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Ubiquitin-proteasome signaling in lung injury

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Abstract

Cell homeostasis requires precise coordination of cellular proteins function. Ubiquitination is a post-translational modification that modulates protein half-life and function and is tightly regulated by ubiquitin E3 ligases and deubiquitinating enzymes. Lung injury can progress to acute respiratory distress syndrome that is characterized by an inflammatory response and disruption of the alveolocapillary barrier resulting in alveolar edema accumulation and hypoxemia. Ubiquitination plays an important role in the pathobiology of acute lung injury as it regulates the proteins modulating the alveolocapillary barrier and the inflammatory response. Better understanding of the signaling pathways regulated by ubiquitination may lead to novel therapeutic approaches by targeting specific elements of the ubiquitination pathways.

INTRODUCTION

Maintenance of tissue homeostasis requires rigorous protein level regulation. Post-translational modifications, among which ubiquitination is critical, regulate most cellular processes, including protein stability, receptor internalization, cell cycle, apoptosis, DNA repair, and signal transduction.[1,2] Degradation of ubiquitinated proteins is mediated by the 26S proteasome, a proteolytic complex designed to carry out selective protein hydrolysis.[1,3–6] The human ubiquitinome comprises ~19,000 sites in ~5000 proteins.[7] Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are life-threatening diseases and their pathobiology is incompletely understood.[8–11] Approximately 200,000 people per year develop ALI in the United States, with mortality rates of 30%–40%.[8,12–14] In this review, we summarize the ubiquitin system role in the pathobiology of ARDS.

THE UBIQUITIN SYSTEM

The addition of the highly conserved 76 amino acid ubiquitin peptide to a target protein occurs via an adenosine triphosphate (ATP)-dependent process.[1,5,15,16] First, the ubiquitin-activating enzyme (E1), in an ATP-dependent manner, forms a transient high-energy thiol with ubiquitin's C-terminal glycine residue, followed by the transfer of ubiquitin to the active-site cysteine residue on the ubiquitin conjugation enzyme (E2). Ubiquitination is then

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completed when the E2 binds with an ubiquitin ligase (E3) conjugating ubiquitin to the target substrate.[4] Given that there is one or two E1, dozens E2, and several hundreds of E3, the process specificity increases as the enzymatic cascade progress.[5,17] Ubiquitinated proteins are recognized by ubiquitin-binding domains encoded within proteins.[18–20] There are three classes of E3 ubiquitin ligases: (a) homologous to the E6-AP C-terminus (HECTs), (b) Really Interesting New Genes (RINGs), and (c) RING-between-RING (RBRs), classified according to their catalytic mechanism.[21–23] The RING domain ligases (the largest family) transfer the ubiquitin from the E2 to the substrate whereas the HECTs have a C-terminal domain that forms an intermediary with ubiquitin before substrate modification.[19,23–26] The RBR ligase family transfers the ubiquitin using a RING–HECT hybrid mechanism. First, ubiquitin is bound to the RING1 domain of the enzyme; then, similar to RINGs ligases mechanism, ubiquitin is transferred to the RING2 domain forming a ubiquitin–thioester intermediate; finally, ubiquitin is transferred from the RING2 to the substrate.[27–30] Ubiquitin modifications can be edited or erased by proteases and deubiquitinases, which regulate the nature and duration of the signal.[5,31–33] By degrading ubiquitin chains, deubiquitinases generate free ubiquitin, thus, replenishing the ubiquitin pool and maintaining the ubiquitin homeostasis.[34] These enzymes add an extra layer in the regulation of cellular functions.

Monoubiquitination results from the attachment of a single ubiquitin molecule to a Lysine (Lys) residue in the target protein. It is also possible that multiple lysine residues become modified with one ubiquitin (multi-monoubiquitination). Conjugation of a single ubiquitin is a weak proteolytic signal and only small fraction of monoubiquitinated proteins are targeted to the proteasome for degradation.[35] However, monoubiquitination is important in transcriptional regulation, DNA damage repair, membrane-associated endocytosis as well as chromatin regulation, protein sorting and trafficking.[6] Ubiquitin can itself be ubiquitylated using any of its seven Lys residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, and Lys63) or in the N-terminal methionine (Met1)[36,37] (Fig 1). The process generates different chain topologies, which provide substantial signal diversity.[37,38] Moreover, ubiquitin can be also conjugated with ubiquitin-like modifiers such as SUMO (small ubiquitin-like modifier) or small molecules such as phosphate.[5] The cellular abundance of the different internal linkages has been defined using mass spectrometry and it is depicted in Fig 1.[7,39,40] Among all conjugation possibilities, Lys48-linked chains are the predominant linkage sites (Fig 1), targeting proteins to the 26S proteasome for degradation.[1,15,16] In contrast, Lys63, the next most common linkage, is involved in autophagic proteolysis[5] and in nondegradative functions such as DNA damage repair,[41] protein trafficking,[42] transcriptional regulation,[43] and kinase signaling.[44] The function of ubiquitin molecules linked through Lys6, Lys11, Lys27, Lys29, or Lys33[36] is a focus of intense studies and is less understood. It has been described that Lys6-linked chains are associated with mitochondrial homeostasis and mitophagy[45]; Lys11-linked ubiquitination is a regulator of cell division,[46] whereas Lys29- and Lys33-linked polyubiquitin chains regulate adenosine monophosphate protein kinase (AMPK)-related kinases activity[47] (Fig 1).

Met1-linked chains, also referred to as linear ubiquitin chains, are formed by the conjugation of the ubiquitin C-terminal Gly to N-terminal α-amino group of the Met of other ubiquitin.[48] Only one E3 ligase has been described to assemble linear chains: the linear ubiquitin chain assembly complex (LUBAC), a member of the RBR ligase family.[19,29,30,49] LUBAC

is formed by the ubiquitin ligases hemeoxidized IRP2 ubiquitin ligase 1L (HOIL-1L), the HOIL1-interacting protein (HOIP), and the regulatory protein SHANK-associated RH domain-interacting protein (SHARPIN).[50–52] These proteins contain multiple domains for interactions within the complex, for ubiquitin binding, and for catalytic activity.[53–55] Within the complex, although HOIL-1L also expresses a RING domain, HOIP is the one responsible for the catalytic activity.[27,48,56] However, independent of LUBAC, the RBR of HOIL-1L has been described to add Lys48 polyubiquitin chains to target proteins.[57–59] Even though it cannot be ruled out that LUBAC-formed Met1 chains tagged proteins for degradation, most of the studies support a nonproteolytic function in signaling pathways activated downstream of several cytokines receptors and toll-like receptors (TLR).[48,51,54,60,61] The role of LUBAC in tumor necrosis factor α-receptor (TNFα-R) downstream signaling is the best described function of LUBAC.[48,54,60,61] After TNFα binding, several adapter molecules are recruited to form a signaling complex. The complex called TNFR1-signaling complex (TNFR1-SC) is composed of TNFR type 1-associated death domain protein, TNFR-associated factor 2 (TRAF2), receptor-interacting serine-threonine kinase 1 (RIPK1), the cellular inhibitor of apoptosis (cIAP), and ubiquitin-conjugating 13 (UBC13)[62–64] LUBAC and other E3-ligases present in the complex ubiquitylate several targets of the TNFR1-SC, including themselves, promoting the propagation of the signal.[48,61]

Linear ubiquitination of NF-kB essential modulator (NEMO), a component together with IKKα and IKKβ of the IκB kinase (IKK) complex, plays an important role in the activation of NF-κB.[54] In turn, NEMO-IKK phosphorylates the NF-κB inhibitor, IκBα, leading to its proteasomal degradation and translocation of NF-κB to the nucleus where it engages in gene transcription. As NEMO's UBAN domain (ubiquitin binding in ABIN and NEMO) has a high affinity for linear chains, its ubiquitylation facilitates the recruitment of additional IKK complexes to the TNFR1-SC.[48,60,61] Stably docked IKK complexes result in the phosphorylation and activation of proximal IKKα and IKKβ.[49,50,52,60] It has been recently described that K63- and Met1-ubiquitin chains are attached covalently to one another and that the formation of K63-polyubiquitin chains during cytokine stimulation is a prerequisite for the formation of Met1-ubiquitin chain.[26,65,66] In vitro, HOIP binds specifically to K63-ubiquitin chains suggesting that the formation of K63-/Met1-polyubiquitin hybrids permits the recruitment of transforming growth factor-β-activated kinase 1 (TAK1) and the canonical IKK complex to the same ubiquitin chains, facilitating the transforming growth factor-β-activated kinase 1-catalyzed phosphorylation and activation of the IKKs.[65] The formation of heterotypic ubiquitin chains represents an additional level signaling complexity.

UBIQUITINATION AND PROTEOLYSIS DURING ARDS

The alveolocapillary barrier is relatively impermeable and via the large alveolar surface effects gas exchange.[67–72] During ARDS, there is an increase in alveolocapillary barrier permeability resulting in flooding of the alveolar space and impairment of gas exchange.[10,73,74] In the majority of patients with ARDS, the alveolar fluid clearance is impaired and needs to be reabsorbed for the patients to improve.[75] Edema fluid clearance, resolution of inflammation, and lung repair are required for the restoration of normal lung function and patient survival.[71,76–79] The driving force for lung edema clearance is active Na^+ transport

across the alveolar epithelium[10,80] where sodium enters alveolar epithelial cells presumably through apical epithelial sodium channel (ENaC) and is actively extruded by basolateral Na,K-ATPase.[69,76,81–83] Among the processes regulated by ubiquitin signaling and the ubiquitin-proteasome system during ARDS is the activity of the transporters effecting fluid clearance, the inflammatory response, and the function of junctional proteins.[70,84–86] Besides the compromised lung function, a common complication of ARDS and critical illness is severe skeletal muscles weakness and atrophy that persists long after lung injury has resolved.[87–90] It has been reported that the ALI-associated muscle wasting is controlled through ubiquitin-mediated proteasomal and autophagy-lysosomal pathways.[87,91,92]

ION TRANSPORT AND REGULATION OF FLUID BALANCE

In the context of ARDS, hypoxemia occurs as a consequence of the impaired gas exchange in the lungs.[13,69,70] During hypoxia, the falling oxygen concentrations inhibit the mitochondria electron transport chain, thus decreasing ATP production.[93–95] Cells respond to the surrounding hypoxia via stabilization of the transcription factor hypoxia-inducible factor (HIF)-1α, which promotes energy generation by enhancing anaerobic glycolysis while decreasing ATP-consuming enzymes, thereby preserving cell metabolism.[94,95] The Na,K-ATPase pumps sodium out of cells while pumping potassium in, and under basal conditions utilizes ~30% of the cell's ATP to maintain the Na^+ and K^+ concentration gradients across the cell membrane necessary for cellular homeostasis.[93,94,96,97]

In animal models and in vitro, short-term severe hypoxia causes a decrease in Na,K-ATPase activity in alveolar epithelial cells due to reduction of the number of active Na,K-ATPase molecules at the plasma membrane and a subsequent degradation of the internalized molecules as depicted in Fig 2, A.[96,98,99] Protein kinase C-zeta (PKCζ)-dependent phosphorylation of the Na, K-ATPase catalytic subunit Ser-18 triggers the endocytic process leading to the ubiquitination of the Na,K-ATPase at the plasma membrane.[98,100] Further studies revealed that hypoxia-induced ubiquitination and endocytosis were prevented if the four Lys residues immediately flanking the Ser-18 ($KK^{18}SKK$) were mutated to arginine.[100,101]

Hypoxia-induced Na,K-ATPase degradation could be prevented by inhibiting its ubiquitination at the plasma membrane and by lysosome inhibitors, suggesting that although the Na,K-ATPase is ubiquitinated at the plasma membrane, its degradation occurs in the lysosome.[100,101] The Na,K-ATPase is internalized through a clathrin-dependent mechanism and the attachment of ubiquitin molecules at the plasma membrane serves as a signal both for recognition by the endocytic machinery and for subsequent intracellular trafficking through endosomal compartments and delivery to the lumen of the lysosome.[100,101] For other protein substrates, it has been proposed that phosphorylation generates a docking site for the ubiquitination machinery.[100,102] It was also described that the Na,K-ATPase catalytic subunit is polyubiquitinated leading to proteasomal degradation.[103] The difference in the intracellular degradation sites may be due to the fact that one study assessed only the active plasma membrane enzyme, whereas in the other study, the total pool of the catalytic subunit was studied, which includes unassembled Na^+ pumps located in the ER. The Na,K-ATPase-E3 ligase has not been described; however in human epithelial cells, it was reported that von

Hippel-Lindau protein, a key regulator of HIF-1α, was required albeit indirectly for the degradation of the Na,K-ATPase during hypoxia.[104,105] Because the Na, K-ATPase plays a key role in the regulation of alveolar fluid reabsorption, the identification of its ligase may lead to a clinically relevant druggable target to improve the clearance of lung edema.

It was also reported that as hypoxia persists, the excessive Na,K-ATPase endocytosis and degradation may be deleterious for cellular homeostasis and results in cell death.[97,106] To maintain homeostasis during hypoxia, cells utilize HIF to induce HOIL-1L independently of the other components of LUBAC (Fig 2, B). The newly synthesized HOIL-1L molecules are not part of the LUBAC complex and they promote the Lys48 ubiquitination of PKCζ targeting it for proteosomal degradation, which results in the stabilization of the Na,K-ATPase at the plasma membrane. The trans-location of PKCζ to the membrane and its interaction with HOIL-1L PKCζ requires AMPK-induced PKCζ phosphorylation.[58,59,106] This mechanism represents a noncanonical cell adaptive mechanism to hypoxia where HOIL-1L acts as the PKCζ E3 ligase to protect against lung injury.

Ubiquitination also plays a role in the regulation of ENaC, which is responsible for Na^+ transport across the lung epithelium.[76,107,108] As is the case for the Na, K-ATPase, the number of Na^+ channels on the epithelial cells apical surface regulates Na^+ transport.[85,86,107,109,110] It is well established that polyubiquitination of the ENaC subunits is prerequisite for endocytosis and degradation at the proteosome.[85,86,111] Binding of PY motifs located in the β and γ ENaC subunits to the WW domains of the E3-ubiquitin ligase Nedd4–2 (neural precursor cell expressed developmentally downregulated protein) leads to ubiquitination and internalization of the channel and degradation by the proteasome.[85,111] Nedd4–2 shares a modular structure with the other members of Nedd4 family of HECT-ubiquitin ligases in which a C2 calcium-dependent phospholipid binding domain targets the protein to the membrane and is involved in the binding to the substrates.[107] Nedd4–2 null mice develop a sterile lung inflammation that resembles the clinical manifestations of ARDS, which can be due to the effects of Nedd4–2 not only on ENaC but on other proteins that contribute to the inflammatory response as well. Accordingly, it was reported that Nedd4–2 ubiquitination of lysophosphatidic acid receptor 1, a proinflammatory G-protein-coupled receptor, promotes its proteosomal degradation, thus preventing the interaction with the naturally occurring bio-lipid lysophosphatidic acid and attenuating the release of the chemotactic factor interleukin (IL)-8.[112]

UBIQUITINATION OF CELL JUNCTIONS

Ubiquitination and degradation of cell junction proteins are important in alveolocapillary barrier integrity.[71,113] The epithelial and endothelial sides of the barrier components adherens junctions (AJ) comprise the transmembrane cadherins, whereas the tight junctions (TJ) include ocluddin, claudins, junction adhesion molecules, and other transmembrane proteins.[114] TJ seal the space between adjacent epithelial cells controlling the paracellular transport and when damaged cause epithelial barrier breakdown.[114] Catenins (comprising α, β, γ, and p120) are also a critical component of AJ, providing a link between cadherin and the cytoskeleton[114] serving as anchors connecting the cytoskeleton of neighboring cells.[113]

Cadherins regulation allows dynamic plasticity of the cellular barrier. The E3 ubiquitin ligase, Hakai, targets tyrosine phosphorylated E-cadherin, regulating its expression.[115] Phosphorylation of E-cadherin prevents the binding to p120 and increases Hakai binding leading to endocytosis and degradation.[113] It has been reported that p120 protects against ventilation-induced lung injury by stabilizing both AJ and TJ, and the dissociation of p120 from E-cadherin is the main factor in AJ destabilization during cyclic stretch.[116,117] In lipopolysaccharide (LPS)-challenged mouse lungs, decreased levels of p120 correlate with the severity of inflammation.[116] p120 is ubiquitinated and degraded; however, the E3 ligase for p120 has not been identified.[113] VE-cadherin is ubiquitinated by K5, a member of the membrane-associated RING-CH or MARCH E3 ligase family.[118] Regarding the TJs, claudin-3, −4, and −18 are the most expressed in the lung epithelium, whereas claudin-5 is predominantly expressed in endothelial TJ.[9,113,119] The permeability of the lung epithelium is modulated by claudins, where claudin-4 promotes a tight epithelium and claudin-3 a looser one.[9,120] Influenza infection inhibits claudin-4 expression in the alveolar epithelium leading to barrier dysfunction and lung injury.[73] Because of its potential as a therapeutic strategy, identification of the ubiquitin ligases that target the junction proteins is of great interest.

UBIQUITINATION AND THE REGULATION OF LUNG INFLAMMATION

Innate immunity provides the first line of host defense against pulmonary pathogens. Pro- and anti-inflammatory chemokines increase in the bronchoalveolar lavage fluid and circulating plasma of patients at different stages of ARDS.[8] Alveolar macrophages are central to the development of the inflammatory response recruiting neutrophils and circulating macrophages to the site of injury.[121,122] These cells secrete chemokines, reactive oxygen species, proteases, and other mediators that modulate the inflammatory responses and injure the alveolocapillary barrier. TNF-α and IL-1β are important proinflammatory cytokines in the pathogenesis of ARDS.[123] After their receptor activation, cIAP-mediated K63-ubiquitination of RIPK1 and the TRAF proteins leads to the recruitment of LUBAC.[49,54,59] This activation leads to the phosphorylation of IκB, allowing the recognition and ubiquitination of FBW1, a component of the E3 ligase Skp1–Cullin–F-box protein (SCF) complex.[124] Degradation of the ubiquitinated IκB at the proteasome allows the NF-κB dimer to translocate to the nucleus and activate gene transcription.[124] Ubiquitination is involved in several steps of NF-κB activation participating in the pathobiology of ARDS induced by septic shock, hemorrhage, mechanical ventilation, and allograft rejection.[125–129] In a mouse model of sepsis, it has been reported that the E3 ubiquitin ligase Cbl-b, which plays an important role in inflammation and autoimmunity, controls TLR4-mediated acute lung inflammation by modulating the activation of NF-κB.[130] Cblb acts by regulating TLR4 surface expression immediately after stimulation and regulates the interaction between TLR4 and MyD88, attenuating the MyD88-dependent TLR4 signaling pathway.

Glycogen synthase kinase-3 (GSK3) is described as the “busiest kinase” due to the number of substrates it phosphorylates, including the SCF E3 ligases involved in proteasomal degradation.[131] GSK3-mediated phosphorylation generates the degradation motif necessary for the recognition by SCF E3 ubiquitin ligases.[132] Phosphorylation of ST2L, the IL-33 receptor, is an example of the GSK3-induced phosphorylation in sepsis. IL-33, a member of

the IL-1 family, is released by damaged host cells as a proinflammatory signal.[133] Once phosphorylated, ST2L can be recognized and polyubiquitinated by the SCF family member FBXL19. ST2L degradation by the proteasome limits the inflammatory effects of IL-33 and ameliorates the severity of lung injury in mouse models of pneumonia.[132] The regulation of GSK3 can also have an anti-inflammatory effect. FBXO17 has been described as an F-box protein subunit that recognizes and mediates GSK3 polyubiquitination and degradation to attenuate inflammatory responses in lung epithelial cells after LPS injury.[134] Another example of the ubiquitin-proteasomal pathway regulating inflammatory signaling is during the interplay between the proinflammatory FBXO3 and the anti-inflammatory FBXL2 E3 ligases during sepsis.[135] In a human epithelial cell line, FBXL2 constitutively ubiquitinates TRAFs leading to its degradation. However, in response to bacterial endotoxin, FBXO3 expression increases and it ubiquitinates FBXL2 for degradation with resultant accumulation of TRAF. The increases of TRAF-mediated cytokine release lead to changes in lung permeability, alveolar edema and multiorgan failure.[135] As TRAFs proteins are crucial mediators of TLRs and cytokines receptors, therapeutics designed to selectively downregulate their abundance by targeting the E3 ligases may have beneficial effects by modulating the inflammatory response.

PROTEASOME AND LUNG INJURY

The proteasome is the major eukaryotic intracellular degradation machinery, degrading ~90% of intracellular proteins, thus contributing to protein quality control. The 26S proteasome is a multicatalytic ATP-dependent protease complex composed of a catalytic 20S core and two 19S regulatory complexes on either end of the barrel, which recognize ubiquitinated proteins and unfold and guide them to the proteolytic 20s core.[136,137] The role of the proteasome in the pathophysiology of chronic lung diseases and ARDS has been described.[137–139] Proteasomal dysfunction has been reported in chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF).[132,140–142]

The immunoproteasome, a proteasome variant induced during the immune response by the TNF-α or interferon gamma (IFN-ɣ) release from proinflammatory cells, processes antigenic peptides involved in major histocompatibility complex I antigen presentation.[136,137,143] It has been associated with autoimmune and inflammatory disorders, cancers,[144] as well as lung injury.[145] Dysfunction of the immunoproteasome pathway may lead to the accumulation of misfolded proteins observed in chronic lung diseases such as cystic fibrosis,[145,146] where the lack of cystic fibrosis transmembrane conductance regulator (CFTR) protein in the cell surface is due to the activity of the E3 ligases CHIP and RMA1. These ligases recognize and trigger the proteasomal degradation of the mutated CFTR,[147] whereas c-cbl plays a role in the regulation of the normal CFTR expression at the membrane.[148] In asthma, the E3 ligases Itch and Cbl-b were described to participate in the antigen-specific hyperactive helper T-cell type 2 immune response, increasing airway IL-12 and IFN-ɣ, respectively.[149] Cbl-b also downregulates the inflammatory response during ARDS[150] and the neutrophil-mediated release of TNF-α and IFN-ɣ induces both immunoproteasome and standard proteasome expression in response to cigarette smoke.[151] Cigarette smoke-induced immunoproteasome dysfunction may contribute to prolonged infections and exacerbations in COPD and IPF.

As the understanding of the ubiquitin-proteasome pathway increases, also do the recognition of potential therapeutic targets and the development of new drugs. Nedd8 (neural precursor cell expressed developmentally downregulated protein 8) is one of the most studied ubiquitin-like molecules and the process of neddylation is similar to ubiquitination.[152] Neddylation of cullins, a component of the cullin-RING ubiquitin ligases (CLRs), is required for CLR-dependent ubiquitination.[153] It has been described that CLRs are responsible for the ubiquitination of 20% of the proteins tagged for degradation at the proteasome.[153] MLN4924 (Pevonedistat) is a potent neddylation inhibitor that blocks the covalent attachment of Nedd8 to cullins by inhibition of the Nedd8-activating enzyme and plays a role in tumorigenesis.[150] Recently, it has been described that MLN4924 improves lung function in the early inflammatory stage of pulmonary fibrosis and prevents the release of proinflammatory cytokines like monocyte chemoattractant protein-1 (MCP1) secreted in response to LPS, suggesting a role as an anti-inflammatory drug.[154]

CONCLUSIONS

Ubiquitination is a reversible protein modification that regulates many cellular functions, and the ubiquitin-proteasome pathway plays an important role in the pathogenesis of ARDS. Upon stimulation, the ubiquitination of target proteins with a specific type of ubiquitin chain occurs in cellular domains and requires defined adaptors. We have reviewed some of the relevant aspects of ubiquitin signaling pathway in the pathobiology of lung injury. Better understanding of the mechanisms of this pathway and specifically the interaction between E3-ligases and their substrates may lead to the identification of druggable targets and novel therapeutic approaches.

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Abbreviations:

AJ	adherens junctions
ALI	acute lung injury
AMPK	adnosine monophosphate activated protein kinase
ARDS	acute respiratory distress syndrome
ATP	adenosine triphosphate
CFTR	cystic fibrosis transmembrane conductance regulator
cIAP	cellular inhibitor of apoptosis
CLR	cullin-RING ubiquitin ligase
ENaC	epithelial sodium channel
GSK3	glycogen synthase kinase-3

HECT	homologous to the E6-AP C-terminus
HIF-1α	hypoxia-inducible factor 1α
HOIL-1L	Hemeoxidized IRP2 ubiquitin ligase 1L
HOIP	HOIL-1L interactin protein
IFN-γ	interferon gamma
IKK	IκB kinase
IL	interleukin
LUBAC	linear ubiquitination assembly complex
Met1	N-terminal methionine
Nedd	neural precursor cell expressed developmentally downregulated protein
NEMO	NF-κB essential modulator
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
PKCζ	protein kinase C-zeta
RBR	ring in between ring
RING	really interesting new genes
RIPK1	receptor-interacting serine-threonine kinase 1
SCF	skp1–Cullin–F-box protein
SHARPIN	SHANK-associated RH domain interacting protein
SUMO	small ubiquitin-like modifier
TAK1	transforming growth factor-β-activated kinase 1
TJ	tight junctions
TNFα-R	tumor necrosis factor α-receptor
TNFR1-SC	TNFR1-signaling complex
TLR	toll like receptors
TRAF2	TNFR-associated factor 2
Ub	ubiquitin
UBC13	ubiquitin-conjugating13

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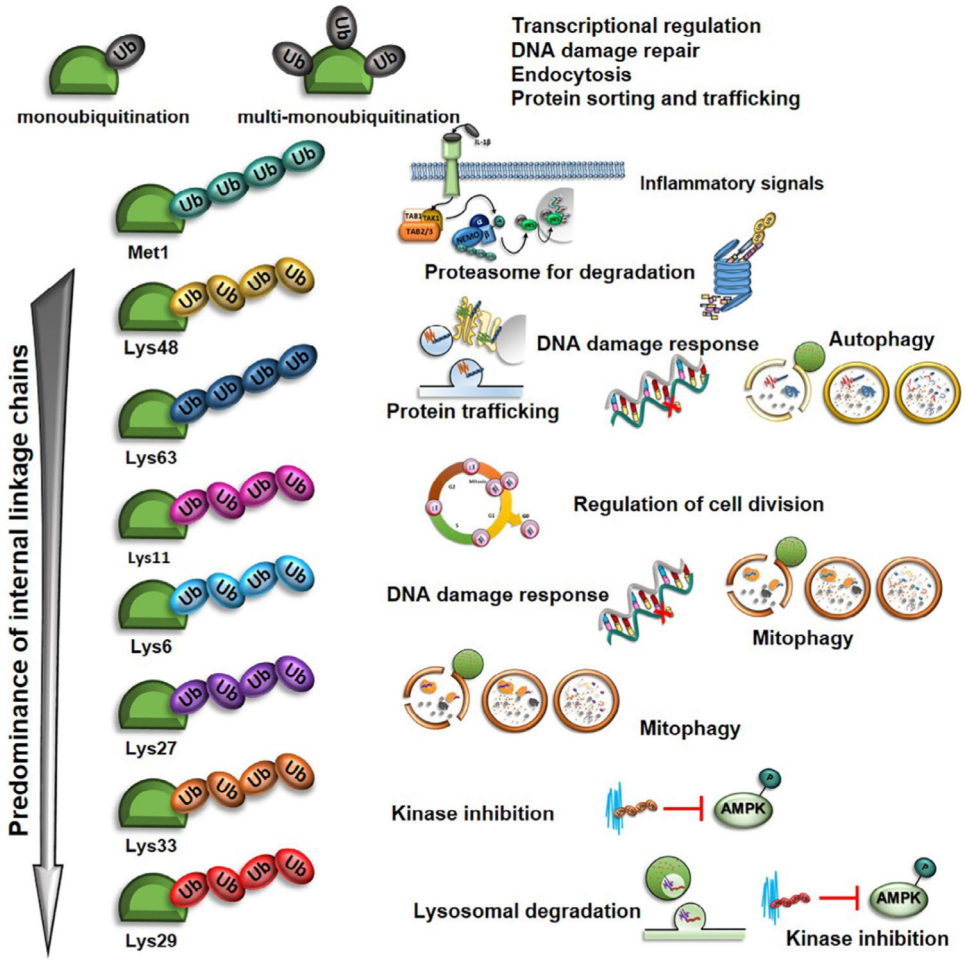

Fig 1.

Biological processes associated with the different types of ubiquitin linkages. Substrates can be monoubiquitinated, poly-monoubiquitinated or targeted with homotypic or heterotypic polyubiquitin chains as ubiquitin can itself be conjugated with another ubiquitin in any of the seven Lys residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, and Lys63) or, alternatively, Met1. Prevalence of the different internal linkages is depicted. AMPK, adenosine monophosphate-activated protein kinase; Met1, N-terminal methionine; Ub, ubiquitin.

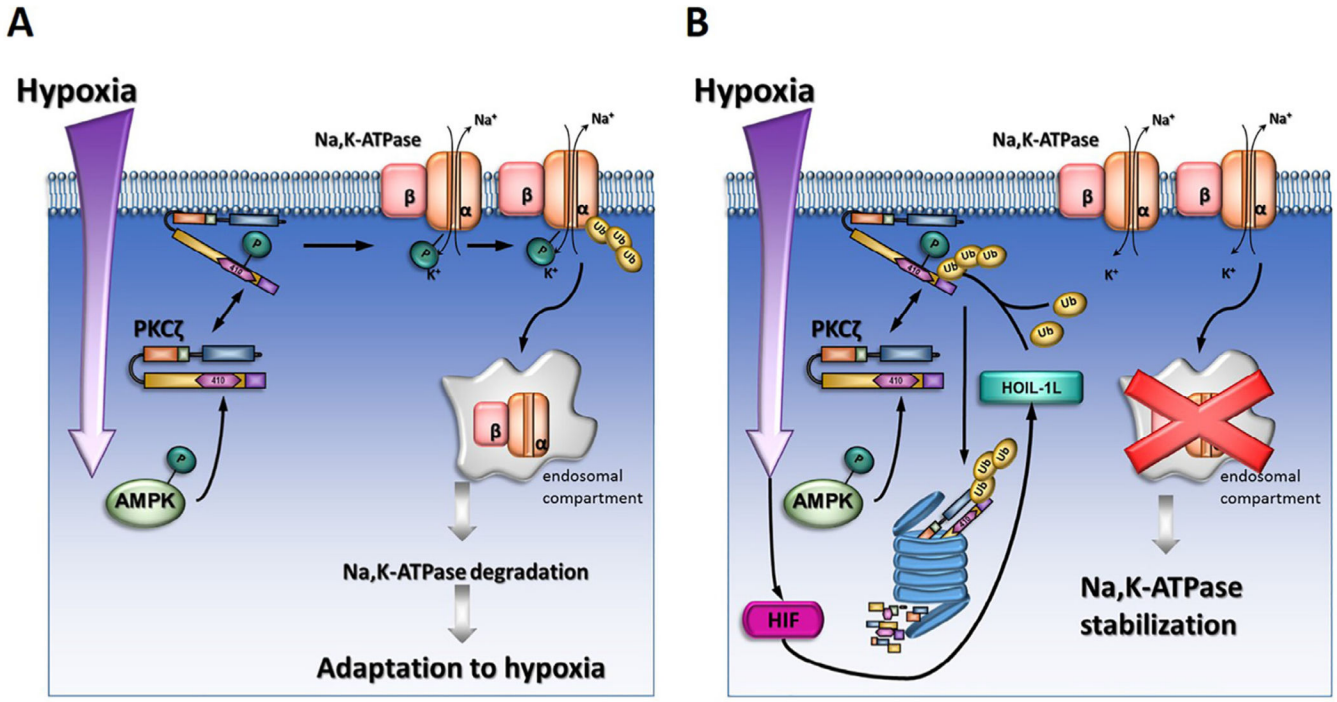

Fig 2.

Proposed mechanism regulating Na,K-ATPase stability at the plasma membrane during hypoxia. **A**, Phosphorylation of Na,K-ATPase by PKCζ is required for ubiquitination which leads to the endocytosis and degradation of the enzyme at the lysosome. **B**, Na,K-ATPase stabilization during prolonged hypoxia. Hypoxia upregulates the E3 ligase HOIL-1L, which ubiquitinates PKCζ, triggering its proteasomal degradation to prevent excessive downregulation of Na,K-ATPase as a mechanism of adaptation to hypoxic conditions. PKCζ, protein kinase C-zeta; HOIL-1L, hemeoxidized IRP2 ubiquitin ligase 1L; AMPK, adenosine monophosphate-activated protein kinase; HIF, hypoxia-inducible factor; Ub, ubiquitin.