



Minireview

Emerging Paradigm of Crosstalk between Autophagy and the Ubiquitin-Proteasome System

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Cellular protein homeostasis is maintained by two major degradation pathways, namely the ubiquitin-proteasome system (UPS) and autophagy. Until recently, the UPS and autophagy were considered to be largely independent systems targeting proteins for degradation in the proteasome and lysosome, respectively. However, the identification of crucial roles of molecular players such as ubiquitin and p62 in both of these pathways as well as the observation that blocking the UPS affects autophagy flux and *vice versa* has generated interest in studying crosstalk between these pathways. Here, we critically review the current understanding of how the UPS and autophagy execute coordinated protein degradation at the molecular level, and shed light on our recent findings indicating an important role of an autophagy-associated transmembrane protein EI24 as a bridging molecule between the UPS and autophagy that functions by regulating the degradation of several E3 ligases with Really Interesting New Gene (RING)-domains.

Keywords: autophagy, crosstalk, EI24, RING-domain, ubiquitin proteasome system

INTRODUCTION

After the determination of the structure of DNA in 1953 (Watson and Crick, 2003) followed by the elucidation of the central dogma of life that established proteins as cellular

work-horses (Crick, 1970), people began to wonder how proteins are destined for degradation and what the last moments in a protein's life look like (Pickart, 2004). Aaron Ciechanover, Avram Hershko, and Irwin Rose answered this crucial question with their discovery of ubiquitin-mediated proteolysis, for which they were deservedly awarded the Nobel Prize in Chemistry in 2004 (Hershko, 2005). Their groundbreaking work portrayed cellular protein degradation not as a random act of indiscriminate slaughtering but as a highly regulated process centered around a 76 amino-acid long molecule called ubiquitin (Ub) (Schmidt and Finley, 2014). Ubiquitin chains act as passwords providing poly-ubiquitinated target protein access to enter the proteolytic environment of the proteasome where they are degraded and recycled as amino acids (Labbadia and Morimoto, 2015). Covalent attachment of a polyubiquitin chain to a lysine residue of the target protein is carried out via the concerted action of three different ubiquitin enzymes (E1, E2, and E3) at the expense of ATP (Hershko et al., 1983). A chain of four or more Ubs is generally both necessary and sufficient to be transformed into a targeting signal for proteasome delivery (Thrower et al., 2000). The 26S proteasome, also known as "the proteasome," is a barrel-shaped proteolytic organelle comprised of a 20S central catalytic complex and two 19S lid complexes. The 19S complexes play regulatory roles by binding to cargo-loaded shuttling proteins, deubiquityling the substrates, and channeling them into the six proteolytic sites of the inner core of the 20S central subunit where the target

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proteins are degraded and recycled (Budenz et al., 2017; Collins and Goldberg, 2017; Livneh et al., 2016; Nandi et al., 2006).

While the proteasome is extremely efficient in degrading smaller polyubiquitinated proteins, large misfolded proteins and damaged organelles cannot enter the proteasome, owing to its small barrel-shaped structure (Groll and Huber, 2003). These larger substrates are delivered to and degraded in lysosomes, which are responsible for autophagy (Klionsky et al., 2008). The history of autophagy is several decades older than that of the ubiquitin-proteasome system (UPS); however, owing to the lack of clear mechanistic studies, this field remained dormant for some time and was instead dominated by UPS research (Dikic, 2017). Finally, autophagy returned to its glory days through the elegant works of Yoshinori Ohsumi, who single-handedly identified and characterized key autophagy genes in yeast in the 1990s (Suzuki and Ohsumi, 2007). He was awarded the Nobel Prize in Physiology or Medicine in 2016 for his work (Tooze and Dikic, 2016). The autophagy process is initiated by the formation and elongation of a double-layered phagophore that later develops into an autophagosome by enwrapping a portion of cytoplasm (Lamb et al., 2013). Autophagy, closely resembling the UPS, is carried out by the combined action of several autophagy-related genes (Atg) that are responsible for forming molecular complexes that work in sequential order to deliver the cytosolic cargo to the lysosomes (Ohsumi, 2014). For instance, the unc-51-like autophagy activating kinase 1 (ULK1)-ATG13-RB1-inducible coiled-coil 1 (RB1CC1), also known as FIP200-ATG101 complex is responsible for the induction of autophagosome formation downstream of the mechanistic target of rapamycin (mTOR) signaling pathways (Dikic, 2017). The phosphatidylinositol 3-phosphate kinase (PI3K) complex III that constitutes vacuolar protein sorting 34 (VPS34), BECLIN1, ATG14L, VPS15, and Autophagy/Beclin-1 regulator 1 (AMBRA1) participates in vesicle nucleation (Feng et al., 2014). One of the conjugation systems forms an ATG5-12 conjugate that is regulated by E1-like ATG7 and E2-like ATG10. The second system conjugates ATG8 (also known as LC3) to the lipid phosphatidylethanolamine (PE) that is mediated by ATG7 and E2-like ATG3. LC3 remains attached to the lipid while the ATG5-12 conjugate is removed after autophagosomes are formed, making LC3 a reliable autophagosomal marker in measuring rates of autophagosome formation and degradation (Nakatogawa, 2013). After being transported along microtubules, soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-like protein complexes facilitate the fusion of autophagosomes and lysosomes into autophagolysosomes, where the cargo is degraded by lysosomal hydrolases (Moreau et al., 2013).

AUTOPHAGY-UPS CONNECTION

Until recently, the UPS and autophagy were considered two parallel protein degradation machineries with no point of intersection (Korolchuk et al., 2009b). This idea was fostered partly because autophagy and the UPS have separate molecular machinery and substrate preferences (Korolchuk et

al., 2010). Autophagy is a vesicular trafficking pathway that specializes in the delivery of long-lived proteins and damaged organelles to the lysosome (Klionsky et al., 2008). The degradation of soluble, short-lived regulatory proteins by the UPS, on the other hand, occurs in the cytosol (Streich and Lima, 2014). According to the classical definition, the UPS is a selective degradation process for cellular proteins that require temporal control, such as regulatory and cell cycle-related proteins (Ciechanover, 2005). Autophagy, in contrast, is viewed as a cellular response that serves to scavenge nutrients when cells are subjected to starvation (Russell et al., 2014). However, from the cellular point of view, it would make sense that the two major protein-degradation machineries, with their implications in cellular homeostasis, communicate with each other. In line with this theory, studies conducted in the last decade have irrefutably confirmed this paradigm by unraveling the interplay between autophagy and the UPS at the molecular and functional levels (Dikic, 2017).

Possibly the strongest link between the UPS and autophagy comes from the observation that several molecules are shared as either regulators or substrates of both these pathways (Lilienbaum, 2013). One of the most crucial molecules that marks target proteins for degradation in both these pathways is ubiquitin (Tai and Schuman, 2008). In the UPS, E1-E2-E3 work together to conjugate polyubiquitin chains to the protein to be degraded (Nandi et al., 2006). In autophagy, the polyubiquitin chain is recognized by proteins such as p62 to recruit targets into autophagosomes (Komatsu et al., 2007). This form of autophagy that specifically degrades a ubiquitinated substrate is referred to as selective autophagy (Schreiber and Peter, 2014). A question then arises: how is the decision to degrade a particular ubiquitinated substrate either by autophagy or the UPS made? The nature of polyubiquitin chains determines the mode of degradation, with K48-linked chains being preferably degraded by the UPS and K63-linked chains as well as damaged mitochondria or monoubiquitinated substrates by autophagy (Kirkin et al., 2009; Kwon and Ciechanover, 2017). Autophagy adaptor proteins such as p62, a neighbor of BRCA gene 1 (NBR1), and histone deacetylase 6 (HDAC6) act as bridging molecules to link ubiquitin to the autophagosome (Cohen-Kaplan et al., 2016). These proteins contain ubiquitin-associated (UBA)-domains specialized for binding to ubiquitin and an additional domain dedicated to linkage to autophagosomes, such as the LC3-interacting region (LIR) that facilitates adaptor protein binding to LC3 (Kirkin et al., 2009). Thus, the presence of two domains, both specialized in their own ways in connecting autophagy and ubiquitin, makes adaptor proteins very important nodes of communication between the UPS and autophagy (Lilienbaum, 2013). Chaperone proteins such as c-terminus of HSP-70-interacting protein (CHIP) and BCL-2-associated anthanogenes (BAG1 and 3) also determine the fate of protein degradation either by autophagy or the UPS. The tetratricopeptide repeat domain in CHIP directs substrates for degradation by the UPS and U-box domain by the lysosome (Zhou et al., 2014). BAGs on the other hand interact with CHIP and channel protein degradation by the UPS (Kriegenburg et al., 2014).

Autophagic degradation by BAGs is facilitated by their interaction with HSPB8, a small heat shock protein (Gurusamy et al., 2009). Parkin, a known E3 ligase, also plays a critical role at the interface between the UPS and autophagy by mediating proteasomal degradation of a subset of mitochondrial substrates, whereas another subset undergoes autophagic degradation (Chan et al., 2011). In one of the most interesting discoveries of a direct connection between autophagy and the UPS, it was recently reported that autophagy could degrade the proteasome in a process called proteaphagy. Using *Arabidopsis* as a model system, the authors demonstrated regulatory particle non-ATPase 10 (RPN10)-mediated degradation of GFP-tagged of inactive 26S proteasomes by autophagy (Marshall et al., 2015).

The strongest evidence of the functional interconnection between autophagy and the UPS came from the observation that UPS inhibition affects the autophagy pathway and *vice versa* (Lilienbaum, 2013). It is well known that the autophagy pathway is activated to compensate for reduced UPS activity to relieve cells from the cytotoxic effects of accumulated proteins (Shen et al., 2013). Using experimental models in *Drosophila*, it was verified that upregulation of autophagy can confer a protective effect against cell death caused by proteasome inhibition (Pandey et al., 2007). Activation of autophagy in the case of proteasome inhibition is mediated by the unfolded protein response (UPR) that results in the activation of transcription factor ATF4 causing the upregulation of autophagy genes (B'Chir et al., 2013). In another parallel pathway, proteasome inhibition results in the activation of the IRE1-JNK1 pathway that releases the inhibitory brake of Bcl2 on Beclin1 through phosphorylation (Wei et al., 2008). Several evidences also suggest a role for the master tumor suppressor p53 in mediating crosstalk between the UPS and autophagy. Upon blockage of the UPS, p53 protein accumulates, resulting in the p53-mediated activation of AMP-activated protein kinase (AMPK) and the subsequent inhibition of mTOR and induction of autophagy through damage-regulated autophagy modifier (DRAM) (Crighton et al., 2006).

Mitochondria and endoplasmic reticulum (ER) are the two cellular organelle sensors of reduced proteasome activity. Accumulation of unfolded and damaged proteins due to inactive UPS results in alterations to the mitochondrial proteome, in turn leading to a burst of mitochondria-originating ROS, which triggers autophagy through activated AMPK (Zhao et al., 2016). Since mitochondria are specialized in producing ATP, the state of cellular energy reserve is also an important factor that mediates autophagy-UPS crosstalk. When energy is depleted, cellular ATP levels are reduced, which increases the concentration of AMP, activates AMPK, and suppresses mTOR, resulting in the activation of autophagy (Gomes et al., 2011). Thus, a special kind of autophagy called mitophagy clears those mitochondria that are damaged and unable to produce ATP (Youle and Narendra, 2011). Further strengthening the claim that the mitochondrion acts as a crosstalk organelle between autophagy and the UPS, it was recently reported that mitochondrial E3 ligase RNF185 regulates autophagy (Tang et al., 2011). The UPS, on the other hand, works smoothly when cells have

sufficient ATP reserves (Hershko, 2005). Therefore, the levels of ATP—representing energy status—determines whether a cell goes through autophagy or proteasome-mediated protein degradation. A recent study reported the import of misfolded proteins into mitochondria, suggesting that this organelle could be a shuttling hub for UPS substrates to be targeted for degradation by mitophagy when the UPS machinery is overwhelmed (Ruan et al., 2017).

Failure to properly fold newly synthesized proteins can also act as a trigger to initiate communication between autophagy and the UPS. After proteins are synthesized, they are correctly folded in the endoplasmic reticulum (ER) (Araki and Nagata, 2011). An accumulation of misfolded proteins in ER results in the retrotranslocation of proteins to the cytosol where they are ubiquitinated and degraded by the proteasome (Meusser et al., 2005). In cases of UPS overloading that compromises the ER-associated degradation (ERAD) pathway, however, compensatory autophagy is activated (Houck et al., 2014). In another parallel pathway, ER-stress caused by UPS inhibition results in the dissociation of the nuclear factor like 2 (NRF2)-Kelch-like ECH-associated protein 1 (KEAP1) complex and upregulation of Nrf2 target genes, several of which induce autophagy (Jiang et al., 2015). Crosstalk between autophagy and a special type of UPS-mediated proteolysis degrades proteins by the N-end rule pathway, based on the N-terminal arginylation of target proteins (Sriram et al., 2011). In cases of ER stress, arginylated glucose regulated protein (GRP78/BiP) associates simultaneously with misfolded proteins and p62 in the stressed cytosol leading to allosteric activation, aggregation, and delivery of cargo-loaded p62 to autophagosomes (Chamolstad et al., 2015). p62, thus mediates the crosstalk between the ubiquitin-proteasome system and autophagy through binding Nt-Arg and other N-degrons (Chamolstad et al., 2017).

While UPS inhibition activates autophagy, evidence for compensatory activation of the proteasome when autophagy is blocked is not available (Wang and Wang, 2015). Nonetheless, several studies have pinpointed the fact that the UPS cannot act as the backup protein degradation machinery when autophagy is blocked (Liu et al., 2016). The general consensus on an explanation for this observation is that most of the autophagosomal substrates are too large to be channeled through the barrel of the proteasome (Park and Cuervo, 2013). In line with this theory, it was reported that reduced autophagy impairs the clearance of p62 and that the resulting accumulation of p62 and subsequent sequestration of ubiquitinated proteins delays delivery to the proteasome (Korolchuk et al., 2009a). This notion was further verified in mouse models lacking essential autophagy genes (Atg5, Mizushima and Levine, 2010; or Atg7, Komatsu et al., 2005) that accumulate ubiquitinated protein aggregates. The critical role that p62 plays as a negative factor in UPS activity when autophagy is blocked is due to its intrinsic ability to oligomerize, thus, being unable to be channeled through the proteasome (Liu et al., 2016). At the physiological level, autophagy inhibition also reportedly abrogates DNA-repair mechanisms by inhibiting the function of p62 to recruit FLNA (Filamin A) and recombinase RAD51 to

the double-strand break sites (Hewitt et al., 2016).

While most studies describing autophagy-UPS crosstalk have focused on the compensatory and complementary relationship between these pathways, there are comparatively few examples of autophagy and the UPS being simul-

taneously required for a particular function. In one such study, it was reported that both the UPS and autophagy contribute to muscle atrophy in fasting (Zhao et al., 2007). The list of molecules that have been reported to mediate UPS-autophagy crosstalk is summarized in Table 1.

Table 1. Molecules facilitating UPS-autophagy crosstalk

Gene ID	Symbol	Description	Functions between UPS and autophagy	Reference	PMID
468	ATF4	Activating transcription factor 4	Upregulating autophagy genes when proteasome is inhibited	B'Chir et al. (2013)	23804767
9474	ATG5	Autophagy related 5	Accumulates ubiquitylated protein aggregates	Mizushima et al. (2010)	20811354
10533	ATG7	Autophagy related 7		Komatsu et al. (2005)	15866887
9532	BAG	BCL-2-associated athanogene	Interacts with CHIP and channels protein degradation by UPS, interact with HSPB8 and facilitate autophagic degradation	Kriegenburg et al. (2014)	24497846
664	BNIP3	BCL2 interacting protein 3	Mitochondrial receptor for Parkin-mediated mitophagy	Zhang et al. (2009)	19229244
672	BRCA1	BRCA1, DNA repair associated	Contain ubiquitin-associated (UBA)-domain specialized for binding to ubiquitin and an additional domain dedicated to linkage to autophagosomes	Cohen-Kaplan et al. (2016)	27448843
10241	CALCOCO2	Calcium binding and coiled-coil domain 2	Autophagy adaptor protein	Nozawa et al. (2017)	28848034
10645	CaMKK β	Calcium/calmodulin dependent protein kinase kinase 2	Senses impaired proteasome activity to regulate autophagy	Deshmukh, et al. (2015)	26227473
867	CBL	Cbl proto-oncogene		Sandilands et al. (2011)	22138575
10273	CHIP (STUB1)	STIP1 homology and U-box containing protein 1	Tetratricopeptide repeat domain in CHIP directs substrates for degradation by UPS and U-box domain by the lysosome	Zhou et al. (2014)	24497840
55332	DRAM1	DNA damage regulated autophagy modulator 1	Mediates stress response working together with KEAP1 and p62	Mrschik et al. (2016)	27046253
9538	EI24	EI24, autophagy associated transmembrane protein	Facilitates autophagy-UPS crosstalk by mediating autophagy-dependent degradation of RING-domain E3 ligases	Devkota et al. (2016)	27541728
1965	EIF2S1	Eukaryotic translation initiation factor 2 subunit alpha	Binding of misfolded proteins	B'chir et al. (2013)	23804767
2308	FOXO1	Forkhead box O1	Senses impaired proteasome activity to regulate autophagy	Milan et al. (2015)	25858807
139341	FUNDC1	FUN14 domain containing 1	Kinase working with Parkin for mitophagy	Chen et al. (2015)	27050458
2932	GSK-3 β	Glycogen synthase kinase 3 beta	Mitophagy/viral autophagy	Marchand et al. (2015)	25561726
10013	HDAC6	Histone deacetylase 6	Contains ubiquitin-associated (UBA)-domain specialized for binding to ubiquitin and an additional domain dedicated to a linkage to autophagosomes	Cohen-Kaplan et al. (2016)	27448843
3320	HSP90AA1	Heat shock protein 90 alpha family class A member 1	Binding of misfolded proteins	Dokladny et al. (2015)	25714619
3308	HSPA4	Heat shock protein family A (Hsp70) member 4			
3309	HSPA5 (GRP78/BIP)	Heat shock protein family A (Hsp70) member 5	Associates simultaneously with misfolded proteins and p62 in stressed cytosol leading to allosteric activation, aggregation, and delivery of cargo-loaded p62 to autophagosomes	Molstad et al. (2015)	26075355
3064	HTT	Huntingtin	Common substrate for autophagy and UPS	Koyuncu et al. (2017)	28753941
9817	KEAP1	Kelch like ECH associated protein 1	Mediates stress response working together with KEAP1 and p62	Dodson et al. (2015)	26205490
3920	LAMP2	Lysosomal associated membrane protein 2	Lysosomal receptor for chaperone-mediated autophagy	Eskelinen et al. (2002)	12221139
84557	LC3	Microtubule-associated proteins light chain 3	Autophagosome marker, 20S proteasome-dependent proteasomal degradation	Gao et al. (2010)	20061800

(continued)

Table 1. Molecules facilitating UPS-autophagy crosstalk

Gene ID	Symbol	Description	Functions between UPS and autophagy	Reference	PMID
2475	mTOR	Mechanistic target of rapamycin kinase	Senses impaired proteasome activity to regulate autophagy	Kim et al. (2011)	21258367
4077	NBR1	NBR1, autophagy cargo receptor	Autophagy adaptor protein	Kirkin et al. (2009)	19250911
4780	NRF2	Nuclear factor, erythroid 2 like 2	Mediates stress response working together with KEAP1 and p62	Dodson et al. (2015)	26205490
10133	OPTN	Optineurin	Autophagy adaptor protein	Wong et al. (2014)	25294927
8878	p62 (SQSTM1)	Sequestosome 1	Contains ubiquitin-associated (UBA)-domain specialized for binding to ubiquitin and an additional domain dedicated to a linkage to autophagosomes	Cohen-Kaplan et al. (2016)	27448843
5071	Parkin (PRKN)	Parkin RBR E3 ubiquitin protein ligase.	Role at the interface between UPS and autophagy by mediating proteasomal degradation of a subset of mitochondrial substrates	Chan et al. (2011)	21296869
65018	PINK1	PTEN induced putative kinase 1	Kinase working with Parkin for mitophagy	Drapalo et al. (2017)	28803490
5562	PRKAA1	Protein kinase AMP-activated catalytic subunit alpha 1	Senses impaired proteasome activity to regulate autophagy	Kim et al. (2011)	21258367
91445	RNF185	Ring finger protein 185	K63 ubiquitylation of BNIP1 and p62, LC3 recruitment in the autophagosome	Fei et al. (2011)	21931693
6048	RNF5	Ring finger protein 5	Stabilizes ATG4B	Kuang et al. (2012)	23093945
57154	SMURF1	SMAD specific E3 ubiquitin protein ligase 1	Mitophagy/viral autophagy	Orvedahl et al. (2011)	22020285
6622	SNCA	Synuclein alpha	Common substrate for autophagy and UPS	Wani et al. (2017)	28724388
8887	TAX1BP1	Tax1 binding protein 1	Autophagy adaptor protein	David et al. (2015)	26451915
7157	TP53	Tumor protein p53	Transcriptional activation of target genes that induce autophagy	White et al. (2016)	27037419
7189	TRAF6	TNF receptor associated factor 6	Autophagy-mediated clearance of pathogens	Pu et al. (2017)	28825144
10206	TRIM13	Tripartite motif containing 13	Activation of autophagy during ER stress	Tomar et al. (2012)	22178386
7314	Ub (UBB)	Ubiquitin	Tags proteins for UPS/autophagy-dependent degradation	Kraft et al. (2010)	20811356

AUTOPHAGY-UPS CONNECTION THROUGH THE RING-DOMAIN

E3 ligases are major stakeholders in the UPS system, as they are the final executors of ubiquitin tagging (Ardley and Robinson, 2005). The functional domain present in the majority of E3 ligases is the RING-domain that binds to an E2 and facilitates the transfer of ubiquitin to the protein destined to be degraded (Metzger et al., 2014). In this section of the review, we highlight our recent work that demonstrated a critical connection between RING-domain E3 ligases and autophagy machinery (Devkota et al., 2016). We previously reported that EI24 suppresses epithelial-to-mesenchymal transition (EMT) and tumor progression by suppressing RELA proto-oncogene/NF- κ B subunit (RELA/NF κ B p65) activity by promoting autophagy-dependent degradation of RING E3 ligases, including TNF receptor associated factor 2 (TRAF2) and TRAF5 (Choi et al., 2013). In a separate study, we also reported that EI24-induced degradation of tripartite motif containing 41 (TRIM41/RINCK1) results in protein kinase c α (PKC α) stabilization and that this function of EI24 is important for the development of 7,12-dimethylbenz [a]-anthracene (DMBA)-12-O-tetradecanoylphorbol-13-acetate (TPA)-induced skin carcinogenesis in mice (Devkota et al., 2012). Based on these studies illustrating degradation of RING-domain E3 ligases by EI24 and recent reports describing EI24 as an essential autophagy gene in *C. elegans* and mice (Zhao et al., 2012), we hypothesized that EI24 is the communication point between the

UPS and autophagy by virtue of its ability to degrade RING E3 ligases (Fig. 1).

To elucidate the mechanism of RINCK1 degradation by EI24, we first examined whether EI24-mediated degradation of RINCK1 occurs via the UPS or via autophagy. Our results revealed that EI24-mediated degradation of RINCK1 could be relieved in the presence of an autophagy inhibitor but not a proteasome inhibitor. This observation provided the first clue that the central players in the UPS, i.e., E3 ligases, could themselves be the target of the autophagy machinery. Furthermore, domain mapping revealed that the RINCK1-RING domain was required for binding and to be degraded by EI24. Until now, the destiny of E3 ligases was that they are primarily regulated by self-ubiquitination and degradation by the proteasome or recycling (de Bie and Ciechanover, 2011). However, our results indicated that EI24 recognizes the RING domain that is present in the majority of E3 ligases and degrades them using the autophagy pathway, suggesting the existence of another facet of RING-domain E3 ligase regulation. We then extended the E3 ligase screen to include more RING-domain candidates. Out of 20 RING-domain E3 ligases tested, 14 (70%) were found to be degraded by EI24 (TRAF2, TRAF5, RINCK1, RINCK2, TRIM1, TRIM3, TRIM4, TRIM6, TRIM21, TRIM2, TRIM28, TRAF6, CIAP1, and MDM2), whereas 6 (30%) were not (TRIM5, TRIM8, TRIM20, Parkin, XIAP, and CIAP2). Based on the primary screening data, we sought to ascertain whether it would be possible to formulate a generalized rule that gives us the predictive knowledge to determine whether a given RING-

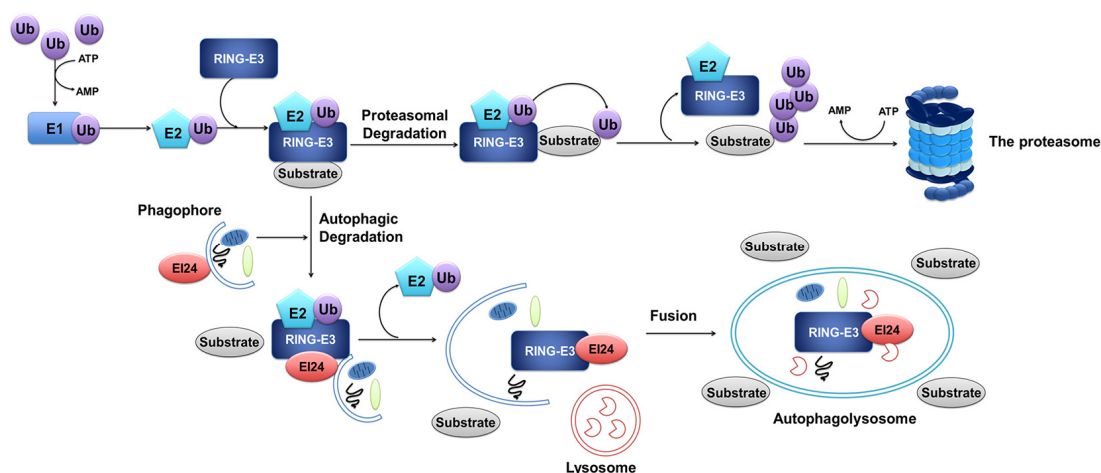


Fig. 1. E124 as a central molecule facilitating communication between autophagy and the UPS. The UPS comprises ATP-dependent concerted action of the E1, E2, and E3 enzyme cascade that results in the ubiquitination of target proteins and degradation in the proteasome. RING-domain E3 ligases are the central molecules of the UPS machinery and function by catalyzing the transfer of ubiquitin chains to target proteins. Recently, we unraveled the novel connection of the UPS to the autophagy pathway through the ability of autophagy-inducing protein E124 to bind to and degrade RING-domain E3 ligases through autophagy machinery. E124 acts as a connecting link to facilitate the recruitment of RING-domain E3 ligases to the autophagosome and their ultimate degradation in the autophagolysosome.

domain E3 ligase can be degraded by E124. For this purpose, the E3 ligases were separated into two groups: those that are susceptible (Group 1) and resistant (Group 2) to E124-mediated degradation. We then searched for gene expression differences between Group 1 and 2 that could potentially contribute to E124-mediated autophagic degradation susceptibility. Using a multi-block partial least square-discriminant analysis (MPLS-DA) (Hwang et al., 2004; Park et al., 2016) with which two different E124 gene expression datasets could be effectively integrated (Boucas et al., 2015; Choi et al., 2013), Group 1 was successfully separated from Group 2 and 161 E3 ligases (predicted Group [pGroup] 1) were predicted to be E124 targets and 64 E3 ligases (pGroup 2) were predicted to be non-targets. Notably, the computationally generated pGroups 1 and 2 correctly categorized the previously tested E3 ligases into their respective experimentally identified Groups. More importantly, the separation of E3 ligases into pGroup 1 and 2 was validated experimentally indicating the high degree of sensitivity and specificity of our model. The fact that the RING domain, which is present in the majority of E3 ligases, acts as an 'eat-me' signal for E124-mediated autophagic degradation strongly supports the idea of integration of the autophagy machinery with the UPS, indicating that these protein degradation pathways are not as independent as previously suggested. Another important revelation was that in addition to the presence of RING-domain, cellular localization of E3 ligases could be also a contributing factor in determining susceptibility to degradation by E124. pGroup 1 members were primarily localized to the organelles that are involved in the autophagy process such as the endosome, ubiquitin ligase complex, vacuole, lysosome, chromatin, and cytoskeleton. Conversely, pGroup 2 was associated with the perinuclear region of the cyto-

plasm and the Golgi apparatus (Devkota et al., 2016). Overall, our data illustrate that in addition to the presence of RING domain, differences in the cellular localization of E3 ligases could be an additional factor that determines the susceptibility of a particular E3 ligase to degradation by E124. Collectively, our proposed model clearly represents a paradigm shift regarding our understanding of E3 ligase fate determination.

One of the pivotal questions that needs to be addressed regarding our finding that E124 binds to and degrades several RING-domain E3 ligases is: what is the biological implication of this degradation? Several studies conducted in our laboratory addressed the implications of E124-mediated degradation of RING-domain E3 ligases in cancer and cellular metabolism. We previously demonstrated that E124 binds and degrades TRIM41, an E3 ligase of PRKCA (Devkota et al., 2012). Consequently, loss of E124 resulted in TRIM41 accumulation and reduced PRKCA protein levels in mice. Because PRKCA is required for skin carcinogenesis, we found that mice with reduced E124 expression had an attenuated response to DMBA-TPA-induced skin carcinogenesis. In a separate study, we reported that E124 degrades TRAF2 and TRAF5 via autophagy, based on its recognition of the E3 ligase RING domain (Choi et al., 2013). Because TRAF signaling lies upstream of the NFKB p65 pathway, reduced E124 expression resulted in NFKB p65 signaling activation, emergence of EMT, and tumor metastasis. In another study, we reported that E124-induced degradation of TRAF2 suppresses MTOR signaling, resulting in the activation of autophagy (Devkota et al., 2016). Autophagy-mediated proteolysis supplies amino acids to the tricarboxylic acid cycle to generate energy that is required for cell survival in nutrient-deprived conditions (Ravikumar et al., 2010). Consistent with

this model, we found that cells with reduced expression of E124 contained decreased ATP levels in HBSS-treated conditions. As a consequence of the inability to replenish ATP, E124 knockdown cells displayed increased cell death in nutrient-deprived conditions. Increased susceptibility of E124 knockdown cells that lack autophagy-inducing activity is consistent with previous reports demonstrating the protective nature of the autophagy process during metabolic stress (Mizushima, 2007). We are currently focused on validating the wealth of data generated during the E3 ligase screen at the functional level, using several mouse models of cancer.

CONCLUSION

Autophagy and the UPS are specialized in the degradation of their respective targets; however, in cases when cells are unable to degrade toxic proteins and damaged organelles that might lead to pathogenesis, it is imperative that these machineries communicate with each other (Lee et al., 2012). Only very recently has the importance of deciphering underlying modes of communication between these pathways been appreciated (Cohen-Kaplan et al., 2016). The question whether the UPS and autophagy are directly/inversely proportional to each other is especially important for clinical applications, since the alteration of one pathway will ultimately have consequences for the other pathway. For example, inhibition of the UPS has been proposed as one of the strategies for treating cancer; however, such inhibition has been shown to result in the upregulation of cytoprotective autophagy, such that cancer cells can escape the proteasome inhibition, ultimately resulting in the failure of therapy (Chude and Amaravadi, 2017). This demonstrates the drawback of conceptualizing the UPS and autophagy as isolated systems and highlights the importance of investigating the UPS and autophagy as cooperative and complementary systems (Dikic, 2017). The identification of critical players that function as nodes of communication between autophagy and the UPS, for example E124-mediated degradation of RING-domain E3 ligases (Devkota et al., 2016), and the elucidation of their physiological roles in *in vivo* systems could be critical to the effective manipulation of these pathways for therapeutic purposes.

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