Minireview

Autophagy: A Critical Regulator of Cellular Metabolism and Homeostasis

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Autophagy is a dynamic process by which cytosolic material, including organelles, proteins, and pathogens, are sequestered into membrane vesicles called autophagosomes, and then delivered to the lysosome for degradation. By recycling cellular components, this process provides a mechanism for adaptation to starvation. The regulation of autophagy by nutrient signals involves a complex network of proteins that include mammalian target of rapamycin, the class III phosphatidylinositol-3 kinase/Bcl-1 complex, and two ubiquitin-like conjugation systems. Additionally, autophagy, which can be induced by multiple forms of chemical and physical stress, including endoplasmic reticulum stress, and hypoxia, plays an integral role in the mammalian stress response. Recent studies indicate that, in addition to bulk assimilation of cytosol, autophagy may proceed through selective pathways that target distinct cargoes to autophagosomes. The principle homeostatic functions of autophagy include the selective clearance of aggregated protein to preserve proteostasis, and the selective removal of dysfunctional mitochondria (mitophagy). Additionally, autophagy plays a central role in innate and adaptive immunity, with diverse functions such as regulation of inflammatory responses, antigen presentation, and pathogen clearance. Autophagy can preserve cellular function in a wide variety of tissue injury and disease states, however, maladaptive or pro-pathogenic outcomes have also been described. Among the many diseases where autophagy may play a role include proteopathies which involve aberrant accumulation of proteins (e.g., neurodegenerative disorders), infectious diseases, and metabolic disorders such as diabetes and metabolic syndrome. Targeting the autophagy pathway and its regulatory components may eventually lead to the development of therapeutics.

INTRODUCTION

Autophagy (from the Greek words meaning “self-eating”), is an evolutionarily-conserved and genetically-regulated pathway that governs the intracellular turnover of subcellular proteins, organelles, and foreign pathogens through the mobilization of lyso-

some-dependent degradation processes. In fulfilling these capacities, autophagy provides a fundamental mechanism to maintain cellular homeostasis under normal and pathophysiological conditions (Yang and Klionsky, 2010a).

Three major subtypes of autophagy have been described: macroautophagy (the most common subtype), microautophagy, and chaperone-mediated autophagy (Ravikumar et al., 2010). Macroautophagy refers to the sequestration of cytosolic material or “cargo” (e.g., proteins, lipids, and organelles) into double-membrane compartments called autophagosomes which subsequently fuse with lysosomes. Autophagic cargoes that are delivered to the lysosome are then digested by lysosomal hydrolases to their basic components (e.g., amino acids, fatty acids), which are released by membrane permeases, to be reutilized for biosynthetic pathways (Mizushima and Komatsu, 2011; Ravikumar et al., 2010). Microautophagy refers to a process by which cytosolic material is directly assimilated into the lysosome by invagination of the lysosomal membrane (Santambrogio and Cuervo, 2011). In chaperone-mediated autophagy, proteins that contain a specific recognition sequence (KFERQ), are targeted to the lysosome by molecular chaperones, (e.g., the 70 kDa heat shock cognate protein, Hsc70) (Kaushik et al., 2011a).

Macroautophagy (hereafter abbreviated as autophagy) proceeds through a series of sequential steps including initiation and autophagosomal nucleation (formation of the phagophore), the elongation of the nascent autophagosomal membrane, the maturation of the double-membraned autophagosome with cargo assimilation, and finally autophagosome-lysosome fusion, which culminate in lysosomal-degradation of cargo (Mizushima et al., 2008) (Fig. 1).

Autophagy is well known as a physiological response to starvation, which prolongs cell survival through the recycling of cellular macromolecules. This process replenishes pools of cellular precursors in response to nutrient depletion (Mizushima and Komatsu, 2011). Autophagy exerts important physiological functions in protein turnover, and in organelle quality control, by maintaining appropriate organelle numbers, and disposing of dysfunctional or damaged organelles (Ravikumar et al., 2010).

In addition to cellular homeostasis, autophagy functions as a general inducible response to cellular stress caused by exposure to a wide variety of chemical and physical agents.

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These include xenobiotics, oxidants, infectious agents and pro-inflammatory states, hypoxia, and chemicals that perturb endoplasmic reticulum (ER) function (Kroemer et al., 2010). During such conditions, the degradative functions of autophagy may be particularly important in clearing subcellular damage. Specifically, autophagy can maintain cellular protein homeostasis (proteostasis) by facilitating the removal of ubiquitinated protein aggregates, (a process termed “aggrephagy”) and thus may serve as an overflow pathway for protein turnover under conditions of impaired proteasomal activity (Lamark and Johansen, 2012). Furthermore, autophagy can clear dysfunctional or depolarizing mitochondria that arise as the result of xenobiotic or environmental stress, in a process called mitophagy (Youle and Narendra, 2011).

Although autophagy is generally considered to represent a survival or adaptive process, its relationship to cell death pathways remains incompletely understood. Autophagy has been variably reported to act as a protagonist or antagonist of apoptosis, depending on experimental context, and also as a cell death effector pathway under conditions of impaired apoptosis (Maiuri et al., 2007). In recent years it has also become clear that autophagy has important roles in innate and adaptive immunity, with diverse functions including pathogen clearance (xenophagy), antigen presentation, and regulation of the inflammatory response (Deretic and Levine, 2009; Levine et al., 2011).

The role of autophagy in diseases is an emerging area of investigation, with recent studies indicating that autophagy may exert multifunctional roles in specific diseases, with the potential for both adaptive and maladaptive outcomes. Furthermore, deficiency or absence in autophagic function may play a pathogenic role in select human diseases (Choi et al., 2013; Levine and Kroemer, 2008; Mizushima et al., 2008; Rubinsztein et al., 2012). This review will cover the basic principles of autophagy regulation and function, with an emphasis on the role of this process in cellular metabolism, as well as its roles in selected diseases, including metabolic diseases, and proteopathies.

MOLECULAR REGULATION OF AUTOPHAGY

Mammalian autophagy responds to regulation by environmental cues through signaling pathways that operate a system of proteins termed the core autophagic machinery. This regulatory framework consists of many autophagy-related gene (ATG) products that are homologues of similar proteins (*Atg*) originally identified in yeast genetic screens (Yang and Klionsky, 2010a; 2010b).

Regulation of autophagy by nutrient signals

The autophagy pathway is inhibited by nutrients and growth factors, and upregulated by starvation or energy exhaustion through the mammalian (or mechanistic) target of rapamycin (mTOR) pathway (Jung et al., 2010)(Fig. 2). mTOR associates with a multi-protein complex (mTOR Complex 1; mTORC1) that includes the regulatory-associated protein of mTOR (Raptor), G-protein β -subunit like protein (G β L), and proline-rich Akt/PKB substrate 40 kDa (PRAS40) (Vander Haar et al., 2008). This multi-protein complex is activated by nutrient-related signals including amino acids and growth factors, and negatively regulates autophagy (Jung et al., 2010).

The mTORC1 inhibits its substrate complex, the ULK1/ATG1 complex, which consists of the mammalian uncoordinated-51-like protein kinase (ULK1) mATG13, ATG101, and RB1CC1/FIP200 (Chan, 2012; Mizushima, 2010). Inhibition of mTORC1

by starvation or energy depletion causes mTORC1 to dissociate from the ULK1 substrate complex, leading to dephosphorylation of ULK1 and mATG13, and the phosphorylation of RB1CC1, resulting in the initiation of autophagy (Ganley 2009; Hosokawa et al., 2009; Wong et al., 2013).

Autophagy is negatively regulated by growth factors (e.g., insulin) through the Class I phosphatidylinositol-3-kinase (PI3K/AKT) pathway. AKT induces mTOR by inactivating its upstream inhibitor, the tuberous sclerosis complex-2 protein (TSC2) (Jung et al., 2010). Autophagy also responds to regulation by depletion of cellular energy charge through the increased activity of the adenosine monophosphate (AMP)-activated protein kinase (AMPK) (Mihaylova and Shaw, 2011). In response to elevated AMP levels, AMPK may inactivate mTORC1 by activating TSC2, by phosphorylating Raptor, and may also phosphorylate ULK1 (Inoki et al., 2003; Jung et al., 2009; Kim et al., 2011).

The Beclin 1 complex

Autophagosome formation is also regulated by the autophagy protein Beclin 1 (homolog of yeast Atg6) (Liang et al., 1999). Beclin 1 interacts with a multi-protein complex that includes hVps34, a class III phosphatidylinositol-3 kinase (PI3KC3), p150, and ATG14L or UVRAG. This complex functions to generate phosphatidylinositol-3-phosphate (PI3P), a second messenger which promotes autophagosomal nucleation (He and Levine, 2010). Beclin 1 associates with either ATG14L or UVRAG to activate autophagy (Itakura et al., 2008). Rubicon inhibits autophagy by interacting with UVRAG. Additional proteins that interact with the Beclin1 complex include Ambra-1 and Bif-1 (which activate autophagy) (He and Levine, 2010), and Bcl-2 family proteins (which inhibit autophagy) (Pattingre, 2005). AKT, a signaling regulator, may directly phosphorylate Beclin 1, and thereby negatively regulates autophagy (Wang et al., 2012).

ATG9 system

Additional transmembrane proteins such as mammalian Atg9 (mAtg9) and vacuole membrane protein 1 (VMP1) have been implicated in autophagosome assembly (Yang and Klionsky, 2010b). mAtg9 translocates to the phagophore assembly site from the trans-Golgi during the activation of autophagy by starvation, in a mechanism requiring ULK1 (Young et al., 2006). Additionally, mATG9 has been detected in membrane vesicles, and in maturing autophagosomes. Though the function of this protein in autophagy remains unclear, mATG9-containing vesicles may participate in the early stages of autophagosome assembly (Yamamoto et al., 2012).

Regulation of autophagosome elongation

The steps of autophagosome elongation and maturation require two ubiquitin-like conjugation systems: the ATG5-ATG12 conjugation system and the microtubule associated protein-1 light chain-3 (LC3-ATG8) conjugation system (Ravikumar et al., 2010) (Fig. 3). ATG12, an ubiquitin-like protein, is conjugated to ATG5 by ATG7/ATG10 activities. ATG5-ATG12 forms a complex with ATG16L, which facilitates autophagosome elongation (Yang and Klionsky, 2010b).

In the second ubiquitin-like conjugation system, the microtubule-associated protein-1 light chain 3 (LC3), (a mammalian homologue of yeast Atg8) is cleaved by ATG4B to generate the cytosolic free form LC3-I (Yang and Klionsky, 2010b). LC3 isoforms (e.g., LC3B) and other mammalian Atg8 homologues such as γ -aminobutyric acid-A receptor-associated protein (GABARAP), are conjugated with phosphatidylethanolamine in a reaction catalyzed by ATG7/ATG3 (Kabeya, 2000; 2004; Yang and

Klionsky, 2010b). The conversion of LC3B-I (unconjugated form) to LC3B-II (lipid-conjugated form) is an indicator of autophagosome formation (Kabeya et al., 2000). After autophagosome-lysosome fusion, LC3B-II is degraded by lysosomal activity, or deconjugated to LC3B-I at the membrane surface by Atg4B (Satoo et al., 2009).

LC3 conversion and autophagosome accumulation are commonly used as experimental indicators that the autophagy process is activated or modulated. However, autophagy is a dynamic process and the observance of these markers does not necessarily indicate completion of the process through lysosomal degradation of substrates (autophagic flux) (Mizushima et al., 2010). In fact, apparent increases in autophagosome numbers may also signify blockage of autophagosome-lysosome fusion and impaired clearance by lysosomal degradation (Boya et al., 2005). To distinguish between activated and blocked autophagy, autophagic flux assays are conducted by monitoring the turnover of a selected autophagic substrate (e.g., LC3) in the absence or presence of a chemical inhibitor of autophagic process (Mizushima et al., 2010).

Selective autophagy cargo adaptors

Previously, autophagy was regarded as a non-specific process for bulk assimilation of cytosol; however increasing evidence suggests that autophagy has a more selective role in the delivery of a wide range of specific cytoplasmic cargo to the lysosome for degradation (Johansen and Lamark, 2011; Shaid et al., 2013). A number of proteins function as selective autophagy cargo adaptors, which participate in the targeting of specific cargo to the autophagosome. Among these, p62/SQSTM1 (p62) can polymerize or aggregate as well as specifically recognize protein substrates (Ichimura and Komatsu, 2010). p62 interacts directly with ubiquitinated proteins through a ubiquitin-associated (UBA) domain. Furthermore, p62 can interact with LC3 through its LIR (LC3-interacting region) motif, thus targeting ubiquitinated proteins to nascent autophagosomes (Shaid et al., 2013).

Another selective autophagy adaptor, neighbor of BRCA1 gene 1 (NBR1), promotes the formation of ubiquitin-positive protein aggregates, facilitating their sequestration and removal by aggrephagy (Yamamoto and Simonsen, 2011). This process involves the 400 kDa, PI3P-binding autophagy-linked FYVE domain protein (ALFY), a p62-interacting protein (Clausen et al., 2010). The cytosolic deacetylase, histone deacetylase 6 (HDAC6), is another ubiquitin-binding protein that can facilitate aggrephagy. HDAC6 facilitates the organization of ubiquitinated proteins into aggresome-like structures, through interactions with dynein microtubule motors and the actin cytoskeleton, and targets them to autophagosomes. HDAC6 can also promote autophagosome-lysosome fusion (Kawaguchi et al., 2003; Lee et al., 2010).

During infection, autophagy assists in immune responses by providing a mechanism for the selective intracellular degradation of invading pathogens (xenophagy) (Deretic and Levine, 2009; Levine et al., 2011). Invading pathogens are ubiquitinated and subsequently targeted to autophagosomes by selective cargo adaptor proteins, including p62 (Johansen and Lamark, 2011), optineurin, (OPTN) (Wild et al., 2011), (SMAD) ubiquitin regulatory factor 1 (Smurf1) (Orvedahl et al., 2011), NBR1 (Kirkin et al., 2009) and nuclear dot protein, 52-kDa (NDP52) (Thurston et al., 2009).

Autophagy in mitochondrial homeostasis

The selective removal of mitochondria by autophagy (mitophagy)

plays an important role in erythrocyte maturation, and the maintenance of cellular homeostasis. While mitochondrial turnover represents a general housekeeping function of autophagy, the accelerated turnover of mitochondria by mitophagy may result from environmental stress or xenobiotic exposure.

Mitophagy can regulate mitochondrial number to match metabolic requirements. Mitochondria are removed during erythrocyte maturation by the BH3-only protein, Nix /Bnip3L1. Nix localizes to the outer mitochondrial membrane and directly interacts with mammalian Atg8 homologs (e.g., GABARAP-L1), through its LIR motif (Shaid et al., 2013).

In contrast, the mobilization of damaged and dysfunctional mitochondria to the autophagosome for removal by mitophagy is initiated by the phosphatase and tensin homolog deleted in chromosome 10 (PTEN)-induced putative kinase 1 (PINK1) and Parkinson protein-2 (Parkin) (Geisler, 2010; Narendra et al., 2008; Youle and Narendra, 2011). Mutations in the corresponding PINK1 and PARK2 genes are associated with recessive familial forms of Parkinson's disease (Morris, 2005). In mice, PINK1 and PARK2 deletions are associated with mitochondrial dysfunction (Trancikova et al., 2012).

Loss of mitochondrial membrane potential and the increased production of mitochondrial reactive oxygen species (ROS) represent initiating signals for mitophagy. Mitochondrial-derived ROS are produced as byproducts of oxidative phosphorylation, and may increase in response to toxins, or altered oxygen tension. If not removed by cellular antioxidant defenses, ROS can potentially cause mitochondrial DNA and protein damage leading to mitochondrial dysfunction. PINK1, a transmembrane Ser/Thr kinase, is stabilized on damaged or depolarized mitochondria. Following the decline of mitochondrial membrane potential, which can be elicited by certain xenobiotics, PINK1 recruits cytosolic Parkin, an E3 ubiquitin protein ligase, to the mitochondria (Geisler et al., 2010; Narendra et al., 2008; Vives-Bauza et al., 2010).

Parkin initiates the formation of polyubiquitin chains which mark depolarized mitochondria for degradation. Parkin ubiquitinates mitochondrial outer membrane proteins, including the voltage-dependent anion-selective channel protein 1 (VDAC1) and other potential substrates. Ubiquitinated mitochondria are subsequently recognized and targeted to autophagosomes by the autophagic cargo adaptor protein p62 (Geisler, 2010; Narendra et al., 2008; Youle and Narendra, 2011). Loss of PINK1 function can promote oxidative stress and trigger mitochondrial fragmentation and autophagy (Dagda et al., 2009). Conversely, overexpression of PINK1 causes mitochondrial clustering and excessive accumulation of autophagosome-like structures (Vives-Bauza et al., 2010).

Recent studies have uncovered other factors potentially acting as selective mitophagy cargo adaptors (Schreiber and Peter, 2013). These include the outer mitochondrial membrane proteins BNIP1, and FUN14 domain containing protein-1 (FUNDC1) which may regulate mitophagy under hypoxic conditions (Liu et al., 2012; Zhang et al., 2008). Additionally, the cytosolic HECT-type E3 ubiquitin ligase SMURF1 has been implicated in regulating mitophagy via its NH₂-terminal C2 domain (Orvedahl et al., 2011).

Recent experiments highlight the roles of core autophagy proteins in mitochondrial homeostasis. Macrophages isolated from autophagy-deficient *Becn1*^{-/-} or *LC3B*^{-/-} mice displayed elevated mitochondrial ROS production associated with enhanced secretion of inflammasome-associated cytokines (e.g., IL-1 β), mitochondrial depolarization, and mitochondrial DNA release, under pro-inflammatory conditions (Nakahira et al., 2011).

Fig. 1. Autophagy pathway. The autophagic pathway proceeds through a series of sequential steps. These begin with the initiation of the pathway and autophagosome nucleation (formation of a pre-autophagosomal structure leading to an isolation membrane or phagophore). This is followed by autophagosomal elongation, a step mediated by two ubiquitin-like conjugation systems (i.e., ATG8, and the ATG5/ATG12 systems). The next steps involve autophagosome maturation, including the sequestration of cytosol or specific substrates, and autophagosome/lysosome fusion. In the final stage, autophagosomal cargoes are digested by lysosomal enzymes, and the contents released for metabolic recycling.

Autophagy in pathogen clearance

Autophagy can exert newly recognized and pleiotropic roles in diverse aspects of innate and adaptive immune system function. Among these, autophagy has been implicated in the regulation of inflammatory processes, including the regulation of inflammasome activation, interferon production, T-cell selection, B cell survival and antibody production, efferocytosis, and antigen presentation. These novel functions of autophagy have been extensively reviewed elsewhere (Deretic and Levine, 2009; Levine et al., 2011; Yano and Kurata, 2011). Of major importance, autophagy can act as a defense mechanism for the clearance of invading intracellular pathogens, in a process called xenophagy. A number of medically-relevant pathogens have now been identified as potential substrates for autophagy, and these include bacteria (e.g., *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, group A *Streptococcus pyogenes*, *Salmonella enterica*, *Listeria monocytogenes*, and others) viruses such as herpes simplex virus type 1 (HSV-1), and parasites (e.g., *Toxoplasma gondii*) (Deretic and Levine, 2009; Rubinstein et al., 2012).

Nevertheless, the function of autophagy can also be manipulated by invading pathogens, and in some instances these pathogens can convert autophagic organelles into growth-

supporting compartments (Deretic and Levine, 2009). Among the pathogens which can exploit the autophagic pathway for survival and replication include various strains of bacteria [e.g., *Brucella abortus* (Starr et al., 2012), *Coxiella burnetii* (Gutierrez et al., 2005), *Francisella tularensis* (Checroun et al., 2006) and viruses such as human immunodeficiency virus (HIV) (Kyei et al., 2009), hepatitis-B virus (Li et al., 2011; Tian et al., 2011), hepatitis C virus, and others (Ke and Chen, 2011)]. Among these, autophagy has been shown variably inhibit (Campbell and Spector et al., 2011) or promote (Kyei et al., 2009) HIV infection.

Autophagy in proteopathies

Several human diseases known as conformational diseases or proteopathies are associated with the accumulation of misfolded protein, as the consequence of mutation or impaired clearance mechanisms. Among the best studied examples of proteopathies in which autophagy may play a role, include the major neurodegenerative diseases (e.g., Alzheimer's, Parkinson's, and Huntington's disease), as well as certain lung and liver diseases.

By providing a selective pathway for the clearance of aggregate-prone proteins, autophagy may represent an adaptive pro-

Fig. 2. Regulation of autophagy by nutrient signals. Autophagy responds to regulation by nutrient signals, which operate a signaling pathway involving mammalian (or mechanistic) target of rapamycin (mTOR). Autophagy is negatively regulated by the Class I phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway, which activates mTOR in response to insulin and other growth factors. Akt may also negatively regulate autophagy by phosphorylating Beclin 1. The adenosine 5'-monophosphate-activated protein kinase (AMPK) which is regulated by AMP levels, negatively regulates mTOR, and also phosphorylates ULK1, thereby acting as a positive regulator of autophagy in response to energy exhaustion. mTOR is a major component of the mTOR signaling complex (mTORC1), which regulates the ULK1 complex, consisting of the mammalian uncoordinated-51-like protein kinase ULK1, ATG13, ATG101, and FIP200. Under nutrient rich conditions, mTORC1 inhibits ULK1 kinase activity, thereby inhibiting the activation of autophagy. Autophagy is also regulated by the Beclin 1 interactive complex, consisting of Beclin 1, class III phosphatidylinositol-3-kinase (VPS34 or PI3KC3) and ATG14L. Stimulation of this complex generates phosphatidylinositol-3-phosphate (PI3P), which triggers autophagosomal membrane nucleation. Several interacting factors may participate in this regulation, including UVRAG and Bif-1, which substitute for ATG14L, Ambra1, the negative regulator Rubicon, and Bcl-2 family proteins.

cess in neurodegenerative diseases and other proteopathies. p62/SQSTM1 (p62), an adaptor of selective autophagy, exerts an crucial function in the recognition of cytoplasmic protein inclusion bodies, which are hallmark features of neurodegenerative disorders (Ichimura, 2008).

Autophagy is typically found to be dysregulated in neurodegenerative disorders (Metcalf et al., 2012; Wong and Cuervo, 2010). In mouse models, genetic deficiencies of autophagy proteins promote age-dependent neurodegeneration associated with protein-aggregate accumulation (Hara et al., 2006; Komatsu et al., 2006; Pickford et al., 2008). Genetic studies using neuronal-specific deletion of autophagy proteins (Atg5 or Atg7) demonstrate a role for basal autophagy in the turnover turnover of soluble, cytosolic proteins; and in the clearance of abnormal neuroproteins whose accumulation is associated with neurodegeneration (Hara et al., 2006; Komatsu et al., 2006). The induction of autophagy using genetic manipulation or pharmacological agents can inhibit symptoms of neurodegeneration in mouse models (Ravikumar et al., 2004; Schaeffer et al., 2012; Spencer et al., 2009).

Alzheimer's disease may be associated with autophagic dysfunction, as evidenced by increased autophagosome number in human brains with Alzheimer's disease (Ma et al., 2010). In the pathogenesis of Alzheimer's disease hyperphosphorylated forms

of microtubule-associated protein Tau accumulate in neurons, leading to the formation of neurofibrillary tangles and the accumulation of β -amyloid peptide ($A\beta$) in neural plaques (Jellinger, 2010). $A\beta$ can impair lysosomal function and thereby inhibit its own autophagic clearance. Furthermore, the autophagosome, which contains γ -secretase and related enzymes involved in the generation of $A\beta$ from precursor forms, may also represent a source of $A\beta$ under conditions of impaired autophagosome-lysosome fusion (Yu et al., 2005).

Huntington's disease and associated polyglutamine disorders involve the accumulation of mutant proteins with polyglutamine rich extensions (Imarisio et al., 2008). Mutant huntingtin, which forms protein aggregates and inclusion bodies in neurons of Huntington's disease patients, can be degraded through the autophagic pathway (Ravikumar et al., 2004). Recent studies suggest that mutant huntingtin interferes directly with autophagy protein function, and autophagosomal cargo recognition, thus rendering the autophagic pathway inefficient (Martinez-Vicente et al., 2010; Shibata et al., 2006).

Sporadic Parkinson's disease involves the accumulation of α -synuclein aggregates in neural cytoplasmic inclusions (Lewy bodies). The accumulation of α -synuclein in neurons promotes mitochondrial dysfunction (Hsu et al., 2000). Genetic defects in mitophagy-associated genes have been associated with reces-

Fig. 3. Regulation of autophagosome elongation. Autophagosome membrane elongation is regulated by two ubiquitin-like conjugation systems. The ubiquitin-like protein ATG12 is conjugated to ATG5 by ATG7 (E1-like) and ATG10 (E2-like) enzymes. The resulting ATG5-ATG12 forms a complex with ATG16L1, which participates in elongation of the autophagic membrane. A second conjugation system requires the ubiquitin-like protein microtubule-associated protein-1 light chain 3 (LC3, ATG8). LC3 and its homologues are modified with the cellular lipid phosphatidylethanolamine (PE). The precursor form of LC3 is cleaved by the protease ATG4B to generate the LC3-I form, with an exposed lipid conjugation site at the C-terminal glycine residue. Conjugation of PE with LC3-I occurs from the sequential action of ATG7 (E1-like) and ATG3 (E2-like) activities. In mammals, the conversion

of LC3-I (free form) to LC3-II (PE-conjugated form) is a key regulatory step in autophagosome formation.

sive familial forms of human Parkinson's disease. Although α -synuclein is an autophagic substrate, the accumulation of this protein impairs autophagy thus interfering with its own clearance (Winslow et al., 2010). In conclusion, autophagy may provide an adaptive mechanism in neurodegenerative disorders. However, evidence also suggests that this process could be inhibited by pathological accumulation of substrates, and may also contribute to disease progression (Wong and Cuervo, 2010).

Alpha1-antitrypsin (α 1-AT) represents an example of a proteopathy affecting systemic tissues. Early onset emphysema is associated with a pathogenic mutation in the alpha1-antitrypsin (α 1-AT) gene (PiZ mutation), which causes newly synthesized α 1-AT polypeptide chains to misfold and aggregate in the ER. α 1-AT is a serine protease inhibitor that is synthesized in the liver and secreted into the circulation, where it controls tissue degradation by the enzyme neutrophil elastase (Marciniak and Lomas 2010; Ranes and Stoller, 2005). Aggregates of α 1-AT in the ER result in the reduced export of α 1-AT protein from hepatocytes into the bloodstream, leading to unopposed protease activity in the lung (Ranes and Stoller, 2005) contributing to early-onset emphysema.

As a protective cellular mechanism to maintain ER function, mutated α 1-AT is either sequestered into ER-associated inclusion bodies (Granell et al., 2008) or targeted for degradation by autophagy (Kamimoto et al., 2006; Marciniak and Lomas, 2010). α 1-AT protein aggregates co-localize with LC3. The degradation of α 1-AT has been shown to be impaired by genetic or chemical inhibition of autophagy (Kamimoto et al., 2006), suggesting that mutant α 1-AT in the liver may be selectively removed by autophagy. The activation of autophagy with high doses of the antiepileptic agent, carbamazepine, reversed the liver pathology observed in α 1-AT PiZ over-expressing transgenic mice (Hidvegi et al., 2010).

Previously, we have shown that α 1-AT deficient patients, as well as smokers, and patients with cigarette smoke-induced emphysema, have increased ratios of LC3B-II/LC3B-I in lung

tissue, suggesting that the regulation of autophagy in the lungs of these patients may also be altered (Chen et al., 2008). Dysregulated autophagy in these patients may occur as a result of increased neutrophil elastase burden, causing unchecked proteolytic activity and impaired proteostasis leading to emphysema in the lung (Bodas et al., 2012; Min et al., 2011). Moreover, mutant PiZ- α 1-AT protein aggregates can be detected in alveolar macrophages as well as in bronchioalveolar lavage fluid and lung biopsies of α 1-AT patients with emphysema (Eliot et al., 1998; Mornex et al., 1986). The clearance of these aggregates by autophagy within alveolar macrophages might be impaired, particularly in smokers. Continued investigations into the role of autophagy in α 1-AT protein deficiency may lead to a better understanding of emphysema development in this disorder and the development of improved therapy.

Cystic fibrosis, a genetic disorder caused by mutation in the cystic fibrosis transmembrane conductance regulator (CFTR), presents with aberrant airway mucous production, pulmonary dysfunction, and secondary infections. Recent preclinical studies implicate cystic fibrosis as a disease of impaired clearance of aggregated protein (aggrephagy). Human airway epithelial cells or nasal biopsies derived from cystic fibrosis patients displayed dysfunctional aggrephagy, evidenced by accumulation of polyubiquitinated proteins and decreased clearance of aggregates which accumulated mutant CFTR (Luciani et al., 2010).

AUTOPHAGY IN DISORDERS OF METABOLISM

A relationship between autophagy and cellular metabolism is suggested by the autophagy-dependent regeneration of metabolic precursors such as amino acids and lipids from cellular macromolecules (Rubinsztein et al., 2012). Lipids are stored in the cell in the form of lipid droplets that consist of cholesterol and triglycerides and function to supply free fatty acids needed to sustain mitochondrial β -oxidation and ATP generation (Liu and Czaja, 2013). Chemical or genetic disruption of autophagy

proteins promotes the storage of triglycerides into lipid droplets in the liver, suggesting that autophagy can regulate lipid metabolism and storage (Singh et al., 2009a). LC3 can be recruited to lipid droplets, suggesting the existence of a lipid-selective form of autophagy (lipophagy) (Singh et al., 2009a). The mechanisms by which lipid droplets are targeted to autophagosomes may involve the soluble NSF attachment protein receptors (SNAREs) (Liu and Czaja, 2013).

During exercise, autophagy is increased in cardiac and skeletal muscle, adipose tissue and pancreatic β -cells. Exercise-induced autophagy was shown to protect mice against glucose-intolerance associated with high fat diet (He et al., 2012). Furthermore, autophagy-deficient mice displayed decreased endurance and altered glucose metabolism during acute exercise, and lost the beneficial effects of chronic exercise on glucose intolerance (He et al., 2012).

Hepatic autophagy was also shown to be downregulated in the liver in mouse models of obesity and insulin resistance (Yang et al., 2010). Genetic knockdown of Atg7 in lean mice caused ER stress, and impaired insulin signaling; whereas restoration of Atg7 improved insulin signaling in obese mice (Yang et al., 2010). In contrast, adipose-specific deletion of autophagy proteins led to altered adipose tissue homeostasis and differentiation and promoted insulin sensitivity (Singh et al., 2009b). Recent studies suggest that genetic deletion of autophagy proteins (e.g., Atg7) in the brain can lead to dysregulation of food intake (Kaushik et al., 2011b). Starvation induced autophagy was shown to regulate the production of agouti-related peptide, a signaling mediator for food intake, in the hypothalamus, through a mechanism involving lipid metabolism and the enhanced generation of free fatty acids (Kaushik et al., 2011b).

Recent studies have also implicated roles for autophagy in the pathogenesis of Type I and Type II diabetes (Gonzales et al., 2011). Mice with a β -cell specific deletion of Atg7 develop a phenotype resembling Type-II diabetes when fed a high fat containing diet. Atg7-mutant mice showed reduced β -cell mass and pancreatic insulin content, and evidence of mitochondrial dysfunction, as well as evidence of hypoinsulinemia and hyperglycemia (Jung and Lee, 2010).

In a mouse model of diabetes caused by pro-insulin misfolding, stimulation of autophagy with rapamycin ameliorated ER stress associated with protein aggregate accumulation, and prevented pancreatic β -cell apoptosis (Bachar-Wikstrom et al., 2013).

Interestingly, rapamycin was found to aggravate β -cell toxicity and hyperglycemia, in a mouse model of streptozotocin induced Type I diabetes (Zhou et al., 2010). Further studies are needed to understand metabolic changes incurred by autophagy stimulation impact the progression of human diseases such as diabetes.

CONCLUSIONS

Autophagy represents a primordial cellular mechanism that prolongs cell survival during starvation. Additionally, autophagy has important functions in organelle maintenance, protein turnover, and the cellular stress response.

Autophagy is generally regarded as an adaptive cellular response to stress, which may provide protective functions during tissue injury, infection, and disease pathogenesis. These protective functions include, but are not limited to cell survival, mitochondrial homeostasis, protein aggregate turnover, and pathogen clearance. These adaptive functions of autophagy

are likely to be of general relevance to a broad range of pathogenic conditions and organ systems. Nevertheless, autophagy has also been associated with pro-pathogenic effects, such as the enhanced survival of cancer cells, facilitation of pathogen replication, and in some contexts, promotion of apoptosis and cell death. The dual nature of autophagy in disease complicates the design of therapies. Increased understanding of the relative role of autophagy in the pathogenesis of specific diseases may be required before targeting elements of this pathway for therapy. Further elucidation of the molecular mechanisms that regulate autophagy may uncover new targets for therapeutic manipulation.

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