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The Role of Ubiquitin System in Autophagy

Yi-Ting Wang and Guang-Chao Chen

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Abstract

Autophagy is a highly conserved lysosomal degradation pathway, which has been shown to play a pivotal role during normal physiological and pathological conditions. Many proteins and signaling pathways have been shown to regulate autophagy during different stages of the process. Modifying autophagy-related proteins (Atg) by posttranslational modification (PTM) is an important way to control proper autophagic activity. Ubiquitination is one of the PTM that has a crucial role in controlling protein stability and functions. Proteins can be conjugated with ubiquitin chains with different topologies that are associated with different outcomes. Many autophagy regulators are found to be substrates for ubiquitin E3 ligases or deubiquitinating enzymes (DUBs). Ubiquitination modifications of these autophagy regulators result in autophagy induction or termination. Moreover, ubiquitin is also involved in selective autophagy by acting as a degradation signal. Here, we are going to review how E3 ligases and DUBs function in autophagy regulation and discuss the recent findings about ubiquitination regulation in autophagy-related processes and diseases.

Keywords: autophagy, ubiquitin, E3 ligase, deubiquitinating enzyme

1. Introduction

Proteome dynamics and complexity are tightly regulated to maintain normal cellular function and homeostasis. The ubiquitin-proteasome system (UPS) and autophagy are the two main intracellular degradative machineries in eukaryotes [1, 2]. UPS mainly degrades specific short-lived proteins, whereas autophagy is responsible for the bulk degradation of long-lived

proteins and damaged organelles. Autophagy is a lysosome-mediated catabolic process by which cytoplasmic components are degraded and recycled for cellular homeostasis [3, 4]. It is tightly controlled by complex signaling pathways and serves as a cytoprotective mechanism in response to environmental stresses such as nutrient deprivation, reactive oxygen species (ROS), and pathogen invasion [5]. Dysregulation of autophagy pathway has been implicated in various human diseases [6, 7], including myopathies, aging, neurodegeneration, and cancer, as well as in heart, liver, and kidney diseases. To date, more than 35 autophagy-related (Atg) genes have been implicated in regulating the autophagic process [8]. The core Atg proteins are highly conserved and can be assembled into different complexes such as the autophagy-initiating Atg1/Ulk protein kinase complex, Beclin1-class III PI3K complex, the Atg5-Atg12 and Atg8/LC3 ubiquitin-like conjugation systems, and the Atg9 recycling system [8, 9].

Protein posttranslational modification (PTM) plays a pivotal role in increasing proteome complexity and determining the fates of proteins [10–13]. It is a widespread mechanism that involves the addition of a functional group covalently to a protein. The major types of PTMs include phosphorylation, glycosylation, acetylation, ubiquitination, methylation, and lipidation. The diversity of PTM provides enormous flexibility for control of protein structure, localization, activity, and function; this modification can be reversible or irreversible. Recent studies have identified various forms of PTMs in the regulation of autophagy [14–16]. Some PTMs regulate autophagy by affecting the enzymatic activity of Atg proteins. For example, the Ulk1 kinase (the key initiator of autophagy) can be phosphorylated by upstream regulators such as AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR), which result in the activation or inactivation of Ulk1 kinase activity, respectively [17–19]. Additionally, Ulk1 can also be acetylated at K162 and K606 by acetyl transferase KAT5/TIP60, and the PTM acetylation is vital to the activation of Ulk1 [20]. PTMs can also regulate autophagy by changing the interacting partners of Atg proteins [21, 22]. It has been shown that the posttranslational modification of Beclin1 affects its interaction with Vps34 complex. Zalckvar et al. showed that death-associated protein kinase (DAPk)-mediated phosphorylation of Beclin1 promotes the association between Beclin1 and Vps34 complex [23]. On the contrary, Beclin1 phosphorylation by CDK1 leads to the dissociation of Beclin1 from Vps34 [24].

Accumulating evidence indicates that protein ubiquitination and deubiquitination play multiple roles in the regulation of protein stability and signaling during autophagy [15, 16]. Several ubiquitin E3 ligases and deubiquitinating enzymes (DUBs) have been shown to regulate autophagy at different stages. However, the detailed mechanisms of the E3 ligases and the DUBs in controlling both "on" and "off" signals of autophagy remain unclear. The ubiquitin system is also essential for the recognition and removal of damaged organelles and invading pathogens during selective autophagy processes. Moreover, two ubiquitin-like conjugation systems are found to be crucial for the expansion of the elongation and expansion of autophagosomal membrane. Here, we will discuss recent advances on the role of ubiquitin systems in autophagy.

2. Ubiquitin modification and protein fate determination

2.1. Ubiquitin modification

Ubiquitination is an ATP-dependent enzymatic process that involves the covalent conjugate a highly conserved 8-kDa ubiquitin (Ub) peptide to lysine residues of target proteins [25]. The ubiquitination reaction requires three classes of enzyme: ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and substrate-specific ubiquitin ligases (E3) [25, 26]. In mammals, there are more than 500 E3 ligases, 30 E2-conjugating enzymes, and two E1-activating enzymes [26–28]. The E1 enzyme activates free ubiquitin by forming a thioester linkage between the C-terminal carboxyl group of ubiquitin and the E1 cysteine sulfhydryl group in an ATP-dependent manner. The activated ubiquitin is then transferred from E1 to the catalytic cysteine of E2, which can determine the type of ubiquitin chain formed. Finally, the E3 binds both the Ub-charged E2 and substrates to catalyze and transfers the C-terminus of Ub to the lysine residue of substrates. The E3 ligase transfers ubiquitin to specific protein targets and is critical for conferring the substrate specificity. Because of the large number of E2 and E3 enzymes, a broad range of substrates can be modified by distinct ubiquitin chain configurations.

Ubiquitination of a substrate can be classified according to the number of ubiquitins, linkage of ubiquitin chains, and chain length [27]. It has been shown that the attachment of an ubiquitin molecule at one site in the substrate causes monoubiquitination [28, 29]. Whereas monoubiquitin is conjugated on several lysine residues of the substrate results in multiubiquitination. Mono- and multiubiquitination regulate processes that range from histone modification to membrane-receptor endocytic trafficking [30]. In addition, the E2/E3 complexes can catalyze further cycles of ubiquitination on the substrate-conjugated ubiquitin, resulting in substrate polyubiquitination [28, 29]. Ubiquitin links to another ubiquitin molecule to form polyubiquitin chain via one of its seven lysines (K6, K11, K27, K29, K33, K48, and K63) or the N-terminal methionine residue (M1) [27, 31]. The polyubiquitin chain can elongate using the same lysine residue on each ubiquitin (homogeneous ubiquitin chain) or the polyubiquitin chain can form through conjugation with mixed topology (heterogeneous ubiquitin chain). The different ubiquitination modification patterns provide a way to increase the diversity of protein regulation and functions. For example, the polyubiquitin chain linked through Lys48 (K48-linked polyubiquitin) provides a signal for protein degradation by the 26S proteasome, thereby regulating the stability of proteins [32]. The K11-linked polyubiquitin can trigger the degradation of cell cycle regulators via the ubiquitin-proteasome pathway during mitosis [33, 34]. Moreover, ubiquitination modification also regulates protein-protein interaction, enzyme activity, and the cellular localization of proteins. The K63-linked polyubiquitin was reported to be involved in signal transduction [35], kinase activation [36, 37], and protein-protein interaction [21]. Together, these findings indicate that ubiquitin modification affects diverse cellular processes by regulating the stability and the function of proteins. The temporal and spatial control of ubiquitin signaling plays a pivotal role to maintain normal cellular functions.

2.2. Deubiquitinating enzymes (DUBs)

Protein ubiquitination is a reversible posttranslational modification process. The process to cleave ubiquitin from proteins and other molecules is called deubiquitination, and the process is catalyzed by a large group of ubiquitin-cleaving proteases, the deubiquitinating enzymes (DUBs) [38–41]. DUBs can be classified into five families: ubiquitin-specific proteases (USPs), ubiquitin C-terminal hydrolase (UCHs), ovarian tumor proteases (OTUs), Machado-Joseph disease domain proteases (MJDs), and Jab1/Mpn/Mov34 metalloenzymes (JAMMs) and play several critical roles in the ubiquitin pathway [38, 40]. First, they are responsible for processing inactive ubiquitin precursors. The ubiquitin is translated in the form of linear polyubiquitin chain or fusion with ribosomal proteins. DUBs are needed to generate free ubiquitin monomer. Second, DUBs can antagonize E3 ligases by removing the ubiquitin molecule or trimming the ubiquitin chain form substrates, thereby changing the ubiquitin signaling or stability of targeted proteins. Finally, DUBs are required for the recycling of ubiquitin molecule. The ubiquitin molecules cleaved from substrates or ubiquitin chains can re-enter to the free ubiquitin pool. The coordination of E3 ligases and DUBs leads to conjugating, trimming, and removing ubiquitin modification of target proteins for various biological processes.

3. The role of ubiquitin modifications in autophagy regulation

Recent investigations have implicated the involvement of complex signaling pathways during different stages of autophagic process. Modifying autophagy-related proteins (Atg) by posttranslational modification is one of the important mechanisms to control proper autopha-
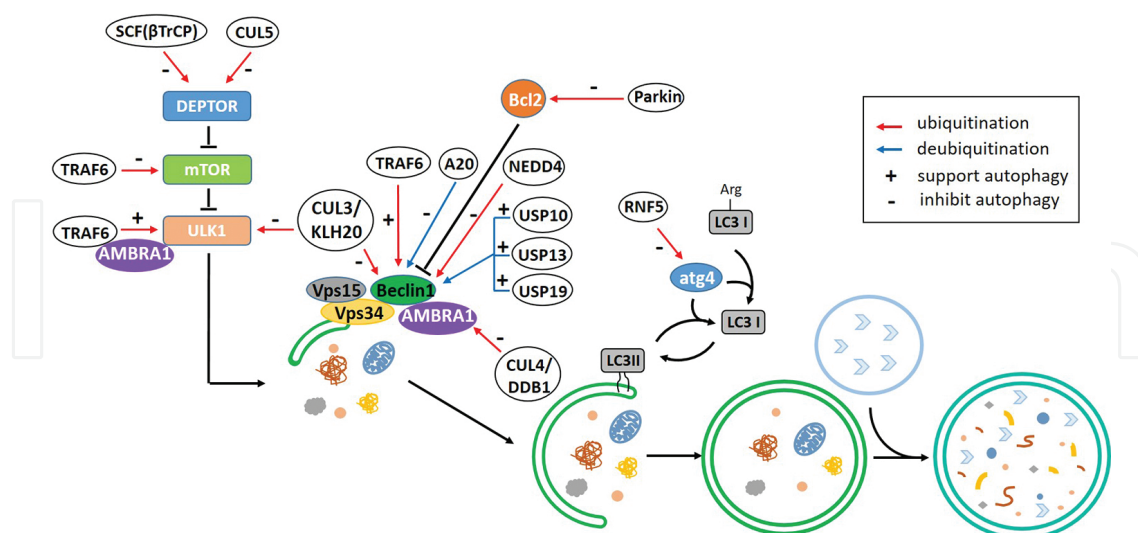

Figure 1. Functional role of ubiquitin-related enzymes (E3 ligases and DUBs) in autophagy regulation. An overview shows the involvement of the ubiquitination events during different steps of autophagy. Ubiquitination modifications by E3 ligases are shown in red arrows, and deubiquitination by DUBs are shown in blue arrows. "+" indicates that enzymes play positive regulatory roles (USP10, USP13, and USP19), and "−" indicates those thought to play negative roles (SCF$^{\beta TrCP}$, CUL5, CUL3/KLH20, A20, NEDD4, CUL4/DDB1, Parkin, and RNF5) in autophagy. TRAF6 can function as positive or negative regulator in autophagy via ubiquitination modification of different substrates.

gic activity [15, 16]. Many autophagy regulators are found to be substrates for ubiquitin E3 ligases or deubiquitinating enzymes (DUBs) [42, 43]. Ubiquitination modification of these autophagy regulators controls autophagy induction, nucleation, maturation, or termination. Here, we are going to review recent findings on the role of E3 ligases and DUBs in the regulation of autophagy (**Figure 1**).

3.1. Ubiquitin systems and autophagy initiation

Many signaling pathways participate in the induction of autophagy. The inhibition of mTOR function and the activation of Ulk1 complex are two major mechanisms in the initiation of autophagy [8]. Several ubiquitination enzymes have been shown to participate in the induction of autophagy by modifying the initiators of autophagy [44–47]. It has been reported that mTOR inhibitor, DEPTOR, can be ubiquitinated by SCF$^{\beta TrCP}$ E3 ubiquitin ligase and CUL5, and led to the degradation of DEPTOR. In response to growth signals, DEPTOR is phosphorylated by the downstream components of mTOR pathway such as RSK1 and S6K1, and the phosphorylated DEPTOR is then targeted for ubiquitination by SCF$^{\beta TrCP}$ E3 ligase for degradation [44, 45]. Interestingly, Antonioli et al. recently showed that CUL5 can also catalyze the DEPTOR ubiquitination and promote its degradation under normal conditions. The degradation of DEPTOR leads to the activation of mTOR, which acts as an inhibitor of autophagy. Upon autophagy stimulation, the CUL5-mediated degradation of DEPTOR is inhibited by Ambra1 in an Ulk1-dependent manner and promotes the onset of autophagy [46, 47].

The mTOR activity can also be regulated by ubiquitination through the TRAF6 E3 ligase [48]. Upon amino acids stimulation, TRAF6 is recruited to mTOR complex 1 (mTORC1) through p62 and catalyzes K63-linked polyubiquitination of mTOR which is required for mTORC1 translocation to the lysosome and its subsequent activation. Moreover, TRAF6 has also been shown to promote K63-linked polyubiquitination of Ulk1, which results in Ulk1 stabilization, self-association, and autophagy induction [49]. The TRAF6-mediated Ulk1 ubiquitination depends on Ambra1 which is also a substrate target of Ulk1 during autophagy induction. These findings together indicate that ubiquitin system and ubiquitin-related enzymes play a critical role in autophagy initiation.

3.2. Ubiquitin systems and autophagy nucleation

The Beclin1 and Vps34, a class III phosphoinositide 3-kinase (PI 3-kinase), are the key regulators in the nucleation step of autophagy [8]. Beclin1 acts as an adaptor, which recruits cellular components such as Ambra1 and UVRAG to form different Beclin1-Vps34 complexes that are responsible for modulating the activity of Vps34. Recent studies have shown that the interaction between Beclin1 and its binding partners can be regulated by PTMs including ubiquitination [21].

It was reported that, upon lipopolysaccharide (LPS) stimulation, TRAF6 catalyzes K63-linked polyubiquitination of Beclin1 at K117 [50]. The TRAF6-mediated Beclin1 ubiquitination leads to the disassociation of Beclin1 with Bcl2 and promotes autophagy. On the contrary, the deubiquitinating enzyme A20 antagonized the TRAF6-mediated Beclin1 ubiquitination and

abrogated autophagy induction. Recently, Chen et al. showed that the E3 ubiquitin ligase Parkin, which is found to be involved in the neurodegenerative Parkinson's disease (PD), can also catalyze the monoubiquitination of Bcl2 [51]. Parkin-mediated Bcl2 ubiquitination increases the steady-state levels of Bcl2 and enhances the interactions between Bcl2 and Beclin1, leading to the inhibition of autophagy. Moreover, E3 ubiquitin ligase Nedd4 (neural precursor cell expressed developmentally down-regulated protein 4) promotes Beclin1 degradation through proteasomal system in the absence of Vps34 interaction [52]. Nedd4 controls the stability of Beclin1 via K11-linked polyubiquitination. Through the degradation of Beclin1, Nedd4 acts as a negative regulator of autophagy. Besides E3 ligases, Liu et al. showed that USP10 and USP13 DUBs also participate in the autophagy nucleation by regulating the stability of Beclin1-Vps34 complex components including Vps34, Beclin1, Vps15, and Atg14L [53]. Unexpectedly, Beclin1-Vps34 complex also promotes the stability and activity of USP10 and USP13 [53, 54]. Recently, the deubiquitinating enzyme USP19 was found to stabilize Beclin1 by removing the K11-linked ubiquitin chains of Beclin-1 at lysine 437 and act as a positive regulator of autophagy [55]. Moreover, USP19 inhibits RIG-I-mediated type I interferon (IFN) signaling and antiviral immune responses by blocking RIG-I-MAVS interaction in a Beclin-1-dependent manner. In addition, the deubiquitinating enzyme USP33 is also involved in autophagy induction by deubiquitinating the RAS-like GTPase RALB under starvation conditions [56]. RALB interacts with the exocyst components EXO84. Upon nutrient deprivation, the USP33-mediated deubiquitylation of RALB induces the assembly of RALB-EXO84-Beclin1 complex and the initiation of autophagy.

By interfering the interaction or by controlling the stability of the Vps34-Beclin1 complex components, the ubiquitin system provides a flexible and diverse way to regulate autophagy nucleation.

3.3. Ubiquitin systems and autophagosome elongation/expansion

The Atg8 (LC3 and GABARAP in mammals) and Atg12 ubiquitin-like conjugation systems are two major pathways involved in the regulation of autophagosomal elongation and expansion [57]. Similar to ubiquitination, Atg12 is conjugated to the lysine residue in Atg5 by the E1 enzyme Atg7 and the E2 enzyme Atg10. The Atg12-Atg5 conjugate subsequently forms a complex with Atg16 for phagophore membrane elongation. On the other hand, Atg8 is first processed at the C-terminus by the cysteine protease Atg4 and then activated by Atg7 (E1) and Atg3 (E2) for the conjugation of the lipid phosphatidylethanolamine (PE). Atg4 is also required for the cleavage of Atg8 from PE on the autophagic membrane after the completion of autophagosome formation. As Atg4 plays a critical role in the phagophore expansion and autophagosome completion, depletion of Atg4 inhibits the processing of Atg8 paralogues and autophagy. It has been shown that the membrane-associated E3 ligase RNF5 regulates autophagy by ubiquitinating Atg4b and promoting the proteolytic degradation of Atg4b [58].

3.4. Ubiquitin systems and autophagy termination

Like the initiation of autophagy, the termination of autophagy is also tightly regulated after completion of each run. The failure of autophagy termination under prolonged starvation will

lead to unrestrained cellular degradation and cell death [59]. In addition to mTOR activation induced by the regeneration of intracellular nutrients, recent studies revealed that the proteasomal degradation of autophagy components also plays a critical role in controlling autophagy termination [46, 59]. It has been shown that CUL4 controls autophagy termination by promoting Ambra1 ubiquitination and regulating Ambra1 protein levels [47]. Under high nutrient conditions, DDB1/CUL4 mediates Ambra1 ubiquitination and maintains Ambra1 at low level. Upon starvation, CUL4 dissociates with Ambra1, and Ambra1 is stabilized by Ulk1 phosphorylation. The phosphorylated Ambra1 inhibits CUL5-mediated degradation of DEPTOR and further downregulating mTOR activity. Under prolonged stress conditions, DDB1/CUL4 re-establishes its interaction with Ambra1 and promotes the ubiquitination and degradation of Amba1, which in turn leads to autophagy termination [46].

Recently, Liu et al. reported that CUL3 also participates in the termination of autophagy [59]. KLHL20/CUL3 recruited autophosphorylated Ulk1 for ubiquitination and degradation under stress conditions. Moreover, KLHL20/CUL3 also promotes ubiquitination of phagophore-residing VPS34 and Beclin1, and the ubiquitination leads to their degradation. KLHL20/CUL3 plays a crucial role in autophagy termination by regulating the turnover of Ulk1 and VPS34 complex to restrain the amplitude and duration of autophagy.

To date, many ubiquitination events have been shown to participate in autophagy regulation. The ubiquitin-mediated modification functions at different steps of autophagy and targets at different substrates in response to distinct stress stimulation. Moreover, some of the ubiquitination modifications are antagonized by the deubiquitinating enzymes. Therefore, the ubiquitin system provides flexible, diverse, and effective ways to control the onset and the termination of autophagy.

4. Ubiquitination modification and selective autophagy

Although autophagy was originally thought to be a non-selective pathway which appears to randomly sequester cytosolic components for lysosomal degradation, it is now recognized that autophagy also acts in selective processes that involves specific receptors to target certain cargos [60, 61]. Accumulating evidence indicates that many intracellular degradation events are processed through selective autophagy, including the turnover of damaged organelles such as mitochondria (mitophagy) [62, 63] and peroxisomes (pexophagy) [64, 65], removal of protein aggregates (aggrephagy) [66], and elimination of intracellular pathogens (xenophagy) [67, 68].

Upon the induction of selective autophagy, phagophore is enriched with specific cargos in a process dependent on cargo receptors [61]. These cargo receptors can interact with both target proteins and the autophagic vesicle components such as LC3/Atg8 family proteins, which result in the enclosure of selective cargos to the autophagosome and promote the autophagic degradation of cargos. Like nonselective autophagy, selective autophagy also plays an important role in cellular homeostasis and has been associated with a variety of human diseases [63, 69].

4.1. The role of ubiquitin in selective autophagy

Ubiquitination has long been recognized as a key regulator to determine protein fate by tagging proteins for proteasomal degradation [60]. Ubiquitination of cargo proteins plays a crucial role in selective autophagy process. In selective autophagy, cargos are ubiquitinated and recognized by ubiquitin-binding receptors to transport cargos for lysosomal degradation [70]. Therefore, ubiquitin acts as a degradation signal for selective autophagy. Protein aggregates, damaged organelles, or pathogens can be tagged and targeted for degradation through the lysosome machinery to maintain cellular homeostasis. In this section, we will illustrate the mechanism and importance of ubiquitination in selective autophagy (**Figure 2**).

Recent studies have shown that selective autophagy is responsible for delivering a wide range of cargos to the lysosome for degradation [70–72]; however, the detailed mechanisms of selective degradation by lysosome remain largely unknown. Several types of adaptor proteins such as p62, NDP52, optineurin (OPTN), NBR1, and HDAC6, which contain the ubiquitin-binding motif, have been reported to target ubiquitinated cargos for lysosomal degradation under stress conditions [70, 71]. Besides the ubiquitin-binding motif, these cargo receptors often also contain a LC3-interacting region (LIR) or Atg8 interaction motif to interact with the LC3/Atg8 family members [60, 70]. Therefore, through binding to ubiquitinated cargos and LC3 simultaneously, these receptors can deliver selective cargos to the autophagosome and promotes the autophagic degradation.
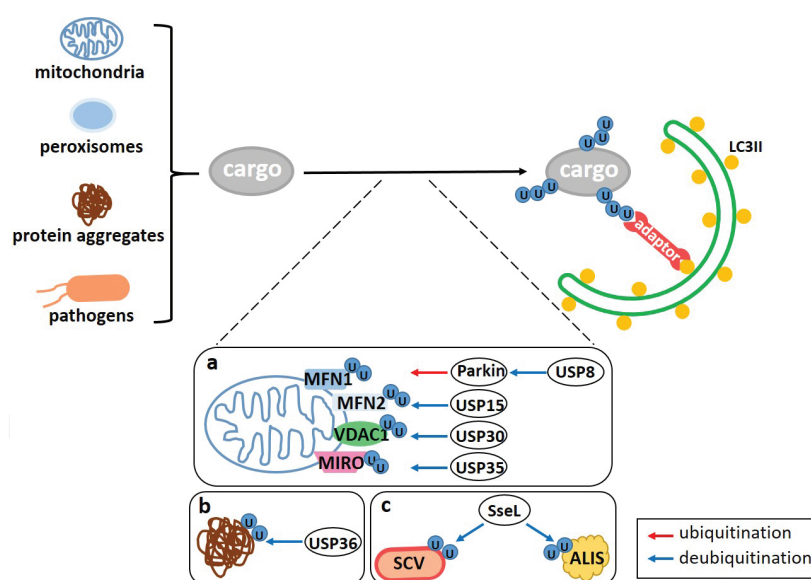

Figure 2. The involvement of ubiquitin-related enzymes (E3 ligases and DUBs) in selective autophagy. Selective autophagy is a process that depends on the ubiquitin signals and the ubiquitin recognition adaptor proteins. (a) The E3 ligase (Parkin) promotes mitophagy by catalyzing the ubiquitination of mitochondrial proteins, and DUBs (USP15, USP30, and USP35) inhibit mitophagy by removing the ubiquitin signals of mitochondrial proteins. USP8 participates in mitophagy by removing non-canonical K6-linked ubiquitin chains from Parkin, a process required for the efficient recruitment of Parkin to depolarized mitochondria. (b) USP36 removes the ubiquitin markers from protein aggregates, which inhibits aggrephagy. (c) The DUB SseL which is secreted by Salmonella can remove the ubiquitin tags on Salmonella-containing vacuole (SCV) and aggresome-like induced structures (ALIS) in order to escape xenophagy. "U" means ubiquitin (this involves different types of ubiquitination); the red arrows and the blue arrows indicate ubiquitination events and deubiquitination events, respectively.

Ubiquitinated cytosolic proteins can undergo degradation via proteasome or the lysosome. Proteins conjugated with K48-linked polyubiquitin chains often are recognized by UBD (ubiquitin-binding domain) containing proteasomal receptors and degraded by proteasome [73]. On the other hand, the ubiquitin-binding autophagy adaptors have been shown to interact with cargos containing K63-linked polyubiquitin chains [74–77]. Cargos modified with K63 polyubiquitination are preferentially targeted via the autophagy/lysosomal degradation pathway.

4.2. E3 ligases and DUBs in selective autophagy

Given that ubiquitin plays a critical role by acting as a tag for substrates recognition in selective autophagy, it is important to understand the regulatory mechanism of ubiquitin system during this process. E3 ubiquitin ligases and deubiquitiylating enzymes (DUBs) involved in the cargos ubiquitination are crucial in the selective autophagy regulation.

Autophagy of the mitochondria, also known as mitopahagy, depends on a set of ubiquitination modification on mitochondrial outer membrane proteins [63]. Upon the induction of mitophagy, Parkin, an E3 ubiquitin ligase that is also involved in the pathogenesis of Parkinson's disease, is recruited to the depolarized mitochondria [77, 78] to ubiquitinate several mitochondrial proteins, including MFN1, MFN2, VDAC1, and MIRO [79–81]. How is Parkin recruited to the damaged mitochondria? Recently, three studies showed that Pink1-mediated phosphorylation of ubiquitin at Ser65 activates Parkin [82–84]. The accumulation of ubiquitinated mitochondrial proteins then recruits the autophagy adaptors NDP52 and optineurin, which then promote the formation of mitophagy [85, 86]. Mitophagy is also modulated by a number of DUBs. It has been shown that USP15 [87], USP30 [88, 89], and USP35 [89] reduce the ubiquitin levels from the ubiquitinated mitochondrial proteins, thereby preventing the recognition by autophagy adaptors and blocking mitophagy. Moreover, TRAF6 [90] and USP8 [91] can also participate in mitophagy by regulating the ubiquitination of Parkin.

The selective degradation of protein aggregates requires aggregative proteins to be labeled with K63-linked ubiquitin chains which then are recognized by autophagy adaptors including p62 and NBR1 and HDAC6 [92]. Taillebourg et al. recently showed that DUB USP36 can act as a negative regulator to inhibit the selective autophagy of protein aggregates by removing the ubiquitin signals [93]. However, the specific ubiquitin ligases involved in aggrephagy remain to be identified.

The process of selective autophagy also plays a crucial role in host defense. It has been shown that the intracellular pathogen Salmonella Typhimurium can be eliminated by selective autophagy [94]. After infection, Salmonella Typhimurium grows in a membranous compartment, the Salmonella-containing vacuole (SCV). The bacterial infection often induces immune and nonimmune cells forming aggresome-like induced structures (ALIS). The host cell can eliminate SCV and ALIS by ubiquitination and xenophagy. However, S. Typhimurium can remove the ubiquitin signals by secreting the deubiquitinating enzyme SseL, which leads to lower autophagy flux due to the failure of autophagy-receptor recognition [94].

The ubiquitin-mediated selective autophagy plays an important role in maintaining cellular homeostasis and in the elimination of invading pathogens. Therefore, it is critical to further identify the E3 ubiquitin ligases and DUBs involved in selective autophagy under physiological and pathological conditions.

5. Ubiquitin system and autophagy in human health and disease

Like the ubiquitin-proteasome system, autophagy is a tightly regulated lysosomal degradation pathway which has been implicated in various human pathological and physiological processes. Basal autophagy is essential for removing misfolded proteins and damaged organelles; therefore, autophagy is also important for maintaining normal cellular processes in all tissues [95, 96]. Since the ubiquitin system serves as a central regulator to modify the autophagic activity and functions, it is no doubt that the protein modification by ubiquitination and deubiquitination also play crucial roles in autophagy-related diseases [97–100]. However, the relationship between the ubiquitin system and the autophagy-related pathological processes remain unclear. In this section, we will discuss the recent findings and progresses in the field.

5.1. Ubiquitin system and autophagy in cancer

Cancer is one of the first human diseases identified to be associated with autophagy malfunction [101]. Some autophagy genes mutation or deletion can lead to cancer. For instance, monoallelic deletion of Beclin1 gene has been detected in 40–75% of human breast, ovarian, and prostate cancer [102]. Besides Beclin1, many autophagy genes are found to be involved in human cancer, including UVRAG [103], Atg5, and Atg7 [104]. Accumulating evidence has indicated that autophagy also plays a crucial role in cancer cell progression. Autophagy likely plays distinct roles during different stages of cancer development [105]. It has been shown that autophagy has a preventive effect against tumorigenesis and the cancer occurrence during early cancer formation. However, autophagy provides a protective mechanism and supports the tumor growth once cancer progresses [105–107].

Although the role of autophagy in cancer progression remains elusive, several recent studies have shown that the inhibition of autophagic pathway can enhance the efficacy of anticancer drugs [108, 109]. Shao et al. showed that autophagy inhibitor-1 (spautin-1), an inhibitor of USP10 and USP13, can enhance Imatinib mesylate (IM)-induced cell death in chronic myeloid leukemia (CML) in a Beclin1-dependent manner [102]. Since autophagy plays a role in IM resistance and spautin-1 inhibits IM-induced autophagy in CML cells, inhibition of autophagy with the DUBs inhibitor spautin-1 may provide a promising approach to increase the efficacy of IM for patients with CML [110]. In another study, Yang et al. showed that knockdown of the regulator of CUL1 (ROC1) suppresses the growth of liver cancer cells through the induction of autophagy and senescence [103]. The triggering of autophagic response in ROC1 silencing cells is through the accumulation of the mTOR inhibitory protein DEPTOR [111]. Another link of ubiquitination regulation in autophagy and cancers is that the ubiquitin modification of

Beclin1 and p53 by E3 ligases or DUBs can balance the interaction between Beclin1 and p53, and their interaction is thought to regulate the cellular decision between apoptosis and autophagy in embryonal carcinoma cells [43].

As ubiquitination modification in autophagy regulation plays a notable role in cancer cells, it is crucial to further investigate the detailed mechanisms of how ubiquitin system regulates autophagic function during cancer progression. Findings from these studies will provide new insights into cancer biology as well as novel approaches in cancer prevention and treatment.

5.2. Ubiquitin system and autophagy in neurodegenerative diseases

In recent years, there is increased attention on the role of autophagy in neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) [112, 113]. These neurodegenerative disorders are usually characterized by the presence of protein inclusions and aggregates in neurons, which result from the failure of protein degradation, and these protein aggregates may be one cause of the progressive degeneration and/or death of neuronal cells [113, 114]. The ubiquitin-proteasome and autophagy-lysosome pathways are the two major pathways to degrade misfolded proteins and damaged organelles [60]. Accumulating evidence indicates that the dysfunction of autophagy may result in the accumulation of abnormally folded protein aggregates, which may contribute to neurodegenerative disorders [112, 114]. Although there are growing studies indicating the importance of autophagy in the neurodegenerative diseases, the molecular mechanisms of how autophagy or selective autophagy functions in these disorders are still not completely understood.

Ubiquitination of cellular proteins and organelles has been shown to promote the autophagic clearance of cargos associated with neurodegenerative diseases [76, 112, 115]. Parkin is a multifunctional ubiquitin ligase that has been found to be mutated in sporadic and familial early onset Parkinson's disease [116]. The involvement of Parkin and DUBs such as USP15, USP30, and USP35 in mitophagy has also been demonstrated to be critical in neurodegeneration related to PD [43, 116]. Besides its role in regulating mitochondrial homeostasis, it has been shown that Parkin catalyzes the ubiquitination modification of misfolded proteins, which then promotes the degradation of these substrates via proteasome or autophagy pathway [116]. The dysfunction of Parkin leads to the accumulation of protein aggregates and causes some neurodegenerative diseases. It was also reported that the C-terminus of Hsc70 interacting protein (CHIP) E3 ubiquitin ligase can promote the ubiquitination of denatured proteins and play an important role in neurodegeneration. By binding to different E2 enzymes, CHIP can catalyze K48- and K63-linked polyubiquitin chains which promote proteins degradation via chaperone-dependent ubiquitin-proteasome system and autophagy-lysosome pathway, respectively [117].

5.3. Ubiquitin system and autophagy in infectious diseases

Activation of autophagy provides a promising approach in the treatment of infectious diseases. Recent studies have shown that this cellular process can either selectively target microorgan-

isms for lysosomal degradation (referred to as xenophagy) or promote the delivery of microbial nucleic acids and antigens to endo/lysosomal compartments for innate and adaptive immunity activation [118–120]. Accumulating evidence indicates that autophagy activity is higher upon the pathogen infection [119], and it is also known that autophagy can facilitate the intracellular antigen-processing events [118]. Moreover, the autophagy pathway can cross talk with immunity pathways [50, 120]. Interestingly, several reports also indicate that autophagy may provide a pathway for pathogens to escape from host defense and help them to invade host tissues [97].

The role of autophagy in immunity has been further confirmed by the finding of the connections between autophagy and several immune diseases. For example, Atg16 mutations are associated with increased risk of an inflammatory bowel disease, Crohn disease, which affects the gastrointestinal tract from the mouth to the anus [98]. Moreover, several studies have linked single-nucleotide polymorphisms (SNPs) in ATG5 to systemic lupus erythematosus (SLE) susceptibility [119]. Ubiquitination regulation of autophagy regulators was also found to participate in the infectious events. The ubiquitin E3 ligase TRAF6 has been shown to catalyze the K63-linked polyubiquitination of Beclin1 upon LPS stimulation and is critical for TLR4-triggered autophagy in macrophages [50]. And the deubiquitinating enzyme A20 antagonizes TRAF6-mediated ubiquitination of Beclin1 and limits the induction of autophagy in response to TLR signaling. The balanced activity of TRAF6 and A20 is required for the inflammatory response [50]. Another indication that ubiquitination modification regulates inflammation through autophagy involves the E3 ligase RNF216 (ring finger protein 216). RNF216 was reported to inhibit autophagy in macrophages by catalyzing K48-linked polyubiquitination of Beclin1, which induces the degradation of Beclin1 [121]. Manipulating RNF216 expression may provide a therapeutic approach for treatment of inflammatory diseases. In addition, the ubiquitination modification processes participated in xenophagy also plays important roles for the bacterial infection. The DUB SseL regulates the ubiquitin modification of SCVs and ALIS and is important for the removal of pathogens [115]. Kuang et al. showed that RNF5 promotes the ubiquitination and the degradation of Atg4b limits the basal levels of autophagy and influences susceptibility to bacterial infection [58].

In addition to the diseases discussed earlier, there are also disorders related to autophagy dysfunction, including developmental defect, muscle atrophy, heart diseases, liver disease, and aging [95]. However, it remains unclear whether the ubiquitin system also plays a role in these diseases.

6. Conclusion

Protein ubiquitination is considered as one of the most important reversible posttranslational modifications and has been implicates in various cellular signaling processes. Increasing evidence indicates that the ubiquitin system plays a pivotal role in the regulation of autophagy pathway. Recent studies have explored and highlighted the important functions of ubiquitin system in the pathogenesis of autophagy-related diseases such as tumorigenesis,

neurodegeneration, and pathogen infection. Further investigations to identify novel E3 ligases and DUBs involved in autophagy and to determine their underlying mechanisms will not only contribute to our understanding on how autophagy is controlled by the ubiquitin system but also provide a rationale for novel therapeutic interventions in autophagy-related diseases.

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