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Silke Meiners

Ludwig-Maximilians University

Catherine M. Greene

Royal College of Surgeons in Ireland, cmgreene@rcsi.ie

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Protein Quality Control in Lung Disease – it's all About Cloud-Networking

Silke Meiners¹ and Catherine M. Greene²

¹ Comprehensive Pneumology Center (CPC), University Hospital, Ludwig-Maximilians University, Helmholtz Zentrum München, Munich, Member of the German Center for Lung Research (DZL), Germany.

² Dept. Medicine, Royal College of Surgeons in Ireland, Education and Research Centre, Beaumont Hospital, Dublin 9, Ireland.

Correspondence to:

Silke Meiners, PhD

Comprehensive Pneumology Center, Ludwig-Maximilians-Universität, Asklepios Klinik Gauting und Helmholtz Zentrum München, Max-Lebsche-Platz 31, 81377 München, Germany

Phone: 0049 89 3187 4673

Fax: 0049 89 3187 194673

Email: silke.meiners@helmholtz-muenchen.de

Protein quality control involves the comprehensive management of protein function in the cell and is called 'proteostasis' [1]. It ranges from translation and chaperone-assisted three-dimensional folding, interaction with protein partners, signal-induced post-translational modifications to disposal by the proteasome or the autophagy pathways.

Dysfunctional protein quality control is emerging as a key pathomechanism for chronic lung diseases. Two major hereditary conformational disorders of the lung, cystic fibrosis (CF) and alpha-1 antitrypsin (AAT) deficiency as well as some familial forms of IPF are caused by the expression of mutant and misfolded proteins which disrupt protein homeostasis and drive the onset of pulmonary diseases [2, 3]. Disturbed protein homeostasis also causes sporadic respiratory diseases [1, 4]. Cigarette smoke-induced protein misfolding, aberrant proteasomal protein degradation and induction of autophagy have been observed in COPD patients and in smoked mice [4, 5]. Dysregulation of autophagy and ER stress have also been implicated in CF, pulmonary arterial hypertension (PAH), IPF and other lung diseases [6, 7]. Impairment of protein quality control pathways exacerbates the detrimental effects of harmful exposures of the lung in lung pathogenesis [1].

The ERS Research Seminar on "Protein Quality Control in Lung Disease", held on the 1st and 2nd March, 2014 at Lake Starnberg in Germany, brought together international experts to develop a comprehensive view of protein quality control in general, and in the lung in particular. Understanding the complex interplay of protein misfolding, ER homeostasis and protein degradation as interrelated components of adaptive proteostasis will identify novel therapeutic targets for treatment of pulmonary diseases as outlined below.

Protein misfolding

Proteins are synthesized as an amino acid chain which folds into a well-defined three-dimensional structure. A network of molecular chaperones assists protein folding and maintains functional states [8]. Destabilizing missense mutations or stress-induced misfolding challenge the proteostasis network and target protein destruction via proteasomal degradation or autophagy. When chaperone-assisted refolding or degradation fails, protein aggregation may occur. Aggregates exert proteotoxicity by interfering with normal proteins and cellular function [8].

In CF, the F508del-mutation prevents exit of the CFTR protein from the ER, and leads to its premature degradation by the ER quality control systems. Rescuing F508del-CFTR has involved corrector compounds such as VX-809 (Vertex). Disappointingly, the results of clinical trials are modest [9]. As pointed out by Margarida Amaral synergistic F508del-CFTR correction may be achieved by other compounds that target distinct conformational sites, later trafficking stages or cellular checkpoints of the proteostasis network as recently shown using a druggable genome siRNA library [10]. Imbalancing the proteostasis network by a single mutant protein may also apply to novel rare AAT variants with altered protein structure as suggested by Mila Ljubic [11]. Mark Hipp from the Max-Planck-Institute of Biochemistry in Munich provided evidence for a complete collapse of protein homeostasis: above a certain threshold, misfolded aggregated proteins indirectly inhibited overall protein degradation by the proteasome [12]. Misfolded proteins also sequester chaperones which are then missing for other essential folding and transport functions [13]. William Balch elegantly extended the current protein quality control network concept by proposing dynamic and multi-dimensional proteostasis network 'clouds'. A cloud represents a quinary level (Q-state) of structural control to locally and dynamically modify structure and interactions of a protein [14]. On the cellular level, such "Q-clouds" are present in Q-bodies which represent local and dynamic cytoplasmic compartments containing chaperone-complexed misfolded proteins as well as degradation components such as the proteasome [15]. This concept adds a novel spatial and dynamic level to the proteostasis network: Upsetting the "Q-cloud" by single point mutated proteins may result in a chronic proteotoxic crisis contributing to the pathology of chronic lung diseases.

ER homeostasis

A number of conformational disorders of the lung involve the expression of mutant secretory and membrane proteins such as surfactant protein (SP)-C, SP-A, AAT and CFTR triggering the unfolded protein response (UPR) of the ER [16]. The three UPR sensors – IRE1 α , ATF-6 and PERK cooperate to restore protein homeostasis in the ER by slowing down translation, activating the folding capacity and increasing degradation of misfolded proteins by proteasomal or autophagic pathways. Stefan Marciniak showed that some AAT mutants activate the UPR, while others induce ER overload suggesting that the ER has some degree of freedom in its response to misfolded proteins. Additional stress then tips the balance and makes ER-overloaded cells more sensitive to ER stress responses [17]. Adjustment of protein synthesis to match the folding capacity of the ER is a key event to allow adaptive restoration of protein homeostasis and involves fine-tuned PERK-mediated phosphorylation versus GADD34-induced dephosphorylation of the translation initiation factor eIF2 α [19]. Loss of this negative feed-back loop contributes to tumour growth and is detrimental in conditions of chronic ER stress yet offers novel targets for experimental chemotherapeutics [20].

Defects in the processing cascade of secretory proteins represent another challenge to ER homeostasis. Andreas Günther provided novel evidence that impaired processing of proSP-B in alveolar epithelial cell type II (AECII) contributes to maladaptive ER stress in sporadic IPF. Reduced expression of the proteases napsin-A and cathepsin-H hampered processing of proSP-B which then accumulated in its unprocessed form and induced ER stress.

Whilst UPR is one way to cope with misfolded or unprocessed secretory proteins, autophagolysosomal disposal of the ER is another option. Michael Beers showed that autophagy function is impaired in AECII cells of IPF patients in response to missense and splicing mutants of SP-C. In cell culture, abnormalities in autophagosomal protein clearance affected organelle homeostasis not only of the ER but also of mitochondria involving mitophagy - a specialised form of mitochondrial autophagy.

Simon Tavernier from Bart Lambrecht's group in Ghent added a novel aspect to our understanding of the ER stress response: The IRE-1 α -XBP-1 axis regulates homeostasis of

CD8+ dendritic cells in the absence of UPR signalling [21] proposing a role of UPR effectors in the regulation of cell differentiation far beyond mediating a canonical ER stress response.

Protein degradation

Two protein disposal systems, the ubiquitin-proteasome and the autophagy pathways not only perform “housekeeping” functions of normal protein turnover but also “quality control” tasks that ensure rapid disposal of dysfunctional proteins [22, 23]. For conformational disorders of the lung, the proteasome is the effector disposal system for mutant proteins but its regulation in chronic lung disease has not been investigated in detail yet. Silke Meiners from the Comprehensive Pneumology Center, introduced the complexity of the proteasome system and how the proteolytic 20S proteasome is activated by several regulators in a compartment-specific and dynamic manner [23]. The catalytic activities of the proteasome can be acutely impaired by cigarette smoke exposure [24] leading to a partial collapse of proteostasis that exaggerates protein damage and contributes to lung pathologies. In contrast, proteasome function is enhanced upon myofibroblast differentiation in pulmonary fibrosis proposing targeted proteasome inhibition as a novel therapeutic approach for IPF.

A novel perspective on autophagy in chronic lung diseases was provided by emerging data beyond the obvious role of autophagy in protein quality control on its interaction with cilia function. Patrice Codogno from Paris presented recent data on how the autophagy pathway regulates ciliary growth by degradation of proteins required for cilia function [25]. These data suggest a close interplay between autophagic protein degradation and cellular nutrient signalling beyond the mTOR pathway. Suzanne Cloonan from the lab of Augustine Choi, extended this concept of autophagic removal of ciliary proteins, i.e. “ciliophagy”, to ciliated pulmonary cells and defective mucociliary clearance in COPD patients [26] [27]. Disturbed autophagosome function also involves defective mitochondrial clearance via mitophagy emphasizing the interaction of the different autophagosomal disposal systems in the proteostasis network. Ivan Dikic pointed out that proteasomal and autophagic disposal systems closely interact as both pathways use the ubiquitin-tag for their substrate selection and critically depend on the solubility of the protein, association with chaperones, its

ubiquitination state, or binding to shuttle molecules such as p62 and HDAC6 [28]. Moreover, the ubiquitin-like LC3 protein that orchestrates autophagic vesicle formation serves as a bridging factor for endosome vesicle formation indicating a molecular connection between vesicle trafficking and autophagy pathways [29].

Conclusion

Effective protein quality control is central to lung health. Dysfunctional proteostasis underpins almost all chronic lung diseases and, as such, protein homeostasis represents an essential component of the healthy lung. One key aspect emerging from this ERS workshop is that protein quality control in the lung needs to be understood as a dynamic and multi-dimensional proteostasis network (Figure 1): The mesh-like proteostasis network connects cloud-like cellular organelles, e.g. the ER, the nucleus and the mitochondria, but also Q-bodies which serve as local and dynamic quality control sites. Proteotoxic stress dynamically affects multiple cellular compartments and components of the proteostasis network (e.g. parts of the ER, the nucleus and the mitochondrion) and activates protein quality control in Q-bodies coping with misfolded, insoluble and aggregated proteins. Identifying the central hubs that are dysregulated upon proteotoxic stress will be the key to interfere with dysregulated protein homeostasis that drives pathology of chronic lung diseases. With this new knowledge comes the additional challenge of identifying the idiosyncratic triggers and reactions responsible for specific diseases associated with dysfunctional proteostasis in the lung.

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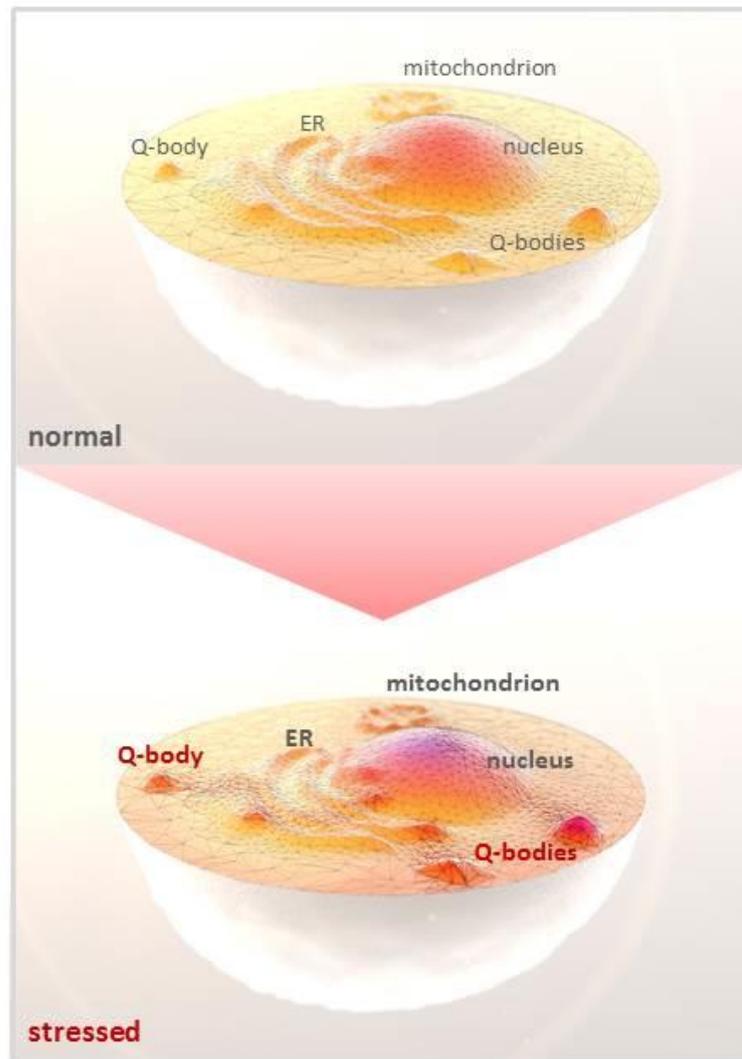


Figure 1