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Fine-tuning the ubiquitin-proteasome system to treat pulmonary fibrosis

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Abstract

Idiopathic pulmonary fibrosis is an extremely aggressive lung disease that develops almost exclusively in older individuals, carries a very poor prognosis, and lacks any truly effective therapies. The current conceptual model is that IPF develops because of an age-related decline in the ability of the lung epithelium to regenerate after injury, largely due to death or senescence of epithelial progenitor cells in the distal airways. This loss of regenerative capacity is thought to initiate a chronic and ineffective wound healing response, characterized by persistent, low-grade lung inflammation and sustained production of collagen and other extracellular matrix materials. Despite recent advances in our understanding of IPF pathobiology, there remains a pressing need to further delineate underlying mechanisms to develop more effective therapies for this disease. In this review, we build the case that many of the manifestations of IPF result from a failure of cells to effectively manage their proteome. We propose that epithelial progenitor cells, as well as immune cells and fibroblasts become functionally impaired, at least in part, because of an accumulation or a loss in the expression of various crucial proteins. Further, we propose that central to this defect is the dysregulation of the ubiquitin proteasome system, which is the major protein degradation system in eukaryotic cells. Lastly, borrowing concepts from other fields, we discuss how targeting the UPS system could be employed as a novel treatment for IPF and perhaps for other fibrotic lung diseases as well.

Keywords

Pulmonary fibrosis; idiopathic pulmonary fibrosis; proteostasis; ubiquitin proteasome system

Introduction.

Pulmonary fibrosis refers to a heterogeneous group of conditions that scar the distal airspaces of the lung. In most cases, pulmonary fibrosis develops as a consequence to some identifiable pulmonary insult, such as a viral infection, exposure to inorganic or organic dusts or treatment with chemotherapeutic agents or thoracic radiation [1, 2]. However, in a significant minority of cases, pulmonary fibrosis develops without any identifiable cause, and in these cases is considered idiopathic in origin. Although several types of idiopathic

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fibrotic lung conditions are known to exist, by far the most common of these is the highly aggressive respiratory disorder called idiopathic pulmonary fibrosis (IPF).

IPF is often classified as an age-related lung disorder because it develops almost exclusively after 50 years of age and its incidence increases almost exponentially after the seventh decade of life. In the US alone, it is estimated that over 100,000 people are diagnosed with IPF each year, and recent evidence indicates that the incidence of this condition has climbed substantially over the last few decades. In addition to age, various environment factors have been linked to the development of the disease, including chronic cigarette smoking, viral infections and a wide variety of environmental and occupational exposures [2]. A favored conceptual model is that IPF develops from an age-related decline in the ability of lung epithelium to regenerate after injury. Chronic environmental exposures, such as those described above, combined with a genetic susceptibility, are believed to contribute to loss of epithelial regenerative capacity by inducing premature death or senescence of epithelial progenitor cells. As result, these terminal cell fates drive chronic inflammatory and wound healing responses that not only further injure the lung but also severely impede respiratory gas exchange and other respiratory functions [3].

Consistent with the mechanisms described above, there are several groups of cells that are believed to play an important role in the pathobiology of IPF. As just mentioned, the primary cell contributing to disease is the distal epithelial progenitor cell, also known as the type II alveolar (AE2) epithelial cell [4, 5, 6]. Similar to other age-related diseases, AE2 cells adopt many hallmarks of aging tissues, including shortened telomeres, genomic instability, mitochondrial dysfunction and loss of proteostasis [7]. Indeed, the accumulation of misfolded and aggregated proteins is one of the earliest manifestations of epithelial dysfunction in the lung of IPF patients [8]. Moreover, abnormal protein accumulation has also been described in the alveolar epithelium of patients with various other fibrotic lung diseases, suggesting that loss of proteostasis might be a unifying factor contributing to the formation of many fibrotic lung conditions [9]. Although in some rare cases mutations causing the misfolding of surfactant proteins (e.g. surfactant C mutations) have been linked to protein misfolding in IPF, in most cases the cause of protein aggregation is entirely unknown [10, 11, 12].

The second cell type critical to the development of IPF is the immune cell, and in particular the alveolar macrophage [13]. While clinical trials suggest that globally suppressing the immune system is not an effective treatment for IPF, it is still firmly accepted by most experts in the field that inflammatory cells, in particular macrophages, contribute significantly to driving fibrotic responses through their secretion of numerous repair and remodeling proteins [14, 15, 16]. Lastly, the fibroblast also plays a central role in the pathobiology of IPF. These cells undergo a wide range of changes in the IPF lung, including adopting a hyperproliferative and anti-apoptotic phenotype, and produce most, in not all, of the extracellular matrix proteins that comprise pulmonary scar tissues [4, 17, 18]. Although only marginally effective, existing therapies for IPF aim to ameliorate disease by suppressing fibroblast activation and the production of extracellular matrix [19, 20, 21].

Although epithelial cells, immune cells and fibroblasts all play very different roles in the pathogenesis of IPF each population exhibits a remarkable change in their proteome at every stage of disease. In addition to lung epithelial cells accumulating misfolded proteins in IPF, these cells also display reduced levels of various key proteins involved in cell-cell junctional connection, antioxidant defenses and other homeostatic processes such as macroautophagy [22, 23]. Moreover, macrophages and fibroblasts in IPF also display major changes to their proteome. In these cells, proteins levels for numerous pro-inflammatory and pro-fibrotic factors are upregulated, presumably due to increased protein translation. However, regardless of the mechanism contributing to changes in protein levels in IPF, it seems reasonable to assume that targeting processes regulating proteostasis might be effective in the treatment of this disease [24, 25]. In this review, we discuss the major mechanisms by which proteins are targeted for destruction in eukaryotic cells, namely the ubiquitin proteasome system (UPS). We also discuss emerging evidence linking dysfunction of this system to IPF, and propose how targeting components of the UPS could, at some future point, be used as a treatment for this disease.

The importance of maintaining the proteome.

Proteins are highly versatile macromolecules that are involved in essentially every biological process in and outside of a cell. The lifespan of a protein begins with the translation of mRNA on ribosomes and ends with its destruction through one of several degradation mechanisms. Generally speaking, all proteins are synthesized and degraded on a continuous basis, although the rate of turnover of any individual protein can vary significantly from several hours to weeks, or even months to years [25]. While this continuous re-synthesis and degradation process may seem somewhat wasteful, it is absolutely essential to limit the accumulation of damaged or misfolded proteins, and to ensure that only newly synthesized and properly configured proteins are controlling essential functions in a cell [26]. While every cell contains several proteolytic systems to carry out the degradation process, the major mechanism by which intracellular proteins are degraded is through the UPS. Importantly, the UPS system is highly regulated, and contains multiple mechanisms to ensure specificity so that only proteins meant to be degraded are ultimately targeted for destruction [27, 28]. However, this system is not infallible and even small increases or decreases in protein degradation can have important consequence that deleteriously affect the functioning of a cell, and ultimately contribute to the development of disease. Indeed, abnormalities in the UPS have already been linked to a wide range of disorders, including Parkinson's disease, Alzheimer's disease, cancer, heart failure and, relevant to this review, various forms of pulmonary fibrosis [29, 30, 31, 32, 33].

The Ubiquitin-Proteasome System

In most mammalian cells, the primary mechanism by which proteins are degraded is through the UPS; this system is estimated to degrade 80–90% of all proteins in eukaryotic cells [28, 34]. In addition to this system, proteins can also be degraded by macroautophagy, which is a multistep process that clears proteins (and other cellular components) by enveloping cellular materials in double membrane vesicles that are then sent to lysosomes for ultimate destruction. Although both processes are important to cells, it is now apparent that no part of

the cell is out of reach of the UPS, including the nucleus, mitochondrial and endoplasmic reticulum. Moreover, it is also appreciated that most misfolded, oxidized and damaged proteins are cleared through the UPS rather than through macroautophagy, making the UPS absolutely essential for the health and proper functioning of cells [35, 36].

Protein degradation by the UPS begins with the covalent linkage of ubiquitin, a highly conserved 76 amino acid protein, to a lysine 48 (K48) residue on the substrate protein (Figure 1). Ubiquitination is an ATP-dependent process carried out by three classes of enzymes. In first step, an ubiquitin activating enzyme, also called an E1 enzyme, forms a thio-ester bond with the ubiquitin protein, thereby permitting the binding of ubiquitin to the E2 ubiquitin conjugating enzyme. This second step is followed by the formation of an isopeptide bond between the carboxy-terminus of ubiquitin and a lysine residue on the target protein. Notably, this last step is carried out by the E3 ubiquitin ligase, which has very high specificity for individual substrates. Importantly, for some E3 ligases, the ubiquitin-binding and target protein binding domains exist on different polypeptides, requiring specialized adaptor proteins or cullins to bring these binding sites together [37, 38].

As mentioned, E3 ubiquitin ligases have enormous specificity, thereby limiting any indiscriminate targeting of proteins for destruction. To date, many E3 ligases have been identified and they have much more specificity when compared to either E1 or E2 ligases, making them ideal candidates as therapeutic targets. Importantly, E3 ubiquitin ligases can be a single peptide, such Mouse double minute 2 homolog (MDM2), or can be comprised of multiple components as is the case with the SKP1-Cullin 1-F-box (SCF) proteins. Of note, most E3 ligases contain additional linker proteins and collectively fall into one of three main groups: 1) Homologous to the E6-AP C-terminus (HECTs). 2) Really interesting new genes (RINGs) and 3) RING between RING (RBRs) [39, 40, 41, 42].

Once proteins are tagged (ubiquinated) for destruction, they are then delivered to the 26S proteasome, a large, 2.5-MDa molecular protein killing machine, which is extremely well-designed for carrying out proteolysis. Located in the cytoplasm, the 26S proteasome is comprised of a barrel proteolytic core called the 20S particle (core particle, CP), which has ATP-dependent activity, and a regulatory 19S complex (regulatory particle, RP), which recognizes, binds and unwinds substrate proteins before degradation. The 19S RP is divided into two subcomplexes; the lid and the base. The lid consists of nine Rpn subunits and functions to remove ubiquitin molecules, while the base has six regulatory AAA ATPase subunits (Rpt1-Rpt6) and four non-ATP subunits. The AAA family (ATPases associated with various cellular activities) is a large group of ATPases found in all biological kingdoms and is characterized by the presence of one or two conserved ATP-binding domains of a type called the AAA motif. In contrast to the 19S, the 20S CP is made up of duplicating seven α subunits and seven β subunits that form four axially stacked heteroheptameric rings [34]. The outer-alpha rings contain seven subunits (alpha1- alpha7) forming a pore that regulates the entrance and removal of degraded proteins from the proteasome complex. Similarly, the inner-beta rings contain seven subunits (beta1- beta7), and within this site is where protein breakdown occurs, releasing amino acids and small peptide fragments into the cytoplasm. To date, the proteasome has been shown to exhibit three types of protein lysis activities: 1) chymotrypsin-like (CT-L); 2) trypsin-like (T-L) and 3) caspase-like (C-L) [43].

When put together, the proteasome complex is comprised of 33 individual proteins all encoded by different genes [44]. Currently, it remains unclear whether these genes can all be regulated by one master transcription factor or require many distinct regulatory proteins to control gene expression. So far, studies have identified Rpn4 as an important transcription factor of many proteasome genes in various cell systems, and other transcription factors, such as nuclear factor erythroid 2-related factor 1 (Nrf1) appear to also drive gene expression in response to proteasome inhibition [45, 46, 47] (Figure 2). Furthermore, one recent study showed that proteasome biogenesis can be triggered by activating the mechanistic target of rapamycin (mTORC1), a major anabolic factor in mammalian cells. These latter findings suggest that increased protein destruction by the UPS may be important for supplying free amino acids in response to the activation of mTOR-mediated pro-growth pathways. Consistent with this notion, mTORC1 activation is known to be a potent inhibitor of macroautophagy, highlighting the fact that growing cells may be more dependent on the UPS to acquire the amino acids needed for growth and proliferation [48].

The importance of the UPS in mitochondrial homeostasis.

In addition to controlling cytoplasmic protein quality control, the UPS plays a critical role in regulating the proteome of individual organelles. Perhaps no better example of this is in mitochondria, which depend on a constant influx of cytoplasmic proteins [49, 50]. Mitochondria are double-membrane organelles that serve many essential functions, including the generation of ATP and biosynthesis of amino acids, nucleotides and NAD. In addition, mitochondria control cell fate through their release of ROS and other pro- and anti-apoptotic factors, making mitochondrial homeostasis a key determinant of life and death in cells [51].

Mitochondrial dysfunction is now emerging as an important pathogenic player in a wide range of age-related diseases, including IPF. Recently, Bueno et al. provided the first important piece of evidence linking mitochondrial dysfunction to IPF [52]. These investigators showed that large, dysmorphic mitochondria accumulate in AE2 cells in the IPF lung. They also went on to demonstrate that these findings associated with a marked reduction in levels of PTEN-induced putative kinase 1 (PINK1), a protein important for the clearance of dysfunctional mitochondria from the cytoplasm. Furthermore, targeted deletion of the PINK1 gene in mice was found not only to lead to mitochondrial dysfunction but also to cause protein aggregation in the endoplasmic reticulum (ER) (also known as ER stress) and enhance susceptibility to developing bleomycin-induced pulmonary fibrosis [52]. Together, these findings indicate a mechanistic link among mitochondrial dysfunction, protein aggregation and susceptibility to pulmonary fibrosis in the mouse lung. Since these observations, several groups have shown that various other proteins important for proper functioning of mitochondria are downregulated in pulmonary fibrosis, including those involved in oxidative phosphorylation (electron transport chain proteins), mitochondrial DNA repair (8-oxoguanine) and anti-oxidant defense (sirtuins).

Although the mechanisms by which mitochondrial dysfunction causes protein aggregation in IPF are not understood, a mechanistic link between these pathological processes can easily be appreciated if one understands how the mitochondrial proteome is actually formed.

Despite containing their own genome, mitochondrial DNA encodes for only a handful of genes (37 to be exact) mostly related to oxidative phosphorylation and the synthesis of tRNAs. This means that the rest of the ~1,200 proteins needed for proper functioning of mitochondria are derived from nuclear DNA, requiring synthesis outside of the organelle and import into the organelle for ultimate usage [53]. As a result, the mitochondria proteome is dependent on the UPS for not only limiting the entry of misfolded proteins but also for degrading misfolded proteins that accumulate after entry into the organelle [22].

The best evidence implicating the UPS in mitochondrial protein degradation comes from proteomic analysis of isolated mitochondria showing that over 100 mitochondrial proteins contain ubiquitin binding sites, including many involved in key mitochondrial functions such as ATP production and fatty acids synthesis. Different mechanisms have been described for how the UPS mediates mitochondrial protein degradation. By far, the easiest to understand is how the UPS removes damaged proteins before entry into the organelle. In these scenarios, targeted destruction likely occurs through mechanisms similar to other cytoplasmic proteins; first, they undergo ubiquitination by specific ligases and then they are sent to the 26S proteasome for degradation. However, the mechanisms by which proteins are degraded after already arriving in mitochondria is less well understood. Recently, several groups have shown that defective proteins on the outer membrane of mitochondria (OMM) can be polyubiquitinated by E3 ligases and then extracted from the membrane by an AAA-ATPase p97 to be delivered to the proteasome for degradation. One example of this is the OMM protein called Mc11, a pro-survival protein of the Bcl-2 family. In addition to degrading OMM proteins, several lines of evidence suggest that the UPS can also facilitate destruction of other, more internally located, mitochondrial proteins. Examples of this include endonuclease G, a protein found in the inner mitochondrial space, and two inner mitochondrial proteins called uncoupling proteins 2 and 3. However, the mechanistic details of how proteins within inner mitochondrial compartments are ubiquitinated and retro-translocated out of the organelle have not yet been established. That said, recent work indicates that at least 9 of the known 54 human RING-containing E3 ligases are present in mitochondria, indicating that targeting proteins for destruction likely begins within the organelle [54].

As already mentioned, large dysmorphic mitochondria accumulate in the epithelium of the IPF lung. Mitochondrion are unique organelles in that they are constantly undergoing structural changes, including changes in size, shape and position within the cell. Importantly, many of these changes are influenced by the ability of mitochondria to undergo fission (division of a single organelle into two or more independent structures) and fusion (the opposing reaction) events. Notably, myofibroblasts in pulmonary fibrosis have been shown to exhibit an increase in mitochondrial fission events and pharmacologically inhibiting this activity with Astaxanthin has been shown to ameliorate lung parenchymal distortion and collagen deposition by inducing apoptosis [55]. During the fission process, various proteins accumulate on the OMM, such as Drp1 and Fis1, to help mediate the process. Meanwhile, mitochondrial fusion proteins (Mfn1 and Mfn2) are targeted for degradation by the UPS. Conversely, when mitochondrial fusion occurs there is an increase in Mfn1 and Mfn2, and fission proteins are ubiquitinated and targeted for destruction. Interestingly, Radke et al. reported that pharmacological inhibition of the 26S proteasome alone was capable of

dramatically increasing mitochondrial fission in cells [56]. Moreover, Tar et al. demonstrated that high levels of Blm10/PA200, a proteasome activator, increased the degradation of mitochondrial fission proteins and augmented mitochondrial fusion events [57, 58]. Together, these observations highlight the critical role of the UPS in regulating mitochondrial dynamics and the potential for targeting this system to ameliorate aggressive fibroblast behaviors.

Proteasome dysfunction in IPF.

The proteome is severely dysregulated in the epithelium of IPF patients. In addition to the accumulation of misfolded and aggregated proteins in the endoplasmic reticulum, it has also been shown that levels of many other important proteins are downregulated in the IPF lung epithelium. This includes cell-cell junctional proteins, various kinases (PTEN) and many other antioxidant and DNA repair proteins, suggesting that restoring the proteome in the IPF lung epithelium will require more than just clearing misfolded proteins [59, 60, 61]. These findings also suggest that functional decline in the lung epithelial in IPF is not just about protein accumulation but also about a global dysregulation of proteostasis. Currently, it is unknown whether activity of the UPS is actually upregulated or downregulated in the epithelium of IPF patients.

Like the epithelium, the proteome of fibroblasts is severely altered in IPF as manifested by both increases and decreases in the expression of many different proteins. Since TGF β 1 secretion represents a crucial element in the pathophysiology of IPF it has been suggested as a possible therapeutic target for the disease [62, 63]. Importantly, levels of many downstream mediators of TGF β 1 are controlled through the UPS [64, 65, 66]. That said, Semren et al. reported that levels of the 19S regulatory Rpn6 subunit were markedly increased in fibroblasts from IPF patients, and that levels of K48 ubiquitination were also increased in human lung fibroblasts in response to TGF β exposure. Consistent with these findings, Baker et al. demonstrated that levels of several proteasome proteins were increased in the lungs of patients with IPF when compared to normal donors and COPD patients [67], although these studies were not performed in isolated fibroblasts. Furthermore, Mutlu et al. reported that Bortezomib prevents bleomycin-induced lung fibrosis in mice. These investigators also showed that these findings were associated with an increase peroxisome proliferator-activated receptor gamma levels in fibroblasts, which is known to repress TGF β 1 production. Altogether, these findings suggest that inhibiting proteasome activity might be effective in the treatment of pulmonary fibrosis by suppressing TGF β 1 signaling and fibroblast activation [68].

Another protein whose levels changes significantly in pulmonary fibrosis is sirtuin 3 (SIRT3), which is a mitochondrial protein deacetylase that regulates antioxidant responses and mitochondrial homeostasis. For example, Sundaresan et al. reported that levels of SIRT3 were markedly decreased in several major organs developing age-related fibrosis and this associated with a marked downregulation in SIRT3 expression in tissue fibroblasts [69]. Consistent with this, Sosulski et al. showed that SIRT3 expression was reduced in the lungs of old versus young mice, and was also downregulated in two mouse models of pulmonary fibrosis [70]. Moreover, analysis of SIRT3 expression in the lungs of patients with

pulmonary fibrosis revealed low SIRT3 staining within the fibrotic regions and decreased SIRT3 expression in human lung fibroblasts was found to promote fibroblast to myofibroblast differentiation [71, 72]. Similarly, reduction in SIRT3 levels has been reported in AE2 cells from mouse and human fibrotic lungs and decreased expression was shown trigger these cells to undergo apoptosis in culture [70, 73]. Notably, in both fibroblasts and alveolar epithelial enforced expression of SIRT3 was found to be protective. Although sirtuins are regulated through diverse types of post-translational modifications, including phosphorylation, methylation and SUMOylation it is also appreciated that ubiquitination remains a vital mechanism for targeting these proteins for degradation. These findings suggest the possibility that increased UPS activity might contribute to reduced SIRT3 in pulmonary fibrosis and that targeting this mechanism could have beneficial effects on both fibroblasts and epithelial cells in IPF.

Although activity of the UPS seems to be increased in pulmonary fibrosis, at least in fibroblasts, the mechanisms contributing to its upregulation remain unknown. One theoretical mechanism could be through the upregulation of angiotensin II (AT-2) (Figure 2). AT-2 is a small peptide that is the main regulatory factor of the renin-angiotensin system. However, AT-2 has also been shown to play an important role in driving fibrotic responses in a wide range of tissues, including the lung [74]. Although AT-2 is believed to promote organ fibrosis through stimulating TGF β 1 signaling and extracellular matrix production, Sanders et al. demonstrated that AT-2 can also directly stimulate the proteasome system in a wide range of tissues [75]. Moreover, Uhal et al. recently showed that AT-2 levels were dramatically increased in the mouse lung in response to bleomycin and similar findings have been described in human with pulmonary fibrosis [74]. Together, these findings support the notion that UPS activation might be driven by AT-2 in IPF and that targeting this peptide could attenuate lung fibrosis through multiple avenues [74].

Type I collagen is the principle component of pulmonary scar tissues. Collagen proteins are known to be degraded by the UPS. In addition, extracellular collagen can be degraded by enzymes known as matrix metalloproteinases (MMPs) [76]. Specifically, it is known that MMP1 mediates the degradation of type I collagen in some model systems, functioning as a key element in degradation of pulmonary scars [77]. In this context, studies have reported that TGF β 1 leads this process by upregulating type I collagen, while downregulating MMP1 gene transcription [78]. Interestingly, Goffin et al. showed that inhibition of the UPS with bortezomib enhanced MMP1 production while also decreasing type I collagen synthesis in human lung fibroblast [79]. Similar results have also been described in cardiac fibroblasts using MG132 as the proteasome inhibitor, again suggesting that modifying the UPS could be effective for reducing tissue remodeling in pulmonary fibrosis [80].

Therapeutic targeting of UPS.

There is no doubt that the UPS plays a critical role in the functioning of cells and their adaptation to stress. Moreover, it seems reasonable to assume that fine-tuning this system, particularly in situations where there has been a functional decline in UPS due to genetic mutations, injury or advanced age, could improve cellular function and possibly ameliorate disease. The best example of targeting the UPS as a treatment for disease is in the cancer

field in which global inhibitors of the UPS have been shown to be cytotoxic to multiple myeloma cells [81, 82]. However, unlike cancer treatments, cytotoxic outcomes are not desired when treating most other conditions, particularly benign diseases such as pulmonary fibrosis. As such, this makes non-selective inhibitors of the UPS system likely a poor long-term treatment strategy. Thus, ongoing research will be needed to further delineate the proteins whose expression is altered in diseased IPF lung tissues and are also believed to significantly contribute to the pathobiology of disease. Armed with this information, it will be important to determine whether restoring expression of these proteins using more selective approaches can enhance critical cellular functions and undo or dismantle pro-fibrotic responses.

One obvious approach to selectively target the UPS would be to inhibit the activity of individual E3 ligases responsible for targeting specific proteins for destruction (see Table 1). Since E3 ligases responsible for targeting most proteins have not been identified the first step will be to catalog the E3 ligases responsible for controlling protein expression in relevant cell populations in the lung. Once achieved, the next step will be to develop small molecules capable of augmenting or inhibiting the activity of specific E3 ligases, like has recently been done for specific E3 ligases in the cancer field [83, 84, 85]. Alternatively, one could also develop small molecules that bind preferentially to specific substrates or to their ancillary proteins rather than to an E3 ligase. However, this theoretical approach is also limited at this time due to the absence of critical information related to the key structural interactions between E3 ligases and their substrate proteins. Finally, if these approaches are employed we will need to pay careful attention to the effects on other tissues to avoid unwanted extra-pulmonary side effects. That said, the lung is unique in that drugs could theoretically be administered through inhalation in order to minimize unwanted systemic side effects.

Conclusion.

Proteins are highly versatile macromolecules that are involved in essentially every biological process in and outside cells. However, organ homeostasis is not just dependent on the production of proteins but also on precise control of their degradation. It is now appreciated that the UPS is the major degradation system to minimize the toxic effects of damaged or misfolded proteins in cells. Although just a theoretical possibility in the lung, targeting the UPS is emerging as a valid approach for restoring health to cells and ameliorating the severity of disease. Because IPF is a disease characterized by loss of proteostasis, it seems reasonable that UPS targeted approaches may one day be effective in treating this disease. Until then, future studies are clearly needed to better define and establish the mechanisms by which protein dysregulation contributes to the onset and progression of this deadly disease.

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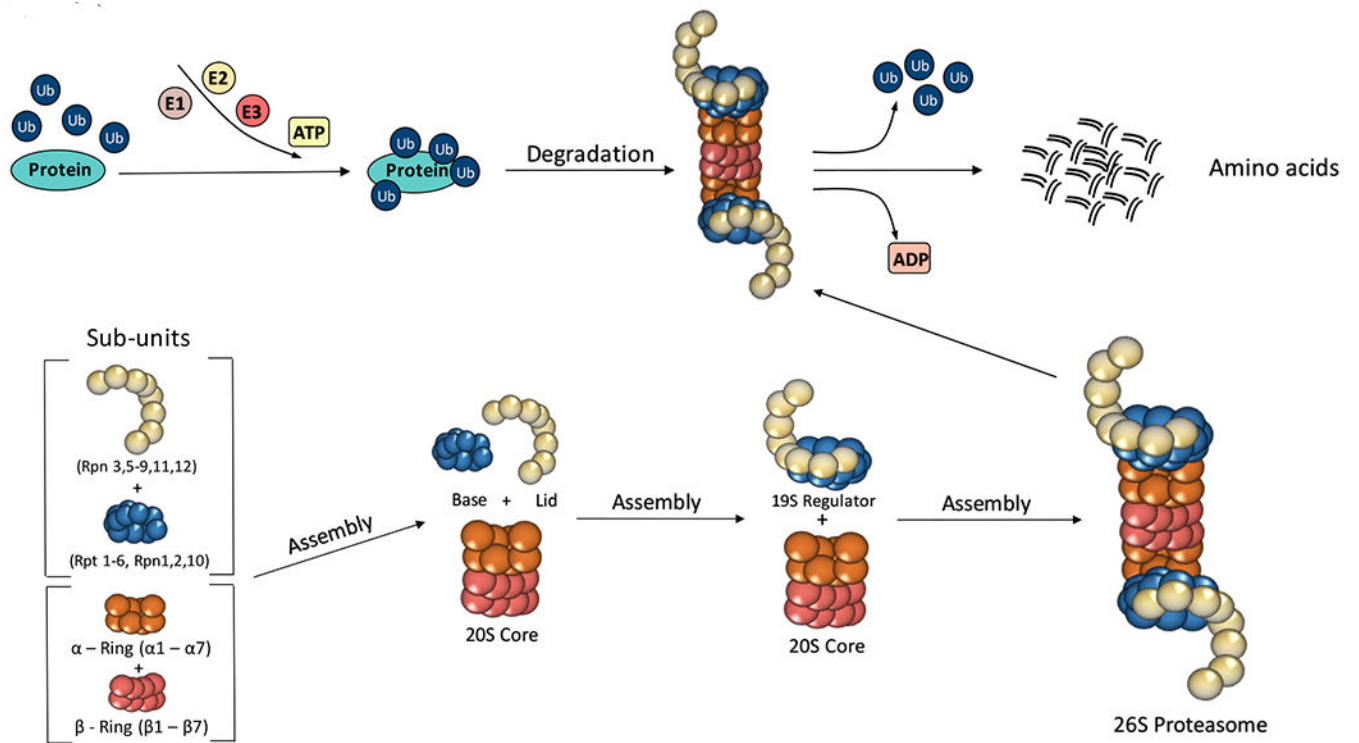


Figure 1: Ubiquitin-Proteasome system. Sub-units of the 26S proteasome are assembled into 19S regulatory and 20S core complex to become the barrel-shaped proteolytic machine. The 26S recognizes ubiquitin-conjugated proteins, a process catalyzed by three ligases known as E1, E2 and E3. Once breakdown ensues, amino acids are released into the cytoplasm and recycled.

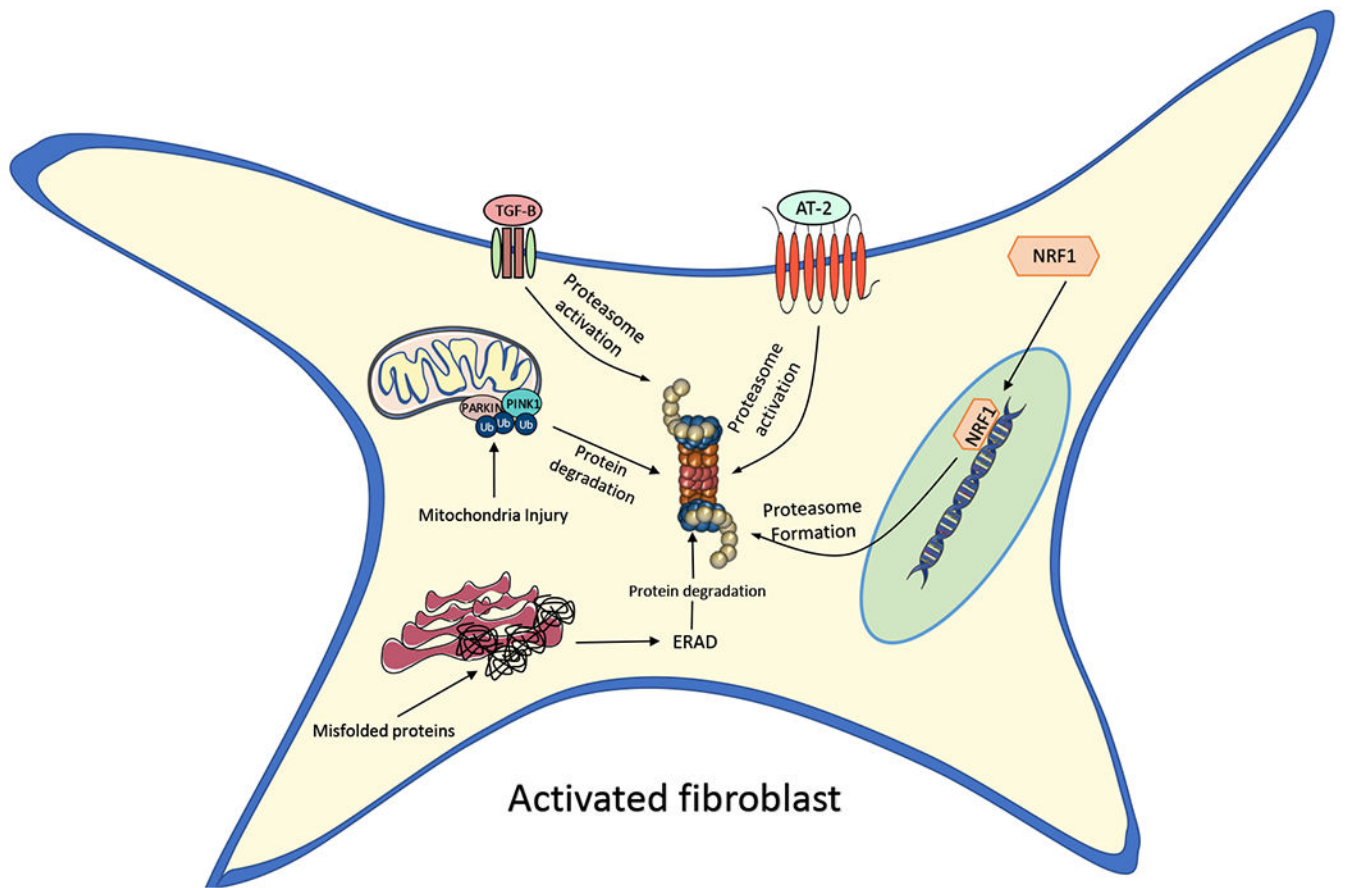


Figure 2: Proposed mechanisms by which the UPS activates in lung fibroblasts during pulmonary fibrosis. Angiotensin 2 (AT-2) and transforming-growth factor beta 1 (TGFβ1) could directly stimulate an increase in proteasome activity. Nuclear receptor factor 1 (NRF1) and other transcriptions can drive the production of proteasomal protein subunits.

Table 1.

Fine-tuning the ubiquitin-proteasome system to treat pulmonary fibrosis

Cell type	Target protein for UPS degradation	E3 ubiquitin-ligase	Drug
AEC	↓PINK1 ↓SIRT3 ↓PGC1α ↑MMP7 / MMP1 ↑CTGF ↑PDGF ↑CXCL12	Parkin ¹ SKP2 ² RNF34 ³ / PARIS ⁴ / Fbxw7 ⁵ Unidentified Smurf2 ⁶ c-Cbl / Cbl-b ⁷ WWP1 / ITCH (AIP4) ⁸	LRPPRC CG-12 / SKP2-C25 ----- Heclin ----- 1,4-Naphthoquinones
Macrophage	↑TGF-β ↑Galectin-3	Smad ⁹ TRIMs ¹⁰	SIS3 HCl
Fibroblast	↑UCP2 ↑NOX4 ↑TGF-β	Unidentified STUB1 (CHIP) ¹¹ Smad ⁹	----- ----- SIS3 HCl

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