The Emerging Role of the Ubiquitin Proteasome in Pulmonary Biology and Disease

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Derangements in normal cellular homeostasis at the protein level can cause or be the consequence of initiation and progression of pulmonary diseases related to genotype, infection, injury, smoking, toxin exposure, or neoplasm. We discuss one of the fundamental mechanisms of protein homeostasis, the ubiquitin proteasome system (UPS), as it relates to lung disease. The UPS effects selective degradation of ubiquitinated target proteins via ubiquitin ligase activity. Important pathobiological mechanisms relating to the UPS and lung disease have been the focus of research, with inappropriate cellular proteolysis now a validated therapeutic target. We review the contributions of this system in various lung diseases, and discuss the exciting area of UPS-targeting drug development for pulmonary disease.

Keywords: ubiquitin; proteasome; pulmonary disease; drug development; E3 ligase

INTRODUCTION

Normal pulmonary physiology can be disrupted by direct contact with the environment, exposure to potentially noxious inhalants, and infection with ensuing inflammatory cell activation. This delicate balance from normal homeostasis is tipped toward inflammatory injury in patients with chronic obstructive pulmonary disease (COPD), cystic fibrosis, acute respiratory distress syndrome (ARDS), acute lung injury (ALI), and pneumonia. Insights into the molecular pathophysiology of these diseases have greatly increased. In this review, we highlight emerging discoveries regarding selective regulation of protein degradation in the lung by the ubiquitin (Ub)–proteasome system (UPS), and how this regulation at the protein level affects critical functions of lung cells with ramifications that can maintain or threaten the vitality of the organism.

Selective Protein Degradation and Cellular Function: E3 Ubiquitin Ligase as a Physiological Switch

Maintenance of any healthy tissue requires stringent quality control at the protein level. All cellular proteins undergo tightly regulated turnover in the cell to prevent improper activity or unnecessary accumulation of dysfunctional proteins. Protein degradation also changes critical cellular protein concentrations in response to chemical signals or for important cellular events such as mitosis. Major protein regulatory systems include the autophagic/lysosomal pathways and the more prevalent UPS (1). Ubiquitin covalently interacts with other proteins whereby a single ubiquitin attaches to one lysine (monoubiquitination) or to multiple lysine residues on the target (multimonoubiquitination), or a ubiquitin chain can be produced at a single lysine residue (polyubiquitination). Ubiquitin contains seven lysine residues, which results in eight different interubiquitin linkage types, in turn leading to binding sites for other ubiquitin molecules and distinct branched ubiquitin chains that determine function. Substrate ubiquitination occurs in an ATP-dependent fashion, through an elaborate enzymatic cascade that adds the ubiquitin protein to, first, a ubiquitin-activating enzyme (E1), and then to a ubiquitin-conjugating enzyme (E2), and last to a specific target protein, a critical event that is catalyzed by a ubiquitin E3 ligase. One or more Ub molecules are thus added to the substrate, with monoubiquitination usually tagging the substrate for endocytic sorting, whereas polyubiquitination tags the substrate for recognition by the proteasome, often resulting in target protein degradation by the 26S proteasome protein complex, which is composed of one 20S and two 19S proteolytic subunits (2) (Figure 1). Overall, this process consumes large amounts of cellular energy, is represented by the largest family of enzymes present in eukaryotes, and accounts for about 5% of the genome. Given the physiological importance of the UPS system to cellular and tissue physiology, failure of the UPS is only rarely seen, but some mutations in proteins identified as UPS E3 ligases result in human familial diseases including Angelman syndrome, Parkinson's disease, and von Hippel–Lindau (vHL) syndrome (3), and changes in UPS function have been implicated in disorders of the cardiovascular (4), neurological (5), and pulmonary (6) systems.

The system is hierarchical, with one or two E1 enzymes described in mammalian cells, about 40 E2 enzymes, and more than 1,000 E3 ligases described. The targeting, ubiquitination, and degradation of proteins occur in a highly regulated and specific manner, with the E3 ligase usually binding target proteins with some post-translational modification that harbors a specific structural motif, termed a "degron" (7). Two major families of E3 ligases orchestrate ubiquitin addition to target proteins and ultimately determine substantial shifts in cellular behavior. The RING (really interesting new gene) finger and RING-related proteins comprise the largest E3 family, whose members function either independently as monomers or dimers, or in a multisubunit complex to ubiquitinate a broad range of substrates. The HECT (homologous to the E6-AP carboxy terminus) domain–containing proteins are a smaller E3 ligase family whose members are important for regulating many proteins, some of which include the transmembrane surface proteins. There is also a family of "U-box" E3 ligases, which do not have enzymatic activity and mostly

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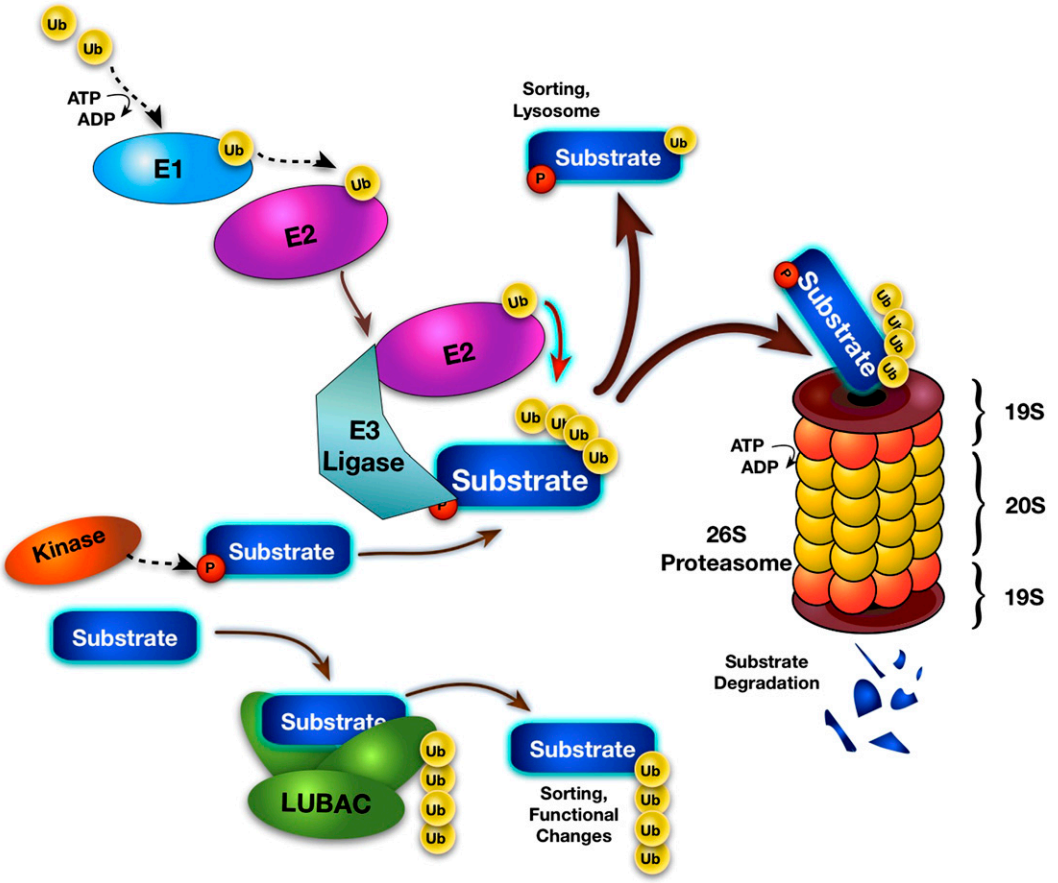

Figure 1. Schematic of the ubiquitin (Ub)–proteasome system. Protein degradation is a regulated, multistep process. Ubiquitin is loaded onto an E1 activating enzyme in an ATP-dependent fashion, and then transferred to an E2 conjugating enzyme. The same E2 can bind many E3 ligases, which in turn can ubiquitinate several target substrate proteins. E3 ligases bind specific substrate proteins based on substrate degron motifs usually consisting of a post-translational modification, such as phosphorylation. Adduction via the K48 residue on Ub tags the substrate for sorting, lysosomal destruction, or proteolytic cleavage and degradation by the 26S ubiquitin proteasome. Linear ubiquitination via the M1 residue by the linear ubiquitination complex (LUBAC) (HOIL-1L, HOIP, and SHARPIN) changes the cellular localization and activity of substrates, including RIP and NEMO for NF-κB signaling. HOIL-1L = heme-oxidized IRP2 (iron-responsive element–binding protein-2) ubiquitin ligase-1; HOIP = HOIL-1L–interacting protein; NEMO = nuclear factor-κB essential modulator; NF-κB = nuclear factor-κB; P = phosphate; RIP = receptor-interacting protein; SHARPIN = SHANK (SH3 and multiple ankyrin repeat domains-2)–associated RH [RBCK1 homology] domain–interacting protein; Ub = ubiquitin.

bridge catalytically active E2 enzymes, facilitating the transfer of ubiquitin to the target protein. The linear ubiquitination complex (LUBAC) is another multisubunit E3 ligase that polyubiquitinates substrates for sorting and/or degradation by adduction of chains of Ub monomers joined end-to-end (8). LUBAC contains three important components: HOIL-1L (heme-oxidized IRP2 [iron-responsive element–binding protein-2] ubiquitin ligase-1), HOIP (HOIL-1–interacting protein), and SHARPIN (SHANK [SH3 and multiple ankyrin repeat domains-2]–associated RH [RBCK1 homology] domain-3–interacting protein). The E3 ligases are diverse, represented by hundreds of genes in humans, and are highly represented in all eukaryotes (9).

SCF Ligase and the Role of F-Box Proteins

Among RING E3 multisubunit ligases, the Cullin–RING family and anaphase-promoting complex/cyclosome (APC/C) are the best characterized. The largest family of Cullin–RING E3 ligases is the Skp1–Cullin–F-box protein (SCF) family, a canonical multimodule E3 ubiquitination complex often causing target proteasomal degradation. The F-box proteins (FBPs) are interchangeable and confer substrate specificity through recognition of post-translationally modified degron motifs within substrates via target-binding domains, and tether substrate proteins to the Cullin protein of the SCF complex via F-box domains (10). Each FBP causes degradation of a specific set of target proteins by unique degron recognition. Sixty-eight FBPs have been identified in humans, designated FBXL (containing a leucine-rich domain), FBXW (containing a WD-40 domain), or FBXO (containing neither leucine-rich nor WD-40 domains) (11). Overall, only a small fraction of FBP biology is known, and multiple laboratories are describing the diverse and critical activities of FBPs in physiology and disease.

IMPORTANCE OF THE PROTEASOME AND UBIQUITIN E3 LIGASES IN LUNG HOMEOSTASIS AND DISEASE

Oxygen Sensing and Pulmonary Vascular Disease

One example of E3 ligase molecular regulation related to respiration is the oxygen-sensing role of hypoxia-inducible factor (HIF)-1α, a stress response transcriptional activator of chemokines, growth factors, and proteases. In normoxia, HIF-1α is hydroxylated through the action of prolyl hydroxylase domain protein-2 (PHD2), using oxygen as substrate, and the hydroxylated HIF-1α is bound by the vHL protein, which recruits a ubiquitin ligase for HIF-1α polyubiquitination and proteasomal degradation (12). During hypoxia, however, PHD2 activity (and therefore HIF-1α hydroxylation) is decreased, and vHL no longer prevents HIF-1α activation of transcription. Such molecular oxygen sensing is important for normal embryonic development and growth but is a major pathway "hijacked" by cancer cells with limited local oxygen supply to stabilize HIF-1α and augment local tumor growth and metastatic potential (13). In translational biomedical research, the vHL protein and HIF-1α signaling axis was described initially in vHL disease, caused by ineffective vHL; affected patients have polycythemia, pulmonary arterial hypertension, and respiratory insufficiency, attributed to increased HIF-1α signaling.

TABLE 1. E3 LIGASES AND THEIR TARGETS RELEVANT TO LUNG DISEASE

Disease/Condition	E3 Ligase/Subunit	Target	Biological Effect
Pulmonary hypertension	vHL	HIF-1α	Prevents HIF-1α transcription of proliferative and invasive/angiogenic genes
Legionella pneumonia	Lpp2082*	ParvB	Prevents ParvB degradation and establishes permissive cellular environment for bacteria
Adenoviral respiratory infection	E4orf6 and E1B55K†	p53	Disrupts p53 quality control mechanisms to enhance viral replication
Cystic fibrosis	CHIP and RMA1	CFTR ΔF508	Premature UPS degradation of CFTR, preventing surface expression
	C-CBL	CFTR WT	Endosomal internalization and UPS destruction of CFTR
Pulmonary edema	NEDD4-2	ENaC	Normal ENaC degradation; NEDD4-2$^{-/-}$ mice develop CF phenotype; overexpression impairs fluid clearance
	vHL	Na-K-ATPase	Decreased Na-K-ATPase activity; impaired sodium and fluid clearance from epithelia and interstitium
	HOIL-1L	PKCζ	
Airway inflammation	β-Trcp (FBXW1)‡	IκB	De-repression of NF-κB and production of multiple proinflammatory cytokines
	FBXL2‡	TRAFs 1–6	Decreased inflammatory signal transduction and decreased NF-κB activity
	FBXO3‡	FBXL2	Increased TRAF activity and inflammatory signal transduction
	Itch	JunB, c-Jun, Notch, PKC, PLCγ, ErbB	Decreased Th2 cytokine production and immunological tolerance; Itch KO results in loss of tolerance; nonfunctional SNP causes lung and multiorgan inflammatory syndrome
	Mule	Miz1	Disinhibits TNF induced inflammatory signaling
Asthma	Cbl-b	Uncharacterized	Decreased Th1 cytokine production and immunological tolerance; Cbl-b KO results in loss of tolerance
	MID1	PP2A	Increased NF-κB activity after antigen exposure
COPD	RLIM§	HDAC2	Acetylated histones leave chromatin open for transcription of inflammatory genes
ALI	Cbl-b	Uncharacterized	Decreased ALI inflammatory response; Cbl-b KO results in increased inflammation and TLR expression
	FBXL19‡	ST2 (IL-33R)	Decreased IL-33 signaling and inflammation in ALI and pneumonia
Lung disease–associated myopathy	MuRF1	Myosin	Skeletal muscle wasting; MuRF1 knockdown in ALI prevents associated muscle wasting
Lung cancer	β-Trcp (FBXW1)‡	IκB	NF-κB derepression with increased cellular activation, proliferation, and invasion
		β-Catenin	Impaired cell differentiation through Wnt signaling
	SKP2 (FBXL1)‡	p27, Fox01, p21, and p57	Loss of tumor suppressor protein activity
	FBXW7‡	Cyclin E1, c-Myc, c-Jun, Notch	Tumor suppression via degradation of oncoproteins
	c-CBL	Receptor tyrosine kinases	Reduced proliferation; c-CBL overexpression decreased tumor burden
	FBXL2‡	Cyclin D2, cyclin D3, Aurora B	Reduced proliferation; FBXL2 overexpression decreased tumor burden
Surfactant homeostasis	NEDD4-2	SP-C	Normal protein sorting and processing; SP-C disease mutants are ubiquitinated but form aggregates in familial ILD
	FBXL2	CCTα	Reduced membrane/surfactant phospholipid synthesis
	β-Trcp (FBXW1)‡	LPCAT1	Impaired surfactant phospholipid remodeling

Definition of abbreviations: ALI = acute lung injury; C-CBL = C-casitas B-lineage lymphoma E3 ligase; CCT = CTP:phosphocholine cytidylyltransferase; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane regulator; CHIP = C terminus of Hsc70-interacting protein; COPD = chronic obstructive pulmonary disease; ENaC = epithelial sodium channel; FBXL, FBXW, FBXO = F-box protein containing a leucine-rich domain, a WD-40 domain, or neither a leucine-rich nor WD-40 domain, respectively; HDAC2 = histone deacetylase-2; HIF-1α = hypoxia-inducible factor-1α; HOIL = heme-oxidized IRP2 ubiquitin ligase-1; IκB = inhibitor of NF-κB; IL-33R = IL-33 receptor; ILD = interstitial lung disease; KO = knockout; LPCAT1 = lysophosphatidylcholine acyltransferase-1; Lpp2082 = *Legionella pneumophila* (strain Paris) F-box protein; MID1 = E3 ubiquitin ligase midline-1; MuRF1 = muscle RING finger-1; NEDD = neural precursor cell expressed developmentally down-regulated protein; NF-κB = nuclear factor-κB; ParvB = parvin B; PKC = protein kinase C; PLC = phospholipase C; RLIM = RING finger LIM domain–binding protein; RMA1 = RING finger protein with membrane anchor-1; SKP2 = S-phase kinase-associated protein-2; SNP = single-nucleotide polymorphism; SP-C = surfactant protein C; Th2 = helper T-cell type 2; TNF = tumor necrosis factor; TRAF = TNF receptor–associated factor; UPS = ubiquitin proteasome system; vHL = von Hippel–Lindau protein; WT = wild type.

* *Legionella* bacteria–derived F-box protein.

† Adenovirus-derived E3 ligase.

‡ Part of the SCF (Skp1–Cullin–F-box protein) multisubunit Cullin–RING E3 ligase.

§ Specific mechanism not fully characterized in lung disease.

Host Defense

Immunoproteasome and extracellular alveolar proteasomes. The proteasome becomes more specialized in the setting of infection or inflammation. For example, tumor necrosis factor (TNF) or IFN release from proinflammatory cells leads to the conversion of 19S elements in the proteasomal machinery to form an "immunoproteasome," which generates peptides that are trafficked preferentially through antigen-processing machinery and ultimately to the type I major histocompatibility complex to be presented to T lymphocytes that bolster immunity to pathogens (14).

Studies demonstrate the presence of proteasomes outside the cellular environment, such as lung alveolar fluid. Proteasomes in lung fluid are only shown to have the 20S subunit and cleave proteins without requirement for E3 ligases for protein-specific recognition or ubiquitination. It has been proposed that extracellular proteasomes are a consequence of cell lysis and spillage of cellular contents; alternatively, there may be packaging and exocytosis (i.e., active secretion) of intact 20S proteasomes. Regardless of the mechanisms of release, extracellular proteasomes are increased in the setting of acute infection and inflammation, and

may play a role in antigen presentation to activate immunity against extracellular microbes the host encounters (15).

Proteasome dysregulation as a microbial pathogenic mechanism. Pathogens can exploit host cell UPS machinery to their advantage. For example, *Legionella pneumophila* bacteria produce their own FBP, Lpp2082, which is required for infection. This FBP binds and competes the substrate ParvB away from degradation, apparently creating a permissive cellular environment (16). Human adenovirus creates two E3 ligase proteins that cause degradation of the p53 protein, allowing production of viral proteins and genetic material without p53-mediated host cell apoptosis (17). *Pseudomonas aeruginosa* secretes a toxin, Cif, in vesicles that increases ubiquitination and degradation of cystic fibrosis transmembrane regulator (CFTR) (18), thus making the airway secretions more tenacious. The coronavirus that causes severe acute respiratory syndrome possesses a Ub-like protein that increases pathogenicity; also, proteasome inhibitor pretreatment reduced viral replication and improved survival in mice (19), implicating some role for the UPS in severe acute respiratory syndrome.

Pulmonary Ion Transport and Fluid Balance

Cystic fibrosis. Cystic fibrosis is due to insufficient CFTR cell surface expression, causing impaired chloride secretion in the airway lumen, with reduced airway surface liquid, conglomeration of proteins, impaired ciliary clearance, and enhanced susceptibility to infection. Cystic fibrosis is most commonly due to CFTR mutation at the position 508 phenylalanine residue (ΔF508); this mutant protein is translated, but intercepted in the endoplasmic reticulum by E3 ligases CHIP and RMA1, ubiquitinated, and degraded by the proteasome before reaching the cell surface (20). C-terminal CFTR deletions are processed normally, but rapidly shuttled to the proteasome for degradation (21), while normal CFTR membrane expression is regulated by E3 ligase C-CBL, mediating ubiquitination and endosomal internalization (22).

Pulmonary edema. In pulmonary edema, epithelial sodium channel activity regulates apical Na^+ entry into the cell, from where it is actively transported out of the cell via the Na-K-ATPase as the critical mechanism for fluid balance in the lungs (23). In addition to its regulation of HIF-1α protein concentrations discussed previously, vHL protein also controls edema clearance during hypoxia, where it mediates degradation of Na-K-ATPase (24). Here, it appears that reactive oxygen species participate in the regulation of the Na-K-ATPase via PKCζ and a member of the LUBAC, HOIL-1L, which leads to impaired lung fluid clearance. Thus, the steady state of both the epithelial sodium channel and Na-K-ATPase are highly regulated by the UPS to critically maintain epithelial function to effect lung fluid balance and normal breathing.

Airway Inflammation

Perhaps the most prominently implicated signal in pulmonary inflammation is the activity of the nuclear factor of κ light polypeptide gene enhancer in B cells, NF-κB (25). When active, this transcription factor master regulator of inflammation leads to expression of cytokines, chemokines, adhesion molecules, matrix metalloproteases, and leukocyte growth factors, among others. The negative regulator of NF-κB is IκB, which usually binds and sequesters NF-κB in the cytosol (26). IκB is degraded by the ubiquitin proteasome via the FBP β-transducin repeat–containing protein (β-Trcp, now designated FBXW1). When IκB is phosphorylated, it is recognized by SCF^{FBXW1} for ubiquitination and degradation, leaving NF-κB unrestricted to initiate the inflammatory cascade. IκB phosphorylation is in turn regulated by kinases, which are each activated by ligation of receptors, or the activity of protein second messengers, such as the TNF receptor–associated factor (TRAF) proteins.

LUBAC has been described to have an important role in regulating inflammation (27). LUBAC is now known to be part of the TNF receptor signaling complex and participates in signaling processes by end-to-end polyubiquitination of TNF receptor signal modulators RIP1 and NEMO, apparently increasing signal transduction by this particular ubiquitination scheme (28). LUBAC also targets IL-1β, CD40 ligand, and several Toll-like receptors (TLRs). SHARPIN mutant mice develop a proliferative dermatitis, and patients with mutations of HOIL-1L and thus LUBAC deficiency have protracted inflammatory disorders and invasive bacterial infections (29).

Studies indicate that TRAF proteins are targets of the SCF^{FBXL2} E3 ligase (30). TRAF degradation after overexpression of FBXL2 globally suppresses inflammatory responses in response to endotoxin. Interestingly, another E3 ligase, SCF^{FBXO3}, targets FBXL2 for its degradation; FBXO3 depletion in cells increases FBXL2 and decreases TRAF protein levels, blunting inflammatory cytokine release *in vitro*. A human FBXO3 polymorphism with a relatively high (∼6%) frequency among individuals of European descent exists, and this mutant FBXO3 decreases FBXL2 ubiquitination; humans with this polymorphism hospitalized with sepsis have lower serum concentrations of inflammatory cytokines (30).

The E3 ligase Itch causes degradation of multiple inflammatory transcription factors and signaling molecules (31), and Itch mutations in mice cause multiorgan inflammatory disease with pulmonary interstitial inflammation (32). A 2010 study of an Amish family with a multiorgan inflammatory–autoimmune syndrome characterized by severe pneumonitis and premature death identified an autosomal recessive mutation in the Itch E3 ligase (33), almost completely recapitulating the disease phenotype initially observed in knockout mice. Miz1 appears to have checkpoint function in the regulation of LPS-induced inflammation where the cytoplasmic Miz1 suppresses LPS- or TNF-induced production of proinflammatory cytokines through inhibition of JNK (c-Jun N-terminal kinase) activation (34). It has been reported that the HECT domain–containing E3 ligase Mule catalyzes TNF-α–induced Miz1 K48-ubiquitination degradation, which is of importance in TNF-α–induced JNK activation and cell death (35). Interestingly, the interaction between Mule and Miz1 occurred TNF-α independently of the pox virus and zinc finger domain of Miz1, which is of potential relevance in the inflammatory pathways during pulmonary infections.

Asthma. In allergic asthma, an antigen-specific hyperactive helper T-cell type 2 (Th2) immune response causes airway obstruction and hyperresponsiveness. Bronchodilators or immunomodulators including steroids represent the initial therapy, with allergen-specific immunotherapy used to promote immunological tolerance and durable symptom relief. Immune tolerance is mediated, in part, by the Ub E3 ligase Itch, which degrades the phosphorylated JunB, a Th2-specific transcription factor. Itch deficiency causes high JunB levels with increased production of the Th2 cytokines (36). In asthma animal models, Itch knockout mice fail to develop tolerance to ovalbumin antigen–specific immunomodulatory therapy. Likewise, in another ovalbumin-induced asthma study of mice lacking the E3 ligase Cbl-b, immune tolerance was disrupted, although with increased airway neutrophils and Th1 cytokines IL-12 and IFN-γ (37). Itch and Cbl-b therefore each seem to play a role in maintaining immune tolerance in different effector arms of the T-cell system. Another E3 ligase, Midline 1 (MID1), is up-regulated after antigen stimulation with the common asthma allergen house dust mite (38). MID1 targets protein phosphatase-2A (PP2A), an endogenous

inhibitor of cytokine signaling that deactivates NF-κB; hence small inhibitory RNA MID1 knockdown suppressed allergic inflammation *in vivo* in mice and *in vitro* in human lung cells.

COPD. With tobacco exposure, some smokers have a sustained inflammatory phenotype, and many develop COPD. UPS activity is dysregulated in this setting, with increased UPS components (39) and impairment of proteasome activity after cigarette smoke administration (40, 41). The epigenetic regulator, histone deacetylase-2 (HDAC2), is degraded by the UPS after cigarette smoke exposure secondary to HDAC2 phosphorylation (42). Although the E3 ligase RING finger LIM domain–binding protein has been shown to target HDAC2 in other systems (43), its role in the lung has not been described; regardless, loss of HDAC2 causes aberrant inflammatory gene transcription and feed-forward inflammation in some smokers, with HDAC2 levels correlating inversely with COPD severity (44).

Acute Respiratory Distress Syndrome

In ALI, many physiological changes involve the UPS (45). TLRs sense pathogen-associated molecular patterns and initiate inflammatory responses. E3 ligase Cbl-b down-regulates TLR signaling, with Cbl-b deficiency potentiating the inflammatory response (46).

IL-33 is a strong inflammatory activator during asthma and ALI through the receptor ST2L. Phosphorylated ST2 is bound and ubiquitinated by SCF^{FBXL19}, causing UPS degradation. Ectopically expressed FBXL19 decreased ST2 and reduced inflammation while improving survival in animal models of ALI (47). In other work, depletion of the proinflammatory FBP FBXO3, which targets the TRAF inhibitor FBXL2 for its disposal, restores FBXL2 protein levels, improves survival, lowers cytokine release, and lessens inflammation histologically in mice in a *Pseudomonas* and LPS model of ARDS (30).

Lung Disease–associated Myopathy

In severe ARDS and COPD, diaphragmatic and peripheral muscle wasting are common (48). In a mouse ALI model displaying comorbid muscle wasting the E3 ligase muscle RING finger-1 (MuRF1) mediates muscle breakdown as MuRF1 knockdown prevented ALI-associated muscle wasting (49).

Lung Cancer

Many components within the UPS participate in neoplastic processes, including cancer-promoting FBPs β-Trcp (FBXW1) and SKP2 (S-phase kinase-associated protein-2; also known as FBXL1) (50). β-Trcp disinhibits inflammatory NF-κB activity, causing expression of cell-activating cytokines, growth factors, and proteases that augment tumor proliferation and invasion. β-Trcp also targets the β-catenin protein, which mediates cell differentiation through the Wnt signaling pathway. Loss of β-catenin could impair cell differentiation, typical of aggressive malignancies (51). FBXW7, however, is a p53-dependent tumor suppressor that facilitates degradation of oncoproteins (52). FBXL2 destabilizes proteins critical to cell cycle progression, thereby inhibiting lung tumor cell growth (53–55).

In lung cancer, SKP2 is considered a proneoplastic factor that degrades protective p27 and increases tissue invasiveness (56). Studies show that increased SKP2 protein levels in biopsy specimens are associated with increased metastasis. Moreover, decreased p27 in $SKP2^{hi}$ specimens is robustly correlated with shorter survival (57, 58).

Surfactant Metabolism

Pulmonary surfactant is composed of key surfactant-associated proteins and phospholipids, the components of which participate in the innate immune response and maintenance of alveolar stability by lowering surface tension. Mutations of the surfactant protein C (SP-C) gene have been associated with a familial form of usual interstitial pneumonia and pulmonary fibrosis (59, 60). SP-C processing and cell secretion require distinct steps, including ubiquitination by the E3 ligase NEDD4-2 (61, 62); many familial ILD-associated SP-C alleles involve the C terminus of the protein, with ubiquitinated and aggregated SP-C within perinuclear inclusions of one such mutation (63), demonstrating defective trafficking after E3 ligase association.

CTP:phosphocholine cytidylyltransferase (CCTα) is an essential lipogenic enzyme needed for surfactant phospholipid synthesis. CCTα ubiquitination is catalyzed by the SCF^{FBXL2} E3 ligase complex (64), and FBXL2 depletion stabilizes CCTα levels and stimulates surfactant biosynthesis. Another surfactant enzyme, lysophosphatidylcholine acyltransferase (LPCAT1), is targeted for ubiquitination and degradation by β-Trcp (65). Thus, it is likely that the Ub E3 ligases regulate surfactant components to modulate lung homeostasis.

PHARMACOLOGICAL TARGETING OF THE PROTEASOME

Bortezomib and Nonselective Proteasome Inhibitors

The first U.S. Food and Drug Administration (FDA)–approved drug that targets the proteasome is bortezomib (Velcade), a reversible 20S proteasome inhibitor. Bortezomib has emerged as an effective agent in the treatment of multiple myeloma, a malignancy previously linked with a dismal prognosis (66, 67). Only one other proteasome inhibitor has been approved by the FDA for use in humans, carfilzomib, as second-line therapy for multiple myeloma and for non-Hodgkin's lymphoma.

New Proteasome Inhibitors

The success of bortezomib has established the proteasome as a viable target in the current era of drug development, with reports on five "second-generation" proteasome inhibitors that target overlapping aspects of cell signaling *in vitro*. The preclinical and *in vivo* targets of these drugs include hematologic malignancies as well as the solid tumors (68). Four of these agents have entered into phase 1 and 2 clinical trials in subjects with solid tumors as well as hematologic malignancies. Neuropathy is a class-wide side effect of proteasomal inhibitors, likely from accumulation of ubiquitin-laden proteins in dorsal root ganglia of patients receiving therapy. This effect could be avoided by targeting factors upstream of the proteasome (Figure 2). The first such compound to be tested in humans is MLN4924, which targets the NEDD8 activating enzyme required for activation of the Cullin–RING proteins (69); this drug globally suppresses ubiquitination through Cullin–RING ligases, which includes all SCF E3 ligase family members and others. *In vitro* activity of this agent reduces tumor burden in multiple models via tumor cell apoptosis or autophagy. Four phase 1 clinical trials for MLN4924 safety testing are now underway.

Another newly characterized drug is CC0651, the first small-molecule E2 ligase inhibitor, with high potency and specificity for the E2 ligase Cdc34 (70). This drug suppresses ubiquitination through Cullin–RING E3 ligases that depend on Cdc34. CC0651 and MLN4924 would therefore theoretically have highly overlapping pharmacology, and may have synergistic effects.

The small molecule tosyl-L-arginine methyl ester (TAME) was described as an inhibitor of the APC E3 ligase required for dismantling the spindle assembly checkpoint and completion of mitotic division (71). TAME prevents depletion of cyclin B1, thereby leading to mitotic arrest in metaphase. The next

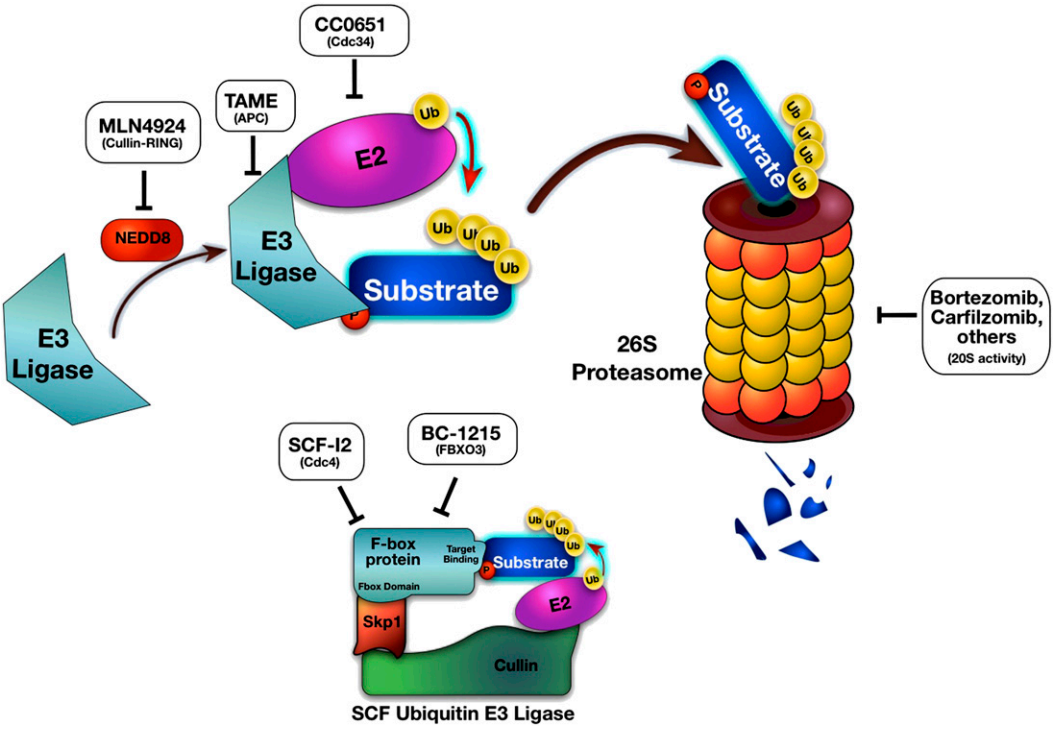

Figure 2. Drugs targeting the ubiquitin–proteasome system (UPS). Bortezomib and carfilzomib are U.S. Food and Drug Administration–approved proteasome inhibitors that block cleavage of substrate proteins through the 26S proteasome. Upstream inhibitors CC0651 and MLN4924 block ubiquitination by inhibition of the Cullin–RING E3 ligases by inhibition of the Cdc34 E2 enzyme and the NEDD8 activator of Cullin–RING E3 ligases, respectively, to temper cell proliferation. TAME blocks APC E3 ligase activity and causes mitotic arrest. The F-box specific inhibitor SCF-I2 blocks the yeast Cdc-4 F-box protein–dependent ubiquitination of substrate proteins (but is not active on human FBPs), and BC-1215 prevents SCF^{FBXO3}-mediated substrate ubiquitination and degradation of the FBXL2 protein, enhancing SCF^{FBXL2}-dependent TRAF degradation, decreasing inflammation. APC = anaphase-promoting complex; FBP = F-box protein; FBXL, FBXO = F-box protein containing a leucine-rich domain, or neither a leucine-rich nor WD-40 domain, respectively; NEDD = neural precursor cell expressed developmentally down-regulated protein; P = phosphate; RING = really interesting new gene; SCF = Skp1–Cullin–F-box protein; TAME = tosyl-L-arginine methyl ester; TRAF = tumor necrosis factor receptor–associated factor; Ub = ubiquitin.

putative selective target of the ubiquitination–proteasome pathways is to target individual subunits of the E3 ligases. Although no drugs with this activity have entered clinical trials, there is significant research in this area.

FBP-specific inhibitors. The first report of an E3 ligase–targeting drug identified SCF-I2, discovered by small-molecule interrogation of the SCF^{Cdc4} complex by its ability to displace the SCF from its phosphodegron in a yeast system. This molecule antagonized SCF^{Cdc4}, but not SCF complexes with related FBPs nor the human ortholog, FBXW7 (72).

Another small molecule inhibits SCF^{FBXO3} by targeting the FBXO3 C-terminal ApaG domain, a bacteria-like domain found in only two other genes in humans. This compound, BC1215, shows potent blockade of FBXO3 activity with a robust decrease in TRAFs and downstream inflammatory mediators released from endotoxin-stimulated human blood monocytes. BC-1215 effectively reduced inflammation and lung injury in preclinical models of sepsis and ALI (30). This demonstration of a targeted, and specific inhibitor to a single FBP requires further testing, but if successful, has far-reaching implications and may set the stage for a new genus of antiinflammatory drugs.

CONCLUSIONS

In summary, the UPS in lung biology is a fundamental area of research and discovery. The activities of many E3 ligases and FBPs remain unknown, and many more discoveries await us in the years to come. However, it is becoming clear that protein processing via the UPS plays a central role in most of the principal types of lung disease (Table 1). Along with these newly described mechanisms of pathobiology come significant advances in our strategies for intervention to expand our arsenal of potential therapies. Although treatment with UPS-targeting medications has thus far been limited to hematologic malignancy, antiinflammatory agents that act on some UPS component may become commonplace in the next decade, especially as more selective compounds with fewer side effects are identified. It is difficult to ascertain how these innovations will impact medicine in the evolution of UPS-targeting therapy; however, it seems that new generations of drugs acting on this important system will become the mainstay of therapy for some diseases.

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