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Liver autophagy: physiology and pathology

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Autophagy has long been thought of as a bulk degradation system in which cytoplasmic components are sequestered by double-membrane structures called autophagosomes, and the contents are then degraded after autophagosomes fuse with lysosomes. Genetic experiments in yeast identified a set of *Autophagy-related (ATG)* genes that are essential for autophagy. We have since elucidated many of the molecular underpinnings of autophagy and the physiologic roles of these processes in various systems. This review summarizes the physiologic roles of autophagy with a particular focus on liver autophagy based on analyses of knockout mice lacking *Atg* genes.

Keywords: Atg/autophagy/hepatic tumour/liver/p62.

Abbreviations: aPKC, atypical protein kinase C; *ATG*, *autophagy-related* genes; DEHP, diethylhexyl phthalate; DFCP1, double FYVE-containing protein 1; ER, endoplasmic reticulum; hsc70, heat shock cognate protein of 70 kDa; LAMP-2A, lysosome-associated membrane protein type 2A; LIR, LC3-interacting region; mTORC1, mammalian target of rapamycin complex 1; PAS, phagophore assembly sites; PE, phosphatidylethanolamine; PI3K, phosphatidylinositol 3-kinase; PI3P, phosphatidylinositol 3-phosphate; Uba, ubiquitin associated; UVRAG, UV irradiation resistance-associated gene; WIPI, WD-repeat protein interacting with phosphoinoside.

In the lysosomal pathway, degradation of plasma membrane proteins and extracellular proteins is dependent on endocytosis, whereas cytoplasmic components are degraded via three types of autophagy: microautophagy, chaperone-mediated autophagy and macroautophagy (*1*). Microautophagy is characterized by membrane invagination in lysosomes or endosomes, which results in sequestration and degradation of cytoplasmic components close to the lysosome or endosome (Fig. 1A). Chaperone-mediated autophagy targets specific cytosolic proteins that are bound by heat shock cognate protein of 70 kDa (hsc70), translocated into the lysosomal lumen via interactions with lysosome-associated membrane protein type 2A (LAMP-2A) and rapidly degraded (Fig. 1A). Macroautophagy (hereafter referred to as autophagy) is the best-characterized type of autophagy, in which isolated membranes or phagophores engulf a portion of cytoplasm into double-membrane vesicles called 'autophagosomes'. These structures then transport the contents to lysosomes. Fusion of an autophagosome with a lysosome triggers breakdown of the inner autophagosomal membrane followed by degradation of internal contents (Fig. 1B).

This review paper briefly discusses the molecular machinery underlying mammalian autophagy and examines the pathophysiologic roles of liver autophagy, including starvation-induced, constitutive and selective autophagy, assessed using liver-specific autophagy-deficient mouse models.

Molecular Mechanisms of Mammalian Autophagy

The molecular underpinnings of autophagosome formation have been mainly uncovered in studies of the yeast *Saccharomyces cerevisiae* (*2*,*3*). To date, genetic studies of *S. cerevisiae* have revealed more than 30 *Autophagy-related* (*ATG*) genes, 18 of which are core *ATG* genes that are essential for autophagosome formation. Importantly, core *ATG* genes are well conserved in mammals, where the functions of the encoded proteins markedly overlap with the corresponding proteins in yeast, although a few mammal-specific proteins have been identified (*4*). Atg proteins have been categorized into several subclasses, which function sequentially and cooperatively to regulate autophagosome formation (Figs. 1B and 2).

ULK1 complex

Blood levels of amino acids, insulin and/or glucose regulate autophagic activity. Mammalian target of rapamycin complex 1 (mTORC1), which is downstream of each of these signals, serves as an important checkpoint in autophagy. ULK1 complex is composed of ULK1 (a serine/threonine protein kinase), Atg13, Atg101 and RB1-inducible coiled-coil 1/FAK family-interacting protein of 200 kDa (RB1CC1/FIP200), and plays a central role in inducing autophagosome formation and regulating mTORC1 (Fig. 2A). Under nutrient-rich conditions, mTORC1 is incorporated into ULK1 complex, and subsequently phosphorylates ULK1 and Atg13, which suppress autophagic activity (*5*, *6*). In response to starvation, mTORC1 dissociates from ULK1 complex, resulting in dephosphorylation of ULK1 Ser 757, which phosphorylates FIP200 to initiate autophagosome formation (*5*, *6*) (Fig. 2A). Moreover, autophagy is also induced when Ser 317 and Ser 777 of ULK1 are directly phosphorylated by AMP-activated protein kinase, which is activated in response to

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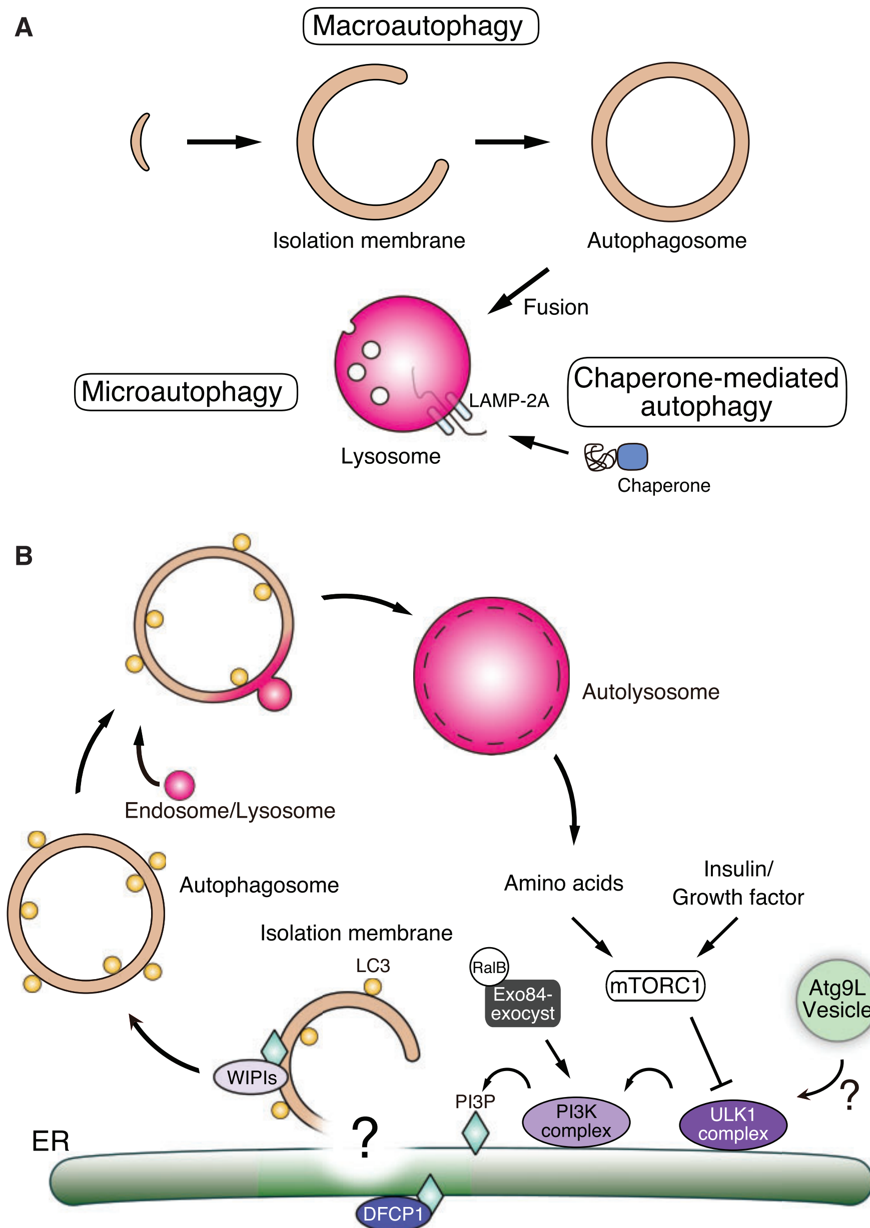

Fig. 1 Autophagy. (**A**) Macroautophagy is the prototypical autophagic process in which cytoplasmic materials, including organelles, are sequestered inside membranes or phagophores. The resulting autophagosomes fuse with lysosomes, resulting in complete degradation of the sequestered cytoplasmic components by lysosomal hydrolases. Microautophagy is characterized by membrane invagination in lysosomes or endosomes to sequester and degrade nearby cytoplasmic material. Chaperone-mediated autophagy targets specific cytosolic proteins that contain a KFERQ-like pentapeptide sequence. The proteins are trapped by hsc70, and translocated into the lysosomal lumen via interactions with LAMP-2A, before they are rapidly degraded. (**B**) Autophagosome formation in mammalian cells. Induction of autophagy activates ULK1 complex, which translocates to locations near the ER. ULK1 complex regulates class III PI3K complex, which is assisted by RalB and an Exo84-containing exocyst complex. The multimembrane spanning protein Atg9L is involved in an early stage of autophagosome formation. Formation of PI3P recruits DFCP1 and promotes the formation of the omegasome. Other PI3P-binding WIPIs, Atg12–Atg5–Atg16L complex and LC3–PE conjugate play important roles in the elongation and closure of the isolation membrane. Finally, the complete autophagosome fuses with endosomes or lysosomes to form autolysosomes. In the lysosomes, cytoplasmic components that have been engulfed by the autophagosomes are degraded by resident hydrolases. The resulting amino acids and other basic cellular materials are reused by the cell; at high levels, these building blocks reactivate mTORC1 and suppress autophagy.

decreased ATP levels in a low glucose state (*7*, *8*) (Fig. 2A).

Class III phosphatidylinositol 3-kinase

Autophagy requires phosphatidylinositol 3-kinase (PI3K) to generate phosphatidylinositol 3-phosphate (PI3P). In mammals, two types of PI3K are involved in autophagy: classes I and III PI3K. Whereas class I PI3K is principally involved in modulating signalling cascades (Fig. 2A), class III PI3K complexes regulate organelle biogenesis. Class III PI3K can regulate autophagy positively or negatively at the different steps depending on various interaction partners. Class III PI3K complex consisting of p150, Vps34, Beclin 1 and Atg14 (complex I) is involved in early autophagosome formation and regulated by ULK1 complex via RalB and an

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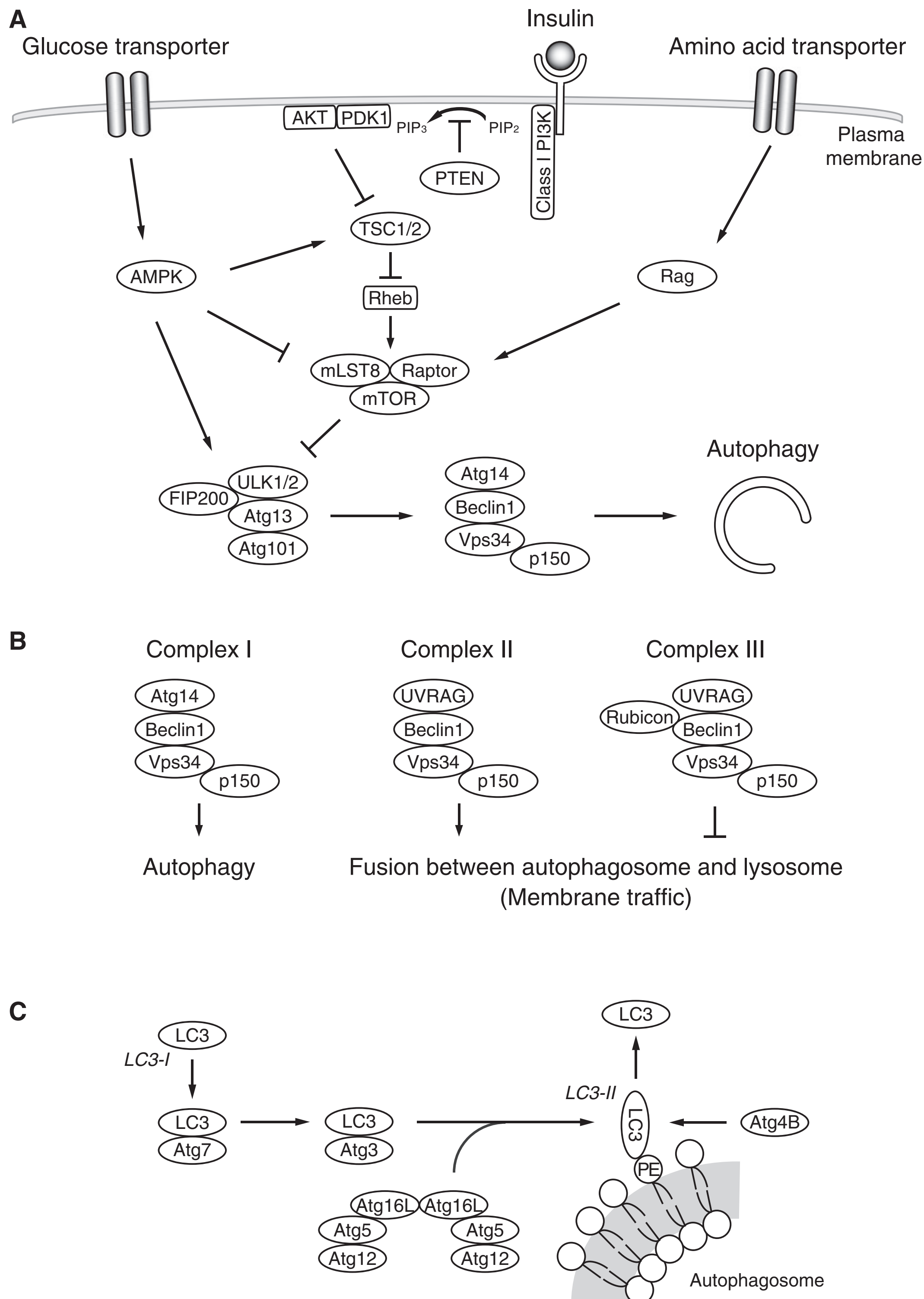

Fig. 2 Atg proteins in mammalian cells. (**A**) As a terminal target of insulin, amino acid and glucose signalling, mTORC1 controls the activity of ULK1 complex. (**B**) Autophagy-related class III PI3K complexes. PI3K regulates different steps of autophagy positively or negatively based on various interaction partners. (**C**) Ubiquitin-like conjugation systems essential for autophagy. The ubiquitin-like modifiers Atg12 and LC3 are activated by Atg7 and transferred to Atg10 and Atg3, respectively. Atg12 forms an isopeptide bond with Atg5 followed by formation of Atg12–Atg5–Atg16L complexes. LC3 forms an amide bond with PE (LC3-II), which is dependent on Atg12–Atg5–Atg16L complex. Finally, LC3-II on the inner membrane is degraded after autophagosomes fuse to lysosomes, whereas LC3-II on the outer membrane is released from autophagosomes via the specific cysteine protease Atg4B.

Exo84-containing exocyst complex (*9–11*) (Figs 1B and 2B). Other PI3K complex composed of p150, Vps34, Beclin1 and UV irradiation resistance-associated gene (UVRAG) (complex II) facilitates autophagosome and endosome maturation (*12*, *13*) (Fig. 2B). In contrast, when RUN domain- and cysteine-rich domain-containing Beclin 1-interacting protein (Rubicon) associates with a subpopulation of UVRAG-containing PI3K

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complex, the complex (complex III) negatively regulates a later step of autophagy and the endocytic pathway (*14*, *15*) (Fig. 2B). Recruitment of Beclin 1 to PI3K complex is sensitive to starvation conditions. Beclin 1 forms a complex with endoplasmic reticulum (ER)-associated Bcl-2 under nutrient-rich conditions and is released after Bcl-2 is phosphorylated by c-Jun N-terminal kinase 1 (*16*). PI3P generated by complex I recruits the PI3P-binding protein double Fab1, YotB, Vac1p, and EEA1 (FYVE)-containing protein 1 (DFCP1) to preautophagosomal structures or phagophore assembly sites (PASs). DFCP promotes the formation of the omegasome from which autophagosomes are generated though it is not essential for autophagosome formation (*17*) (Fig. 1B). Other PI3P-binding WD-repeat protein interacting with phosphoinoside (WIPI) proteins are also crucial for maturation of the isolation membrane and the associated omegasome (*18*) (Fig. 1B).

Atg9

Most Atg proteins are cytosolic and transiently associate with PAS via other Atg components and/or lipids. Mammalian Atg9 moves between the trans-Golgi network and Rab7- and Rab9-positive endosomes under normal conditions (*19*). In response to starvation, Atg9 binds to p38IP, which increases levels of Atg9 dispersed throughout the cytosol (*20*). Atg9 subsequently localizes to PAS, which is dependent on p38IP, ULK1, Atg13 and PI3K (*20*) (Fig. 1B). Atg9 returns to endosomes via p38α, which negatively regulates the interaction between Atg9 and p38IP (*20*). The motility of Atg9 during the assembly and disassembly of structures at the site of autophagosome formation implies that Atg9-containing membrane structures may be at least one source of membranes for autophagosomes. Alternatively, this structure may supply and/or remove some critical factors or lipid components essential for autophagosome formation (*21*).

Ubiquitin-like conjugating systems

The ubiquitin-like modifiers Atg12 and microtubule-associated protein 1 light chain-3 (LC3) are activated by the E1-like enzyme Atg7 and transferred to two different E2-like enzymes: Atg10 and Atg3, respectively. Whereas Atg12 forms an isopeptide bond with Atg5, LC3 forms an amide bond with phosphatidylethanolamine (PE) in a reaction that is dependent on the Atg12–Atg5 conjugation (*22*, *23*) (Fig. 2C). Atg16L forms a high molecular weight complex with Atg12–Atg5 (*24*) (Fig. 2C). The Atg12–Atg5–Atg16L complex functions as an E3-like enzyme, determining the site of LC3 lipidation (*25*) (Fig. 2C). Although the Atg12–Atg5–Atg16L complex is required for elongation of the isolation membrane (*26*), PE-bound LC3 (LC3-II) is thought to be important for membrane biogenesis and/or closure of the isolation membrane (*27–29*) (Fig. 2C).

Physiologic Roles of Liver Autophagy

In late 1950s and early 1960s, after the discovery of the lysosome by Christian de Duve (*30*), autophagy was identified as a machinery that degrades cytoplasmic components. Later, electron microscopic analyses identified autophagosomes in isolated liver tissues subjected to stressful conditions (*e.g.*, livers perfused with glucagon) (*31*, *32*), suggesting an important role for these structures in responses to starvation. In 1977, Mortimore's group examined this potential function using perfused rat livers and showed that autophagy is induced in response to decreased amino acid levels (*33*) and that glucagon drives autophagy, which directly contrasts the activity of insulin (*34*). During this period, Seglen and colleagues used a primary rat hepatocyte culture system to show that depriving cells of certain amino acids (*e.g.*, leucine, phenylalanine, tyrosine, tryptophan, histidine, asparagine and glutamine) leads to autophagy (*35*), and that the inhibitor of class III PI3K 3-methyladenine suppresses autophagy (*36*). Like other research fields, studies of autophagy took advantage of rodent liver and primary culture hepatocytes. For a long time, studies of autophagy were limited to biochemical and morphologic approaches. The emergence of genetic techniques led to the identification of *ATG* genes and subsequent analyses of autophagy in various model organisms, including mice. During the last decade, mouse strains with liver-specific deletions of *Atg* genes have uncovered a number of physiologic roles for liver autophagy in addition to responses to starvation conditions (Fig. 3).

Starvation adaptation

In response to prolonged fasting, the liver begins to break down internal proteins to supply the body with amino acids. In wild-type mice, for example, 48 h of fasting results in catabolization of 30–40% of liver proteins (*37*). Whereas amino acid levels in liver tissue and blood are transiently elevated after 24 h of fasting, mice with *Atg7* specifically knocked out in the liver exhibit neither fasting-induced decreases in liver protein levels nor transient increases in amino acid levels (*38*). Importantly, blood glucose levels are stable after the transient elevations in amino acid levels in the wild-type mice, but the mutant mice become hypoglycemia, suggesting that glucogenic amino acids are created via liver autophagy and subsequent gluconeogenesis (*38*) (Fig. 3A). Liver autophagy also helps to supply free fatty acids in response to fasting, which induces increased circulating levels of fat tissue-derived free fatty acids. The free fatty acids are rapidly captured by various cell types, including hepatocytes and hypothalamic neurons, and esterified into triglycerides in lipid droplets. These lipid droplets are selectively broken down by autophagy (lipophagy) to provide endogenous free fatty acids for energy production through β-oxidation (*39*, *40*) (Fig. 3A). Consistent with reduced β-oxidation and excess accumulation of cellular lipid droplets in cultured hepatocytes in which autophagy is suppressed, liver-specific knockout of *Atg7* in mice causes marked accumulation of triglycerides and cholesterol in lipid droplets (*39*). However, the molecular mechanisms by which autophagosomes selectively sequester lipid droplets are unclear, and defective autophagy significantly affects intracellular metabolic pathways via secondary effects (our unpublished data). Further studies are needed to

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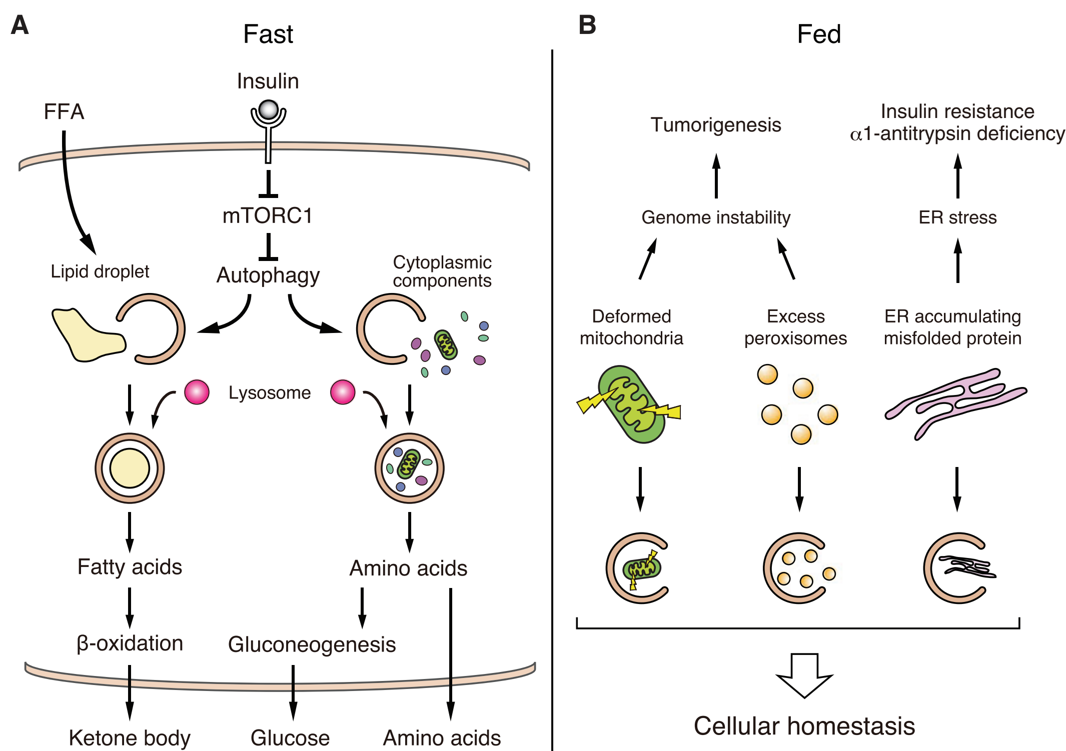

Fig. 3 Physiologic roles of liver autophagy. (**A**) In response to fasting, liver autophagy results in nonselective catabolization of cytoplasmic components into such building blocks as amino acids and free fatty acids, which are used in glyconeogenesis and β-oxidation. (**B**) Low levels of autophagy also occur constitutively even under nutrient-rich conditions to mediate global turnover of cytoplasmic materials, regulate selective turnover of abnormal proteins and organelles, and prevent disease onset.

evaluate the direct contribution of autophagy to lipolysis.

Quality control

Regardless of the nutritional conditions, basal levels of autophagy result in the metabolization of cytoplasmic components to prevent the accumulation of degenerating proteins and organelles (Fig. 3B). For example, loss of *Atg7* in mouse hepatocytes causes marked accumulation of swollen and deformed mitochondria, concentric membranous structures consisting of ER, increased number of peroxisomes and lipid droplets, and formation of protein aggregates (*41*). The mutant mice exhibit severe hepatomegaly and hepatocytic hypertrophy, which are followed by inflammation, hepatitis and tumourigenesis (*41*, *42*). Similar phenotypes have also been observed in mouse livers lacking Vps34, which is essential for autophagosome formation (*43*). Chaperone-mediated autophagy also contributes to global protein turnover in hepatocytes because persistent activation of this process in the liver prevents age-associated declines in cellular maintenance and hepatic function (*44*).

Autophagic surveillance of cytoplasmic material in hepatocytes is likely to be more important under stressful or pathologic conditions (Fig. 3B). The phthalate ester diethylhexyl phthalate (DEHP) is a peroxisome proliferator and hepatic carcinogen. Increased peroxisome proliferation in response to DEHP is thought to initiate the neoplastic transformation of hepatocytes by increasing intracellular levels of DNA-damaging reactive oxygen species. Liver autophagy contributes to selective clearance of peroxisomes (pexophagy) that develop after treatment with DEHP (*45*) (Fig. 3B), suggesting that pexophagy is antitumourigenic. In obese mice, autophagic activity in hepatocytes is reduced owing to significantly decreased levels of Atg proteins, including Beclin 1, Atg5 and Atg7. This results in ER stress, probably due to impaired ER homeostasis in hepatocytes, leading to insulin resistance (*46*). Surprisingly, only adenovirus vector-mediated *Atg7* overexpression in livers of obese mice ameliorated ER stress, enhanced hepatic insulin activity and systemically improved glucose tolerance (*46*) (Fig. 3B).

The classic form of α_1-antitrypsin deficiency is caused by a point mutation, resulting in accumulation of misfolded α_1-antitrypsin in the ER and the formation of intrahepatic inclusions that cause cirrhosis. This activates autophagy to degrade the inclusions. Importantly, carbamazepine, which increases autophagy and the disposal of aggregation-prone proteins, reduces the severity of liver disease in a mouse model of α_1-antitrypsin deficiency by enhancing the degradation of misfolded α_1-antitrypsin (*47*) (Fig. 3B). Moreover, autophagy activation using rapamycin—an inhibitor of mTOR—eliminates Mallory-Denk bodies that form as acidophilic cytoplasm aggregates, a hallmark of alcoholic liver injury (*48*). Although the removal of the degenerating and/or excessive organelles and proteins described above seems to be selective, the molecular underpinnings largely remain unclear.

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Prevention of tumourigenesis

As shown in Fig. 2A, mutation or deletion of oncogenes and tumour suppressor genes involved in insulin signalling inhibits autophagy. This suggests that reduced autophagy owing to constantly activated mTORC1 may participate in neoplasia. In fact, liver-specific *phosphatase and tensin homolog (PTEN)* or *tuberous sclerosis 1 (TSC1)* knockout mice develop hepatocellular carcinoma and exhibit constitutively activated mTOR and decreased autophagic activity (*49–51*). How impaired autophagy contributes to neoplasia, however, was unknown because mTOR controls multiple physiologic processes, including protein translation, autophagy and gene transcription. In 1999, *Beclin 1* was identified as a tumour suppressor gene (*52*), suggesting a tumour suppressive role for autophagy. *Beclin* $1^{+/-}$ mice significantly reduced autophagic activity and increased the risk for cancer; these mice develop spontaneous tumours, including hepatocellular carcinoma, and more frequently develop liver tumours after hepatitis B virus infections (*53*, *54*). Moreover, the Beclin 1-interacting protein UVRAG suppresses the proliferation and tumourigenicity of human colon cancer cells (*12*). Long-term deletion of autophagy-specialized gene (*e.g.*, *Atg5* or *Atg7*) resulted in multiple tumours in the livers, demonstrating a tumour-suppressive effect for autophagy, at least in mouse livers. Micro tumours form in the livers of systemically mosaic *Atg5*-deficient mice and hepatocyte-specific *Atg7* knockout mice when the mice are 7–9 months old (*42*, *55*). Importantly, *Atg5* mosaic knockouts develop tumours only in liver tissues (*55*). As the mice get older, the number and size of the tumours increase, and the livers are almost covered by tumours when the mice reach 16–19 months. *Atg5*- or *Atg7*-deficient hepatocytes/tumour cells show enlarged mitochondria and a large number of peroxisomes, probably due to impaired organelle quality control; protein oxidation and oxidative stress have also been observed in these cells (*42*, *55*). As a result, cells in autophagy-deficient livers are thought to have genomic instability, resulting in spontaneous tumourigenesis (Figs. 3B and 4B). Tumours in mutant livers are monoclonal with regular arrangements and patterns, and metastasis is not observed in other tissues, suggesting that these tumours are benign adenomas (*42*, *55*). These results suggest that, in addition to suppressing tumourigenesis in livers, autophagy contributes to the malignant transformation of hepatic adenomas.

Liver Disorders and the Autophagy-Specific Substrate p62

p62/SQSTM1/A170 (hereafter referred to as p62) is a stress-inducible intracellular protein that is conserved in most multicellular organisms, but not plants or fungi (*56*, *57*). Mutations in the ubiquitin-associated (Uba) domain of p62 give rise to inherited Paget's disease of bone (*58*). p62 is targeted to the autophagosome formation site on the ER; targeting is dependent on the N-terminal Phox and Bem1p (PB1) domain, which is responsible for self- and hetero-oligomerization (*59*) (Fig. 4A) and directly interacts with LC3 through the LC3-interacting region (LIR) (*60*, *61*) (Fig. 4A). Subsequently, p62 is incorporated into the autophagosome and degraded by autophagy. Interestingly, p62 is also thought to be a receptor for ubiquitinated cargos to deliver them into autophagosomes (*57*, *62*, *63*). This section focuses on the pathophysiologic significance of autophagic turnover of p62.

p62 and aggregate formation

Suppression of autophagy results in marked accumulation of p62 followed by formation of large aggregates positive for p62 and ubiquitinated proteins because of the nature of both self-oligomerization and ubiquitin-binding of p62 (*64*, *65*) (Fig. 4B). Notably, similar ubiquitin- and p62-positive aggregates have been identified in various human disorders, including neurodegenerative diseases (Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis), liver disorders (alcoholic hepatitis and steatohepatitis) and cancers (malignant glioma and hepatocellular carcinoma) (*66*). Intriguingly, the aggregates observed in hepatocytes and neurons of mice with liver- or brain-specific knockouts of *Atg7* mice and in human hepatocellular carcinoma cells are not observed when *p62* is knocked out (*42*, *64*). Nbr1 and p62 have similar domain structures—for example, a PB1 domain, zinc finger domain, LC3-interacting region and the Uba domain (Fig. 4A)—and are both selective substrates for autophagy (*67*). Like p62, Nbr1 accumulates in autophagy-deficient hepatocytes, and extensively colocalizes with p62- and ubiquitin-positive aggregates (*67*); these structures—for example, Mallory-Denk bodies in acidophilic body are hallmarks of alcoholic liver injury, and intracytoplasmic hyaline bodies are found in hepatocellular carcinoma—have been identified in human liver diseases, although the role of Nbr1 in aggregate formation has not been elucidated.

p62 as a signalling hub

Because p62 was originally identified as an atypical protein kinase C (aPKC)-interacting protein, p62 has been hypothesized to be involved in nuclear factor-kappaB (NF-κB) signalling and cytoskeletal rearrangement, which are regulated by aPKC proteins (*68*). Moreover, p62 promotes ubiquitination of TNF receptor-associated factor 6 (TRAF6) in response to interleukin 1, Receptor Activator of Nuclear Factor κB (RANK) ligand and nerve growth factor, thereby activating NF-κB to enhance cell survival (*69*) (Fig. 4B). In contrast, when caspase-8 is ubiquitinated in repsonse to tumour necrosis factor (TNF)-related apoptosis-inducing ligand, p62 promotes aggregation of ubiqutinated caspase-8, leading to full activation and processing of the enzyme and committing the cell to apoptosis (*70*) (Fig. 4B). In addition, p62 interacts with the Nrf2-binding site of Keap1, a component of Cullin3-type ubiquitin ligase for Nrf2 that is responsible for the expression of a battery of genes encoding antioxidant proteins and detoxification enzymes (*71*, *72*) (Fig. 4B). Because autophagy strictly regulates p62 protein levels, autophagy may also control NF-κB signalling, activation of apoptosis and responses to

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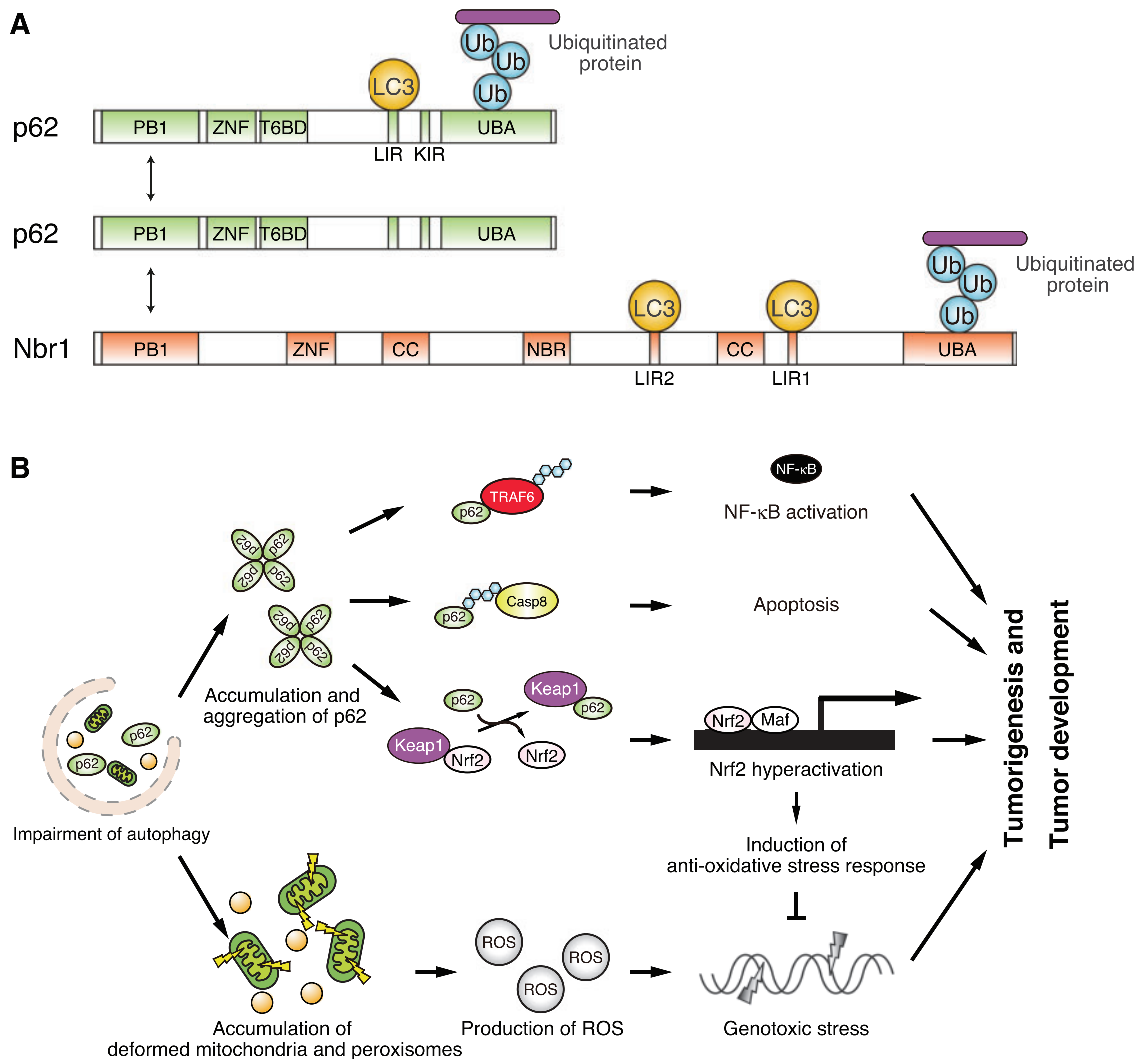

Fig. 4 Selective turnover of p62 by autophagy. (**A**) Domain structures of p62 and Nbr1. The PB1 domain self-oligomerizes and hetero-oligomerizes with other proteins containing PB1 domains, such as Nbr1. LC3 directly binds p62 and Nbr1 through the LIR. KIR, Keap1-interacting region; T6BD, TRAF6-binding domain; UBA, ubiquitin-associated domain; ZNF, zinc finger domain. (**B**) p62 functions as a signalling hub for NF-κB signalling, apoptosis and Nrf2 activation. Defective autophagy is characterized by dysregulation in these signalling pathways as well as impaired organelle homeostasis, pathologic processes that make significant contributions to liver cancers.

environmental stress. If this is the case, impaired autophagy would be accompanied by dysregulation in these signalling pathways.

p62 is a predisposing factor for disease in autophagy-deficient livers

Notably, loss of p62 or Nrf2 markedly attenuates the liver phenotypes that are induced by impaired autophagy, including hepatomegaly, hepatocytic hypertrophy and liver injury (*64*, *71*), suggesting that persistent activation of Nrf2 due to impaired p62 turnover is a major pathogenic response to deficient autophagy. The mechanism by which constitutive Nrf2 activation causes liver disorders is unclear. In contrast, concomitant loss of *p62* did not ameliorate or exacerbate the neurodegeneration phenotype in mice with brain-specific deletions of *Atg7* (*64*). Thus, the cytotoxicity of persistent Nrf2 activation may be tissue specific. A large number of mouse lines with tissue-specific deletions of *Atg* have been generated and analyzed, and loss of autophagy causes various life-threatening diseases (*73*). The pathologic significance of abnormal p62 accumulation for disease onset has been examined only in liver and brain tissue, however.

The role of p62 in tumourigenesis and tumour development

As mentioned above, mice heterozygous for *Beclin 1* mutation, systemically mosaic for *Atg5* deficiency or lacking *Atg7* specifically in liver tissue develop liver tumours probably due to dysregulation of signal transduction pathways as well as impaired organelle quality control (Fig. 4B). Liver adenoma growth in mice with liver-specific knockouts of *Atg7* is markedly suppressed when *p62* is also knocked out (*55*). Because constitutive activation of NF-κB is involved in tumourigenesis, dysregulation of NF-κB signalling due to excess accumulation of p62 is thought

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to predispose the mouse to tumour development. In agreement with this hypothesis, suppressing NF-κB signalling by knocking out *p62* prevents growth and development of Ras-induced lung adenocarcinoma (*74*), and inducing *p62* expression via constitutive activation of Kras contributes to the development of pancreatic ductal adenocarcinoma (*75*). Moreover, dysregulation of NF-κB signalling in autophagy-incompetent cells is due, at least in part, to increased p62 levels, which subsequently enhance tumourigenesis (*76*). On the other hand, Nrf2 activation also participates in tumourigenesis and/or tumour development. Somatic mutations in either *Keap1* or *Nrf2* have been identified in patients with lung, head and neck, or gall-bladder cancers (*77*, *78*). These mutations make tumour cells resistant to oxidative damage and anticancer agents, because the interaction between Keap1 and Nrf2 is disrupted followed by persistent activation of Nrf2. Nrf2 is activated in certain types of cancer even in absence of these somatic mutations. For instance, Keap1 is succinated in type 2 papillary renal cell carcinomas that carry mutations in fumarate hydratase, leading to hyperactivation of Nrf2 (*79*, *80*). In addition, oncogene-driven Nrf2 transcription serves as an early tumourigenic event (*81*). These studies suggest that activation of Nrf2 may be involved in tumourigenesis and tumour development. Interestingly, aggregates positive for p62 and Keap1 are often detected in human cancers, such as hepatocellular carcinoma, and expression of Nrf2 target genes has been observed in most of these tumours (*42*). Together, the evidence indicates that persistent activation of Nrf2 in response to increased levels of p62 contributes to hepatoma development.

Concluding Remarks

Basal levels of selective autophagy in postmitotic cells serve to protect the cells from accumulation of degenerative components, and adaptive autophagy provides cells with building blocks to overcome adverse conditions. In addition to these fundamental roles, a series of analyses with mice with disrupted autophagy specifically in the liver revealed some unexpected cellular functions of autophagy, such as regulation of NF-κB signalling and stress management via p62 turnover. We now know that dysfunction in these pathways is involved in various liver disorders and tumourigenesis. To take advantage of autophagy clinically for liver disease and cancer, however, effective indicators, biomarkers and/or probes of autophagy activity are required, particularly for monitoring the effects of drugs that target different steps in autophagy. Therefore, studies based on collaborations among industry, academia and government institutions are needed to develop autophagy-based diagnostic and therapeutic approaches for various disease states.

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Conflict of Interest

None declared.

References

1. Mizushima, N., Levine, B., Cuervo, A.M., and Klionsky, D.J. (2008) Autophagy fights disease through cellular self-digestion. *Nature* **451**, 1069–1075
2. Nakatogawa, H., Suzuki, K., Kamada, Y., and Ohsumi, Y. (2009) Dynamics and diversity in autophagy mechanisms: lessons from yeast. *Nat. Rev. Mol. Cell. Biol.* **10**, 458–467
3. Xie, Z. and Klionsky, D.J. (2007) Autophagosome formation: core machinery and adaptations. *Nat. Cell. Biol.* **9**, 1102–1109
4. Mizushima, N., Yoshimori, T., and Ohsumi, Y. (2011) The role of Atg proteins in autophagosome formation. *Annu. Rev. Cell. Dev. Biol.* **27**, 107–132
5. Hosokawa, N., Hara, T., Kaizuka, T., Kishi, C., Takamura, A., Miura, Y., Iemura, S., Natsume, T., Takehana, K., Yamada, N., Guan, J.L., Oshiro, N., and Mizushima, N. (2009) Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol. Biol. Cell.* **20**, 1981–1991
6. Jung, C.H., Jun, C.B., Ro, S.H., Kim, Y.M., Otto, N.M., Cao, J., Kundu, M., and Kim, D.H. (2009) ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol. Biol. Cell.* **20**, 1992–2003
7. Egan, D.F., Shackelford, D.B., Mihaylova, M.M., Gelino, S., Kohnz, R.A., Mair, W., Vasquez, D.S., Joshi, A., Gwinn, D.M., Taylor, R., Asara, J.M., Fitzpatrick, J., Dillin, A., Viollet, B., Kundu, M., Hansen, M., and Shaw, R.J. (2011) Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* **331**, 456–461
8. Kim, J., Kundu, M., Viollet, B., and Guan, K.L. (2011) AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell. Biol.* **13**, 132–141
9. Matsunaga, K., Morita, E., Saitoh, T., Akira, S., Ktistakis, N.T., Izumi, T., Noda, T., and Yoshimori, T. (2010) Autophagy requires endoplasmic reticulum targeting of the PI3-kinase complex via Atg14L. *J. Cell Biol* **190**, 511–521
10. Itakura, E. and Mizushima, N. (2010) Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins. *Autophagy* **6**, 764–776
11. Bodemann, B.O., Orvedahl, A., Cheng, T., Ram, R.R., Ou, Y.H., Formstecher, E., Maiti, M., Hazelett, C.C., Wauson, E.M., Balakireva, M., Camonis, J.H., Yeaman, C., Levine, B., and White, M.A. (2011) RalB and the exocyst mediate the cellular starvation response by direct activation of autophagosome assembly. *Cell* **144**, 253–267
12. Liang, C., Feng, P., Ku, B., Dotan, I., Canaani, D., Oh, B.H., and Jung, J.U. (2006) Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nat. Cell. Biol.* **8**, 688–699
13. Liang, C., Lee, J.S., Inn, K.S., Gack, M.U., Li, Q., Roberts, E.A., Vergne, I., Deretic, V., Feng, P., Akazawa, C., and Jung, J.U. (2008) Beclin1-binding UVRAG targets the class C Vps complex to coordinate

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autophagosome maturation and endocytic trafficking. *Nat. Cell. Biol.* **10**, 776–787

14. Matsunaga, K., Saitoh, T., Tabata, K., Omori, H., Satoh, T., Kurotori, N., Maejima, I., Shirahama-Noda, K., Ichimura, T., Isobe, T., Akira, S., Noda, T., and Yoshimori, T. (2009) Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. *Nat. Cell. Biol.* **11**, 385–396
15. Zhong, Y., Wang, Q.J., Li, X., Yan, Y., Backer, J.M., Chait, B.T., Heintz, N., and Yue, Z. (2009) Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex. *Nat. Cell. Biol.* **11**, 468–476
16. Wei, Y., Pattingre, S., Sinha, S., Bassik, M., and Levine, B. (2008) JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. *Mol. Cell.* **30**, 678–688
17. Axe, E.L., Walker, S.A., Manifava, M., Chandra, P., Roderick, H.L., Habermann, A., Griffiths, G., and Ktistakis, N.T. (2008) Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. *J. Cell Biol* **182**, 685–701
18. Polson, H.E., de Lartigue, J., Rigden, D.J., Reedijk, M., Urbe, S., Clague, M.J., and Tooze, S.A. (2010) Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. *Autophagy* **6**, 506–522
19. Young, A.R., Chan, E.Y., Hu, X.W., Kochl, R., Crawshaw, S.G., High, S., Hailey, D.W., Lippincott-Schwartz, J., and Tooze, S.A. (2006) Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. *J. Cell. Sci.* **119**, 3888–3900
20. Webber, J.L. and Tooze, S.A. (2010) Coordinated regulation of autophagy by p38alpha MAPK through mAtg9 and p38IP. *Embo. J.* **29**, 27–40
21. Orsi, A., Razi, M., Dooley, H., Robinson, D., Weston, A., Collinson, L., and Tooze, S. (2012) Dynamic and transient interactions of Atg9 with autophagosomes, but not membrane integration, is required for autophagy. *Mol. Biol. Cell.* **23(10)**, 1860–1873
22. Mizushima, N., Noda, T., Yoshimori, T., Tanaka, Y., Ishii, T., George, M.D., Klionsky, D.J., Ohsumi, M., and Ohsumi, Y. (1998) A protein conjugation system essential for autophagy. *Nature* **395**, 395–398
23. Ichimura, Y., Kirisako, T., Takao, T., Satomi, Y., Shimonishi, Y., Ishihara, N., Mizushima, N., Tanida, I., Kominami, E., Ohsumi, M., Noda, T., and Ohsumi, Y. (2000) A ubiquitin-like system mediates protein lipidation. *Nature* **408**, 488–492
24. Mizushima, N., Kuma, A., Kobayashi, Y., Yamamoto, A., Matsubae, M., Takao, T., Natsume, T., Ohsumi, Y., and Yoshimori, T. (2003) Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate. *J. Cell. Sci.* **116**, 1679–1688
25. Fujita, N., Itoh, T., Omori, H., Fukuda, M., Noda, T., and Yoshimori, T. (2008) The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. *Mol. Biol. Cell.* **19**, 2092–2100
26. Mizushima, N., Yamamoto, A., Hatano, M., Kobayashi, Y., Kabeya, Y., Suzuki, K., Tokuhisa, T., Ohsumi, Y., and Yoshimori, T. (2001) Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells. *J. Cell Biol* **152**, 657–668
27. Sou, Y.S., Waguri, S., Iwata, J., Ueno, T., Fujimura, T., Hara, T., Sawada, N., Yamada, A., Mizushima, N., Uchiyama, Y., Kominami, E., Tanaka, K., and Komatsu, M. (2008) The Atg8 conjugation system is indispensable for proper development of autophagic isolation membranes in mice. *Mol. Biol. Cell.* **19**, 4762–4775
28. Fujita, N., Hayashi-Nishino, M., Fukumoto, H., Omori, H., Yamamoto, A., Noda, T., and Yoshimori, T. (2008) An Atg4B mutant hampers the lipidation of LC3 paralogues and causes defects in autophagosome closure. *Mol. Biol. Cell.* **19**, 4651–4659
29. Kabeya, Y., Mizushima, N., Ueno, T., Yamamoto, A., Kirisako, T., Noda, T., Kominami, E., Ohsumi, Y., and Yoshimori, T. (2000) LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *Embo. J.* **19**, 5720–5728
30. De Duve, C., Pressman, B.C., Gianetto, R., Wattiaux, R., and Appelmans, F. (1955) Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochem. J.* **60**, 604–617
31. Ashford, T.P. and Porter, K.R. (1962) Cytoplasmic components in hepatic cell lysosomes. *J. Cell Biol* **12**, 198–202
32. Novikoff, A.B. and Essner, E. (1962) Cytolysomes and mitochondrial degeneration. *J. Cell Biol* **15**, 140–146
33. Mortimore, G.E. and Schworer, C.M. (1977) Induction of autophagy by amino-acid deprivation in perfused rat liver. *Nature* **270**, 174–176
34. Schworer, C.M. and Mortimore, G.E. (1979) Glucagon-induced autophagy and proteolysis in rat liver: mediation by selective deprivation of intracellular amino acids. *Proc. Natl. Acad. Sci. USA.* **76**, 3169–3173
35. Seglen, P.O., Gordon, P.B., and Poli, A. (1980) Amino acid inhibition of the autophagic/lysosomal pathway of protein degradation in isolated rat hepatocytes. *Biochim. Biophys. Acta.* **630**, 103–118
36. Seglen, P.O. and Gordon, P.B. (1982) 3-Methyladenine: specific inhibitor of autophagic/lysosomal protein degradation in isolated rat hepatocytes. *Proc. Natl. Acad. Sci. USA.* **79**, 1889–1892
37. Mortimore, G.E., Hutson, N.J., and Surmacz, C.A. (1983) Quantitative correlation between proteolysis and macro- and microautophagy in mouse hepatocytes during starvation and refeeding. *Proc. Natl. Acad. Sci. USA.* **80**, 2179–2183
38. Ezaki, J., Matsumoto, N., Takeda-Ezaki, M., Komatsu, M., Takahashi, K., Hiraoka, Y., Taka, H., Fujimura, T., Takehana, K., Yoshida, M., Iwata, J., Tanida, I., Furuya, N., Zheng, D.M., Tada, N., Tanaka, K., Kominami, E., and Ueno, T. (2011) Liver autophagy contributes to the maintenance of blood glucose and amino acid levels. *Autophagy* **7**, 727–736
39. Singh, R., Kaushik, S., Wang, Y., Xiang, Y., Novak, I., Komatsu, M., Tanaka, K., Cuervo, A.M., and Czaja, M.J. (2009) Autophagy regulates lipid metabolism. *Nature* **458**, 1131–1135
40. Kaushik, S., Rodriguez-Navarro, J.A., Arias, E., Kiffin, R., Sahu, S., Schwartz, G.J., Cuervo, A.M., and Singh, R. (2011) Autophagy in hypothalamic AgRP neurons regulates food intake and energy balance. *Cell. Metab.* **14**, 173–183
41. Komatsu, M., Waguri, S., Ueno, T., Iwata, J., Murata, S., Tanida, I., Ezaki, J., Mizushima, N., Ohsumi, Y., Uchiyama, Y., Kominami, E., Tanaka, K., and Chiba, T. (2005) Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. *J. Cell Biol* **169**, 425–434
42. Inami, Y., Waguri, S., Sakamoto, A., Kouno, T., Nakada, K., Hino, O., Watanabe, S., Ando, J.,

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Iwadate, M., Yamamoto, M., Lee, M.S., Tanaka, K., and Komatsu, M. (2011) Persistent activation of Nrf2 through p62 in hepatocellular carcinoma cells. *J. Cell Biol* **193**, 275–284

43. Jaber, N., Dou, Z., Chen, J.S., Catanzaro, J., Jiang, Y.P., Ballou, L.M., Selinger, E., Ouyang, X., Lin, R.Z., Zhang, J., and Zong, W.X. (2012) Class III PI3K Vps34 plays an essential role in autophagy and in heart and liver function. *Proc. Natl. Acad. Sci. USA*. **109**, 2003–2008
44. Zhang, C. and Cuervo, A.M. (2008) Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function. *Nat. Med.* **14**, 959–965
45. Iwata, J., Ezaki, J., Komatsu, M., Yokota, S., Ueno, T., Tanida, I., Chiba, T., Tanaka, K., and Kominami, E. (2006) Excess peroxisomes are degraded by autophagic machinery in mammals. *J. Biol. Chem.* **281**, 4035–4041
46. Yang, L., Li, P., Fu, S., Calay, E.S., and Hotamisligil, G.S. (2010) Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. *Cell. Metab*. **11**, 467–478
47. Hidvegi, T., Ewing, M., Hale, P., Dippold, C., Beckett, C., Kemp, C., Maurice, N., Mukherjee, A., Goldbach, C., Watkins, S., Michalopoulos, G., and Perlmutter, D.H. (2010) An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis. *Science* **329**, 229–232
48. Harada, M., Hanada, S., Toivola, D.M., Ghori, N., and Omary, M.B. (2008) Autophagy activation by rapamycin eliminates mouse Mallory-Denk bodies and blocks their proteasome inhibitor-mediated formation. *Hepatology* **47**, 2026–2035
49. Horie, Y., Suzuki, A., Kataoka, E., Sasaki, T., Hamada, K., Sasaki, J., Mizuno, K., Hasegawa, G., Kishimoto, H., Iizuka, M., Naito, M., Enomoto, K., Watanabe, S., Mak, T.W., and Nakano, T. (2004) Hepatocyte-specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas. *J. Clin. Invest*. **113**, 1774–1783
50. Arico, S., Petiot, A., Bauvy, C., Dubbelhuis, P.F., Meijer, A.J., Codogno, P., and Ogier-Denis, E. (2001) The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. *J. Biol. Chem.* **276**, 35243–35246
51. Menon, S., Yecies, J.L., Zhang, H.H., Howell, J.J., Nicholatos, J., Harputlugil, E., Bronson, R.T., Kwiatkowski, D.J., and Manning, B.D. (2012) Chronic activation of mTOR complex 1 is sufficient to cause hepatocellular carcinoma in mice. *Sci. Signal*. **5**, ra24
52. Liang, X.H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H., and Levine, B. (1999) Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature* **402**, 672–676
53. Qu, X., Yu, J., Bhagat, G., Furuya, N., Hibshoosh, H., Troxel, A., Rosen, J., Eskelinen, E.L., Mizushima, N., Ohsumi, Y., Cattoretti, G., and Levine, B. (2003) Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J. Clin. Invest*. **112**, 1809–1820
54. Yue, Z., Jin, S., Yang, C., Levine, A.J., and Heintz, N. (2003) Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc. Natl. Acad. Sci. USA*. **100**, 15077–15082
55. Takamura, A., Komatsu, M., Hara, T., Sakamoto, A., Kishi, C., Waguri, S., Eishi, Y., Hino, O., Tanaka, K., and Mizushima, N. (2011) Autophagy-deficient mice develop multiple liver tumors. *Genes Dev*. **25**, 795–800
56. Ishii, T., Itoh, K., Takahashi, S., Sato, H., Yanagawa, T., Katoh, Y., Bannai, S., and Yamamoto, M. (2000) Transcription factor Nrf2 coordinately regulates a group of oxidative stress-inducible genes in macrophages. *J. Biol. Chem.* **275**, 16023–16029
57. Johansen, T. and Lamark, T. (2011) Selective autophagy mediated by autophagic adapter proteins. *Autophagy* **7**, 279–296
58. Goode, A. and Layfield, R. (2010) Recent advances in understanding the molecular basis of Paget disease of bone. *J. Clin. Pathol*. **63**, 199–203
59. Itakura, E. and Mizushima, N. (2011) p62 Targeting to the autophagosome formation site requires self-oligomerization but not LC3 binding. *J. Cell Biol* **192**, 17–27
60. Pankiv, S., Clausen, T.H., Lamark, T., Brech, A., Bruun, J.A., Outzen, H., Overvatn, A., Bjorkoy, G., and Johansen, T. (2007) p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J. Biol. Chem.* **282**, 24131–24145
61. Ichimura, Y., Kumanomidou, T., Sou, Y.S., Mizushima, T., Ezaki, J., Ueno, T., Kominami, E., Yamane, T., Tanaka, K., and Komatsu, M. (2008) Structural basis for sorting mechanism of p62 in selective autophagy. *J. Biol. Chem.* **283**, 22847–22857
62. Weidberg, H., Shvets, E., and Elazar, Z. (2011) Biogenesis and cargo selectivity of autophagosomes. *Annu. Rev. Biochem*. **80**, 125–156
63. Kirkin, V., McEwan, D.G., Novak, I., and Dikic, I. (2009) A role for ubiquitin in selective autophagy. *Mol. Cell*. **34**, 259–269
64. Komatsu, M., Waguri, S., Koike, M., Sou, Y.S., Ueno, T., Hara, T., Mizushima, N., Iwata, J., Ezaki, J., Murata, S., Hamazaki, J., Nishito, Y., Iemura, S., Natsume, T., Yanagawa, T., Uwayama, J., Warabi, E., Yoshida, H., Ishii, T., Kobayashi, A., Yamamoto, M., Yue, Z., Uchiyama, Y., Kominami, E., and Tanaka, K. (2007) Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. *Cell* **131**, 1149–1163
65. Nezis, I.P., Simonsen, A., Sagona, A.P., Finley, K., Gaumer, S., Contamine, D., Rusten, T.E., Stenmark, H., and Brech, A. (2008) Ref(2)P, the Drosophila melanogaster homologue of mammalian p62, is required for the formation of protein aggregates in adult brain. *J. Cell Biol* **180**, 1065–1071
66. Zatloukal, K., Stumptner, C., Fuchsbichler, A., Heid, H., Schnoelzer, M., Kenner, L., Kleinert, R., Prinz, M., Aguzzi, A., and Denk, H. (2002) p62 is a common component of cytoplasmic inclusions in protein aggregation diseases. *Am. J. Pathol*. **160**, 255–263
67. Kirkin, V., Lamark, T., Sou, Y.S., Bjorkoy, G., Nunn, J.L., Bruun, J.A., Shvets, E., McEwan, D.G., Clausen, T.H., Wild, P., Bilusic, I., Theurillat, J.P., Overvatn, A., Ishii, T., Elazar, Z., Komatsu, M., Dikic, I., and Johansen, T. (2009) A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. *Mol. Cell*. **33**, 505–516
68. Moscat, J. and Diaz-Meco, M.T. (2000) The atypical protein kinase Cs. Functional specificity mediated by specific protein adapters. *EMBO. Rep*. **1**, 399–403
69. Moscat, J., Diaz-Meco, M.T., and Wooten, M.W. (2007) Signal integration and diversification through the p62 scaffold protein. *Trends Biochem. Sci*. **32**, 95–100

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70. Jin, Z., Li, Y., Pitti, R., Lawrence, D., Pham, V.C., Lill, J.R., and Ashkenazi, A. (2009) Cullin3-based polyubiquitination and p62-dependent aggregation of caspase-8 mediate extrinsic apoptosis signaling. *Cell* **137**, 721–735
71. Komatsu, M., Kurokawa, H., Waguri, S., Taguchi, K., Kobayashi, A., Ichimura, Y., Sou, Y.S., Ueno, I., Sakamoto, A., Tong, K.I., Kim, M., Nishito, Y., Iemura, S., Natsume, T., Ueno, T., Kominami, E., Motohashi, H., Tanaka, K., and Yamamoto, M. (2010) The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat. Cell. Biol.* **12**, 213–223
72. Lau, A., Wang, X.J., Zhao, F., Villeneuve, N.F., Wu, T., Jiang, T., Sun, Z., White, E., and Zhang, D.D. (2010) A noncanonical mechanism of Nrf2 activation by autophagy deficiency: direct interaction between Keap1 and p62. *Mol. Cell. Biol.* **30**, 3275–3285
73. Mizushima, N. and Komatsu, M. (2011) Autophagy: renovation of cells and tissues. *Cell* **147**, 728–741
74. Duran, A., Linares, J.F., Galvez, A.S., Wikenheiser, K., Flores, J.M., Diaz-Meco, M.T., and Moscat, J. (2008) The signaling adaptor p62 is an important NF-kappaB mediator in tumorigenesis. *Cancer Cell* **13**, 343–354
75. Ling, J., Kang, Y., Zhao, R., Xia, Q., Lee, D.F., Chang, Z., Li, J., Peng, B., Fleming, J.B., Wang, H., Liu, J., Lemischka, I.R., Hung, M.C., and Chiao, P.J. (2012) KrasG12D-induced IKK2/beta/NF-kappaB activation by IL-1alpha and p62 feedforward loops is required for development of pancreatic ductal adenocarcinoma. *Cancer Cell* **21**, 105–120
76. Mathew, R., Karp, C.M., Beaudoin, B., Vuong, N., Chen, G., Chen, H.Y., Bray, K., Reddy, A., Bhanot, G., Gelinas, C., Dipaola, R.S., Karantza-Wadsworth, V., and White, E. (2009) Autophagy suppresses tumorigenesis through elimination of p62. *Cell* **137**, 1062–1075
77. Taguchi, K., Motohashi, H., and Yamamoto, M. (2011) Molecular mechanisms of the Keap1-Nrf2 pathway in stress response and cancer evolution. *Genes Cells* **16**, 123–140
78. Hayes, J.D. and McMahon, M. (2009) NRF2 and KEAP1 mutations: permanent activation of an adaptive response in cancer. *Trends Biochem. Sci.* **34**, 176–188
79. Adam, J., Hatipoglu, E., O'Flaherty, L., Ternette, N., Sahgal, N., Lockstone, H., Baban, D., Nye, E., Stamp, G.W., Wolhuter, K., Stevens, M., Fischer, R., Carmeliet, P., Maxwell, P.H., Pugh, C.W., Frizzell, N., Soga, T., Kessler, B.M., El-Bahrawy, M., Ratcliffe, P.J., and Pollard, P.J. (2011) Renal cyst formation in Fh1-deficient mice is independent of the Hif/Phd pathway: roles for fumarate in KEAP1 succination and Nrf2 signaling. *Cancer Cell* **20**, 524–537
80. Kinch, L., Grishin, N.V., and Brugarolas, J. (2011) Succination of Keap1 and activation of Nrf2-dependent antioxidant pathways in FH-deficient papillary renal cell carcinoma type 2. *Cancer Cell* **20**, 418–420
81. DeNicola, G.M., Karreth, F.A., Humpton, T.J., Gopinathan, A., Wei, C., Frese, K., Mangal, D., Yu, K.H., Yeo, C.J., Calhoun, E.S., Scrimieri, F., Winter, J.M., Hruban, R.H., Iacobuzio-Donahue, C., Kern, S.E., Blair, I.A., and Tuveson, D.A. (2011) Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature* **475**, 106–109

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