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Ubiquitination and Proteolysis in Acute Lung Injury

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Ubiquitination is a posttranslational modification that regulates a variety of cellular functions depending on timing, subcellular localization, and type of tagging, as well as modulators of ubiquitin binding leading to proteasomal or lysosomal degradation or nonproteolytic modifications. Ubiquitination plays an important role in the pathogenesis of acute lung injury (ALI) and other lung diseases with pathologies secondary to inflammation, mechanical ventilation, and decreased physical mobility. Particularly, ubiquitination has been shown to affect alveolar epithelial barrier function and alveolar edema clearance by targeting the Na,K-ATPase and epithelial Na^+ channels upon lung injury. Notably, the proteasomal system also exhibits distinct functions in the extracellular space, which may contribute to the pathogenesis of ALI and other pulmonary diseases. Better understanding of these mechanisms may ultimately lead to novel therapeutic modalities by targeting elements of the ubiquitination pathway. ***CHEST 2012; 141(3):763–771***

Abbreviations: ALI = acute lung injury; ARDS = acute respiratory distress syndrome; ELF = epithelial lining fluid; ENaC = epithelial Na^+ channels; NEDD8 = neural precursor cell expressed, developmentally downregulated 8; pVHL = von Hippel Lindau protein; RING = really interesting new gene

The Ubiquitin Proteasome System

The Discovery of the Ubiquitin Proteasome System

Protein turnover plays an important role in a broad spectrum of cellular processes. Notably, approximately 3% to 5% of our cellular proteins are degraded and resynthesized daily.[1] Until the mid-1970s it was widely believed that all intracellular proteins were degraded at the same rate in lysosomes in a nonspecific manner, despite the fact that proteins have a wide range of half-lives.[1] The existence of a novel, highly regulated, and specific degradation pathway was first proposed by Ciechanover and colleagues[2] and Hershko and colleagues,[3] which was in agreement with the discovery of an ATP-dependent proteolytic system in reticulocytes that lack lysosomes.[4] As it was described 2 years later, this novel degradation pathway was mediated by a small protein of 76 amino acid residues, termed ATP-dependent proteolysis factor-1 or ubiquitin.[5,6]

The Ubiquitin Enzymatic Cascade

During ubiquitination, target proteins become "marked" as ubiquitin covalently conjugates via its C-terminal glycine to protein substrates at the ε-amino group of their lysine residues.[7] Conjugation of ubiquitin to a target protein occurs via an enzymatic cascade.[1,7] Ubiquitin is first activated by an ATP-dependent E1 ubiquitin-activating enzyme by the formation of a thiolester bond between the E1 enzyme and ubiquitin. Ubiquitin is then transferred via a transthiolation

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reaction to an E2 ubiquitin-conjugating enzyme, which in turn transfers ubiquitin onto the target protein. This conjugation step occurs in conjunction with E3 ubiquitin ligases, which provide the specificity of the cascade by recognizing the target proteins selected for ubiquitination. Three major E3 classes exist. The so-called zinc finger-containing really interesting new gene (RING) ligases facilitate transfer of ubiquitin from an E2 to substrates. In contrast, the homologous to E6-AP carboxyl terminus (HECT) ligases directly bind ubiquitin and transfer it onto the substrate.[8,9] Recently, a novel E3 ligase family, the U box ubiquitin ligases, has been identified.[10] A schematic representation of the ubiquitin enzymatic cascade is depicted in Figure 1.

The specificity and diversity of ubiquitination is a consequence of the hierarchical cascade of ubiquitin conjugation. Although in most eukaryotes there are only one or two E1s, the number of E2s is significantly larger (~50-100 in mammals); it is estimated that >1,000 different E3 ligases exist, and the system comprises ~5% of the genome.[11] The diversity of the effects of ubiquitination is further enhanced by the various ways ubiquitin can tag a protein.[12] In some cases, a single ubiquitin molecule attaches to one lysine (monoubiquitination) or to multiple lysine residues (multi-monoubiquitination) of the target protein; however, frequently polyubiquitin chains are generated that link several ubiquitin molecules via any of the seven lysine residues present in ubiquitin (K6, K11, K27, K29, K33, K48, and K63). Therefore, dependent on the internal linkages, the chains can be not only of varying lengths but also of varying types, further increasing the versatility of ubiquitination.[13,14] Finally, the activity of approximately 100 deubiquitinating enzymes is responsible for the reversible nature of ubiquitination.[15,16]

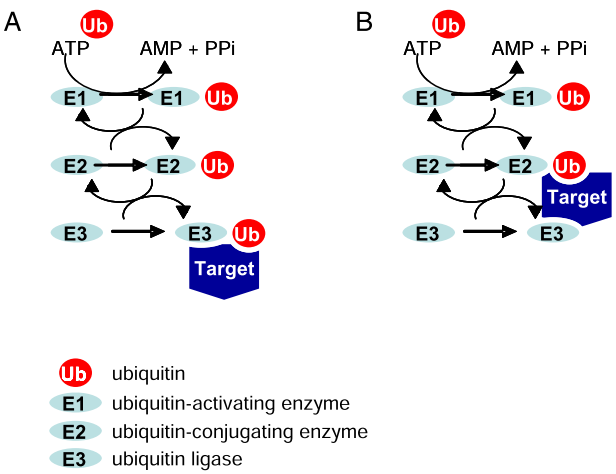

FIGURE 1. Schematic representation of ubiquitin enzymatic cascade. Ubiquitin is first activated by an ATP-dependent E1 ubiquitin-activating enzyme and then transferred to an E2 ubiquitin-conjugating enzyme, which in turn transfers ubiquitin onto the target protein. A, Homologous to E6-AP carboxyl terminus (HECT) HECT E3 ligases bind ubiquitin and subsequently transfer it onto the substrate. B, In contrast, really interesting new gene (RING) and U box ubiquitin ligases facilitate the transfer of ubiquitin from the E2 directly to the target. PPi = inorganic pyrophosphate.

Depending on subcellular localization and the number and topology of ubiquitin molecules conjugated to the target, the fate of the tagged molecule can be degradation or nonproteolytic modification.[17] Degradation of damaged or abnormal proteins, as well as most proteins with short half-lives that control, for example, cell cycle, transcription or DNA repair is mediated by the 26S proteasome,[18] a large 2.5 MDa protein complex consisting of two subcomplexes: the catalytic core 20S proteasome and the regulatory 19S proteasome.[19] The proteolytic activity of the 20S proteasome is constituted by chymotrypsin-, trypsin-, and caspase-like activities.[19] The proteasome cleaves tagged proteins into short oligopeptides while ubiquitin is detached and recycled.[18] The proteasome also plays an important role in generating the major histocompatibility complex class 1-presented peptides by degrading proteins of intracellular pathogens.[20]

Usually, a polyubiquitin chain consisting of four or more ubiquitins confers recognition by the 26S proteasome; however, not all ubiquitinated targets are degraded in the proteasome (Fig 2).[17] For example, a single ubiquitin tag often acts as an intracellular sorting signal affecting trafficking of the target molecule, such as internalization from the plasma membrane, sorting in endosomes, or Golgi-to-endosome trafficking.[21] Moreover, the fate of the target protein is also determined by the type of conjugations between the ubiquitin molecule and the target and between the ubiquitin molecules. As described previously, conjugations between ubiquitin molecules may occur through any of the seven lysine residues inducing various effects; however, K48-linked polyubiquitination usually targets the protein to proteasomal degradation, whereas lysosomal sorting and trafficking are mainly regulated by K63-linked monoubiquitin or multi-monoubiquitin tags.[12]

Role of Ubiquitination in Respiratory Diseases

Role of Ubiquitination in Chronic Inflammation, Fibrosis, and Muscle Dysfunction

Although ubiquitination has been demonstrated to play a role in a myriad of various cellular functions, its function in respiratory disease and particularly in acute lung injury (ALI) remains poorly understood. Only in the last few years have we started to gain insights into some of these mechanisms. We now know that regulation of inflammation by ubiquitination plays an important role in the pathogenesis of asthma

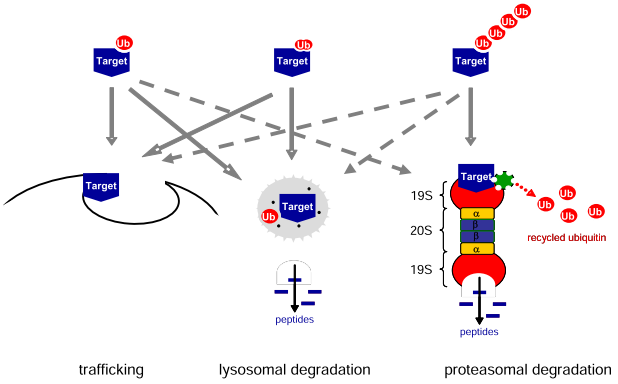

FIGURE 2. Types of ubiquitin tagging and fate of the target protein. A, A single ubiquitin molecule may attach to one lysine (monoubiquitination) of the target protein; or B, A single ubiquitin molecule may attach to multiple lysine residues (multi-monoubiquitination) of the target protein. C, Alternatively, polyubiquitin chains are generated that link several ubiquitin molecules via lysine residues present in ubiquitin. Usually, monoubiquitination and multi-monoubiquitination promote trafficking or lysosomal degradation of the target protein, whereas polyubiquitin chains consisting of four or more ubiquitins confer recognition by the 26S proteasome; however, trafficking or lysosomal degradation of proteins after polyubiquitination and proteasomal degradation after monoubiquitination have also been reported. See Figure 1 legend for expansion of abbreviation.

and COPD. For example, the nuclear factor κ-light-chain-enhancer of activated B cells pathway, which has been implicated with a key role in the pathogenesis of both asthma and COPD by playing a pivotal role in adaptation to noxious stimuli and inflammation, is critically regulated by ubiquitination.[22] Another mechanism that may contribute to chronic inflammation in these patient populations is the dysregulation of histone deacetylation. Histone deacetylation exerts antiinflammatory effects by repressing inflammatory genes. However, in patients with COPD and smoking patients with asthma, oxidative and nitrative stress results in the formation of peroxynitrite, which nitrates tyrosine residues on histone deacetylase-2, thereby resulting in its ubiquitination and degradation and, thus, chronic inflammation.[23] Moreover, regulation of the human platelet-activating factor receptor by ubiquitination has also been implied in the pathogenesis of asthma.[24] Furthermore, increasing evidence suggest that regulation of the transforming growth factor-β signaling cascade by ubiquitination plays an important role in the pathogenesis of lung fibrosis.[25]

Several studies focused on the role of ubiquitination in muscle dysfunction in respiratory diseases. Muscle wasting and impaired muscle function have been well documented in several chronic pulmonary diseases, such as COPD, interstitial lung disease, and cystic fibrosis, and also represent typical consequences of delayed recovery in critically ill patients.[26,27] It is now increasingly evident that muscle weakness during respiratory disease is not a passive event but is rather mediated by ubiquitination and subsequent degradation of muscle fibers.[28] For example, in an experimental rat ICU model, in which animals were mechanically ventilated, sedated, and pharmacologically paralyzed for up to 14 days, severe muscle wasting and paralysis similar to that observed among ICU patients was reported.[28] Interestingly, the marked decrease in muscle fiber force generation capacity was associated with a sequential change in the localization of the muscle-specific RING finger proteins 1/2 leading to degradation and transcriptional down-regulation of the molecular motor protein myosin, suggesting that ubiquitination played a central role in both muscle proteolysis and synthesis.[28]

Furthermore, it is well known that diaphragmatic function is critically important for successful weaning of patients from mechanical ventilation. However, mechanical ventilation itself has been shown to result in a rapid loss of diaphragmatic strength. Notably, several recent studies suggested an involvement of ubiquitination in diaphragmatic weakness in mechanically ventilated patients.[29-31] In a recent study, rapidly progressive diaphragmatic weakness and injury in ventilated patients was reported, which was associated with high expression of ubiquitin and other proteolysis-related factors.[29] Another study also identified a marked increase in the ubiquitin-proteasome activity as well as atrophic protein kinase B-forkhead Box O signaling, leading to degradation of myosin and thus resulting in myofiber atrophy and reduced diaphragm force-generating capacity in patients on mechanical ventilation.[30] Moreover, a recent manuscript reported the potential involvement of the autophagy-lysosomal pathway secondary to activation of the E3 ligase, muscle-specific RING finger protein 1, in diaphragmatic protein degradation in ventilated patients.[31]

Ubiquitination in ALI

Role of Ubiquitination in Acute Lung Inflammation and Surfactant Dysregulation

Ubiquitination may also regulate the inflammatory responses initiating ALI.[32] The E3 ubiquitin ligase Cblb, which plays an important role in inflammation and autoimmunity, has been shown to regulate the Toll-like receptor 4-mediated acute lung inflammation induced by polymicrobial sepsis, thereby impairing lung injury and survival, suggesting that ubiquitination may play a role in the initiation of ALI secondary to sepsis and lung inflammation.[32]

Ubiquitination has also been proposed to play a role in the pathomechanism of ALI by altering surfactant function. In a recent study it has been demonstrated that lipopolysaccharide causes polyubiquitination and subsequent degradation of acyl-CoA:lysophosphatidylcholine acyltransferase, an

enzyme critically involved in the synthesis of the bioactive surfactant phospholipid, dipalmitoylphosphatidylcholine, using the Skp1-Cullin-F-box ubiquitin E3 ligase component, β-transducin repeat-containing protein.[33] Furthermore, in a murine model of *Pseudomonas aeruginosa*-induced surfactant deficiency, which was characterized by the loss of CTP: phosphocholine cytidylyltransferase α, a nuclear enzyme that catalyzes the rate-limiting step in phosphatidylcholine synthesis, lung injury could be prevented by inhibition of the Skp1-Cullin-F-box E3 ligase, which was shown to monoubiquitinate CTP:phosphocholine cytidylyltransferase α leading to its degradation. Thus, ubiquitination may be critically important in surfactant homeostasis and ALI.[34]

Ubiquitination in Alveolar Epithelial Barrier Dysfunction

Ubiquitination has been proposed to play an important role in alveolar epithelial dysfunction during ALI. Accumulating data suggest that ubiquitination may regulate structural components of the alveolar epithelial monolayer. Structural integrity of epithelial cells and intercellular junctions play an important role in the maintenance of alveolar epithelial barrier integrity. For example, keratin intermediate filaments, a major cytoskeletal component of epithelial cells, are critically involved in alveolar barrier function. Interestingly, in response to injury, keratin intermediate filaments may undergo rapid structural reorganization, thereby transmitting signals from the cell surface to intracellular regions of cell.[35] This structural reorganization of keratin intermediate filaments, mediated by the UbcH5 family members and Ubc3 E2 ubiquitin-conjugating enzymes, has been recently shown to lead to disassembly and degradation of the keratin intermediate filaments, thereby contributing to barrier dysfunction.[36] Similarly, it has been recently proposed that both assembly and degradation of tight junction and adherens junction proteins are tightly regulated by ubiquitination.[37-39] Because of the potential therapeutic value, additional research addressing these events in the context of ALI is of high priority.

A key function of the alveolar epithelium is to maintain optimal alveolar fluid balance.[40] Under physiologic conditions, the alveolar epithelial barrier is highly impermeable, and its apical surface is covered by a thin liquid film, the alveolar epithelial lining fluid (ELF), which facilitates maintenance of surface tension, gas exchange and host defense.[41] During ALI and its more severe form, ARDS, which are characterized by an increase in alveolo-capillary barrier permeability resulting in flooding of the alveolar space, a life-threatening impairment of gas exchange occurs.[42] Therefore, effective control of the ELF volume is of critical importance. Optimal ELF volume is achieved by alveolar fluid reabsorption, which is driven by the active transport of Na^+ from the airspaces into the lung interstitium and the pulmonary circulation.[40] This vectorial transport is contributed by the apically located epithelial Na^+ channels (ENaC) and basolateral Na,K-ATPase, which generate an osmotic gradient leading to the movement of water from the alveolar space into the interstitium.[40,43] Importantly, in the majority of patients with ALI/ARDS, the Na^+ transport and thus the capacity to clear pulmonary edema is markedly impaired, which is associated with worse outcomes.[43-45]

Regulation of Na,K-ATPase and ENaC Function by Ubiquitination

Recently, ubiquitination has been implicated with a key role in regulating Na,K-ATPase[46] and ENaC,[47] thereby effecting alveolar epithelial barrier function and integrity. The Na,K-ATPase (also known as the Na^+ pump) is a heterodimeric holoenzyme composed of a catalytic α and a regulatory β subunit.[43] The Na,K-ATPase represents the only transporter by which alveolar epithelial cells actively remove sodium; furthermore, its expression and function are also intimately coupled to that of junctional complexes.[43,48] Therefore, the Na,K-ATPase plays a central role in both prevention of alveolar edema formation and clearance of lung edema fluid. Ubiquitination of the catalytic α_1 and α_2 subunits of the Na,K-ATPase was first reported almost 15 years ago in transformed kidney fibroblast cells, and although its physiologic role was not experimentally demonstrated, the authors hypothesized that ubiquitination may drive degradation of misassembled Na,K-ATPase at the endoplasmic reticulum and/or endocytosis and degradation of Na,K-ATPase located at the plasma membrane.[49] Another study established that oxidative stress-induced Na,K-ATPase degradation could be prevented by inhibiting either lysosomal or proteasomal degradation in kidney proximal tubule cells.[50]

The regulation of Na,K-ATPase function by ubiquitination in the alveolar epithelium has been extensively studied in the context of hypoxia, a hallmark of ALI/ARDS.[43] The first of these studies showed that during hypoxia the α_1 subunit of the Na,K-ATPase underwent polyubiquitination at the plasma membrane leading to Na,K-ATPase degradation that could be prevented by using a mutant of the ubiquitin-activating enzyme E1.[51] Interestingly, Na,K-ATPase degradation could be prevented by both proteasomal and lysosomal inhibitors, suggesting that perhaps both proteasomal and lysosomal degradation pathways are involved in the hypoxia-induced Na,K-ATPase down-regulation.[51,52] Another study further characterized

the nature of ubiquitination and degradation of the Na,K-ATPase and demonstrated the critical involvement of four lysine molecules on the N-terminus of the α_1 subunit of the Na,K-ATPase in its ubiquitination.[53] Interestingly, these lysines were in the immediate proximity of the S18 residue, the phosphorylation site for protein kinase C-ζ, a key regulator of Na,K-ATPase cell surface abundance.[54-57] Furthermore, mutation of the S18 residue to alanine, which prevented phosphorylation of the Na,K-ATPase by protein kinase C-ζ, also prevented the hypoxia-induced ubiquitination, endocytosis, and subsequent degradation of the Na^+ pump, suggesting a cross-talk between phosphorylation and ubiquitination.[53]

Although it is evident that the α subunit of the Na,K-ATPase undergoes ubiquitination upon exposing the alveolar epithelium to hypoxia, the E3 ligase promoting ubiquitination of the Na,K-ATPase remains unidentified. It has been previously reported that Ubc5, an E2 ubiquitin-conjugating enzyme, is necessary for the hypoxia-induced trafficking and degradation of the Na^+ pump. Notably, Ubc5 is the upstream regulator of the E3 ligase, von Hippel Lindau protein (pVHL), a key regulator of hypoxia that is involved in the ubiquitination and degradation of the hypoxia-inducible factor-1, and thus it was hypothesized that pVHL may also serve as the E3 ligase for the Na,K-ATPase.[58] This hypothesis was further supported by the observation that pVHL was required for the degradation of the Na^+ pump upon exposure to hypoxia.[58] However, this study also demonstrated that pVHL did not directly ubiquitinate the Na,K-ATPase, suggesting that pVHL was probably involved in the ubiquitination of an adaptor protein or a protein upstream of the Na,K-ATPase.[58] Thus, the E3 ligase for the Na,K-ATPase remains to be identified. Since the Na,K-ATPase plays a central role in alveolar epithelial barrier integrity, and its dysfunction is associated with worse outcomes in ALI/ARDS, identification of the E3 ligase that mediates Na,K-ATPase degradation may be of high clinical relevance.

In contrast, the E3 ligase mediating ubiquitination of ENaC was identified more than 15 years ago.[59] Two excellent recent reviews by Rotin and Staub[47,60] detailed this discovery together with our current understanding of ENaC regulation by ubiquitination; therefore, we will only mention some key aspects of this important research in the current review. ENaC is comprised of an α, a β, and a γ subunit with a short proline-rich sequence, called the PY motif, present at the C terminus of each subunit, which serves as the binding site for the E3 ligase Nedd4-2.[47,59] It has been demonstrated that Nedd4-2 can ubiquitinate ENaC at the apical plasma membrane of epithelial cells, which leads to internalization of the channel.[60] The nature of ENaC ubiquitination remains unclear. Although most studies suggest that ENaC undergoes either monoubiquitination or multi-monoubiquitination at the cell surface and subsequent endocytosis, some reports also demonstrated that ENaC may be polyubiquitinated and degraded in the proteasome.[47] Of note, proteolytic cleavage of the ectodomain of ENaC by serine proteases leads to activation of the channel by increasing its open probability. Interestingly, ubiquitination seems to regulate the conformation of the extracellular domain and thus the accessibility of protease sites for cleavage. However, since the protease cleavage releases the inhibitory tracts of ENaC, the cleaved subunits are constitutively active, and this activity can only be inhibited if the channel was removed from the cell surface, which is also exhibited by ubiquitination.[60] Therefore, ubiquitination plays a role in both activation of ENaC by promoting its maturation and deactivation/inhibition of the channel by initiating its endocytosis.

The Extracellular Proteasome

Biologic Roles and Potential Therapeutic Value of the Extracellular Proteasome System

Although the classic description of the proteasome degradation pathway is limited to the intracellular environment, recent evidence points to the presence and possible activity of extracellular proteasome.[25,61,62] This has been surmised to represent a marker of certain diseases[63-65]; however, studies have suggested a potential immunomodulatory role for extracellular proteasome in certain groups of patients.[66] In order to demonstrate that the proteasome might have a specific extracellular biologic function, as opposed to being a marker of cell death, multiple studies have shown that proteasome particles are bound to membranes. One found that when lipopolysaccharide was added to a macrophage membrane preparation, it was specifically bound to 20S proteasome attached membranes.[61,67] Other studies have demonstrated that proteasomes bind both endoplasmic reticulum and mitochondrial membranes.[61,68-70] Nakagawa and colleagues[70] have established that FK506-binding protein 38, a member of the immunophilin family of endoplasmic reticulum and mitochondrial membrane proteins, binds multiple subunits of the 26S proteasome and acts to anchor the proteasome at the organelle membrane. In addition, proteasomes have been found on the cell membrane surface, detectable by proteasome subunit-specific antibodies and flow cytometry on the surface of nonpermeabilized human leukemic cell line U937 and human T and B lymphocytes.[61,71,72]

Despite research showing that proteasomes interact with phospholipid membranes, there is no evidence

that explains how proteasomes may be secreted or released from cells, except as a result of cell death. Zoeger and colleagues[73] compared the function and subtype of circulating proteasomes in patients with rheumatoid arthritis, patients with systemic lupus erythematosus, and healthy volunteers. Circulating proteasomes from all groups were found to be intact and enzymatically active. However, when subtypes were analyzed with high-resolution anion exchange chromatography, the subtypes from patients with rheumatoid arthritis and systemic lupus erythematosus were found to be distinct from those from proteasome recovered from healthy volunteer hematologic cells.[73] Related to the finding of increased circulating 20S proteasome, increased serum or plasma levels of ubiquitin have been detected in multiple conditions, including parasitic infection, renal failure and hemodialysis, cirrhosis, type 2 diabetes mellitus, hairy cell leukemia, and sepsis.[61,74,75] In addition, it has been proposed that extracellular ubiquitin and proteasome may have therapeutic potential. One study of swine infused with endotoxin found that exogenous ubiquitin infusion reduced mortality, respiratory failure, fluid requirements, erythema, and edema over placebo.[76] Intriguingly, there is also evidence that extracellular ubiquitin may have antimicrobial properties as well.[77] As there is documented ubiquitin protease activity in several viruses, bacteria, and protozoa,[78] the potential for uncovering a pathogen-host ubiquitin and proteasome pathway interaction is promising.

Extracellular Proteasome Function in Sepsis and ALI

One inflammatory state in which the presence of extracellular proteasome has been evaluated is critical illness. A small case-control study of critically ill patients found significantly higher serum levels of 20S proteasome in 15 patients admitted to the ICU with a diagnosis of sepsis and 13 with a diagnosis of trauma compared with 15 patients admitted for elective abdominal surgery and 15 healthy volunteers.[79] A mouse model of sepsis found that infusion of the proteasome inhibitor MG-132 significantly dampened tumor necrosis factor-α, IL-1, and IL-10 levels and prolonged survival compared with placebo.[80] This finding may suggest that circulating proteasome interacts with the inflammatory response in sepsis, implying that the proteasome may have important immunomodulatory functions in patients with critical illness such as sepsis.

Research has also focused on extracellular proteasome activity in patients with the ALI. A small study illustrates the presence and activity of proteasome in the alveolar space.[81] All three proteasome activities were identified in a cell-free supernatant of BAL fluid and were inhibited by epoxomicin. Cell lysis was also ruled out as a potential source of alveolar proteasome. Finally, BAL supernatant successfully cleaved albumin in an ATP- and ubiquitin-independent manner.[81] These data point to the presence and biologic activity of proteasome in the alveolar space; furthermore, they imply a unique, ubiquitin-independent role for extracellular proteasome activity. The previous investigation could have profound implications on human pulmonary diseases, particularly those that involve an alveolar filling process that alters intraalveolar protein makeup or oncotic pressure. This was explored in a remarkable study by the same authors in patients with ALI showing a significantly increased alveolar proteasome concentration but 17-fold decreased proteasomal activity compared with healthy control subjects. In addition, alveolar proteasome activity in BAL from healthy patients was found to be inhibited by the addition of BAL from patients with ALI.[82] Thus, an inhibitor of proteasome activity may exist in the alveolar space, which could play a role in the increased intraalveolar protein concentration and oncotic pressure in ALI.

Potential Therapeutic Approaches Targeting Elements of the Ubiquitin System

Since ubiquitination plays a central role in the pathogenesis of several disease states, it is logical that pharmacologic inhibitors of the elements of the ubiquitination cascade may serve as novel therapeutic modalities. The first pharmacologic agents developed targeted the proteasome. These typically short peptide molecules mimic the substrate and inhibit the threonine residue in the 20S active site of the proteasome. Several synthetic proteasome inhibitors are available, which can be distinguished by their chemical properties. For example, MG-132, a cell-permeable peptide aldehyde, which is an effective and reversible inhibitor of the proteasome, is widely used for in vitro studies; however, its nonspecificity (inhibition of serine and cysteine proteases) precludes its clinical application.[25] More importantly, Bortezomib, a boronic acid peptide that specifically and reversibly inhibits the chymotrypsin-like activity of the 26S proteasome in mammalian cells, has recently been approved as the first proteasomal inhibitor by the US Food and Drug Administration for the treatment of multiple myeloma and mantel cell lymphoma.[83] Two novel and irreversible nonpeptide protease inhibitors, carfilzomib (also known as PR-171) and salinosporamide A (also known as NPI-0052), which were derived from lactacystin, a naturally occurring proteasome inhibitor produced by *Streptomyces lactacystinaeus*, are currently in clinical trials.[84]

Notably, not all proteins ubiquitinated undergo proteasomal degradation. Thus, to specifically interfere with ubiquitination of the target protein it appears logical that the interaction between the specific E3 ligase and the substrate must be inhibited. However, targeting E3 ligase-substrate associations remains challenging because of the limited information on the various partnering motifs and the weak nature of these interactions, which often occur along large surfaces.[83]

Most recent studies targeted regulatory elements of the ubiquitin cascade. One such study targeted the neural precursor cell expressed, developmentally downregulated 8 (NEDD8)-activating enzyme, an essential component of the NEDD8 conjugation pathway that controls the activity of the cullin-RING subtype of E3 ligases, the substrates of which have important roles in cancer cell growth. Interestingly, MLN4924, a potent and selective novel inhibitor of the NEDD8-activating enzyme, disrupted the cullin-RING ligase-mediated protein turnover, thereby suppressing tumor growth in mice.[85] Thus, targeting elements of the ubiquitin proteasome system may hold promise for the treatment of various diseases.

Conclusions

Ubiquitination is a posttranslational modification that regulates a wide variety of cellular functions. The versatility of ubiquitination effects relies on timing, subcellular localization, and type of tagging, as well as modulators of ubiquitin binding. Upon ubiquitination, tagged proteins may undergo proteasomal or lysosomal degradation or other nonproteolytic modifications. Ubiquitination appears to play an important role in the pathogenesis of ALI and alveolar epithelial barrier dysfunction upon injury. Particularly, the regulation of alveolar edema clearance by targeting ENaC and the Na,K-ATPase has been a focus of intense research in the last few years. Interestingly, the proteasomal system also exhibits distinct functions in the extracellular space, thereby contributing to ALI and other pulmonary diseases, which may have therapeutic implications. This is a relatively new field in the research of pulmonary and critical care diseases and provides a platform for better understanding of mechanisms, which may lead to novel therapeutic modalities by targeting elements of the highly specific ubiquitination pathway.

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