddington M, Novak EK, Swank RT: Survival and lung pathology of mouse models of Hermansky-Pudlak syndrome and Chediak-Higashi syndrome, Proc Soc Exp Biol Med 1999, 220:162-168

[175] Yoshioka Y, Kumasaka T, Ishidoh K, Kominami E, Mitani K, Hosokawa Y, Fukuchi Y: Inflammatory response and cathepsins in silica-exposed Hermansky-Pudlak syndrome model pale ear mice, Pathology international 2004, 54:322-331

[176] Young LR, Gulleman PM, Bridges JP, Weaver TE, Deutsch GH, Blackwell TS, McCormack FX: The alveolar epithelium determines susceptibility to lung fibrosis in Hermansky-Pudlak syndrome, American journal of respiratory and critical care medicine 2012, 186:1014-1024

[177] Stoka V, Turk B, Schendel SL, Kim TH, Cirman T, Snipas SJ, Ellerby LM, Bredesen D, Freeze H, Abrahamson M, Bromme D, Krajewski S, Reed JC, Yin XM, Turk V, Salvesen GS: Lysosomal protease pathways to apoptosis. Cleavage of bid, not pro-caspases, is the most likely route, The Journal of biological chemistry 2001, 276:3149-3157

[178] Della Latta V, Cecchettini A, Del Ry S, Morales MA: Bleomycin in the setting of lung fibrosis induction: From biological mechanisms to counteractions, Pharmacological research 2015, 97:122-130

[179] Tanaka Y, Ishitsuka Y, Hayasaka M, Yamada Y, Miyata K, Endo M, Kondo Y, Moriuchi H, Irikura M, Tanaka K, Mizushima T, Oike Y, Irie T: The exacerbating roles of CCAAT/enhancer-binding protein homologous protein (CHOP) in the development of bleomycin-induced pulmonary fibrosis and the preventive effects of tauroursodeoxycholic acid (TUDCA) against pulmonary fibrosis in mice, Pharmacological research 2015, 99:52-62

[180] Oikonomou N, Thanasopoulou A, Tzouvelekis A, Harokopos V, Paparountas T, Nikitopoulou I, Witke W, Karameris A, Kotanidou A, Bouros D, Aidinis V: Gelsolin expression is necessary for the development of modelled pulmonary inflammation and fibrosis, Thorax 2009, 64:467-475

[181] Gunther A, Schmidt R, Nix F, Yabut-Perez M, Guth C, Rosseau S, Siebert C, Grimminger F, Morr H, Velcovsky HG, Seeger W: Surfactant abnormalities in idiopathic pulmonary fibrosis, hypersensitivity pneumonitis and sarcoidosis, The European respiratory journal 1999, 14:565-573

[182] Schmidt R, Meier U, Markart P, Grimminger F, Velcovsky HG, Morr H, Seeger W, Gunther A: Altered fatty acid composition of lung surfactant phospholipids in interstitial lung disease, American journal of physiology Lung cellular and molecular physiology 2002, 283:L1079-1085

[183] Korfei M, Ruppert C, Mahavadi P, Koch M, Markart P, Witt H, Lang G, Seeger W, Weaver T, Guenther A. Abnormal accumulation of unprocessed surfactant protein (SP)-B and activation of the ER stress pathway in patients with idiopathic pulmonary fibrosis (IPF) and non-specific interstitial pneumonia (NSIP). *Am J Respir Crit Care Med* 2007;175:A735.

[184] Yang IV, Burch LH, Steele MP, Savov JD, Hollingsworth JW, McElvania-Tekippe E, Berman KG, Speer MC, Sporn TA, Brown KK, Schwarz MI, Schwartz DA: Gene expression profiling of familial and sporadic interstitial pneumonia, American journal of respiratory and critical care medicine 2007, 175:45-54

[185] Kinnula VL, Myllarniemi M: Oxidant-antioxidant imbalance as a potential contributor to the progression of human pulmonary fibrosis, Antioxidants & redox signaling 2008, 10:727-738
[186] Cantin AM, North SL, Fells GA, Hubbard RC, Crystal RG: Oxidant-mediated epithelial cell injury in idiopathic pulmonary fibrosis, The Journal of clinical investigation 1987, 79:1665-1673
[187] Ye Q, Dalavanga Y, Poulakis N, Sixt SU, Guzman J, Costabel U: Decreased expression of haem oxygenase-1 by alveolar macrophages in idiopathic pulmonary fibrosis, The European respiratory journal 2008, 31:1030-1036
[188] Kuwano K, Nakashima N, Inoshima I, Hagimoto N, Fujita M, Yoshimi M, Maeyama T, Hamada N, Watanabe K, Hara N: Oxidative stress in lung epithelial cells from patients with idiopathic interstitial pneumonias, The European respiratory journal 2003, 21:232-240
[189] Montuschi P, Ciabattoni G, Paredi P, Pantelidis P, du Bois RM, Kharitonov SA, Barnes PJ: 8-Isoprostane as a biomarker of oxidative stress in interstitial lung diseases, American journal of respiratory and critical care medicine 1998, 158:1524-1527
[190] Meyer A, Buhl R, Magnussen H: The effect of oral N-acetylcysteine on lung glutathione levels in idiopathic pulmonary fibrosis, The European respiratory journal 1994, 7:431-436
[191] Markart P, Luboeinski T, Korfei M, Schmidt R, Wygrecka M, Mahavadi P, Mayer K, Wilhelm J, Seeger W, Guenther A, Ruppert C: Alveolar oxidative stress is associated with elevated levels of nonenzymatic low-molecular-weight antioxidants in patients with different forms of chronic fibrosing interstitial lung diseases, Antioxidants & redox signaling 2009, 11:227-240
[192] Lenz AG, Costabel U, Maier KL: Oxidized BAL fluid proteins in patients with interstitial lung diseases, The European respiratory journal 1996, 9:307-312
[193] Cullinan SB, Diehl JA: Coordination of ER and oxidative stress signaling: the PERK/Nrf2 signaling pathway, The international journal of biochemistry & cell biology 2006, 38:317-332
[194] Mazur W, Lindholm P, Vuorinen K, Myllarniemi M, Salmenkivi K, Kinnula VL: Cell-specific elevation of NRF2 and sulfiredoxin-1 as markers of oxidative stress in the lungs of idiopathic pulmonary fibrosis and non-specific interstitial pneumonia, APMIS : acta pathologica, microbiologica, et immunologica Scandinavica 2010, 118:703-712
[195] Malhotra JD, Miao H, Zhang K, Wolfson A, Pennathur S, Pipe SW, Kaufman RJ: Antioxidants reduce endoplasmic reticulum stress and improve protein secretion, Proceedings of the National Academy of Sciences of the United States of America 2008, 105:18525-18530
[196] Jones DP: Extracellular redox state: refining the definition of oxidative stress in aging, Rejuvenation research 2006, 9:169-181
[197] Morla M, Busquets X, Pons J, Sauleda J, MacNee W, Agusti AG: Telomere shortening in smokers with and without COPD, The European respiratory journal 2006, 27:525-528
[198] Hewitt G, Jurk D, Marques FD, Correia-Melo C, Hardy T, Gackowska A, Anderson R, Taschuk M, Mann J, Passos JF: Telomeres are favoured targets of a persistent DNA damage response in ageing and stress-induced senescence, Nature communications 2012, 3:708
[199] Antoniou KM, Samara KD, Lasithiotaki I, Margaritopoulos GA, Soufla G, Lambiri I, Giannarakis I, Drositis I, Spandidos DA, Siafakas NM: Differential telomerase expression in idiopathic pulmonary fibrosis and non-small cell lung cancer, Oncology reports 2013, 30:2617-2624
[200] Raghu G, Brown KK, Bradford WZ, Starko K, Noble PW, Schwartz DA, King TE, Jr.: A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis, The New England journal of medicine 2004, 350:125-133
[201] Molyneaux PL, Cox MJ, Willis-Owen SA, Mallia P, Russell KE, Russell AM, Murphy E, Johnston SL, Schwartz DA, Wells AU, Cookson WO, Maher TM, Moffatt MF: The role of bacteria in the pathogenesis and progression of idiopathic pulmonary fibrosis, American journal of respiratory and critical care medicine 2014, 190:906-913
[202] Endo M, Oyadomari S, Suga M, Mori M, Gotoh T: The ER stress pathway involving CHOP is activated in the lungs of LPS-treated mice, Journal of biochemistry 2005, 138:501-507
[203] Torres-Gonzalez E, Bueno M, Tanaka A, Krug LT, Cheng DS, Polosukhin VV, Sorescu D, Lawson WE, Blackwell TS, Rojas M, Mora AL: Role of endoplasmic reticulum stress in age-related susceptibility to lung fibrosis, American journal of respiratory cell and molecular biology 2012, 46:748-756
[204] Chambers RC: Procoagulant signaling mechanisms in lung inflammation and fibrosis: novel opportunities for pharmacological intervention?, British journal of pharmacology 2008, 153 Suppl 1:S367-378
[205] Schwaiblmair M, Behr W, Haeckel T, Markl B, Foerg W, Berghaus T: Drug induced interstitial lung disease, The open respiratory medicine journal 2012, 6:63-74
[206] Walters DM, Kleeberger SR: Mouse models of bleomycin-induced pulmonary fibrosis, Curr Protoc Pharmacol 2008, Chapter 5:Unit 5 46
[207] Degryse AL, Tanjore H, Xu XC, Polosukhin VV, Jones BR, McMahon FB, Gleaves LA, Blackwell TS, Lawson WE: Repetitive intratracheal bleomycin models several features of idiopathic pulmonary fibrosis, American journal of physiology Lung cellular and molecular physiology 2010, 299:L442-452
[208] Claussen CA, Long EC: Nucleic Acid recognition by metal complexes of bleomycin, Chemical reviews 1999, 99:2797-2816
[209] Chaudhary NI, Schnapp A, Park JE: Pharmacologic differentiation of inflammation and fibrosis in the rat bleomycin model, American journal of respiratory and critical care medicine 2006, 173:769-776
[210] Wang L, Song Y, Li X, Guo H, Zhang G: Role of thioredoxin nitration in bleomycin-induced pulmonary fibrosis in rats, Canadian journal of physiology and pharmacology 2015, 1-6
[211] Ask K. UPR and CHOP-mediated macrophage apoptosis protects against bleomycin-induced pulmonary fibrosis. Abstract ICLAF2014, published in internet.
[212] Yao Y, Wang Y, Zhang Z, He L, Zhu J, Zhang M, He X, Cheng Z, Ao Q, Cao Y, Yang P, Su Y, Zhao J, Zhang S, Yu Q, Ning Q, Xiang X, Xiong W, Wang CY, Xu Y: Chop Deficiency Protects Mice Against Bleomycin-induced Pulmonary Fibrosis by Attenuating M2 Macrophage Production, Molecular therapy: the journal of the American Society of Gene Therapy 2016 [Epub ahead of print]

[213] Zhao H, Qin HY, Cao LF, Chen YH, Tan ZX, Zhang C, Xu DX: Phenylbutyric acid inhibits epithelial-mesenchymal transition during bleomycin-induced lung fibrosis, Toxicology letters 2014, 232:213-220

[214] Zhao H, Wu QQ, Cao LF, Qing HY, Zhang C, Chen YH, Wang H, Liu RY, Xu DX: Melatonin inhibits endoplasmic reticulum stress and epithelial-mesenchymal transition during bleomycin-induced pulmonary fibrosis in mice, PloS one 2014, 9:e97266

[215] Schwaiblmair M, Berghaus T, Haeckel T, Wagner T, von Scheidt W: Amiodarone-induced pulmonary toxicity: an under-recognized and severe adverse effect?, Clinical research in cardiology : official journal of the German Cardiac Society 2010, 99:693-700

[216] Mahavadi P, Henneke I, Ruppert C, Knudsen L, Venkatesan S, Liebisch G, Chambers RC, Ochs M, Schmitz G, Vancheri C, Seeger W, Korfei M, Guenther A: Altered surfactant homeostasis and alveolar epithelial cell stress in amiodarone-induced lung fibrosis, Toxicological sciences : an official journal of the Society of Toxicology 2014, 142:285-297

[217] Oh SH, Lim SC: Endoplasmic reticulum stress-mediated autophagy/apoptosis induced by capsaicin (8-methyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin is regulated by the extent of c-Jun NH2-terminal kinase/extracellular signal-regulated kinase activation in WI38 lung epithelial fibroblast cells, The Journal of pharmacology and experimental therapeutics 2009, 329:112-122

[218] Teckman JH, Perlmutter DH: Retention of mutant alpha(1)-antitrypsin Z in endoplasmic reticulum is associated with an autophagic response, American journal of physiology Gastro-intestinal and liver physiology 2000, 279:G961-974

[219] Kouroku Y, Fujita E, Tanida I, Ueno T, Isoai A, Kumagai H, Ogawa S, Kaufman RJ, Kominami E, Momoi T: ER stress (PERK/eIF2alpha phosphorylation) mediates the polyglutamine-induced LC3 conversion, an essential step for autophagy formation, Cell death and differentiation 2007, 14:230-239

[220] Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, Yokoyama M, Mishima K, Saito I, Okano H, Mizushima N: Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice, Nature 2006, 441:885-889

[221] Ding WX, Ni HM, Gao W, Hou YF, Melan MA, Chen X, Stolz DB, Shao ZM, Yin XM: Differential effects of endoplasmic reticulum stress-induced autophagy on cell survival, The Journal of biological chemistry 2007, 282:4702-4710

[222] Mahavadi P, Knudsen L, Venkatesan S, Henneke I, Hegermann J, Wrede C, Ochs M, Ahuja S, Chillappagari S, Ruppert C, Seeger W, Korfei M and Günther A. Regulation of macroautophagy in amiodarone induced pulmonary fibrosis. 2014. (Accepted manuscript online: 11 APR 2015 in The Journal of Pathology: Clinical Research, DOI: 10.1002/cjp2.20).

[223] Martin WJ, 2nd, Kachel DL, Vilen T, Natarajan V: Mechanism of phospholipidosis in amiodarone pulmonary toxicity, The Journal of pharmacology and experimental therapeutics 1989, 251:272-278

[224] Okayasu K, Takeda Y, Kojima J, Yoshizawa A, Kobayashi N, Sugiyama H, Kudo K: Amiodarone pulmonary toxicity: a patient with three recurrences of pulmonary toxicity and consideration of the probable risk for relapse, Intern Med 2006, 45:1303-1307

[225] Diagnosis and initial management of nonmalignant diseases related to asbestos, American journal of respiratory and critical care medicine 2004, 170:691-715

[226] Kamp DW, Liu G, Cheresh P, Kim SJ, Mueller A, Lam AP, Trejo H, Williams D, Tulasiram S, Baker M, Ridge K, Chandel NS, Beri R: Asbestos-induced alveolar epithelial cell apoptosis. The role of endoplasmic reticulum stress response, American journal of respiratory cell and molecular biology 2013, 49:892-901

[227] Liu G, Beri R, Mueller A, Kamp DW: Molecular mechanisms of asbestos-induced lung epithelial cell apoptosis, Chemico-biological interactions 2010, 188:309-318

[228] Ryan AJ, Larson-Casey JL, He C, Murthy S, Carter AB: Asbestos-induced disruption of calcium homeostasis induces endoplasmic reticulum stress in macrophages, The Journal of biological chemistry 2014, 289:33391-33403

[229] Laing S, Wang G, Briazova T, Zhang C, Wang A, Zheng Z, Gow A, Chen AF, Rajagopalan S, Chen LC, Sun Q, Zhang K: Airborne particulate matter selectively activates endoplasmic reticulum stress response in the lung and liver tissues, American journal of physiology Cell physiology 2010, 299:C736-749

[230] Soukup JM, Becker S: Human alveolar macrophage responses to air pollution particulates are associated with insoluble components of coarse material, including particulate endotoxin, Toxicology and applied pharmacology 2001, 171:20-26

[231] Watterson TL, Hamilton B, Martin R, Coulombe RA, Jr.: Urban particulate matter causes ER stress and the unfolded protein response in human lung cells, Toxicological sciences : an official journal of the Society of Toxicology 2009, 112:111-122

[232] Baek HA, Kim do S, Park HS, Jang KY, Kang MJ, Lee DG, Moon WS, Chae HJ, Chung MJ: Involvement of endoplasmic reticulum stress in myofibroblastic differentiation of lung fibroblasts, American journal of respiratory cell and molecular biology 2012, 46:731-739

[233] Zimmerman KA, Graham LV, Pallero MA, Murphy-Ullrich JE: Calreticulin regulates transforming growth factor-beta-stimulated extracellular matrix production, The Journal of biological chemistry 2013, 288:14584-14598

[234] Conte E, Gili E, Fruciano M, et al.. PI3K p110gamma overexpression in idiopathic pulmonary fibrosis lung tissue and fibroblast cells: In vitro effects of its inhibition. Lab Invest 2013;93:566-576

[235] Sisson TH, Maher TM, Ajayi IO, et al.. Increased survivin expression contributes to apoptosis-resistance in IPF-fibroblasts. Adv Biosci Biotechnol 2012;3:657-664

[236] Roberts AB: Molecular and cell biology of TGF-beta, Mineral and electrolyte metabolism 1998, 24:111-119

[237] Murphy J, Kolb PS, Ayaub E, Mahmood S, Naiel S, Gravina M, Dickhout JG, Ask K. Ire1 mediated Xbp1 splicing is critical for pulmonary fibroblast proliferation and differentiation. American journal of respiratory and critical care medicine 2015; 191:A4932

[238] Semren N, Welk V, Korfei M, Keller IE, Fernandez IE, Adler H, Gunther A, Eickelberg O, Meiners S: Regulation of 26S Proteasome Activity in Pulmonary Fibrosis, American journal of respiratory and critical care medicine 2015 [Epub ahead of print]

[239] Kypreou KP, Kavvadas P, Karamessinis P, Peroulis M, Alberti A, Sideras P, Psarras S, Capetanaki Y, Politis PK, Charonis AS:

Altered expression of calreticulin during the development of fibrosis, Proteomics 2008, 8:2407-2419
[240] Khan MI, Pichna BA, Shi Y, Bowes AJ, Werstuck GH: Evidence supporting a role for endoplasmic reticulum stress in the development of atherosclerosis in a hyperglycaemic mouse model, Antioxidants & redox signaling 2009, 11:2289-2298
[241] Fang L, Xie D, Wu X, Cao H, Su W, Yang J: Involvement of endoplasmic reticulum stress in albuminuria induced inflammasome activation in renal proximal tubular cells, PloS one 2013, 8:e72344
[242] Belmont PJ, Chen WJ, Thuerauf DJ, Glembotski CC: Regulation of microRNA expression in the heart by the ATF6 branch of the ER stress response, Journal of molecular and cellular cardiology 2012, 52:1176-1182
[243] Van Duyn Graham L, Sweetwyne MT, Pallero MA, Murphy-Ullrich JE: Intracellular calreticulin regulates multiple steps in fibrillar collagen expression, trafficking, and processing into the extracellular matrix, The Journal of biological chemistry 2010, 285:7067-7078
[244] Rishikof DC, Ricupero DA, Liu H, Goldstein RH: Phenylbutyrate decreases type I collagen production in human lung fibroblasts, Journal of cellular biochemistry 2004, 91:740-748
[245] Engin F, Hotamisligil GS: Restoring endoplasmic reticulum function by chemical chaperones: an emerging therapeutic approach for metabolic diseases, Diabetes, obesity & metabolism 2010, 12 Suppl 2:108-115
[246] Qi W, Mu J, Luo ZF, Zeng W, Guo YH, Pang Q, Ye ZL, Liu L, Yuan FH, Feng B: Attenuation of diabetic nephropathy in diabetes rats induced by streptozotocin by regulating the endoplasmic reticulum stress inflammatory response, Metabolism: clinical and experimental 2011, 60:594-603
[247] Kusaczuk M, Bartoszewicz M, Cechowska-Pasko M: Phenylbutyric Acid: simple structure - multiple effects, Current pharmaceutical design 2015, 21:2147-2166
[248] Korfei M, Skwarna S, Henneke I, MacKenzie B, Klymenko O, Saito S, Ruppert C, von der Beck D, Mahavadi P, Klepetko W, Bellusci S, Crestani B, Pullamsetti SS, Fink L, Seeger W, Kramer OH, Guenther A: Aberrant expression and activity of histone deacetylases in sporadic idiopathic pulmonary fibrosis, Thorax 2015, 70:1022-1032
[249] Ajayi IO, Sisson TH, Higgins PD, Booth AJ, Sagana RL, Huang SK, White ES, King JE, Moore BB, Horowitz JC: X-linked inhibitor of apoptosis regulates lung fibroblast resistance to Fas-mediated apoptosis, American journal of respiratory cell and molecular biology 2013, 49:86-95
[250] Xie Q, Khaoustov VI, Chung CC, Sohn J, Krishnan B, Lewis DE, Yoffe B: Effect of tauroursodeoxycholic acid on endoplasmic reticulum stress-induced caspase-12 activation, Hepatology 2002, 36:592-60
[251] Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, Gorgun CZ, Hotamisligil GS: Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes, Science 2006, 313:1137-1140
[252] Qin L, Wang Z, Tao L, Wang Y: ER stress negatively regulates AKT/TSC/mTOR pathway to enhance autophagy, Autophagy 2010, 6:239-247
[253] Yorimitsu T, Klionsky DJ: Endoplasmic reticulum stress: a new pathway to induce autophagy, Autophagy 2007, 3:160-162
[254] Gonzalez-Rodriguez A, Mayoral R, Agra N, Valdecantos MP, Pardo V, Miquilena-Colina ME, Vargas-Castrillon J, Lo Iacono O, Corazzari M, Fimia GM, Piacentini M, Muntane J, Bosca L, Garcia-Monzon C, Martin-Sanz P, Valverde AM: Impaired autophagic flux is associated with increased endoplasmic reticulum stress during the development of NAFLD, Cell death & disease 2014, 5:e1179
[255] Wang H, Sun RQ, Zeng XY, Zhou X, Li S, Jo E, Molero JC, Ye JM: Restoration of autophagy alleviates hepatic ER stress and impaired insulin signalling transduction in high fructose-fed male mice, Endocrinology 2015, 156:169-181
[256] Bachar-Wikstrom E, Wikstrom JD, Kaiser N, Cerasi E, Leibowitz G: Improvement of ER stress-induced diabetes by stimulating autophagy, Autophagy 2013, 9:626-628
[257] Kim YC, Guan KL: mTOR: a pharmacologic target for autophagy regulation, The Journal of clinical investigation 2015, 125:25-32
[258] Gui YS, Wang L, Tian X, Li X, Ma A, Zhou W, Zeng N, Zhang J, Cai B, Zhang H, Chen JY, Xu KF: mTOR Overactivation and Compromised Autophagy in the Pathogenesis of Pulmonary Fibrosis, PloS one 2015, 10:e0138625
[259] Hidvegi T, Stolz DB, Alcorn JF, Yousem SA, Wang J, Leme AS, Houghton AM, Hale P, Ewing M, Cai H, Garchar EA, Pastore N, Annunziata P, Kaminski N, Pilewski J, Shapiro SD, Pak SC, Silverman GA, Brunetti-Pierri N, Perlmutter DH: Enhancing Autophagy with Drugs or Lung-directed Gene Therapy Reverses the Pathological Effects of Respiratory Epithelial Cell Proteinopathy, The Journal of biological chemistry 2015, 290:29742-29757
[260] Hidvegi T, Mirnics K, Hale P, Ewing M, Beckett C, Perlmutter DH: Regulator of G Signaling 16 is a marker for the distinct endoplasmic reticulum stress state associated with aggregated mutant alpha1-antitrypsin Z in the classical form of alpha1-antitrypsin deficiency, The Journal of biological chemistry 2007, 282:27769-27780
[261] Kamimoto T, Shoji S, Hidvegi T, Mizushima N, Umebayashi K, Perlmutter DH, Yoshimori T: Intracellular inclusions containing mutant alpha1-antitrypsin Z are propagated in the absence of autophagic activity, The Journal of biological chemistry 2006, 281:4467-4476
[262] Deslee G, Woods JC, Moore C, Conradi SH, Gierada DS, Atkinson JJ, Battaile JT, Liu L, Patterson GA, Adair-Kirk TL, Holtzman MJ, Pierce RA: Oxidative damage to nucleic acids in severe emphysema, Chest 2009, 135:965-974
[263] Hidvegi T, Ewing M, Hale P, Dippold C, Beckett C, Kemp C, Maurice N, Mukherjee A, Goldbach C, Watkins S, Michalopoulos G, Perlmutter DH: An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis, Science 2010, 329:229-232
[264] Li J, Pak SC, O'Reilly LP, Benson JA, Wang Y, Hidvegi T, Hale P, Dippold C, Ewing M, Silverman GA, Perlmutter DH: Fluphenazine reduces proteotoxicity in C. elegans and mammalian models of alpha-1-antitrypsin deficiency, PloS one 2014, 9:e87260
[265] Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrecke EH, Blagosklonny MV, Dawson TM, Dawson VL, El-Deiry WS, Fulda S, Gottlieb E, Green DR, Hengartner MO, Kepp O, Knight RA, Kumar S, Lipton SA, Lu X, Madeo F, Malorni W, Mehlen P, Nunez G, Peter ME, Piacentini M, Rubinsztein DC, Shi Y, Simon HU, Vandenabeele P, White E, Yuan J, Zhivotovsky B, Melino G, Kroemer G: Molecular definitions of cell death subroutines:

recommendations of the Nomenclature Committee on Cell Death 2012, Cell death and differentiation 2012, 19:107-120

[266] Chen ZH, Kim HP, Sciurba FC, Lee SJ, Feghali-Bostwick C, Stolz DB, Dhir R, Landreneau RJ, Schuchert MJ, Yousem SA, Nakahira K, Pilewski JM, Lee JS, Zhang Y, Ryter SW, Choi AM: Egr-1 regulates autophagy in cigarette smoke-induced chronic obstructive pulmonary disease, PloS one 2008, 3:e3316

[267] Chen ZH, Lam HC, Jin Y, Kim HP, Cao J, Lee SJ, Ifedigbo E, Parameswaran H, Ryter SW, Choi AM: Autophagy protein microtubule-associated protein 1 light chain-3B (LC3B) activates extrinsic apoptosis during cigarette smoke-induced emphysema, Proceedings of the National Academy of Sciences of the United States of America 2010, 107:18880-18885

[268] Papandreou I, Denko NC, Olson M, Van Melckebeke H, Lust S, Tam A, Solow-Cordero DE, Bouley DM, Offner F, Niwa M, Koong AC: Identification of an Ire1alpha endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma, Blood 2011, 117:1311-1314

[269] Wang L, Perera BG, Hari SB, Bhhatarai B, Backes BJ, Seeliger MA, Schurer SC, Oakes SA, Papa FR, Maly DJ: Divergent allosteric control of the IRE1alpha endoribonuclease using kinase inhibitors, Nature chemical biology 2012, 8:982-989

[270] Ghosh R, Giacomini MM, Backes BJ, Sheppard D, Oakes SA, Papa FR. Inhibition of Ire1α activity attenuates ER Stress-induced pulmonary fibrosis. American journal of respiratory and critical care medicine 2012; 185:A3923

[271] Ghosh R, Wang L, Wang ES, Perera BG, Igbaria A, Morita S, Prado K, Thamsen M, Caswell D, Macias H, Weiberth KF, Gliedt MJ, Alavi MV, Hari SB, Mitra AK, Bhhatarai B, Schurer SC, Snapp EL, Gould DB, German MS, Backes BJ, Maly DJ, Oakes SA, Papa FR: Allosteric inhibition of the IRE1alpha RNase preserves cell viability and function during endoplasmic reticulum stress, Cell 2014, 158:534-548