Chapter 8

General discussion

Summary and general discussion

Main findings

Stress responses have been shown to play a central role in health and disease in the lung. For instance, acute activation of the integrated stress response (ISR; chapter 2, Figure 1) upon viral infection reduces viral replication and enhances appropriate cytokine release including that of interferons. On the other hand, chronic activation of the integrated stress response, for example by cigarette smoke exposure, may lead to enhanced cell death (reviewed in chapter 2). Until last year, the importance of both the ISR as well as the unfolded protein response (UPR; chapter 1, Figure 2) in bacterial infection received little attention. We examined how virulence factors of *Pseudomonas aeruginosa* elicit both stress responses in bronchial epithelial cells (chapter 4). The induction of endoplasmic reticulum (ER) stress, which was mediated via the TAK1-p38 MAPK pathway, was caused by secreted virulence factors of *P. aeruginosa*. Pyocyanin and alkaline protease were the main contributors to the observed effect. To evaluate the ER stress response and subsequent UPR, we made use of our newly developed primers presented in chapter 3. In this way, we were able to monitor the splicing of *XBP1* mRNA in a reliable and quantitative way. The induction of *GADD34* was caused by the ability of the *Pseudomonas* supernatant to sequester iron leading to iron deficiency for the epithelial cells *in vitro*, which was mediated exclusively by the eIF2α kinase HRI and the ISR. This induction of *GADD34* expression appeared to be cytoprotective for bronchial epithelial cells exposed to conditioned medium of *P. aeruginosa*.

Whereas the study of the ISR remained a relatively small field, research in the role of the ER stress response and UPR in the pathophysiology of lung diseases expanded massively in the past five years. In particular conformational diseases, such as Alzheimer's and Parkinson's diseases, amyloidosis and Z α_1-antitrypsin deficiency, gained much attention due to the structural (conformational) change of a mutant protein leading to intracellular polymerisation and eventually disease (1). A subclass of conformational diseases that result from a point mutation in a protein member of the serpin superfamily

of serine proteinase inhibitors, like Z α_1-antitrypsin deficiency, are also referred to as serpinopathies. Central questions in the study of serpinopathies are whether protein polymerisation causes ER stress and related cell death (toxic gain of function); how these serpins are related to increased inflammation found in cells expressing mutants (loss of function); and how ER stress and inflammation contribute to the pathophysiology of the associated diseases. In case of Z α_1-antitrypsin deficiency, much of the answers to these questions were derived from liver samples or overexpressing models. These studies showed that overexpression of Z α_1-antitrypsin leads to accumulation and polymerisation inside the ER, but does not elicit the UPR (2). Remarkably, polymers do prime cells to generate an exaggerated ER stress response upon a so-called second hit (3, 4). Furthermore, polymer formation activates caspases-4 (and -12) and enhances the NF-κB response (2). After the discovery of polymers in bronchoalveolar lavage fluid and lung tissue (5, 6), even 10 years after liver transplantation (7), it was hypothesised that these polymers and the accompanying cellular consequences would contribute massively to the pathophysiology of the lung disease. And so the search for the responsible cell type emerged. Therefore, we investigated whether two of the major sources of α_1-antitrypsin in the lung, bronchial epithelial cells (chapter 5) and macrophages (chapter 7), were the cells responsible for the production of these polymers. In chapter 5 we determined the "critical Z α_1-antitrypsin concentration"; the minimal concentration of α_1-antitrypsin producted by a cell in order to be able to form polymers. We showed that both primary bronchial epithelial cells (chapter 5) and monocyte-derived-macrophages (chapter 7) are able to secrete detectable amounts of α_1-antitrypsin, however, they do not reach the concentration threshold to form polymers. Furthermore, monomeric Z α_1-antitrypsin does not elicit an exaggerated ER stress response upon a second hit in these cells. Importantly, even in the absence of these polymers, primary bronchial epithelial cells do exhibit an enhanced NF-κB response, dependent of the EGF receptor. This might also explain the absence of this exaggerated response in monocyte-derived macrophages (chapter 7). Since macrophages constitute a heterogeneous population, we studied the two extremes of a continuum of macrophage phenotypes, the pro- (mφ-1) and anti-inflammatory (mφ-2) macrophages, and examined

differences in its α_1-antitrypsin production (chapter 6 and 7).

Based on my main findings, this discussion is subdivided into three main topics. First, I will describe macrophage heterogeneity in COPD and its possible implications for α_1-antitrypsin deficiency. Then, I will discuss my experiences with measuring the ER stress response, thereafter I address potential therapeutic options for ER stress associated diseases and α_1-antitrypsin deficiency.

Macrophage heterogeneity

Macrophage diversity enables these cells to possess a wide variety of functions in the innate immune response, such as phagocytosis, the production of effector molecules and antigen presentation to T cells (8). However, the broad range of different macrophage phenotypes is not only explained by their anatomical site, but is also a result of the local environment within one anatomical compartment (8, 9). Furthermore, once differentiated into a subset, a macrophage is still able to adapt to changing conditions, altering its functions, which is termed macrophage plasticity. Although monocytes can be differentiated into distinct macrophage subtypes *in vitro*, the plasticity and dependence on the local milieu are features that make it difficult to mimic *in vivo* monocyte-into-macrophage differentiation (9). Therefore, results derived from two polarised subtypes *in vitro*, the mφ-1 and mφ-2, might not always represent the phenotype of e.g. alveolar macrophages *in vivo*. This was demonstrated in chapter 6, where mφ-1 macrophages produce approximately 4-fold more α_1-antitrypsin compared to the mφ-2 macrophages *in vitro*, and both increase production after LPS treatment. However, alveolar macrophages, which display more a mφ-2 phenotype according to their IL-10 production and complete lack of IL-12p40 secretion (chapter 6 and (10)), produce intermediate amounts of α_1-antitrypsin *in vitro*, which is unresponsive to LPS stimulation. However, it needs to be noted that marked differences exist in the characteristics of alveolar macrophages between patients with and without COPD (11), illustrating the complexity of defining the ultimate alveolar macrophage phenotype.

Nevertheless, access to locally differentiated alveolar macrophages can be

limited, for instance when it is unethical or too dangerous to perform bronchoscopy. Interestingly, some altered characteristics found in alveolar macrophages can also be detected in monocyte-derived macrophages and even in monocytes. Prieto *et al*. (12) showed that circulating monocytes of COPD patients exhibit decreased phagocytic capacity to *Escherichia coli*, and a similar defect was seen in alveolar macrophages (13). More recently, different studies showed the same phagocytic impairment of the more relevant pathogen *Haemophilus influenzae* by both monocyte-derived macrophages as alveolar macrophages of patients with COPD (13-15). The same impairment was found in the ability to phagocyte apoptotic cells (16, 17); however, the phagocytosis of latex beads did not seem to be altered (15, 17). Often smoking has been proposed to be the underlying cause, since healthy controls are better able to phagocytose apoptotic cells compared to smokers and the same holds for ex-smokers with COPD compared to smoking COPD patients (16). Furthermore, macrophages isolated from induced sputum of smoking COPD patients were shown to have predominantly a mφ-1 phenotype (18), whereas in healthy lungs it would resemble a mφ-2 macrophage and thus would contain an increased ability to phagocytose compared to mφ-1. In line, smoking cessation in COPD has been associated with a phenotype shift back towards the mφ-2 phenotype (19). On the other hand, the same reduced phagocytic capacity in monocytes and monocyte-derived macrophages of COPD patients suggests an inherent defect rather than a consequence of local differentiation or environmental exposure of macrophages in the lung.

Since α_1-antitrypsin is known to exhibit also anti-inflammatory properties ((20-22) and chapter 5), a genetic defect could provide additional insights in altered macrophage functions in chronic lung diseases. Indeed, α_1-antitrypsin deficiency is associated with rapidly growing mycobacterial infections, and monocyte-derived macrophages treated with α_1-antitrypsin appeared to be less prone to infection with *Mycobacterium abscessus* (23). However, whether this is due to a reduced phagocytic capacity needs to be investigated. Furthermore, to my knowledge, it is not known whether the phenotype-shift of alveolar macrophages found in COPD is also found in α_1-antitrypsin deficiency.

Interestingly, it has been shown that *in vitro* and *in vivo* treatment with azithromycin improves phagocytic function in alveolar macrophages from COPD patients. Therefore, it would be highly relevant to explore the effect of such treatment on alveolar macrophage function in α_1-antitrypsin deficiency, and to study the clinical benefit of such treatment.

Monitoring ER stress

The ER stress field developed rapidly with many investigators becoming interested in the UPR. Therefore, many experimental methods have been described to study the UPR. However, some of them are very laborious and others extremely difficult because of the lack of (proper) antibodies or low endogenous expression of certain proteins. Here, I will discuss my experience with different tools to measure and interpret the activation of each arm of the UPR.

PERK is an eIF2α kinase mediating the activation of one of the three branches of the UPR, and is known to have a poor endogenous expression in cells, which makes detection of its phosphorylation (as well as that of IRE1α) hard to measure. Moreover, PERK becomes highly phosphorylated upon activation, so its mobility is altered on SDS-PAGE resulting in an activation-dependent mobility shift (24). When specific questions require the detection of PERK activation, it is important to perform immunoprecipitation of PERK from cell lysates before immunoblotting (24). However, this method is very difficult and both time- and material-consuming. Once PERK is studied in the context of the ISR, and the involvement of this kinase in the activation of the ISR, then the use of knock-out MEFs or silencing RNA interference (RNAi) is advised. Alternatively, one can use downstream markers to evaluate the activation status of this pathway in general. There are good antibodies available to detect the phosphorylation of eIF2α (see Chapter 4), however, it is recommended to treat the cells with care and to replenish the media with pre-warmed (37°C) media one hour before treatment. Moreover, stimulation should be started when the cells are only 50% confluent and the use of an acute stressor as a positive control, such as thapsigargin, is advised to detect a notable change. For the detection of the nuclear proteins ATF4 and CHOP (25, 26), large quantities of nuclear extracts are needed, and

therefore, in my opinion, too expensive to use in primary cell cultures. Additionally, until recently, no appropriate antibodies were available for GADD34. Instead, CHOP, as well as GADD34, can be measured quantitatively at mRNA levels by quantitative RT-PCR (qPCR). Taken together, in most cases, phosphorylation of eIF2α, together with *CHOP* and *GADD34* mRNA levels are sufficient to verify the activation of this pathway. However, detection of these downstream mediators does not allow the identification of PERK or another kinase as being responsible for eIF2α phosphorylation.

Like PERK, phophorylation of IRE1 (the starting point for activation of another branch of the UPR) is difficult to measure, but fortunately detection of its most important downstream target, the splicing of *XBP1* mRNA, is more straight forward. Until 2006, the splicing of *XBP1* mRNA was mainly assessed by detection of PCR fragments following RT-PCR and gel electrophoresis (27). By this method, it was not only difficult to quantify the *spliced XBP1*, it was also very laborious and challenging to obtain a conclusive result. In 2006, Hirota *et al*. (28) introduced a new method by which the spliced form could be quantified by cleavage of the RT-PCR product of double stranded (unspliced) *XBP1* with the restriction enzyme PstI. This method requires the addition of the restriction enzyme after 2-4 cycles of annealing-elongating during the qPCR, and is therefore complex and prone to errors. In our search for reliable and simple methods to screen for the involvement of the UPR, we developed a specific primer for the quantitative detection of *spliced XBP1* by qPCR (Chapter 3). *Spliced XBP1* is highly transcriptionally active and regulates the transcription of UPR target genes via direct binding to the UPR element (UPRE) and ER stress response element (ERSE) when in complex with NF-Y (27). ATF6 is the sensor of the third branch of the UPR, and this transcription factor binds to and acts via the ERSE as well (29). Therefore, separation of the UPR target genes belonging to *spliced XBP1* specifically is difficult. Using *Xbp1*$^{-/-}$ and *Atf6*$^{-/-}$ MEFs treated with tunicamycin, Lee *et al*. (30) identified ERdj4, a heat shock protein 40 family member that interacts with ERAD (31) and chaperone p58^{IPK} as specific downstream targets of *spliced XBP1*.

Interestingly, the same group did not identify a specific marker for ATF6. However, both Kaufman's lab (32) and Mori's lab (33, 34) performed transcriptional profile analysis

and microarray analyses on *ATF6α*$^{-/-}$ MEFs. Both groups, independently, pointed towards Derlin-3, a functional component of the ERAD, as a sole downstream target. It is worth mentioning that in both screens *p58*^{IPK} was significantly reduced in *ATF6α*$^{-/-}$ treated with tunicamycin, which suggests that p58^{IPK} may not be specific for detection of *spliced XBP1*. Due to this shared binding site, namely the ERSE, the opposite might be true, with so-called ATF6 specific genes also being responsive to *spliced XBP1*. For instance, bronchial epithelial cells transfected with the commonly used specific ATF6 luciferase reporter plasmid (p5xATF6-luc), showed a 15-fold increase in luciferase activity when treated with tunicamycin for 6 hours. Remarkably, in my hands, this increase was totally abolished when the cells were co-stimulated with 4μ8C (35), a specific IRE1 inhibitor (unpublished data).

A rather general, but important indicator of ER stress is the detection of the KDEL sequence (Lys-Asp-Glu-Leu). This sequence keeps soluble ER resident proteins, such as Glucose Regulated Proteins 78 and 94 (GRP78; also referred to as BiP and GRP94, respectively) and Protein Disulphide Isomerase (PDI) retained inside the ER. Especially the chaperone GRP78 is a key regulator in ER stress, since the activation of the UPR is dependent on its release from the three ER stress transducers. Activation of the three branches of the UPR eventually lead to the induction of UPR target genes, amongst which GRP78. Therefore, GRP78 up regulation is indicative for an ER undergoing stress.

Therapeutic approaches

ER stress mediated diseases

As reviewed in **Chapter 2**, there is increasing evidence of the involvement of the ISR in various chronic lung diseases and lung cancer, which has mainly been associated with an increased PERK activity. Chronic ER stress is in most cases detrimental for the cell, leading to cell death and worsening of the disease, whereas in cancer, increased PERK signalling favours tumour progression and cancer cell survival (36, 37). For that reason, the inhibition of the PERK-arm is currently receiving much attention and significant advances in therapeutical approaches to block this kinase have been made over the last years. However, ER stress-mediated cell death is not only induced via PERK, but can also be triggered via the IRE1α-JNK signalling cascade. Therefore, inhibition of this arm might be relevant as well. In 2012, the laboratorium of David Ron (35) used high-throughput screening to identify the aforementioned 4μ8C as a potent inhibitor of IRE1. Interestingly, this compound not only inhibits the ER stress kinase IRE1α, but is also predicted to be able to inhibit the second isoform, IRE1β (38, 39). The expression of this second IRE1 was initially reported to be restricted to the epithelial cells in the gastrointestinal tract (40). It has long been suggested that this kinase regulates ER homeostasis of goblet cells via an interaction with mucin production. Furthermore, it was assumed that this kinase might also be expressed in lung goblet cells to regulate mucin expression. However, no real evidence existed until very recently, when Tsuru *et al*. (39) showed that *IRE1β*$^{-/-}$ mice showed increased *mucin 2* (*MUC2*) mRNA stability and accumulation of MUC2 protein in the ER of intestinal goblet cells. Four months later, Martino *et al*. (41) presented the same findings for airway goblet cells. In line with this finding, I found that fully differentiated primary bronchial epithelial cells treated with 4μ8C showed reduced expression *MUC5AC* and *MUC2* mRNA and decreased secretion of mucin 5AC (unpublished data). Therefore, it is a potential therapeutic target for patients with ER stress-associated lung diseases and mucus overproduction, like COPD and cystic fibrosis.

Also during chronic infections, ER stress and activation of the UPR are important

causes of cell death. For *P. aeruginosa*, *spliced XBP1* has recently been shown to be crucial in N-(3-oxo-dodecanoyl) homoserine lactone (C12)-mediated apoptosis (42). Blocking IRE1 might be relevant to counteract this effect. In contrast, in chapter 4, we have shown that *GADD34* serves a cytoprotective role in human bronchial epithelial cells and mouse fibroblasts upon exposure to secreted virulence factors of *P. aeruginosa*. Inhibition of *GADD34* would in this case be detrimental. We have not investigated the presence and contribution of C12 in our conditioned medium from *P. aeruginosa* to the observed effects, which should be done in the future as this might be relevant to further reduce the cytotoxicity of *P. aeruginosa* in bronchial epithelial cells. Another approach to treat *P. aeruginosa* infection that needs to be considered is the inhibition of individual virulence factors to reduce the interference with the physiological ISR and ER stress response of the host. However, we were unable to ascribe the observed effect to a single virulence factor, making this approach more complex. Future studies will give us more insights in the contribution of each virulence factor, possibly opening up new therapeutic targets.

Although the development of many ER stress inhibitors, such as the PERK inhibitor (43) and the IRE1α inhibitor 4μ8C (35), are still in the early stages, it has demonstrated the importance of the right balance in ISR activation, and further development of inhibitors might lead to therapeutic options in the different lung diseases. Recently, encouraging results were obtained using a PERK inhibitor that inhibited neurodegeneration in prion-infected mice (44). However, one should bear in mind that only the stressed cells need targeting, since a physiological and functional ISR is crucial to maintain healthy tissue.

Alpha$_1$-antitrypsin deficiency

The discovery of the association between mutants of α_1-antitrypsin and early-onset lung emphysema made the protease-antiprotease imbalance hypothesis the dominant model for the development of the disease. However, restoring this imbalance with intravenous α_1-antitrypsin augmentation therapy did not cure the disease nor had its expected effects on disease progression (45). For this reason, α_1-antitrypsin augmentation therapy remains unsupported for the treatment of α_1-antitrypsin deficiency in several

countries, amongst which the Netherlands and the United Kingdom.

Treating the defect

The group of David Lomas at the University of Cambridge (now relocated to University College London) made major contributions in the development of novel strategies to treat α_1-antitrypsin deficiency. They showed that small molecules that block surface cavities of α_1-antitrypsin could inhibit polymerisation *in vitro* and *in vivo* (46). However, this treatment also inactivated α_1-antitrypsin as a neutrophil elastase inhibitor (46). Moreover, the effective concentrations were expected to be too toxic to use in patients. Due to the enormous potential of this approach as a treatment, future research must certainly provide us new small molecules that are less toxic.

The polymers found in the lung are thought to arise from local production, as they have been reported to be still present in bronchoalveolar lavage fluid ten years after liver transplantation (7). Since we showed in chapter 5 and 7 that both cultured primary bronchial epithelial cells and monocyte-derived macrophages from ZZ patients do not produce these polymers, the origin of these polymers is still unknown. The main question to be answered, in my opinion, is whether only one specific type of cells is responsible for the polymer formation or whether α_1-antitrypsin secreted locally by various cells is retained in the interstitium and epithelial lining fluid, and that at these sites the critical Z α_1-antitrypsin concentrations is reached that is required for extracellular polymer formation. Another explanation could be that the extracellular milieu favours polymer formation, and thus lower secreted concentrations might allow polymerisation. Once we know the answer, we might be able to use the small molecules also as a potential lung therapy.

A second approach for developing a curative treatment for α_1-antitrypsin deficiency is the use of genetic corrections and induced pluripotent stem cells (iPS). Rashid *et al*. (47) presented the derivation of iPS from fibroblasts of skin biopsies of α_1-antitrypsin deficiency patients. Genetic correction of the point mutation underlying the Z mutation (E342K) restored α_1-antitrypsin production and its proper activity in iPS-

derived liver-like cells (48). The challenge remains to differentiate these corrected liver cells to fully mature hepatocytes and to restore the mutations that resulted from the iPS development. Another approach was used by the Birmingham group, who showed that α_1-antitrypsin secretion from monocytes carrying the Z mutation can be corrected with small DNA fragments encoding M α_1-antitrypsin (49). Whether this approach can form the basis for development of new treatments for α_1-antitrypsin deficiency is unclear at present.

Blocking the MEK-EGFR pathway

Based on the studies described in this thesis (chapter 5 and 7), we could argue that bronchial epithelial cells are the main contributors to the local inflammation in the lungs of α_1-antitrypsin deficiency patients. Even in the absence of polymers, they exhibit an increased inflammatory response as demonstrated by increased ERK1/2 signalling and subsequent cytokine release. Interestingly, we were able to reduce these effects by treating the cells with extracellular M α_1-antitrypsin. This might implicate that inhaled α_1-antitrypsin augmentation therapy could diminish local inflammation through an effect on for example neutrophil influx and survival, and local macrophage differentiation. In my opinion, the primary outcome of a first clinical proof-of-concept study using inhaled α_1-antitrypsin should therefore be the measurement of inflammatory markers, such as IL-8, in bronchoalveolar lavage and/or sputum. Subsequent more long-term studies would be needed to explore the effect on lung function (including FEV_1, VC, CO diffusion and single-breath nitrogen test) and alveolar pathology as assessed by densitometric CT scans. Secondary outcomes could comprise bacterial colonisation and exacerbation rates. However, I would like to stress that these read-outs are very complex and not solely dependent on increased inflammation, and therefore possibly not conclusive. Furthermore, the increased ERK1/2 phosphorylation was dependent on the 'classical' MEK-EGFR pathway, which is shared with non-small cell lung carcinoma and certain breast cancers (50, 51). Major therapeutic advances have already been made in the cancer field, and the use of existing MEK inhibitors, EGFR monoclonal antibodies or tyrosine kinase

inhibitors should also be considered as a treatment for α_1-antitrypsin deficiency related emphysema. Further research has to be done to confirm these therapeutic options as actual treatment in α_1-antitrypsin deficiency. However, it needs to be noted that a safety and efficacy trial with an EGFR inhibitor in COPD patients did not show convincing evidence for an effect on mucin stores and furthermore was associated with adverse effects (52).

Future directions

This thesis started with the initial question about the role of polymers and its relation to ER stress in the development of Z α_1-antitrypsin deficiency related emphysema. When the years progressed, it became evident that polymers might not be produced locally by lung cells and that monomeric Z α_1-antitrypsin does not prime cells for an exaggerated ER stress response upon a second hit. In contrast, we can conclude that there is a prominent role for ER stress in other lung related diseases and bacterial infections. Two important questions have been raised by these studies, but still remain to be answered in full:

1) Stress responses during bacterial infection:

- What is the role of the each virulence factor in the induction of ER stress and the ER stress-independent ISR by *P. aeruginosa* and can we use that for future treatment?
- Are these mechanisms specific for virulence factors of *P. aeruginosa*, or are they applicable to other (Gram negative) respiratory pathogens as well?

2) Alpha$_1$-antitrypsin deficiency:

- Is there local production of Z α_1-antitrypsin polymers in the lung?
- Would aerosolised α_1-antitrypsin be suitable as a therapy to reduce lung inflammation?

References

1. Lomas DA, *et al.* (1992) The mechanism of Z alpha 1-antitrypsin accumulation in the liver. *Nature* 357(6379):605-607.
2. Hidvegi T, *et al.* (2005) Accumulation of mutant alpha1-antitrypsin Z in the endoplasmic reticulum activates caspases-4 and -12, NFkappaB, and BAP31 but not the unfolded protein response. *J Biol Chem* 280(47):39002-39015.
3. Lawless MW, *et al.* (2004) Activation of endoplasmic reticulum-specific stress responses associated with the conformational disease Z alpha 1-antitrypsin deficiency. *J Immunol* 172(9):5722-5726.
4. Ordonez A, *et al.* (2013) Endoplasmic reticulum polymers impair luminal protein mobility and sensitize to cellular stress in alpha1 -antitrypsin deficiency. *Hepatology* 57(5):2049-2060.
5. Mahadeva R, *et al.* (2005) Polymers of Z alpha1-antitrypsin co-localize with neutrophils in emphysematous alveoli and are chemotactic in vivo. *Am J Pathol* 166(2):377-386.
6. Elliott PR, *et al.* (1998) Lung polymers in Z alpha1-antitrypsin deficiency-related emphysema. *Am J Respir Cell Mol Biol* 18(5):670-674.
7. Mulgrew AT, *et al.* (2004) Z alpha1-antitrypsin polymerizes in the lung and acts as a neutrophil chemoattractant. *Chest* 125(5):1952-1957.
8. Gordon S, *et al.* (2011) Diversity and plasticity of mononuclear phagocytes. *Eur J Immunol* 41(9):2470-2472.
9. Sica A, *et al.* (2012) Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest* 122(3):787-795.
10. Schneberger D, *et al.* (2011) Monocyte and macrophage heterogeneity and Toll-like receptors in the lung. *Cell Tissue Res* 343(1):97-106.
11. Hiemstra PS (2013) Altered macrophage function in chronic obstructive pulmonary disease. *Ann Am Thorac Soc* 10 Suppl:S180-185.
12. Prieto A, *et al.* (2001) Defective natural killer and phagocytic activities in chronic obstructive pulmonary disease are restored by glycophosphopeptical (inmunoferon). *Am J Respir Crit Care Med* 163(7):1578-1583.
13. Taylor AE, *et al.* (2010) Defective macrophage phagocytosis of bacteria in COPD. *Eur Respir J* 35(5):1039-1047.
14. Berenson CS, *et al.* (2006) Impaired phagocytosis of nontypeable Haemophilus influenzae by human

alveolar macrophages in chronic obstructive pulmonary disease. *J Infect Dis* 194(10):1375-1384.

15. Marti-Lliteras P, *et al.* (2009) Nontypeable Haemophilus influenzae clearance by alveolar macrophages is impaired by exposure to cigarette smoke. *Infect Immun* 77(10):4232-4242.

16. Hodge S, *et al.* (2007) Smoking alters alveolar macrophage recognition and phagocytic ability: implications in chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 37(6):748-755.

17. Hodge S, *et al.* (2003) Alveolar macrophages from subjects with chronic obstructive pulmonary disease are deficient in their ability to phagocytose apoptotic airway epithelial cells. *Immunol Cell Biol* 81(4):289-296.

18. Frankenberger M, *et al.* (2004) Characterization of a population of small macrophages in induced sputum of patients with chronic obstructive pulmonary disease and healthy volunteers. *Clin Exp Immunol* 138(3):507-516.

19. Kunz LI, *et al.* (2011) Smoking status and anti-inflammatory macrophages in bronchoalveolar lavage and induced sputum in COPD. *Respir Res* 12:34.

20. Jonigk D, *et al.* (2013) Anti-inflammatory and immunomodulatory properties of alpha1-antitrypsin without inhibition of elastase. *Proc Natl Acad Sci U S A* 110(37):15007-15012.

21. Janciauskiene S, *et al.* (2004) Inhibition of lipopolysaccharide-mediated human monocyte activation, in vitro, by alpha1-antitrypsin. *Biochem Biophys Res Commun* 321(3):592-600.

22. Bergin DA, *et al.* (2010) alpha-1 Antitrypsin regulates human neutrophil chemotaxis induced by soluble immune complexes and IL-8. *J Clin Invest* 120(12):4236-4250.

23. Chan ED, *et al.* (2007) Alpha-1-antitrypsin (AAT) anomalies are associated with lung disease due to rapidly growing mycobacteria and AAT inhibits Mycobacterium abscessus infection of macrophages. *Scand J Infect Dis* 39(8):690-696.

24. Harding HP, *et al.* (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* 397(6716):271-274.

25. Vallejo M, *et al.* (1993) C/ATF, a member of the activating transcription factor family of DNA-binding proteins, dimerizes with CAAT/enhancer-binding proteins and directs their binding to cAMP response elements. *Proc Natl Acad Sci U S A* 90(10):4679-4683.

26. Wang XZ, *et al.* (1996) Signals from the stressed endoplasmic reticulum induce C/EBP-homologous protein (CHOP/GADD153). *Mol Cell Biol* 16(8):4273-4280.

27. Yoshida H, *et al.* (2001) XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 107(7):881-891.

28. Hirota M, *et al.* (2006) Quantitative measurement of spliced XBP1 mRNA as an indicator of endoplasmic reticulum stress. *J Toxicol Sci* 31(2):149-156.

29. Yoshida H, *et al.* (2000) 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. *Mol Cell Biol* 20(18):6755-6767.

30. Lee AH, *et al.* (2003) XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol Cell Biol* 23(21):7448-7459.

31. Lai CW, *et al.* (2012) ERdj4 protein is a soluble endoplasmic reticulum (ER) DnaJ family protein that interacts with ER-associated degradation machinery. *J Biol Chem* 287(11):7969-7978.

32. Wu J, *et al.* (2007) ATF6alpha optimizes long-term endoplasmic reticulum function to protect cells from chronic stress. *Dev Cell* 13(3):351-364.

33. Adachi Y, *et al.* (2008) ATF6 is a transcription factor specializing in the regulation of quality control proteins in the endoplasmic reticulum. *Cell Struct Funct* 33(1):75-89.

34. Yamamoto K, *et al.* (2007) Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1. *Dev Cell* 13(3):365-376.

35. Cross BC, *et al.* (2012) The molecular basis for selective inhibition of unconventional mRNA splicing by an IRE1-binding small molecule. *Proc Natl Acad Sci U S A* 109(15):E869-878.

36. Bi M, *et al.* (2005) ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth. *EMBO J* 24(19):3470-3481.

37. Rouschop KM, *et al.* (2013) PERK/eIF2alpha signaling protects therapy resistant hypoxic cells through induction of glutathione synthesis and protection against ROS. *Proc Natl Acad Sci U S A* 110(12):4622-4627.

38. Wang XZ, *et al.* (1998) Cloning of mammalian Ire1 reveals diversity in the ER stress responses. *EMBO J* 17(19):5708-5717.

39. Tsuru A, *et al.* (2013) Negative feedback by IRE1beta optimizes mucin production in goblet cells. *Proc Natl Acad Sci U S A* 110(8):2864-2869.

40. Bertolotti A, *et al.* (2001) Increased sensitivity to dextran sodium sulfate colitis in IRE1beta-deficient

mice. *J Clin Invest* 107(5):585-593.

41. Martino MB, *et al.* (2013) The ER stress transducer IRE1beta is required for airway epithelial mucin production. *Mucosal Immunol* 6(3):639-654.

42. Valentine CD, *et al.* (2013) X-Box Binding Protein 1 (XBP1s) Is a Critical Determinant of Pseudomonas aeruginosa Homoserine Lactone-Mediated Apoptosis. *PLoS Pathog* 9(8):e1003576.

43. Atkins C, *et al.* (2013) Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. *Cancer Res* 73(6):1993-2002.

44. Moreno JA, *et al.* (2013) Oral treatment targeting the unfolded protein response prevents neurodegeneration and clinical disease in prion-infected mice. *Sci Transl Med* 5(206):206ra138.

45. Dickens JA, *et al.* (2011) Why has it been so difficult to prove the efficacy of alpha-1-antitrypsin replacement therapy? Insights from the study of disease pathogenesis. *Drug Des Devel Ther* 5:391-405.

46. Mallya M, *et al.* (2007) Small molecules block the polymerization of Z alpha1-antitrypsin and increase the clearance of intracellular aggregates. *J Med Chem* 50(22):5357-5363.

47. Rashid ST, *et al.* (2010) Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. *J Clin Invest* 120(9):3127-3136.

48. Yusa K, *et al.* (2011) Targeted gene correction of alpha1-antitrypsin deficiency in induced pluripotent stem cells. *Nature* 478(7369):391-394.

49. McNab GL, *et al.* (2007) Modification of gene expression and increase in alpha1-antitrypsin (alpha1-AT) secretion after homologous recombination in alpha1-AT-deficient monocytes. *Hum Gene Ther* 18(11):1171-1177.

50. Paez JG, *et al.* (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 304(5676):1497-1500.

51. Lynch TJ, *et al.* (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 350(21):2129-2139.

52. Woodruff PG, *et al.* (2010) Safety and efficacy of an inhaled epidermal growth factor receptor inhibitor (BIBW 2948 BS) in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 181(5):438-445.