Emerging roles of ATG7 in human health and disease

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Abstract

The cardinal stages of macroautophagy are driven by core autophagy-related (ATG) proteins, whose ablation largely abolishes intracellular turnover. Disrupting ATG genes is paradigmatic of studying autophagy deficiency, yet emerging data suggest that ATG proteins have extensive biological importance beyond autophagic elimination. An important example is ATG7, an essential autophagy effector enzyme that in concert with other ATG proteins, also regulates immunity, cell death and protein secretion, and independently regulates the cell cycle and apoptosis. Recently, a direct association between ATG7 dysfunction and disease was established in patients with biallelic ATG7 variants and childhood-onset neuropathology. Moreover, a prodigious body of evidence supports a role for ATG7 in protecting against complex disease states in model organisms, although how dysfunctional ATG7 contributes to manifestation of these diseases, including cancer, neurodegeneration and infection, in humans remains unclear. Here, we systematically review the biological functions of ATG7, discussing the impact of its impairment on signalling pathways and human pathology. Future studies illuminating the molecular relationship between ATG7 dysfunction and disease will expedite therapies for disorders involving ATG7 deficiency and/or impaired autophagy.

Keywords ATG7; autophagy; disease; neurodegeneration; therapeutics

Subject Categories Autophagy & Cell Death

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See the Glossary for abbreviations used in this article.

Introduction

The degradation of encapsulated cytoplasmic material via the endolysosomal system provides a first-principle definition of autophagy. Numerous specialised autophagy pathways have been discovered and categorised, including macroautophagy, which can be selective or non-selective, and other variants such as chaperone-mediated autophagy (CMA) and microautophagy. CMA recognises protein substrates with KFERQ motifs and translocates these to lysosomes (Kaushik & Cuervo, 2018). Microautophagy involves the direct engulfment and destruction of cytoplasmic substrates by lysosomal membrane invagination (Schuck, 2020). Macroautophagy (hereafter, “autophagy”) remains the most widely studied pathway. During autophagy, a transient double-membrane-bound autophagosome engulfs cytoplasmic constituents, eventually fusing with acidic endolysosomal compartments where hydrolysis degrades cargo (Yorimitsu & Klionsky, 2005).

The fundamental morphological and molecular signatures of autophagy have remained largely unchanged over the past 10 years (Levine & Kroemer, 2008, 2019). Autophagy functions constitutively under basal conditions (Mizushima et al., 2004), but can be induced further by a number of stimuli, including starvation, hypoxia and DNA damage (Kroemer et al., 2010). This triggers the de novo nucleation of a phagophore, a double-membrane cup-shaped structure that matures via the incorporation of supplementary lipids (Nakamura & Yoshimori, 2017). A variety of cytoplasmic cargoes, including organelles, microbes and cytotoxic protein aggregates can be sequestered within this transient structure (Johansen & Lamark, 2020). Autophagy can be a non-selective or selective process (Mizushima & Komatsu, 2011). “Bulk” autophagy involves the non-selective sequestration of cytoplasmic material, ensuring the degradation of long-lived proteins and replenishment of essential building blocks. During selective autophagy, specific cellular components are decorated with specialised signals that recruit the autophagic machinery to the target entity for elimination. Selectivity is conferred upon the pathway by LC3-interacting region (LIR) motifs [(W/F/Y)XX(L/I/V)] that are present within cargo or specialised adaptor proteins, enabling them to interact with ATG8 proteins that are embedded within the inner and outer membrane of the phagophore (Martens & Fracchiolla, 2020). Following cargo sequestration, the leading edges of the double-membrane structure fuse to generate
an autophagosome which merges with acidic compartments of the endolysosomal system. After the inner autophagosomal membrane is degraded, the resident lysosomal hydrolases degrade the autophagic cargo which is then recycled (Koyama-Honda et al, 2017) (Fig 1A).

In the 1990s, pioneering studies using yeast genetic screens helped to define the molecular basis of autophagy (Tsukada & Ohsumi, 1993; Harding et al, 1995). This approach led to the identification of autophagy-related (ATG) genes, and whilst the exact number is debated, approximately 20 of these are “core” ATG genes, conserved across eukaryotes and encoding proteins essential for both non-selective and selective autophagy (Tsukada & Ohsumi, 1993). Auxiliary ATG proteins enhance the autophagic process and/or participate in selective autophagy, though degradative autophagy in any case requires functional lysosomal proteins (Tanaka et al, 2000). One of the key molecular signatures of autophagy is ATG8 lipidation, a process whereby ATG8 is conjugated to phosphatidylethanolamine (PE) embedded in the emerging phagophore, thus enabling ATG8 to become an integral part of the autophagic membrane (Martens & Fracchiolla, 2020). ATG8 lipidation is a particularly important event because ATG8 facilitates several stages of autophagy including phagophore expansion, cargo recruitment, autophagosome transport and lysosomal fusion. In mammals, there are six ATG8 homologues, classified in the LC3 or GABARAP protein subfamilies.

A large body of evidence has demonstrated that ATG proteins contribute to a diverse range of biological processes that extend far beyond autophagy (Levine & Kroemer, 2019). ATG7 is one such multifaceted core ATG protein that drives the cardinal stages of classical degradative autophagy through ATG8 lipidation. ATG7 also makes pivotal contributions to innate immunity via LC3-associated phagocytosis, unconventional protein secretion, receptor recycling, exocytosis of secretory granules and modulation of p53-dependent cell cycle arrest and apoptosis ( Mizushima & Levine, 2020; Mizushima, 2020) (Figs 1 and 2). This review will describe the role of ATG7 in these pathways, and the breakthrough genetic models that have led to our understanding of how ATG7 deficiency affects mammalian physiology. We will explore the association of impaired ATG7 activity with human pathologies including neurodegeneration, cancer and infection and pay particular attention to the recently identified recessive congenital disorder of autophagy caused by inherited ATG7 dysfunction leading to neurological manifestations ( Collier et al, 2021). Recent breakthroughs in delineating the role of ATG7 in cell biology and human disease have important implications for the development of therapeutics that regulate autophagy. Whereas activation of autophagy provides an attractive therapeutic approach to treat human neuropathology where impaired autophagy is implicated, evidence has also emerged that autophagy inhibition can improve cancer treatment outcomes ( Mizushima & Levine, 2020).

Biological functions of ATG7

**Classical degradative autophagy**

ATG7 impairment classically renders cells and tissues as “autophagy deficient”, and the study of autophagy has underpinned the majority of ATG7-focused research (Komatsu et al, 2005; Komatsu et al, 2007a; Matsumoto et al, 2008; Collier et al, 2021). During autophagy, the phagophore membrane is enriched with phosphatidylethanolamine (PE), an abundant phospholipid that has been reported to positively regulate autophagic activity (Rockenfeller et al, 2015). PE is important because it acts as the anchor for recruitment of cytosolic ATG8 to the emerging phagophore membrane (Fig 1B). There are two mammalian ATG8 subfamilies encoding six homologues to yeast atg8 protein. The first, LC3, has three
members, LC3A, LC3B and LC3C, and the second, GABARAP, represents the remaining three homologues. GABARAP, GABARAPL1 and GABARAPL2 (Lee & Lee, 2016). By convention, ATG8 refers to both LC3 and GABARAP subfamilies, and upon lipidation becomes “ATG8-PE”. Whereas conjugated GABARAP is similarly referred to as “GABARAP-PE”, the lipilated form of LC3 is termed “LC3-II”.

Lipidation of ATG8 remains an important marker for evaluating levels of autophagy in tissue and cells (Mizushima et al, 1998, 2010; Ichimura et al, 2000). In mammalian cells, levels of LC3 lipidation in particular are used to estimate autophagic flux via immunoblotting, yet this should not detract from the biological importance of GABARAP proteins.

Figure 1. ATG7 drives the fundamental stages of degradative autophagy.

(A) An overview of classical degradative autophagy showing the early, middle and late stages of the process. (B) Phagophore expansion is stimulated by ATG7, which facilitates ATG8 lipidation through its E1-like enzymatic activity. (C) ATG8 lipidation is also critical for selective autophagy and (D) contributes to the late stages of autophagy.
ATG8 lipidation is a multistep process, driven by the E1-like enzymatic activity of homodimeric ATG7 (Tanida et al., 1999, 2001; Komatsu et al., 2001) (Fig 1B). First, the protease ATG4 exposes the C-terminus glycine residue of ATG8, generating form I (e.g. LC3-I) (Kirisako et al., 1999). This form is then activated by ATG7 via adenylation, before it is transferred to ATG3 where it is conjugated.
to PE to generate form II (e.g. LC3-II) (Tanida et al, 1999; Ichimura et al., 2000; Taherbhoury et al, 2011) which localises to both the inner (IAM) and outer autophagosomal membranes (OAM), and is subsequently degraded upon autolysosome formation (Kabeya et al., 2004). ATG7 is also involved in a second autophagy conjugation reaction that supports ATG8 lipidation. During this reaction, ATG12 is adenylated by ATG7 then transferred to ATG5 via E2-like enzyme ATG10, generating ATG5-ATG12 conjugates (Mizushima et al., 1998; Shintani et al., 1999; Tanida et al., 1999; Yamaguchi et al, 2012) which are restricted to the OAM and removed prior to sealing of the autophagosome (Koyama-Honda et al., 2013). Although LC3-II can be produced in vitro in the presence of ATG3, ATG7, LC3, ATP and liposomes containing PE, ATG5-ATG12 forms a complex with ATG16L that promotes LC3 lipidation in vivo (Mizushima et al., 1999, 2003; Kuma et al., 2002; Hanada et al., 2007; Lystad et al., 2019). Evidence currently suggests that WIPI2 localises to PI3P-rich regions of the phagophore membrane, recruiting the ATG5-ATG12-ATG16L complex that binds ATG3, which transfers activated ATG8 to membrane-bound PE. Oxidation of ATG3 and ATG7 facilitates autophagy inhibition (Fruedt et al., 2018).

Endogenous ATG7 deletion prevents ATG8 lipidation, so ATG7 knockout (KO) models are commonly used to study the biological significance of the autophagy conjugation systems. ATG8 has diverse roles in the autophagy pathway (Lee & Lee, 2016; Johansen & Lamark, 2020). First, mammalian LC3-II is important for autophagosome maturation, with levels of lipitated LC3 correlating with the extent of autophagosome formation (Kabeya et al., 2004) and autophagic structures generated in the absence of ATG8 homologues are smaller (Nguyen et al., 2016). It was recently demonstrated that attachment of ATG8 to the phagophore membrane stimulates membrane deformation, leading to expansion of this structure and underpinning efficient autophagosome formation (Maruyama et al., 2021). Blockade of mammalian ATG8 lipidation through ATG3 deletion caused delayed autophagosome maturation and a significant reduction in the success rate of autophagosome formation (Tsuboyama et al., 2016). In fact, a number of proteins involved in the early and late stages of autophagy have LIRs, emphasising the ability of ATG8 family proteins to coordinate multiple stages of the autophagic process (Martens & Fracchiolla, 2020) (Fig 1D). Beyond autophagic membrane expansion, ATG8 homologues facilitate transport of autophagosomes along microtubules via interactions with motor proteins via adaptor proteins and contribute to the binding of autophagosomes to lysosomes (Lorincz & Juhasz, 2020; Martens & Fracchiolla, 2020). LC3B can also be phosphorylated to regulate autophagosome transport (Nieto-Torres et al., 2021). Moreover, loss of ATG7 impairs inner autophagosomal membrane (IAM) degradation after autophagosome–lysosome fusion (Tsuboyama et al., 2016). Consequently, autophagy is severely impaired by ATG7 deletion as evidenced in yeast, mouse and humans (Tanida et al., 1999; Komatsu et al., 2005; Luhr et al., 2018).

One of the most widely studied aspects of ATG8 lipidation is its requirement for selective autophagy which is currently defined by the recognition, sequestration and elimination of specific cytoplasmic cargo. This selectivity is achieved through the interaction of cargo with ATG8 via LIR motifs within specific receptors that act as “eat-me” signals for damaged or excess cellular components (Johansen & Lamark, 2020) (Fig 1C). Cargo types include organelles such as mitochondria (termed mitophagy), endoplasmic reticulum (ER-phagy or reticulophagy) and peroxisomes (pexophagy), proteins and protein aggregates (aggrephagy), and intracellular pathogens (xenophagy). Consequently, selective autophagy is an important homeostatic mechanism, preventing the accumulation of dysfunctional organelles, cytoplasmic aggregates and providing innate immune support, as well as a developmental tool facilitating the removal of mitochondria from maturing reticulocytes, cardiomyocytes, kidney cells and ocular tissues, for example (Sandoval et al., 2008; Mortensen et al, 2010; McWilliams et al., 2016, 2019; Esteban-Martinez et al., 2017). LIR motifs can be regulated through masking and activating/inhibitory phosphorylation to prevent promiscuous cargo sequestration under conditions where the autophagic degradation of that substrate or organelle has not been stimulated (Chen et al., 2014, 2016; Lv et al., 2017; Wei et al, 2017). Autophagic sequestration of mitochondrial proteins can also be regulated by acetylation (Webster et al., 2013). Although the fundamental mechanisms driving cargo selection are shared between ATG8 homologues, there is evidence of homologue-specific autophagy adaptor proteins (Wirth et al, 2019).

**Autophagy-related functions**

Autophagy-related functions of ATG7 involve membrane trafficking events that are dependent on LC3 lipidation. Consequently, these processes require the activity of other core ATG proteins that drive lipidation of ATG8 homologues, including ATG3, ATG5 and ATG12—members of the autophagy conjugation system (Mizushima, 2020) (Fig 1). This includes the innate immune process LC3-associated phagocytosis (LAP) (Heckmann & Green, 2019; Inomata et al., 2020) (Fig 2). During LAP, extracellular pathogens, dead/dying cells and other extracellular substrates are recognised by cell surface receptors then endocytosed, generating an intracellular single-membrane structure called the phagosome. Then, LC3-II, generated in an ATG7-dependent manner, is recruited to the phagosome in a process dependent on NOX2-derived ROS (Sanjuan et al., 2007; Martinez et al., 2015). This structure, the LAPosome, is now decorated with LC3-II and able to fuse with lysosomes for elimination (Sanjuan et al., 2007). A related process, termed entosis, is also dependent on LC3 lipidation. During entosis, viable cells are engulfed by epithelial cells. This process is regulated by the cell being engulfed, after which this cell undergoes non-apoptotic cell death driven by autophagosomes and lysosomes of the host cell (a process termed “non-cell autonomous autophagy”). When autophagy in the host cell is inhibited, the engulfed cell largely undergoes apoptosis (Florey et al., 2011), whereas others have been observed to divide inside the host cell, or escape into culture (Overholtzer et al., 2007). Tumour cells can also undergo entosis (Overholtzer et al., 2007; Fais & Overholtzer, 2018).

The activities of ATG7 can also be non-degradative. For example, LAP also facilitates Toll-like receptor 9 (TLR9) trafficking and converges with the classical autophagy pathway to regulate IFN-α production (Henault et al., 2012). ATG7-mediated LC3 lipidation is also required for the exocytic release of cathepsin K by osteoblasts (DeSelm et al, 2011), and LC3-positive lysozyme-containing granules are released by Paneth cells upon infection (Bel et al., 2017). Related to this, autophagosomes facilitate the unconventional secretion of proteins including IL1B and ferritin in response to lysosomal damage (Kimura et al., 2017), and loss of ATG7 causes accumulation of mucin granules in Goblet cells (Patel et al, 2013).
Autophagosomes are also able to sequester TBC1D5, thus freeing the retromer complex to mediate the return shuttling of GLUT1 transporters to the plasma membrane from endosomes (Roy et al., 2017). As part of the autophagic machinery, ATG7 is also involved in regulating the switch between apoptosis and necrosis (Goodall et al., 2016). This important study demonstrated that the necrosome (a protein complex that leads to rapid plasma membrane rupture and inflammation) can assemble on autophagosomes at select autophagy receptor p62 sites and that loss of p62 can switch cell death mechanisms towards apoptosis.

**Autophagy-independent functions**

ATG7 also participates in cellular functions that are independent of its E1-like enzymatic activity. Consequently, these functions do not require the other ATG machinery required for autophagy-associated signalling. A large body of evidence, largely attained through studying Mouse models of ATG7 deficiency, has described ATG7 have been described, with two involving modulation of p53 activity. Upon starvation, Atg7 has been reported to interact with p53 to inhibit the expression of pro-apoptotic genes Nos2, Puma and Bax. Accordingly, Atg7−/− mouse embryonic fibroblasts demonstrated augmented DNA damage under basal conditions. The proliferation of Atg7−/− cells proceeded at a far greater rate than control cells due to diminished p53-mediated p21 expression, which usually promotes cell cycle arrest (Lee et al., 2012). In another study, Atg7 was also shown to repress the pro-apoptotic properties of caspase 9 (Han et al., 2014). An isoform of ATG7 that lacks E1-like enzymatic activity has also been discovered, and this variant cannot lipidate ATG8 homologues (Ogmundsdottir et al., 2018). The biological function of this intriguing isoform is unknown, but it may negatively regulate autophagy by potentially disrupting the formation of functional ATG7 homodimers.

**Mouse models of Atg7 deficiency**

A prodigious body of evidence, largely attained through studying mouse genetic models, has demonstrated that faithful ATG7 function is essential for the development, maintenance and adaptation of mammalian tissues (Xiong, 2015). Embryonic Atg7 deletion in mice results in perinatal lethality, and the subsequent characterisation of conditional Atg7 deficiency in mice has illuminated the contribution of ATG7 to mammalian physiology (Table 1). It is notable that manipulation of other core Atg genes causes similar phenotypes to those observed in Atg7 KO models, supporting the role of autophagy in these discoveries. Here, we discuss these key genetic mouse models, exploring the phenotypic and cellular consequences of endogenous inhibition of mammalian ATG7.

**Systemic or whole-body Atg7 deletion**

Similarly to the majority of other core Atg genes, systemic knockout of Atg7 in mice causes death within 24 h after birth (Komatsu et al., 2005). The neonatal lethal phenotype is recapitulated across other core Atg genes, including Atg5 (Kuma et al., 2004). It was then demonstrated that neural reconstitution of Atg5 activity in Atg5-null mice prevents neonatal death (mice die between 8 weeks and 8 months after birth), revealing that neural dysfunction is the primary cause of perinatal death in whole-body knockout animals. This is possible due to a suckling defect (Yoshii et al., 2016), although Atg7−/− and Atg5−/− null mice died before wild-type mice, even under non-suckling conditions (Kuma et al., 2004; Komatsu et al., 2005). Conditional models such as tamoxifen-inducible whole-body Atg7 deletion in adult mice impaired glucose metabolism, causing death 2–3 months post-knockout due to neurodegeneration, and fasting these mice caused fatal hypoglycaemia (low blood glucose levels) and cachexia (muscle wasting) (Karsili-Uzunbas et al., 2014). Amino acid levels were also diminished in Atg7−/− KO mice (Komatsu et al., 2005). A combination of defective LAP and autophagy may underlie the susceptibility of inducible adult Atg7 KO mice to Strep-tococcus infection (Karsili-Uzunbas et al., 2014). Adult mice with concurrent Atg7 and p53 deletion have similar lifespan to p53 KO mice, and the double KO mice died from neurodegeneration without the tumour development that was observed in p53 KO mice (Yang et al., 2020). The double KO mice were more resistant to fasting, and liver and brain injury was decreased due to protection against apoptosis and DNA damage (Yang et al., 2020).

**Central nervous system**

Perhaps the most striking physiological effects of Atg7 deficiency manifest in the central nervous system, where conditional Atg7 KO caused neurodegeneration resulting in premature death (Komatsu et al., 2006). Mice also displayed an ataxic phenotype caused by selective vulnerability of cerebellar Purkinje neurons to Atg7 deficiency (Komatsu et al., 2006; Komatsu et al., 2007b) and behavioural abnormalities that are recapitulated in mice with myeloid-specific Atg7 deletion via impaired microglial synaptic refinement (Kim et al., 2017). Other regions in the brain affected by Atg7 deletion include the hypothalamus through impaired lipolysis and glucose homeostasis (Coupe et al., 2012; Kaushik et al., 2012), the forebrain via phosphorylated tau accumulation (Inoue et al., 2012b; Nilsson et al., 2013), and midbrain dopaminergic neurons through dysregulated presynaptic neurotransmission (Hernandez et al., 2012). Mice with neural stem cell-specific Atg7 KO were resistant to stress-induced cognitive deficits due to impaired cell death (Jung et al., 2020). It was also found that p62-positive neuronal inclusions hallmark defective autophagy in Atg7-deficient brain models (Komatsu et al., 2007a). This discovery cannot be overstated, as it provided a direct link between autophagy and the accumulation of proteinaceous inclusions that are characteristic of human neurodegenerative pathology.

**Liver**

Atg7-deficient mice demonstrated an impaired fasting response and enlarged livers, as well as the accumulation of abnormal organelles and p62 and ubiquitin-positive inclusions (Komatsu et al., 2005, 2007a). Impaired lipid metabolism, leading to increased cholesterol and triglyceride content, also contributes to liver injury (Singh et al., 2009a). Initially, it was thought that autophagy is required for progression of liver tumours to malignancy, as Atg7 KO caused adenoma formation but no cancerous tumours were detected (Takamura et al., 2011). However, a subsequent study reported that liver-specific Atg7 deletion causes hepatocellular carcinoma at 12 months (Lee, Noon, et al., 2018), with the authors commenting that this may be because of differences in genetic background and microbial environment. Accumulation of p62 in liver appears to play an important role in pathology. P62 primarily acts as an adaptor protein in autophagy,
but is also involved in the non-canonical regulation of the activity of oxidative stress-inducible transcription factor NRF2 (Sanchez-Martin & Komatsu, 2018). P62 competitively interacts with KEAP1, which usually binds NRF2 to prevent its nuclear translocation. Thus, p62 accumulation enhances the release of NRF2, initiating an antioxidant transcriptional response (Inami et al., 2011). Although p62 accumulates in both Atg7 KO liver and brain, only hepatic phenotypes are rescued by concurrent p62/SQSTM1 or NFE2L2 (encoding NRF2) deletion, defining tissue-specific mechanisms underlying Atg7-related pathology (Komatsu et al., 2007a, 2010; Takamura et al., 2011). In support, antioxidant gene expression is upregulated in Atg7 KO liver but not brain (Komatsu et al., 2006; Matsumoto et al., 2008). It has also been shown that concurrent deletion of Atg7 and Yap, which like p62 is degraded via autophagy, prevents hepatomegaly and tumorigenesis (Lee et al., 2018b). The p62-KEAP1-NRF2 axis is maintained in these double KO livers, suggesting that even within individual tissues multiple mechanisms may contribute to pathogenesis.

**Skeletal muscle**

Atg7 is required in skeletal muscle for development, basal homeostasis and adaptation. Loss of Atg7 in embryonic or adult skeletal muscle caused loss of muscle mass and strength, with abnormal mitochondria, swollen sarcoplasmic reticulum, internalised nuclei and vacuoles notable (Masiero et al., 2009). Faithful Atg7 function also protected mice against exercise-mediated mitochondrial dysfunction (Lo Verso et al., 2014). Conversely, skeletal muscle-specific Atg7 deletion is protective against diet-induced obesity and diabetic phenotypes. Basal mitochondrial dysfunction in Atg7-deficient skeletal muscle activates the integrated stress response via Atf4 which promotes Fgf21 expression, stimulating fatty acid oxidation (Kim et al., 2013).

**Circulatory system**

Atg7 appears to protect against diabetic cardiomyopathy. High-fat diet feeding of mice with cardiac-specific Atg7 deletion exacerbated lipid accumulation and diastolic dysfunction, leading to systolic dysfunction (Tong et al., 2019). However, Atg7 is dispensable for protection against physiological energetic stress via starvation or ischaemia (Saito et al., 2019). Loss of Atg7 in vascular smooth muscle accentuated basal Ca2+ concentrations and heightened sensitivity to depolarisation (Michiels et al., 2015). Cultured Atg7-deficient smooth muscle cells showed diminished survival and proliferation although they demonstrated increased resistance to oxidative stress-induced cell death, reportedly due to increased NRF2 nuclear translocation and antioxidant gene expression (Groo-taert et al., 2015; Osonoi et al., 2018). Endothelial-specific Atg7 deletion did not affect vessel architecture or capillary density but did

### Table 1. Overview of Atg7-deficient mouse models.

| Knockout | Phenotypes | References |
|----------|------------|------------|
| Whole body (embryonic) | • Perinatal lethal | Komatsu et al (2005) |
| Whole body (adult) | • Neurodegeneration • Susceptibility to infection | Karsli-Uzunbas et al (2014) |
| Central nervous system | • Neurodegeneration • Ataxia • Behavioural abnormalities | Kim et al (2017), Komatsu et al (2006), Komatsu et al (2007b) |
| Liver | • Liver enlargement • Multiple adenomas | Komatsu et al (2007a), Komatsu et al (2005) |
| Skeletal muscle | • Loss of muscle mass and strength • Impaired exercise adaptation | Lo Verso et al (2014), Masiero et al (2009) |
| Circulatory system | • Diabetic cardiomyopathy • Susceptible to ischaemic injury | Saito et al (2019), Tong et al (2019) |
| Pancreas | • Premature death • Hyperglycaemia • Insulin deficiency • Endotoxin-induced chronic pancreatitis | Xia et al (2020), Zhou et al (2017) |
| Adipose | • Loss of white adipose tissue mass • Insulin sensitivity | Singh et al (2009b), Zhang et al (2009b) |
| Haematopoiesis | • Severe anaemia • Insulin deficiency • Inability to reconstitute irradiated mice | Mortensen et al (2010), Mortensen et al (2011) |
| Bone | • Reduced bone mass • Short tibia and femur | Li et al (2018) |
| Intestine | • Susceptible to infection | Inoue et al (2012b) |
| Ear | • Early-onset hearing loss | Zhou et al (2020) |
| Eye | • Retinal degeneration | Zhang et al (2017) |
impair adrenaline-mediated von Willebrand factor release leading to extended bleeding times (Torisu et al., 2013). Atg7-deficient endothelial cells also exhibited diminished fatty acid storage (Altamimi et al., 2019) and augmented endothelial to mesenchymal transition (Singh et al., 2015).

Pancreas
In the pancreas, loss of Atg7 causes premature death due to declining exocrine and endocrine function (Zhou et al., 2017). Atg7 deletion also appears to increase susceptibility to endotoxin-induced chronic pancreatitis (Xia et al., 2020). Atg7 maintains islet architecture, glucose tolerance and serum insulin levels and is important for islet homeostasis and compensatory pancreatic responses to high-fat diet (Ebato et al., 2008; Jung et al., 2008; Himuro et al., 2019). Atg7-deficient beta cells accumulate dysfunctional organelles, and impaired proliferation and increased apoptosis cause hyperglycaemia and insulin deficiency (Jung et al., 2008).

Adipose tissue
Mice with adipose-specific Atg7 deletion demonstrated impaired metabolic homeostasis. Loss of Atg7 led to a reduction of 80% of white adipose tissue mass, which acquired brown adipose tissue features including accumulation of mitochondria (Singh et al., 2009b; Zhang et al., 2009b). Tissue demonstrated increased beta-oxidation and diminished hormone-induced lipolysis, supporting the finding of altered lipid metabolism in other Atg7-deficient models (Singh et al., 2009a) and leading to attenuation of basal fatty acid plasma concentration and increased insulin sensitivity (Singh et al., 2009b; Zhang et al., 2009b). Loss of Atg7 from brown adipose tissue also elevated mitochondrial content and insulin sensitivity (Kim et al., 2019).

Haematopoiesis
The role of Atg7 in the haematopoietic system has been extensively investigated. Atg7 is required for erythroid differentiation by contributing to mitochondrial clearance during erythrocyte maturation (Zhang et al., 2009a; Cao et al., 2016). Although removal of the endoplasmic reticulum and ribosomes was unaffected by Atg7 deletion, subsequent accumulation of damaged mitochondria caused severe anaemia (Mortensen et al., 2010). Atg7 deficiency also caused apoptosis induced by mitochondrial damage in mature T lymphocytes leading to lymphopenia (Mortensen et al., 2010). Another study reported that T-cell-specific Atg7 deletion impairs IL-2 and IFN-γ production and impairs stimulated proliferation but does not induce apoptosis (Hubbard et al., 2010). ER content and calcium stores were increased in Atg7-deficient T cells, impairing cellular calcium influx (Jia et al., 2011). Haematopoietic stem cell-specific Atg7 KO caused death within weeks, with haematopoietic stem and progenitor cells demonstrating increased ROS, mitochondrial mass, proliferation and DNA damage (Mortensen et al., 2011). Lymphoid and myeloid progenitor production was impaired, and Atg7-deficient stem cells were unable to reconstitute the haematopoietic system of irradiated mice (Mortensen et al., 2011). Myeloid-specific Atg7 KO did not affect metabolism but did increase inflammasome activation, ROS production and IL-1β release after palmitic acid and lipopolysaccharide treatment (Lee et al., 2016). Atg7 is required for normal monocyte differentiation and acquisition of phagocytic function by macrophages (Jacquel et al., 2012). In B cells, Atg7 deletion caused selective loss of B1a B cells through impaired self-renewal. Atg7-deficient B1a B cells accumulated dysfunctional mitochondria and exhibited diminished expression of metabolic genes, which was not as severe in the less autophagy-dependent B2 B cells (Clarke et al., 2018).

Bone
Chondrocyte-specific Atg7 deletion induced ER type II procollagen storage, driving the aberrant formation of the Col2 fibrillary network in the extra cellular matrix, despite normal Col2 levels (Cinque et al., 2015). This led to diminished tibial and femoral lengths in Atg7-deficient mice. Interestingly, pharmacological activation of autophagy rescued phenotypes in Fgfr18- or Fgfr4-deficient mice, suggesting that autophagy is activated by FGF signalling in bone (Cinque et al., 2015). Different models have suggested that chondrocyte-specific Atg7 deletion induces apoptosis and decreases chondrocyte proliferation (Vuppalaapati et al., 2015; Kang et al., 2017). CHOP deletion partially reversed impaired chondrocyte dysfunction, implicating the PERK-ATF4-CHOP axis and thus supporting the role of ER-related stress in pathophysiology (Cinque et al., 2015; Kang et al., 2017). Loss of Atg7 from osteoblasts caused a decrease in bone mass during development and adulthood due to diminished osteoblast formation and matrix mineralisation, as well as increased numbers of osteoclasts (Li et al., 2018). Like other Atg7-deficient bone models, ER stress was upregulated. Relief from ER stress using phenylbutyric acid remedied bone-related phenotypes in several Atg7-deficient models, placing endoplasmic reticulum dysfunction at the heart of pathology (Kang et al., 2017; Li et al., 2018).

Intestine
Deletion of Atg7 from the intestinal epithelium disrupts Paneth cell morphology and affects Paneth cell granule size, morphology and number (Cadwell et al., 2009; Wittkopf et al., 2012). Although histological analysis of the small intestine revealed no changes after ATG7 deletion, mice demonstrated increased expression of pro-inflammatory genes and accentuated endotoxin-induced inflammatory responses due to increased NF-kB activity (Cadwell et al., 2009; Fujishima et al., 2011; Inoue et al., 2012a). Atg7-deficient mice were also susceptible to Citrobacter rodentium infection and infected mice displayed increased disease burden, possibly due to impaired LAP (Inoue et al., 2012a). Loss of Atg7 from intestinal antigen presenting cells elevated mitochondrial ROS production and Tn17 inflammation (Ravindran et al., 2016). Related to this, Atg7 deletion from intestinal stem cells increased oxidative stress, altered gut–microbiota interactions, and impaired DNA repair, contributing to the induction of p53-mediated apoptosis (Trentesaux et al., 2020). Consistent with liver and brain models, concurrent p53 deletion reduced cell death (Trentesaux et al., 2020; Yang et al., 2020).

Eye and ear
A number of other important Atg7-deficient mouse models have been studied. Outer hair cell Atg7 deletion resulted in accumulation of dysfunctional mitochondria, causing profound early-onset hearing loss (Zhou et al., 2020), and Atg7 deficiency in retinal pigmented epithelia predisposed mice to retinal degeneration (Zhang et al., 2017). Interestingly, organelle degradation is normal in the developing lens of autophagy-deficient mice and instead depends upon PLA2 (phospholipase A/acyltransferase) phospholipases (Morishita et al., 2021).
Non-mammalian models of atg7 deficiency

Studies using other models of Atg7 deficiency support numerous findings in mouse models. Atg7 KO Drosophila melanogaster (fruit flies) have reduced lifespan, are sensitive to nutrient and oxidative stress and demonstrate an ataxic-like phenotype associated with degenerating neurons (Juhasz et al., 2007). In Caenorhabditis elegans (nematode roundworm), atg7 is required for longevity in the dietary restriction eat-2 mutant (Jia & Levine, 2007), but not in the insulin-resistant daf-2 longevity model (Hashimoto et al., 2009). Morpholino atg7 knockdown in Danio rerio (zebrafish) causes aberrant cardiac morphogenesis, increasing the number of dead cells and attenuating organism survival (Lee et al., 2014).

ATG7 in human disease

Dysfunctional autophagy has been predicted to underpin a number of human diseases (Fig 3). Given that autophagy-deficient mice manifest profound neurodegenerative phenotypes, it has been proposed that impaired autophagy in humans may underlie neurodegenerative conditions including Alzheimer’s disease (AD), Parkinson’s disease (PD) and amyotrophic lateral sclerosis (ALS) (Fujikake et al., 2018). The relationship between autophagy and cancer is particularly complex, and aberrant autophagic activity is proposed to play a context-specific role in human carcinogenesis (Long & McWilliams, 2020). Involvement of ATG7 in cancer is further complicated by its autophagy-independent ability to modulate cell cycle arrest and apoptosis mediated by the tumour suppressor p53 (Lee et al., 2012). The role of ATG7 in LC3-associated phagocytosis suggests that ATG7 deficiency may lead to increased susceptibility to infection, supported by the predisposition of Atg7-deficient mice to infection (Inoue et al., 2012a; Karsi-Uzunbas et al., 2014). Until we recently discovered a series of patients harbouring pathogenic, biallelic ATG7 variants, there was no direct link between ATG7 and human disease. This section will describe the clinical nature of these patients and assess the contribution of ATG7 dysfunction to complex human disorders.

Childhood-onset neurological disease

Congenital disorders of autophagy are an emerging group of inborn errors of metabolism, primarily affecting the central nervous system with common involvement of the cerebellum and corpus callosum (Teinert et al., 2020). Although the genetic aetiology underpinning these conditions is expanding, congenital autophagy disorders remain incredibly rare. In fact, deleterious variants affecting only five core autophagy genes have been reported, including ATG5, WIP12, WDR45, WDR45b and ATG7 following the very recent description of twelve patients from five, unrelated families harbouring deleterious, biallelic ATG7 variants (Haack et al., 2012; Saitsu et al., 2013; Kim et al., 2016; Suleiman et al., 2018; Jelani et al., 2019; Collier et al., 2021).

Patients with recessive ATG7 variants are primarily affected by neurological abnormalities including mild-to-severe intellectual disability, ataxia and tremor (Collier et al., 2021). Brain magnetic resonance imaging (MRI) revealed cerebellar hypoplasia and a thin posterior corpus callosum in all patients who had been assessed, highlighting the selective vulnerability of these regions to ATG7 deficiency. Assessment of patient skeletal muscle with undetectable ATG7 protein revealed myopathic changes, including subsarcolemmal accumulation of p62 and evidence of inflammation. Related to this, the patient cohort also displayed neuromuscular abnormalities including loss of muscle mass and strength. Ocular dysfunction, predominantly optic atrophy, is commonly displayed by patients, and there was evidence of endocrine dysfunction and behavioural abnormalities. In more severe cases, patients have seizures and are wheelchair bound due to spastic paraplegia, and one patient died during childhood.

Biochemical profiling of patient fibroblasts revealed severely diminished ATG7 protein levels. Mechanistically, this manifested with impairments in autophagic flux, evidenced by both diminished LC3-II accumulation and decreased cargo sequestration activity. Complementation of atg7KO Saccharomyces cerevisiae and ATG7 KO mouse embryonic fibroblasts by missense Atg7 variants, which were predicted to interfere with ATG7 homodimerisation, failed to rescue autophagy to wild-type levels, thus consolidating variant pathogenicity. Remarkably, two siblings studied survived into adulthood despite undetectable ATG7 causing a near absence of autophagic flux and severely attenuated long-lived protein degradation, supporting the idea that human life is compatible, in exceptional circumstances, with loss of a nonredundant core autophagy protein. Moreover, ATG7 patients with dramatic reduction in autophagic activity are now approaching population life expectancy.

These findings consolidated the importance of autophagy in human health, providing a direct link between dysfunctional autophagy and neurological disease in multiple families. A key question that has arisen from this study is as follows: How do humans compensate for loss of classical degradative autophagy? Based on logical assumptions derived from studies on mouse Atg5/Atg7, it was thought that loss of ATG7—or any of the nonredundant core ATG genes—would not be compatible with human survival. Studies using autophagy-dependent cancer cell lines have demonstrated that they can adapt to autophagy inhibition through increasing mitochondrial-derived vesicle production and inducing NRF2 signalling (Towers et al., 2019, 2021). Moreover, an ATG7/ATG5-independent autophagy pathway driven by RAB9 has also been described (Nishida et al., 2009). This pathway was also suggested to contribute to mitophagy (Hirota et al., 2015; Saito et al., 2019), and the delineation of other molecular signatures of this pathway will enhance our understanding of its role in human intracellular degradation. It has also been reported that FIP200 clustering enables selective autophagy in the absence of LC3 lipidation (Ohnstad et al., 2020). Continued clinical assessment of these patients will provide further insight into the role of ATG7 in human homeostasis and disease. The future identification of additional individuals with inherited ATG7 impairments will reveal the spectrum of clinical phenotypes associated with ATG7 dysfunction. This is important because the patients described so far do not demonstrate predictable clinical outcomes. This is exemplified by the discrepancy between clinical and biochemical phenotypes whereby patients with the most severe clinical presentations demonstrated the mildest biochemical impairment of autophagy. These approaches will also help to ascertain whether ATG7-deficient individuals are at altered risk of diseases such as neurodegeneration, cancer and infection compared with the general population, improving our understanding of complex human disorders.

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**Adult neurodegeneration**

Neurodegeneration is characteristic of autophagy-deficient mouse models, yet the relationship between defective autophagy and human neurodegeneration has been more challenging to reconcile (Suomi & McWilliams, 2019). Strong evidence supporting the pathological involvement of autophagy includes the presence of large p62/SQSTM1- and ubiquitin-positive inclusions, hallmarks of autophagy deficiency, in AD, PD and ALS brain tissues (Kuusisto et al., 2001; Mizuno et al., 2006). In support, these structures often contain autophagy cargo including TDP-43, hyperphosphorylated tau, SOD1 and alpha-synuclein (Menzies et al., 2017). The discovery of patients harbouring recessive ATG7 variants has resolved a long-suspected convergence of neuropathology between humans and model organisms with defective autophagy (Collier et al., 2021). Longitudinal assessment of patients harbouring these pathogenic variants will inform whether and how their neuropathological status changes.

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**Figure 3. ATG7 in human disease and therapeutics.**

(A) Patients harbouring biallelic ATG7 variants display childhood-onset disease, causing neurological, muscular and ocular dysfunction through impaired autophagy. In contrast, there is no direct link between ATG7 and adult-onset neurodegeneration, infection or cancer, although recent developments in our understanding of these diseases and ATG7 function have implicated aberrant ATG7 activity in their aetiology. (B) This has implications for therapeutic approaches. Whereas neurological phenotypes may be remedied by induction of ATG7 activity, inhibition of ATG7 may help increase the efficacy of anti-cancer treatments.
over time. It is notable that one patient (71 years old) has developed late-onset dementia decline, yet further studies are warranted before this can be attributed to ATG7 deficiency.

Despite this, mutations in several genes that participate at multiple steps of autophagy have been implicated in familial neurodegeneration (Menzies et al., 2017; Suomi & McWilliams, 2019). However, there is evidence that the contribution of selective autophagy proteins to neurodegeneration may extend beyond their involvement in selective mechanisms alone. For example, dysfunction of the mitophagy-associated protein PINK1/PARK6 is implicated in PD generation (Menzies et al., 2017; Suomi & McWilliams, 2019). Rather, there is evidence that divergent mechanisms of impaired immune regulation contribute to acute PD-like phenotypes. An important example is that intestinal infections trigger the manifestation of authentic PD-like characteristics in Pink1 KO mice, with locomotor dysfunction rescued by L-DOPA treatment (Matheoud et al., 2019).

These data support an emerging theory proposing that a combination of triggers, facilitators and aggressors may affect Parkinson’s disease risk (Johnson et al., 2019). In short, triggers (e.g. viral infection) initiate disease but facilitators (e.g. genetic variant) may drive the spread of pathology, before the progressive loss of health due to aggressors (e.g. impaired autophagy). Given the often sporadic nature of other complex neurodegenerative conditions, it is reasonable to postulate that this may be true for these disorders, too, though the underlying mechanisms may differ between them. Although no direct links between ATG7 dysfunction and neurodegeneration have been demonstrated, polymorphisms within the ATG7 promoter region have been suggested to contribute to sporadic Parkinson’s disease, yet their impacts on promoter activity were modest (Chen et al., 2013). Recently, it was reported that ATG7 protein levels are reduced in post-mortem fronto-temporal lobe brain tissue from ALS patients compared with controls, though causality in human subjects has not yet been proven (Donde et al., 2020). ATG7 variants may also provide disease-modifying effects, as it has been reported that the p.Val471Arg (NP_006386.1) polymorphism is associated with earlier onset (by 4–6 years) of Huntington’s disease (Metzger et al., 2010, 2013). These studies support the future consideration of ATG7 dysfunction as an aggravator of neurodegeneration, contributing to disease progression, rather than as a trigger of neurodegeneration. However, further research will be undoubtedly required to define the role of ATG7 in human neurodegeneration.

**Cancer**

In human cancers, autophagy was first thought to be an anti-tumorigenic process with ATG6/BECN1 haploinsufficiency detected in approximately 45 to 70% of breast, ovarian and prostate cancers (White, 2015). Yet, other core autophagy genes, including ATG7, are rarely mutated in human cancers. It has instead become evident that the relationship between cancer and autophagy is undoubtedly complex, underpinned by the context-specific interactions of pro- and anti-tumour properties of autophagy (Long & McWilliams, 2020). Whereas autophagy protects against tumour formation by promoting genomic stability and inhibition of pro-oncogenic inflammatory signalling, it also helps meet the accentuated metabolic demands of tumour microenvironments that result from increased proliferation (Zhong et al., 2016; Hewitt & Korolchuk, 2017). For example, RAS transformed cancers increase autophagic flux, a mechanism that may also be important for invasion and metastasis (Guo et al., 2011; Lock et al., 2011, 2014; Yang et al., 2011).

Because ATG7 promotes autophagy as well as cell cycle arrest mediated by tumour suppressor gene P53 (Lee et al., 2012), there is a logical expectation that altered ATG7 activity may underlie some cancers. Liver-specific Atg7 deletion predisposes mice to liver tumorigenesis, although these tumours were not reported to become malignant (Takamura et al., 2011). However, a subsequent study reported that Atg7-null mice develop hepatocellular carcinoma (Lee et al., 2018b). Together, these data support a context-specific role of ATG7 in carcinogenesis (perhaps driven by genetic and environmental cues including microbial exposure) and highlight that the relationship between autophagy and tumour formation and progression are even more complex that initially thought. In fact, cell non-autonomous mechanisms are also an important consideration (Mizushima & Levine, 2020) and it was recently shown that tumour growth is supported by autophagy via circulating arginine (Poileit-Perez et al., 2018). Elegant research using a Drosophila melanogaster malignant tumour model also supports the role of the non-cell autonomous autophagy which was shown to be induced in the tumour microenvironment and in distal tissues (Katheder et al., 2017). In this model, early-stage tumour growth and invasion was shown to be dependent on local tumour microenvironment autophagy. Studies have also been undertaken using murine cancer models. Conditional inactivation of Atg7 inhibits intestinal pre-cancerous lesion formation in mice with monoallelic deletion of tumour suppressor gene Apc (Levy et al., 2015) and prevents the growth of BrafV600E-driven melanoma and lung tumours (Strohecker et al., 2013; Xie et al., 2015). However, autophagy inhibition drives the accumulation of pre-malignant pancreatic lesions in mice harbouring an activated oncogenic Kras allele upon p53 inactivation (Rosenfeldt et al., 2013), whereas p53 deletion-driven tumour formation (in the absence of Kras activation) is protected against by autophagy inhibition (Yang et al., 2020). Altogether, these studies demonstrated that the role of autophagy may be intrinsically linked to the status of oncogenes and tumour suppressors, as well as the metabolic microenvironment.

In humans, the link between ATG7 dysfunction and cancer formation is only starting to emerge. Recently, familial cholangiocarcinoma (an aggressive cancer of the bile duct) has been associated with ATG7 mutations (preprint: Greer et al., 2019). In this study, a number of individuals harbouring inherited monoallelic ATG7 variants (interestingly including the p.Arg659* (NP_006386.1) variant identified in Family 1 (Collier et al., 2021)) were discovered having developed cholangiocarcinoma. Tumour analysis revealed somatic loss of ATG7 affecting several family members, providing a strong link between cancer formation and ATG7 dysfunction. This discovery is particularly interesting given the prominent liver phenotypes, including tumour formation, observed in Atg7-null mice (Takamura et al., 2011; Lee et al., 2018b). In contrast, there is currently no evidence for increased cancer susceptibility among patients harboring recessive ATG7 variants, nor in their family members with monoallelic ATG7 variants, including those harbouring the p.Arg659* mutation. Among the patients with biallelic ATG7 variants, a 71-year-old patient has developed an acoustic neuroma—a benign brain tumour, but longitudinal studies in other patients will
provide further insight into whether this is related to ATG7 deficiency. Elsewhere, ATG7 polymorphisms associated with protective or pro-carcinogenic properties have been reported (Yu et al, 2018; Wang et al, 2019b), and elevation of ATG7 expression is associated with some bladder and lung cancers (Sun et al, 2016; Zhu et al, 2017). Furthermore, ATG7 levels may also be of prognostic value in breast cancers patients (Desai et al, 2013). In individuals where inherited and somatic ATG7 variants are discovered, preclinical mouse studies suggest that the genetic state of well-characterised oncogenes and tumour suppressors should also be investigated. This may facilitate a deeper understanding of the role of ATG7 in these cancers—whether they underpin tumour growth or play a supportive role in the later stages of disease.

**Infection**

Both autophagy and LC3-associated phagocytosis are involved in the innate immune response that prevents cells from invading pathogens (Levine et al, 2011; Heckmann et al, 2017). Although pathogens have evolved to modulate autophagic activity, even using it to enhance their pathogenesis, evidence in adult mouse models demonstrates that loss of Atg7 increases infection susceptibility (Karsli-Uzunbas et al, 2014). However, the role of ATG7 in human models of infection remains context-specific. ATG7 restricts *mycobacterium tuberculosis* (Singh et al, 2006; Liu et al, 2020) and human papillomavirus infection (Griffin et al, 2013) and limits Chikungunya virus pathogenesis (Joubert et al, 2012). ATG7-dependent autophagy is also stimulated upon infection with influenza A, leading to endogenous presentation of epitope on MHC class II molecules (Deng et al, 2021). ATG7 also limits poliovirus infection: A defect in stimulus-induced autophagy was observed in primary fibroblasts taken from a patient with poliomyelitis after polio infection, with exome sequencing identifying a heterozygous p.A388T (NP_006386.1) poliovirus infection (Griffin et al, 2013) and limits Chikungunya virus pathogenesis (Joubert et al, 2012). ATG7-dependent autophagy is also stimulated upon infection with influenza A, leading to endogenous presentation of epitope on MHC class II molecules (Deng et al, 2021). ATG7 also limits poliovirus infection: A defect in stimulus-induced autophagy was observed in primary fibroblasts taken from a patient with poliomyelitis after polio infection, with exome sequencing identifying a heterozygous p.A388T (NP_006386.1) ATG7 variant (Brinck Andersen et al, 2020). On the other hand, HIV-1 hijacks autophagy to increase viral yield, before HIV protein Nef acts as an anti-autophagic factor to prevent HIV degradation (Kyei et al, 2020). It has also been shown that autophagy selectively degrades Tat to restrict HIV-1 infection (Saghir et al, 2015). This autophagic dichotomy is observed in hepatitis C virus (HCV) infection, too. ATG7 inhibition suppresses HCV replication (Sir et al, 2008), but ATG7 activity enhances the innate immune response in HCV-infected hepatocytes (Shrivastava et al, 2011). Consistent with a role in regulating inflammatory responses, inflammasome activity is enhanced in Atg7 KO mouse infected with *Pseudomonas aeruginosa*, impairing pathogen clearance, thus implicating ATG7 in sepsis pathogenesis (Pu et al, 2017).

**Therapeutic approaches**

Autophagy-modulating therapeutics are of broad interest and have been studied in a number of settings (Fig 3). Evidence suggesting that dysfunctional autophagy contributes to neurodegenerative disorders has led to clinical studies assessing whether autophagy-inducing compounds can improve neurological function and/or delay disease progression. In contrast, essential autophagic activity in cancer cells is predicted to maintain nutrient supply and prevent oxidative stress, thus contributing to treatment resistance. Hence, inhibition of autophagy may improve cancer treatment efficacy. The ability to modulate ATG7 activity directly using drugs may therefore have widespread implications. In cases where inherited ATG7 deficiency underlies disease, alternative approaches may be more beneficial.

**Treating ATG7 deficiency in neurological disorders**

It is not unreasonable to anticipate that the number of patients identified harbouring biallelic, pathogenic ATG7 variants will increase, and be associated with an ever-widening spectrum of clinical presentations. The prevalent neurological phenotypes observed in the patients identified to date suggest that restoring autophagic function in nervous system would provide the optimal therapeutic approach. This is supported by analogous models of autophagy in Atg5-deficient mice wherein neural expression of Atg5 rescues perinatal lethality and extends life up to 8 months (Yoshii et al, 2016). Such an approach in human patients, for example using adeno-associated viral gene therapy, has been widely investigated, but limitations must be overcome before this is a viable option (Wang et al, 2019a). This approach has, however, yielded success for the treatment of spinal muscular atrophy, a progressive motor neuron disease (Mendell et al, 2017). Bypassing the requirement of ATG7 for LC3 lipidation offers an alternative strategy. Infection with vaccinia, the live virus used in the smallpox vaccine, induces LC3 lipidation independently of ATG7 and ATG5 (Moloughney et al, 2011). Identifying the combination of viral and cellular factors driving non-canonical LC3 lipidation under these circumstances could lead to the development of a viable therapeutic. In *Drosophilia melanogaster*, Uba1 functions in an Atg7/Atg3-independent autophagy pathway that is dependent on Atg8, but this has not been described in mammals (Chang et al, 2013). A different strategy would require a deeper understanding of the molecular events that lead to pathology. Studies using mice have demonstrated that liver injury in Atg7-null mice can be remedied by p62/SQSTM1, NFE2L2, Yap or p53 deletion, but the intracellular consequences of endogenous human ATG7 inactivation in patient neural cells remain to be investigated (Komatsu et al, 2007a; Inami et al, 2011; Lee et al, 2018b; Yang et al, 2020). It would also be interesting to uncover whether ATG7-independent degradation pathways may compensate for dysfunction of classical degradative autophagy. Of note, the Rab9-dependent autophagy pathway, termed “alternative autophagy”, does not require ATG7 or LC3 lipidation, yet further work is required to understand both the molecular signatures and physiological importance of this pathway, although progress is being made (Nishida et al, 2009; Shimizu, 2018; Yamaguchi et al, 2020).

Preclinical mouse studies have suggested that enhancing ATG7 activity could help treat neurodegeneration (Donde et al, 2020). In human neurodegenerative conditions where defective autophagy is implicated, clinical trials have largely focussed on the use of compounds that activate autophagy. Some of these enhance autophagy via mTORC1 inactivation, including rapamycin (Mandrioli et al, 2018), idalopirdine (Wilkinson et al, 2014; Matsunaga et al, 2019) and SB-742457 (Maher-Edwards et al, 2010), whereas others (e.g. lithium (Sacca et al, 2015)) deliver remedy through TORC1-independent mechanisms. Novel therapeutics that are able to enhance the selective delivery of cytoplasmic constituents (including mitochondria and mutant Huntington protein) to the autophagosome have also been reported (Li et al, 2019; Takahashi et al, 2019). It is unclear whether such approaches could meet the clinical demands of ATG7-deficient patients.
Targeting ATG7 to enhance cancer treatment

Although aberrant ATG7 activity is not known to commonly underpin human cancers, it has been theorised that disrupting autophagy can improve the potency of anti-cancer therapeutics. A number of clinical trials have used blocker of autophagy hydroxychloroquine in combination with classical cancer therapies, yet results have been mixed (Mulcahy Levy & Thorburn, 2020). Combining autophagy inhibition with proteasomal inhibition has provided promise, with this approach leading to prostate cancer cell death (Zhu et al., 2010). Attenuating ATG7 function to sensitise tumour cells to cancer treatments has also been investigated in preclinical models with some success. ATG7 inactivation enhances the effectiveness of anti-cancer therapeutics in lung and breast cancer cell treatment (Han et al., 2011; Desai et al., 2013; Yue et al., 2013). Moreover, endogenous noncoding RNA molecules miR-17 and miR-137 diminish ATG7 expression, sensitising several cancer cell lines to chemotherapeutics or low ionising radiation (Cominici et al., 2013; Zeng et al., 2015). Modulating the FoxO1/ATG7 axis may also provide a therapeutic opportunity. FoxO1 encodes a tumour suppressor gene that drives cell death in human colon tumours via autophagy (Zhao et al., 2010). In bladder cancer, tumorigenic growth is also attenuated by FoxO1, yet this appears to be stimulated by ATG7 inhibition (Zhu et al., 2017). Indeed, the effectiveness of targeting ATG7 activity may be dependent on the genetic status of tumour suppressors and oncogenes. For example, whether autophagy promotes or reduces pancreatic tumour growth in mice appears to be dependent on p53 status (Rosenfeldt et al., 2013).

It is known that a number of cancer cell lines are highly autophagy-dependent, and although they are generally resistant to loss of autophagic function (e.g. through ATG7 deletion), there is evidence that populations of cells are able to adapt to autophagy inhibition by upregulating different cellular pathways including NRF2 signalling (Towers et al., 2019). Interestingly, loss of LC3-driven mitophagy via ATG7 deletion upregulates formation of mitochondrial-derived vesicles, 70–150 nm structures that laterally bud from mitochondria encapsulating selective cargo that can deliver material to the endolysosomal system for degradation, or to pexisomes (Sugiura et al., 2014; Towers et al., 2021). These results offer druggable targets under circumstances where autophagy-dependent cancers resist autophagy inhibition that attempts to enhance anti-cancer treatments. They may also offer insight into how patients with inherited ATG7 variants are able to survive into adult life. Overall, these findings reinforce the complexity underpinning human cancers and support the delivery of context-specific therapeutic approaches, whilst the development of high specificity pharmacological inhibitors is also encouraging. This was demonstrated recently whereby Vps34 inhibition improved immunotherapy outcomes in preclinical models (Noman et al., 2020).

Concluding remarks

It has now been demonstrated that inherited ATG7 deficiency causes congenital human disease hallmarked by neurodevelopmental deficits (Collier et al., 2021). Remarkably, humans can survive with mild–moderate neurological impairments despite undetectable levels of ATG7 protein. Future investigations will hopefully reveal which cellular pathways compensate for the absence of classical degradative autophagy, contributing to the survival of these patients. Recent advances suggest that mitochondrial-derived vesicles and upregulated NRF2 signalling may be two mechanisms by which autophagy-deficient cells are able to survive (Towers et al., 2019, 2021). Moreover, FIP200 clustering facilitates the bypass of LC3 lipidation in autophagy and promotes selectivity (Ohnstad et al., 2020).

Although the link between ATG7 and complex human disorders remains mostly elusive, the increasing associations between altered autophagy and complex human disorders suggest that directed modulation of ATG7 could provide a promising therapeutic opportunity. Studies in mice suggest that the most appropriate therapeutic approach when inherited ATG7 dysfunction underpins pathology may involve targeted neural restoration of ATG7 expression or alleviating downstream molecular consequences that may drive disease manifestation. In contrast, evidence suggests that disrupting autophagy may improve anti-cancer therapeutics. Currently, inhibiting autophagy largely involves targeting the endolysosomal system leading to a broad impact on cellular homeostasis. Hence, there is a crucial need to develop selective autophagy inhibitors. Given that ATG7 has enzymatic activity, developing drugs that directly target ATG7 represents an attractive therapeutic strategy and may lead to more specific outcomes. For such approaches to be clinically feasible, it will be important to understand how these treatments affect the various biological pathways influenced by ATG7, and more broadly, how these pathways interact across cellular space and time.

Importantly, further analysis of ATG7-independent degradation mechanisms will be key to understanding intracellular turnover in humans and whether these pathways can compensate under circumstances of autophagy dysfunction. The continued development of disease models and sensitive tools to monitor ATG7 activity in vivo will surely drive further progress in this exciting field.

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Conflict of interest
The authors declare that they have no conflict of interest.

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References

Altamimi TR, Chowdhury B, Singh KK, Zhang L, Mahmood MU, Pan Y, Quan A, Teoh H, Verma S, Lopaschuk GD (2019) A novel role of endothelial autophagy as a regulator of myocardial fatty acid oxidation. J Thorac Cardiovasc Surg 157: 185–193.

Bel S, Pendse M, Wang Y, Li Y, Ruhn KA, Hassell B, Leal T, Winter SE, Xavier RJ, Altamimi TR, Chowdhury B, Singh KK, Zhang L, Mahmood MU, Pan Y, Quan A, Teoh H, Verma S, Lopaschuk GD (2019) A novel role of endothelial autophagy as a regulator of myocardial fatty acid oxidation. J Thorac Cardiovasc Surg 157: 185–193.

Brinnk Andersen NS, Jorgensen SE, Skipper KA, Larsen SM, Heinz J, Thomesen MM, Farahani E, Cai Y, Hait AS, Kay L et al (2020) Essential role of autophagy in restricting poliovirus infection revealed by identification of an ATG7 defect in a poliomyltis patient. Autophagy 17: 1–16.

Cadwell K, Patel KK, Komatsu M, Virgin HW, Stappenbeck TS (2009) A common role for Atg16L1, Atg5 and Atg7 in small intestinal Paneth cells and Crohn disease. Autophagy 5: 250–252.

Cao Y, Cai J, Li X, Yuan N, Zhang S (2016) Autophagy governs erythroid differentiation both in vitro and in vivo. Hematologica 21: 225–233.

Chang TK, Shragave BV, Hayes SD, Powers CM, Simin RT, Wade Harper J, Baehrecke EH (2013) Uba1 functions in Atg7- and Atg5-independent autophagy. Nat Cell Biol 15: 1067–1078.

Chen D, Pang S, Feng X, Huang W, Hawley RG, Yan B (2013) Genetic analysis of the ATG7 gene promoter in sporadic Parkinson’s disease. Neurosci Lett 534: 193–198.

Chen M, Chen Z, Wang Y, Tan Z, Zhu C, Li Y, Han Z, Chen L, Gao R, Liu L et al (2016) Mitophagy receptor FUNDC1 regulates mitochondrial dynamics and mitophagy. Autophagy 12: 689–702.

Cinque L, Forrester A, Bartolomeo R, Svelto M, Venditti R, Montefusco S, Polischuk E, Nusco E, Rossi A, Medina DL et al (2015) FGF signalling regulates bone growth through autophagy. Nature 528: 272–275.

Clarke AJ, Riffelmacher T, Braas D, Cornall RJ, Simon AK (2011) Mitophagy dynamics and mitophagy. Autophagy 12: 689–702.

Coupe B, Ishii Y, Dietrich MO, Komatsu M, Horvath TL, Bouret SG (2012) Loss of autophagy in pro-opiomenocortin neurons perturbs axon growth and causes metabolic dysregulation. Cell Metab 15: 247–255.

Deng J, Lu C, Liu C, Oveisli S, Fairrie WD, Lee EF, Bilse P, Puthalakath H, Chen W (2021) Influenza A virus infection-induced macroautophagy facilitates MHC class II-restricted endogenous presentation of an immunodominant viral epitope. FEBS J 288: 3164 – 3185.

Desai S, Liu Z, Yao J, Patel N, Chen J, Wu Y, Ahn EE, Fodstad O, Tan M (2013) Heat shock factor 1 (HSF1) controls chemoresistance and autophagy through transcriptional regulation of autophagy-related protein 7 (ATG7). J Biol Chem 288: 9165 – 9176.

DeSelm CJ, Miller BC, Zou W, Beatty WL, van Meel E, Takahata Y, Klumperman J,Tooze SA, Teitelbaum SL, Virgin HW (2011) Autophagy proteins regulate the secretory component of osteoclastic bone resorption. Dev Cell 21: 966–974.

Donde A, Sun M, Jeong YH, Wen X, Ling J, Lin S, Braunstein K, Nie S, Wang S, Chen L et al (2020) Upregulation of ATG7 attenuates motor neuron dysfunction associated with depletion of TARDBP/TDP-43. Autophagy 16: 672–682.

Ebat C, Uchida T, Arakawa M, Komatsu M, Ueno T, Komiya K, Azuma K, Hirose T, Tanaka K, Kominami E et al (2008) Autophagy is important in islet homeostasis and compensatory increase of beta cell mass in response to high-fat diet. Cell Metab 9: 325–332.

Esteban-Martinez L, Sierras-Fileardi E, McGreal RS, Salazar-Roa M, Marino G, Secco E, Durand S, Enot D, Graña O, Malumbres M et al (2017) Programmed mitophagy is essential for the glycolytic switch during cell differentiation. EMBO J 36: 1688–1706.

Fais S, Overholtzer M (2018) Cell-in-cell phenomena, cannibalism, and autophagy: is there a relationship? Cell Death Dis 9: 95.

Florey O, Kim SE, Sandoval CP, Haynes CM, Overholtzer M (2011) Autophagy machinery mediates macroendocytic processing and entotic cell death by targeting single membranes. Nat Cell Biol 13: 1335–1343.

Frud K, Burgoyme T, Burgoyme JR (2018) Oxidation of Atg3 and Atg7 mediates inhibition of autophagy. Nat Commun 9: 95.

Fujikake N, Shin M, Shimizu S (2018) Association between autophagy and neurodegenerative diseases. Front Neurosci 12: 255.

Fujishima Y, Nishiumi S, Masuda A, Inoue J, Nguyen NM, Irino Y, Komatsu M, Tanaka K, Kusumi H, Azuma T et al (2011) Autophagy in the intestinal epithelium reduces endotoxin-induced inflammatory responses by inhibiting NF-kappaB activation. Arch Biochem Biophys 506: 223–235.

Goodall ML, Fitzwater BE, Zahedi S, Wu M, Rodriguez D, Mulecathy-Ley MJ, Green DR, Morgan M, Cramer SD, Thorburn A (2016) The autophagy machinery controls cell death switching between apoptosis and necroptosis. Dev Cell 37: 337–349.

Greer SJ, Ogmundsdottir MH, Nadeaul LD, Chen J, Lau BT, Delacruz RCC, Sandoval IT, Kristjansdottir S, Jones DA, Haslem DS et al (2019) Genetic risk of cholangiocarcinoma is linked to the autophagy gene ATG7. BioRxiv https://doi.org/10.1101/836767 [PREPRINT].

Griffin LM, Cicchini L, Pyeon D (2013) Human papillomavirus infection is inhibited by host autophagy in primary human keratinocytes. Virology 437: 12–19.

Grootaert MO, da Costa Martins PA, Bitchs N, Pintelon I, De Meyer GR, Martinet W, Schrijvers DM (2015) Defective autophagy in vascular smooth muscle cells accelerates senescence and promotes neointima formation and atherogenesis. Autophagy 11: 2014–2032.

Guo JY, Chen H-Y, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, Kim SA, Meyer E, Kari E et al (2012) Exome sequencing reveals de novo WDR45 mutations causing a phenotypically distinct, X-linked dominant form of NBIA. Am J Hum Genet 91: 1144–1149.
Han J, Hou W, Goldstein LA, Stolz DB, Watkins SC, Rabinowich H (2014) A complex between Atg7 and caspase-9: a novel mechanism of cross-regulation between autophagy and apoptosis. *J Biol Chem* 289: 6485–6497

Han W, Pan H, Chen Y, Sun J, Wang Y, Li J, Ge W, Feng L, Lin X, Wang X et al (2011) EGFR tyrosine kinase inhibitors activate autophagy as a cytoreprotective response in human lung cancer cells. *PloS One* 6: e18691

Hanada T, Noda NN, Satomori Y, Ichimura Y, Fujioka Y, Takaok A, Inagaki F, Ohsumi Y (2007) The Atg12-Atg5 conjugate has a novel E3-like activity for protein lipidation in autophagy. *J Biol Chem* 282: 37298–37302

Harding TM, Morano KA, Scott SV, Klionsky DJ (1995) Isolation and characterization of yeast mutants in the cytoplasm to vacuole protein targeting pathway. *J Cell Biol* 131: 591–602

Hashimoto Y, Ookuma S, Nishida E (2009) Lifespan extension by suppression of autophagy genes in *Caenorhabditis elegans*. *Genes Cells* 14: 717–726

Heckmann BL, Boada-Romero E, Cunha LD, Magne J, Green DR (2017) LC3-associated phagocytosis and inflammation. *J Mol Biol* 429: 3561–3576

Heckmann BL, Green DR (2019) LC3-associated phagocytosis at a glance. *J Cell Sci* 132: 1–6

Henault J, Martinez J, Riggs J, Tian J, Mehta P, Clarke L, Sasai M, Latz E, Brinkmann K, Iwaki A et al (2012) Noncanonical autophagy is required for type I interferon secretion in response to DNA-immune complexes. *Immunity* 37: 986–997

Hernandez D, Torres C, Setlik W, Cebrián C, Mosharov E, Tang G, Cheng H-C, Khodolivov N, Yaragina O, Burke R et al (2012) Regulation of presynaptic neurotransmission by macroautophagy. *Neuron* 74: 277–284

Hewitt G, Korolchuk VI et al (2012) Regulation of presynaptic neurotransmission by macroautophagy. *Neuron* 74: 277–284

Hewitt G, Korolchuk VI (2017) Repair, reuse, recycle: the expanding role of autophagy in genome maintenance. *Trends Cell Biol* 27: 340–351

Himuro M, Miyatake S, Suzuki L, Miura M, Katahira T, Goto H, Nishida Y, Saijo C, Morimoto R (2012) Autophagy is required for dietary restriction-mediated life span extension in *C. elegans*. *Autophagy* 8: 3573–3576

Hirota Y, Yamashita S, Kunihara Y, Jin X, Aihara M, Saigusa T, Kang D, Kanki T (2015) Mitophagy is primarily due to alternative autophagy and requires the MAPK1 and MAPK14 signaling pathways. *Autophagy* 11: 332–343

Hubbard VM, Valdor R, Patel B, Singh R, Cuervo AM, Macian F (2010) Macroautophagy regulates energy metabolism during effector T cell activation. *J Immunol* 185: 7349–7357

Ichimura Y, Kaita S, Takao T, Satomori Y, Shimonsiya Y, Ishihara N, Mizushima N, Tanida I, Kominami E, Ohsumi M et al (2000) A ubiquitin-like system mediates protein lipidation. *Nature* 408: 488–492

Inami Y, Waguri S, Sakamoto A, Kouno T, Nakada K, Hino O, Watanabe S, Ando J, Iwadate M, Yamamoto M et al (2011) Persistent activation of Nrf2 through p62 in hepatocellular carcinoma cells. *J Cell Biol* 193: 275–284

Inomata M, Xu S, Dufies M, Robert G, Gounon P, Lemichez E, Luciano F, Solary E, Aubeger P (2012) Autophagy is required for CSF-1-induced macrophagic differentiation and acquisition of phagocytic functions. *Blood* 119: 4527–4531

Jelen M, Dooley HC, Cubas A, Mohamoud HSA, Khan MTM, Ali Z, Kang C, Rahim F, Jan A, Vadgama N et al (2019) A mutation in the major autophagy gene, WIPI2, associated with global developmental abnormalities. *Brain* 142: 1242–1254

Jia K, Levine B (2007) Autophagy is required for dietary restriction-mediated life span extension in *C. elegans*. *Autophagy* 3: 597–599

Jia W, Pua HH, Li QJ, He YW (2011) Autophagy regulates endoplasmic reticulum homeostasis and calcium mobilization in T lymphocytes. *J Immunol* 186: 1564–1574

Johansen T, Lamark T (2020) Selective autophagy: ATG8 family proteins, LIR motifs and cargo receptors. *J Mol Biol* 432: 80–103

Johnson ME, Stecher L, Labrie V, Brundin L, Brundin P (2019) Triggers, facilitators, and aggravators: redefining parkinson’s disease pathogenesis. *Trends Neurosci* 42: 4–13

Joubert PE, Wernerke SW, de la Celle C, Guivel-Benhassine F, Giodini A, Peduto L, Levine B, Schwartz O, Lenschow DJ, Albert ML (2012) Chikungunya virus-induced autophagy delays caspase-dependent cell death. *J Exp Med* 209: 1029–1047

Juhász G, Erdi B, Sass M, Neufeld TP (2007) Atg7-dependent autophagy promotes neuronal health, stress tolerance, and longevity but is dispensable for metamorphosis in *Drosophila*. *Genes Dev* 21: 3061–3066

Jung HS, Chung KW, Won Kim J, Kim J, Komatsu M, Tanaka K, Nguyen YH, Kang TM, Yoon KH, Kim JW et al (2008) Loss of autophagy diminishes pancreatic beta cell mass and function with resultant hyperglycemia. *Cell Metab* 8: 318–324

Jung S, Cho E, Woo H, Jeong H, An H-K, Moon H, Ryu HY, Yeo BK, Lee YW, Choi H et al (2020) Autophagic death of neural stem cells mediates chronic stress-induced decline of adult hippocampal neurogenesis and cognitive deficits. *Autophagy* 16: S12–S30

Kabeya Y, Mizushima N, Yamamoto A, Oshtani-Okamoto S, Ohsumi Y, Yoshimori T (2004) LC3, GABARAP and GATE16 localize to autophagosome membrane depending on form-II formation. *J Cell Sci* 117: 2805–2812

Kang X, Yang W, Feng D, Jin X, Ma Z, Qian Z, Xie T, Li H, Liu J, Wang R et al (2017) Cartilage-specific autophagy deficiency promotes ER stress and impairs chondrogenesis in PERK-ATF4-CHOP-dependent manner. *J Bone Miner Res* 32: 2182–2141

Karsil–Uzunbas G, Guo JY, Price S, Teng X, Laddha SV, Khor S, Kalaany NY, Jacks T, Chan CS, Rabinowitz JD et al (2014) Autophagy is required for glucose homeostasis and lung tumor maintenance. *Cancer Discov* 4: 914–927

Kathered NS, Khezri R, O'Farrell F, Schultz SW, Jain A, Raham MM, Schink KO, Theodosiou TA, Johansen T, Juhász G et al (2017) Microenvironmental autophagy promotes tumour growth. *Nature* 541: 417–420

Kashik S, Arias E, Kwon H, Lopez NM, Ationvarangkul D, Sahu S, Schwartz GJ, Pessin JE, Singh R (2012) Loss of autophagy in hypothalamic POMC neurons impairs lipolysis. *EMBO Rep* 13: 258–265

Kashik S, Cuervo AM (2018) The coming of age of chaperone-mediated autophagy. *Nat Rev Mol Cell Biol* 19: 365–381

Kim D, Kim JH, Kang YH, Kim JS, Yun SC, Kang SW, Song Y (2019) Suppression of brown adipocyte autophagy improves energy metabolism by regulating mitochondrial turnover. *Int J Mol Sci* 20: 3520

Kim HJ, Cho MH, Shim WH, Kim JK, Jeon EY, Kim DH, Yoon SY (2017) Deficient autophagy in microglia impairs synaptic pruning and causes social behavioral defects. *Mol Psychiatry* 22: 1576–1584

Kim KH, Jeong YI, Oh H, Kim SH, Cho JM, Kim Y-N, Kim SS, Kim DH, Hur KY, Kim HK et al (2013) Autophagy deficiency leads to protection from obesity and insulin resistance by inducing Fgf21 as a mitokine. *Nat Med* 19: 83–92
Kim M, Sandford E, Caticha D, Qiu YU, Liu XU, Zheng Y, Schulman BA, Xu J, Semple I, Ro S-H et al (2016) Mutation in ATG5 reduces autophagy and leads to ataxia with developmental delay. Elife 5: 1–18
Kimura T, Jia J, Kumar S, Choi SW, Gu Y, Mudd M, Dupont N, Jiang S, Peters R, Farzam F et al (2017) Dedicated SNAREs and specialized TRIM cargo receptors mediate secretory autophagy. EMBO J 36: 42–60
Kirisako T, Baba M, Ishihara N, Miyazumi K, Yoshimori T, Noda T, Ohsumi Y (1999) Formation process of autophagosome is traced with Apg8/Aut7p in yeast. J Cell Biol 147: 435–446
Komatsu M, Kurokawa H, Waguri S, Taguchi K, Kobayashi A, Ichimura Y, Sou Y-S, Ueno I, Sakamoto A, Tong KI et al (2010) The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. Nat Cell Biol 12: 213–223
Komatsu M, Tanida I, Ueno T, Ohsumi M, Ohsumi Y, Kominami E (2001) The C-terminal region of an Apg7p/Cvt2p is required for homodimerization and is essential for its E1 activity and E1–E2 complex formation. J Biol Chem 276: 9846–9854
Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, Ezaki J, Mizushima N, Ohsumi Y, Uchiumi Y et al (2005) Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. J Cell Biol 169: 425–434
Komatsu M, Waguri S, Chiba T, Murata S, Iwata J-I, Tanida I, Ueno T, Koike M, Uchiumi Y, Kominami E et al (2006) Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 441: 880–884
Komatsu M, Waguri S, Koike M, Sou Y-S, Ueno T, Harayama T, Mizushima N, Iwata J-I, Ezaki J, Murata S et al (2007a) Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. Cell 131: 1149–1163
Komatsu M, Wang QJ, Holstein GR, Friedrich Jr VL, Iwata J, Kominami E, Chait BT, Tanaka K, Yue Z (2007b) Essential role for autophagy protein Atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration. Proc Natl Acad Sci USA 104: 14489–14494
Koyama-Honda I, Itakura E, Fujiiwara TK, Mizushima N (2013) Temporal analysis of recruitment of mammalian ATG proteins to the autophagosome formation site. Autophagy 9: 1491–1499
Koyama-Honda I, Tsuboyama K, Mizushima N (2017) ATG conjugation-dependent degradation of the inner autophagosomal membrane is a key step for autophagosomal maturation. Autophagy 13: 1252–1253
Kroemer G, Marino G, Levine B (2010) Autophagy and the integrated stress response. Mol Cell 40: 280–293
Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, Mizushima N (2004) The role of autophagy during the early neonatal starvation period. Nature 432: 1032–1036
Kuma A, Mizushima N, Ishihara N, Ohsumi Y (2002) Formation of the approximately 350-kDa Apg12–Apg5–Apg16 multimeric complex, mediated by Apg16 oligomerization, is essential for autophagy in yeast. J Biol Chem 277: 18619–18625
Kuwistos E, Salmenlinna A, Alafuzzoff I (2001) Ubiquitin-binding protein p62 is present in neuronal and glial inclusions in human tauopathies and synucleinopathies. NeuroReport 12: 2085–2090
Kyei GB, Dinkins C, Davis AS, Roberts E, Singh SB, Dong C, Wu Li, Kominami E, Ueno T, Yamamoto A et al (2009) Autophagy pathway intersects with HIV-1 biology and regulates viral yields in macrophages. J Cell Biol 186: 255–268
Lee E, Koo Y, Ng A, Wei Y, Luby-Phelps K, Juraska A, Xavier RJ, Cleaver O, Levine B, Amatruda JF (2014) Autophagy is essential for cardiac morphogenesis during vertebrate development. Autophagy 10: 572–587
Lee HY, Kim J, Quan W, Lee J, Kim MS, Kim SH, Bae JW, Hur KY, Lee MS (2016) Autophagy deficiency in myeloid cells increases susceptibility to obesity-induced diabetes and experimental colitis. Autophagy 12: 1390–1403
Lee HJ, Kawai Y, Ferguson MM, Rovira II, Bishop AJ, Motoyama N, Cao L, Finkel T (2012) Atg7 modulates p53 activity to regulate cell cycle and survival during metabolic stress. Science 366: 225–228
Lee JJ, Sanchez-Martinez A, Zarate AM, Benincas C, Mayor U, Clague MJ, Whitworth AJ (2018a) Basal mitophagy is widespread in Drosophila but minimally affected by loss of Pink1 or parkin. J Cell Biol 217: 1613–1622
Lee YK, Lee JA (2016) Role of the mammalian ATG8/LC3 family in autophagy: differential and compensatory roles in the spatiotemporal regulation of autophagy. BMB Rep 49: 424–430
Lee YA, Noon LA, Akat KM, Ybanez MD, Lee T-F, Berres M-L, Fujiwara N, Goossens N, Chou H-I, Parvin-Nejad FP et al (2018b) Autophagy is a gatekeeper of hepatic differentiation and carcinogenesis by controlling the degradation of Yap. Nat Commun 9: 4962
Levine B, Kroemer G (2008) Autophagy in the pathogenesis of disease. Cell 137: 22–42
Levine B, Kroemer G (2019) Biological functions of autophagy genes: a disease perspective. Cell 176: 11–42
Levine B, Mizushima N, Virgin HW (2011) Autophagy in immunity and inflammation. Nature 469: 323–335
Lévy J, Cacheux W, Bara MA, L’Hermite A, Lepage P, Fraudeau M, Trentescaux C, Lemarchand J, Durand A, Crain A-M et al (2015) Intestinal inhibition of Atg7 prevents tumour initiation through a microbiome-influenced immune response and suppresses tumour growth. Nat Cell Biol 17: 1062–1073
Li H, Li D, Ma Z, Qian Z, Kang X, Jin X, Li F, Wang X, Chen Q, Sun H et al (2018) Defective autophagy in osteoblasts induces endoplasmic reticulum stress and causes remarkable bone loss. Autophagy 14: 1726–1741
Li Z, Wang C, Wang Z, Zhu C, Li J, Sha T, Ma L, Gao C, Yang Y, Sun Y et al (2019) Allele-selective lowering of mutant HTT protein by HTT–LC3 linker compounds. Nature 575: 203–209
Liu K, Hong D, Zhang F, Li X, He M, Han X, Zhang C, Xu G, Stonehouse NJ, Melia TJ, Simonsen A (2019) Distinct functions of ATG16L1 isoforms in autophagy. Nature 575: 2482–2484
Lock R, Kenific CM, Leidal AM, Salas E, Debnath J (2014) Autophagy-dependent production of secreted factors facilitates oncogenic RAS-driven cancer. Cancer Discov 4: 466–479
Lock R, Roy S, Kenific CM, Su JS, Salas E, Ronen SM, Debnath J (2011) Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation. Mol Biol Cell 22: 165–178
Lock M, McWilliams TG (2020) Monitoring autophagy in cancer: from bench to bedside. Semin Cancer Biol 66: 12–21
Lorincz P, Juhasz G (2020) Autophagosomes-lysosome fusion. J Mol Biol 432: 2462–2482
Luhr M, Szalai P, Engedal N (2018) The Lactate dehydrogenase sequestration assay - a simple and reliable method to determine bulk autophagic sequestration activity in mammalian cells. J Vis Exp 1–10
Lv M, Wang C, Li F, Peng J, Wen B, Gong Q, Shi Y, Tang Y (2017) Structural insights into the recognition of phosphorylated FUNDC1 by LC3B in mitophagy. Protein Cell 8: 25–38
Lystad AH, Carlsson SR, de la Ballina LR, Kauffman KJ, Nag S, Yoshimori T, Melia TJ, Simonsen A (2019) Distinct functions of ATG16L1 isoforms in autophagy. Autophagy 15: 231–245
membrane binding and LC3B lipidation in autophagy-related processes. Nat Cell Biol 21: 372 – 383

Mehler-Edwards G, Zvartau-Hind M, Hunter AJ, Gold M, Hopton G, Jacobs G, Davy M, Williams P (2010) Double-blind, controlled phase II study of a 5-HT6 receptor agonist, SB-742457, in Alzheimer’s disease. Curr Alzheimer Res 7: 374 – 385

Mandrioli J, D’Amico R, Zucchi E, Cessani A, Fini N, Fasano A, Caponnetto C, Chio A, Dalla Bella E, Lunetta C et al (2018) Rapamycin treatment for amyotrophic lateral sclerosis: Protocol for a phase II randomized, double-blind, placebo-controlled, multicenter, clinical trial (RAP-ALS trial). Medicine 97: e11119

Marcassa E, Kallinos A, Jardine J, Rusilowicz-Jones EV, Martinez A, Kuehl S, Mandrioli J, D (2020) Autophagy and neurodegeneration: pathogenic mechanisms and therapeutic opportunities. Neuron 93: 1015 – 1034

Metzger S, Saukko M, Van Che H, Tong L, Puder Y, Riess O, Nguyen HP (2010) Age at onset in Huntington’s disease is modified by the autophagy pathway: implication of the V471A polymorphism in Atg7. Hum Genet 128: 453 – 459

Metzger S, Walter C, Riess O, Roos RAC, Nielsen JE, Craufurd D, Network RiToEHSD, Nguyen HP, Laccone F, Didonato S et al (2013) The V471A polymorphism in autophagy-related gene ATG7 modifies age at onset specifically in italian huntington disease patients. PLoS One 8: e68951

Michiels CF, Fransen P, De Munck DG, De Meyer GR, Martenet W (2015) Defective autophagy in vascular smooth muscle cells alters contractility and Ca(2+)-dependent homeostasis in mice. Am J Physiol Heart Circ Physiol 308: H557 – 567

Mizuno Y, Amari M, Takatama M, Aizawa H, Mihara B, Okamoto K (2006) Immunoactivities of p62, an ubiquitin-binding protein, in the spinal anterior horn cells of patients with amyotrophic lateral sclerosis. J Neurol Sci 249: 13 – 18

Mizushima N (2020) The ATG conjugation systems in autophagy. Curr Opin Cell Biol 63: 1 – 10

Mizushima N, Komatsu M (2011) Autophagy: renovation of cells and tissues. Cell 147: 728 – 741

Mizushima N, Kuma A, Kobayashi Y, Yamamoto A, Matsubae M, Takao T, Natsume T, Ohsumi Y, 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

Mizushima N, Levine B (2020) Autophagy in human diseases. N Engl J Med 383: 1564 – 1576

Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, George MD, Klionsky DJ, Ohsumi M, Ohsumi Y (1998) A protein conjugation system essential for autophagy. Nature 395: 395 – 398

Mizushima N, Noda T, Ohsumi Y (1999) Apg16p is required for the function of the Apg12-Apg5 conjugate in yeast autophagy pathway. EMBO J 18: 3888 – 3896

Mizushima N, Yamamoto A, Matsu M, Yoshimori T, Ohsumi Y (2004) In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. Mol Biol Cell 15: 1101 – 1111

Mizushima N, Yoshimori T, Levine B (2010) Methods in mammalian autophagy research. Cell 140: 313 – 326

Moloughney G, Monken CE, Tao H, Zhang H, Thomas JD, Lattime EC, Jin S (2011) Vaccinia virus leads to ATG12-ATG3 conjugation and deficiency in autophagosome formation. Autophagy 7: 1434 – 1447

Morishita H, Eguchi T, Tsukamoto S, Sakamaki Y, Takahashi S, Saito C, Koyama-Honda I, Mizushima N (2021) Organelle degradation in the lens by PLA2 phospholipases. Nature 592: 634 – 638

Mortensen M, Ferguson D, Edelmann M, Kessler B, Morten KJ, Komatsu M, Simon AK (2010) Loss of autophagy in erythroid cells leads to defective removal of mitochondria and severe anemia in vivo. Proc Natl Acad Sci USA 107: 832 – 837

Mortensen M, Soilleux EJ, Djordjevic G, Tripp R, Lutteropp M, Sadighi-Akha E, Starks AJ, Gianville J, Knight S, W. Jacobsen S-E et al (2011) The autophagy protein Atg7 is essential for hematopoietic stem cell maintenance. J Exp Med 208: 455 – 467

Mulcahy Levy JM, Thorburn A (2020) Autophagy in cancer: moving from understanding mechanism to improving therapy responses in patients. Cell Death Differ 27: 843 – 857

Nakamura S, Yoshimori T (2017) New insights into autophagosome-lysosome fusion. J Cell Sci 130: 1209 – 1216

Nguyen TN, Padman BS, Usher J, Dorschot V, Ramg M, Lazarou M (2016) Atg8 family LC3/GABARAP proteins are crucial for
autophagosome-lysosome fusion but not autophagosome formation during PINK2/Parkin mitophagy and starvation. J Cell Biol 215: 857 – 874

Nieto-Torres JL, Shanahan SL, Chassefeyre R, Chaimarit T, Zaretksi S, Landeras-Bueno S, Verhelle A, Encalada SE, Hansen M (2021) LC3B phosphorylation regulates FTYC01 binding and directional transport of autophagosomes. Curr Biol 31: 3440 – 3449 e3447

Nishida Y, Arakawa S, Fujitani K, Yamaguchi H, Mizuta T, Kanaseki T, Nieto-Torres JL, Shanahan SL, Chassefeyre R, Chaiamarit T, Zaretski S, Noman MZ, Parpal S, Van Moer K, Xiao M, Yu Y, Arakelian T, Viklund J, De Ogmundsdottir MH, Fock V, Sooman L, Pogenberg V, Dilshat R, Bindesboll C, Nilsson P, Loganathan K, Sekiguchi M, Matsuba Y, Hui K, Tsubuki S, Tanaka M, Otsu K, Tsujimoto Y, Shimizu S (2009) Discovery of Atg5/Atg7-independent alternative macroautophagy. Nature 461: 654 – 658

Noman MZ, Parpal S, Van Moer K, Xiao M, Yu Y, Arakelian T, Viklund J, De Milto A, Hasmim M, Andersson M et al (2020) Inhibition of Vps34 reprograms cold into hot inflamed tumors and improves anti-PD-1/PD-L1 immunotherapy. Sci Adv 6: eaax7881

Ogmundsdottir MH, Fock V, Sooman L, Pogenberg V, Dilshat R, Bindesboll C, Ogmundsdottir HM, Simonsen A, Wilmanns M, Steingrimsson E (2013) A short isoform of ATG7 fails to lipidate LC3/GABARAP. Sci Rep B: 14391

Ohnstad AE, Delgado NM, North BJ, Nasa I, Kettenbach AN, Schultz SW, Shoemaker CJ (2020) Receptor-mediated clustering of FIP200 bypasses the role of LC3 lipidation in autophagy. EMBO J 39: e104948

Osoini Y, Mita T, Azuma K, Nakajima K, Masuyama A, Goto H, Nishida Y, Miyatsuka T, Fujitani Y, Koike M et al (2018) Defective autophagy in vascular smooth muscle cells enhances cell death and atherosclerosis. Autophagy 14: 1991 – 2006

Overholtzer M, Maillieux AA, Mouneimne G, Normand G, Schnitjer SJ, King RW, Cibas ES, Brugge JS (2007) A nonapoptotic cell death process, entosis, that occurs by cell-in-cell invasion. Cell 131: 966 – 979

Patel KK, Miyoshi H, Beatty WL, Head RD, Malvin NP, Cadwell K, Guan J-L, Saitoh T, Akira S, Seglen PO et al (2013) Autophagy proteins control goblet cell function by potentiating reactive oxygen species production. EMBO J 32: S130 – S144

Poiillet-Perez L, Xie X, Zhan LE, Yang Y, Sharp DW, Hu ZS, Su X, Maganti R, Jiang C, Lu W et al (2018) Autophagy maintains tumour growth through circulating arginine. Nature 563: 569 – 573

Pu Q, Can C, Li R, Li Y, Tan S, Li X, Wei Y, Lan L, Deng X, Liang H et al (2017) Atg7 deficiency intensifies inflammasome activation and pyroptosis in pseudomonas sepsis. J Immunol 198: 3205 – 3213

Ravindran R, Loebbermann J, Nakaya HI, Khan N, Ma H, Cama L, Machaj D, Lawson B, Hakimpour P, Wang Y-C et al (2016) The amino acid sensor GCN2 controls gut inflammation by inhibiting inflammasome activation. Nature 531: 523 – 527

Rockenfeller P, Koska M, Pietrocola F, Minoiu N, Knittelfelder O, Sica V, Franz J, Carmona-Gutierrez D, Kroemer G, Madeo F (2015) Phosphatidylethanolamine positively regulates autophagy and longevity. Cell Death Differ 22: 499 – 508

Rosenfeldt MT, O’Reilly J, Morton JP, Nixon C, MacKay G, Mowinska A, Au A, Rai TS, Zheng L, Ridgway R et al (2013) p53 status determines the role of autophagy in pancreatic tumour development. Nature 504: 296 – 300

Roy S, Leidal AM, Ye J, Ronen SM, Debnath J (2017) Autophagy-dependent shuttling of TBC1D5 controls plasma membrane translocation of GLUT1 and glucose uptake. Mol Cell 67: 84 – 95.e5

Sacca F, Puorro G, Brunetti A, Capasso G, Cervo A, Cocozza S, de Leva M, Marsili A, Pane C, Quarantelli M et al (2015) A randomized controlled pilot trial of lithium in spinocerebellar ataxia type 2. J Neurol 262: 149 – 153

Sagnier S, Daussy CF, Borel S, Robert-Hebmann V, Faure M, Blanchet FP, Beaumelle B, Biard-Piechaczyk M, Espert L (2015) Autophagy restricts HIV-1 infection by selectively degrading Tat in CD4+ T lymphocytes. J Virol 89: 615 – 625

Saito T, Nak J, Oka S-I, Mukai R, Monden Y, Maejima Y, Ikeda Y, Siciarretta S, Liu T, Li H et al (2019) An alternative mitophagy pathway mediated by Rab9 protects the heart against ischemia. J Clin Invest 129: 802 – 819

Saito H, Nishimura T, Muramatsu K, Koderia H, Kumada S, Sugai K, Kasiyi-Yoshida E, Sawaura N, Nishida H, Hoshino AI et al (2013) De novo mutations in the autophagy gene WDR45 cause static encephalopathy of childhood with neurodegeneration in adulthood. Nat Genet 45: 445 – 449

Sanchez-Martín P, Komatsu M (2018) p62/SQSTM1: - steering the cell through health and disease. J Cell Sci 131: 1 – 13

Sandoval H, Thiagarajan P, Dasgupta SK, Schumacher A, Prchal JT, Chen M, Wang J (2008) Essential role for Nix in autophagic maturation of erythroid cells. Nature 454: 232 – 235

Sanjuan MA, Dillon CP, Tait SWG, Moshiaci S, Dorsey F, Connell N, Komatsu M, Tanaka K, Cleveland JL, Witthoff S et al (2007) Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. Nature 450: 1253 – 1257

Schuck S (2020) Microautophagy - distinct molecular mechanisms handle cargos of many sizes. J Cell Sci 133: 1 – 10

Shimizu S (2018) Biological roles of alternative autophagy. Mol Cells 41: 50 – 54

Shintani T, Mizushima N, Ogawa Y, Matsuura A, Noda T, Ohsumi Y (1999) Apg10p, a novel protein-conjugating enzyme essential for autophagy in yeast. EMBO J 18: 5234 – 5241

Shrivastava S, Raychoudhuri A, Steele R, Ray R, Ray RB (2011) Knockdown of autophagy enhances the innate immune response in hepatitis C virus-infected hepatocytes. Hepatology 53: 406 – 414

Singh KK, Lowen F, Pan Yi, Quan A, Ramadan A, Matkar PN, Ehsan M, Sandhu P, Mantella LE, Gupta N et al (2015) The essential autophagy gene ATG7 modulates organ fibrosis via regulation of endothelial-to-mesenchymal transition. J Biol Chem 290: 2547 – 2559

Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ (2009a) Autophagy regulates lipid metabolism. Nature 458: 1131 – 1135

Singh R, Xiang Y, Wang Y, Baikati K, Cuervo AM, Luu YK, Tang Y, Pessin JE, Schwartz GJ, Czaja MJ (2009b) Autophagy regulates adipose mass and differentiation in mice. J Clin Invest 119: 3329 – 3339

Singh SB, Davis AS, Taylor CA, Deretic V (2006) Human IRGA induces autophagy to eliminate intracellular mycobacteria. Science 313: 1438 – 1441

Sir D, Chen WL, Choi J, Wikita T, Yen TS, Ou JH (2008) Induction of autophagic response by hepatitis C virus via the unfolded protein response. Hepatology 48: 1054 – 1061

Strohecker AM, Guo JY, Sir D, Chen WL, Choi J, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ (2009a) Autophagy regulates lipid metabolism. Nature 458: 1131 – 1135

Strohecker AM, Guo JY, Sir D, Chen WL, Choi J, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ (2009a) Autophagy regulates lipid metabolism. Nature 458: 1131 – 1135

Strohecker AM, Guo JY, Sir D, Chen WL, Choi J, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ (2009a) Autophagy regulates lipid metabolism. Nature 458: 1131 – 1135
Suomi F, McWilliams TG (2019) Autophagy in the mammalian nervous system: a primer for neuroscientists. Neuronal Signal 3: NS20180134

Takahayashi AM, Tait SW, Kaiser SE, Williams AH, Deng A, Nourse A, Hammel M, Kurniov I, Rock CO, Green DR et al (2011) Atg8 transfer from Atg7 to Atg3: a distinctive E1–E2 architecture and mechanism in the autophagy pathway. Mol Cell 41: 451–461

Takahashi D, Moriyama J, Nakamura T, Miki E, Takahashi E, Akaite T, Ito-Nakama K, Ariimoto H (2019) AUTACs: cargo-specific degraders using selective autophagy. Mol Cell 76: 797–810 e710

Takamura A, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguê S, Eishi Y, Hino O, Tanaka K, Mizushima N (2011) Autophagy-deficient mice develop multiple liver tumors. Genes Dev 25: 795–800

Tanaka Y, Guede G, Suter A, Eskenlën EL, Hartmann D, Lullmann-Rauch R, Janssen PM, Blanz J, von Figura K, Saftig P (2000) Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. Nature 406: 902–906

Tanida I, Mizushima N, Kyooshua M, Ohsumi M, Ueno T, Ohsumi Y, Kominami E (1999) Atg7p/Cvt2p: a novel protein-activating enzyme essential for autophagy. Mol Biol Cell 10: 1367–1379

Tanida I, Tanida-Miyake E, Ueno T, Kominami E (2001) The human homolog of Saccharomyces cerevisiae Atg7p is a protein-activating enzyme for multiple substrates including human Atg12p, GATE-16, GABARAP, and MAP-LC3. J Biol Chem 276: 1701–1706

Teintur J, Behne R, Wimmer M, Ebrahimi-Fakhari D (2020) Novel insights into the clinical and molecular spectrum of congenital disorders of autophagy. J Inherit Metab Dis 43: 51–62

Tong M, Saito T, Zhai P, Oka SI, Mizushima W, Nakamura M, Ikeda S, Shirakabe A, Sadoshima J (2019) Mitophagy is essential for maintaining cardiac function during high fat diet-induced diabetic cardiomyopathy. Circ Res 124: 1360–1371

Torisu T, Torisu K, Lee IH, Liu J, Malide D, Combs CA, Wu XS, Rovira II, Fergusson MM, Weigert R et al (2013) Autophagy regulates endothelial cell processing, maturation and secretion of von Willebrand factor. Nat Med 19: 1281–1287

Towers CC, Fitzwalter BE, Regan D, Goodspeed A, Morgan MJ, Liu CW, Gustafson DL, Thorburn A (2019) Cancer cells suprègeular NRF2 signaling to adapt to autophagy inhibition. Dev Cell 50: 690–703 e696

Towers CC, Wodetzki DK, Thorburn J, Smith KR, Caino MC, Thorburn A (2021) Mitochondrial-derived vesicles compensate for loss of LC3-mediated mitophagy. Dev Cell 56: 2029–2042 e5

Trentesaux C, Freudeau M, Pitasi CL, Lemarchand J, Jacques S, Duche A, Letourmeur F, Naser E, Bailly K, Schmitt A et al (2020) Essential role for autophagy protein ATG7 in the maintenance of intestinal stem cell integrity. Proc Natl Acad Sci USA 117: 11136–11146

Tsobuyama K, Koyama-Honda I, Sakamaki Y, Koike M, Morishita H, Mizushima N (2016) The ATG conjugation systems are important for degradation of the inner autophagosomal membrane. Science 354: 1036–1041

Tsuchida M, Ohsumi Y (1998) Isolation and characterization of autophagy-defective mutants of Saccharomyces cerevisiae. FEBS Lett 406: 169–174

Vuppalapati KK, Boudierlique T, Newton PT, Kaminsky VS, Wehtje H, Ohlsson C, Zhivotovsky B, Chagin AS (2015) Targeted deletion of autophagy genes Atg5 or Atg7 in the chondrocytes promotes caspase-dependent cell death and leads to mild growth retardation. J Bone Miner Res 30: 2249–2261

Wang D, Tai PWL, Gao C (2019a) Adeno-associated virus vector as a platform for gene therapy delivery. Nat Rev Drug Discov 18: 358–378

Wang Z, Tao L, Xue Y, Xue L, Wang Z, Chong T (2019b) Association of ATG7 polymorphisms and clear cell renal cell carcinoma risk. Curr Mol Med 19: 40–47

Webster BR, Scott I, Han K, Li JH, Lu Z, Stevens MV, Malide D, Chen Y, Samsel L, Connelly PS et al (2013) Restricted mitochondrial protein acetylation initiates mitochondrial autophagy. J Cell Sci 126: 4843–4849

Wei Y, Chiang WC, Sumpter Jr R, Mishra P, Levine B (2017) Prohibitin 2 is an inner mitochondrial membrane receptor. Cell 168: 224–238 e10

White E (2015) The role for autophagy in cancer. J Clin Invest 125: 42–46

Wilkinson D, Windfeld K, Colding-Jørgensen E (2014) Safety and efficacy of idalopirdine, a 5-HT6 receptor antagonist, in patients with moderate Alzheimer’s disease (LADDER): a randomised, double-blind, placebo-controlled phase 2 trial. Lancet Neurol 13: 1092–1099

Wirth M, Zhang W, Razi M, Nyoni L, Joshi D, O’Reilly N, Johansen T, Toozé SA, Mouilleron S (2019) Molecular determinants regulating selective binding of autophagy adapters and receptors to ATG6 proteins. Nat Commun 10: 2055

Wittkopf N, Gunther C, Martini E, Waldner M, Amann KU, Neurath MF, Becker C (2012) Lack of intestinal epithelial atg7 affects paneth cell granule formation but does not compromise immune homeostasis in the gut. Clin Dev Immunol 2012: 278059

Wrighton PJ, Shwartz A, Heo JM, Quenzer ED, LaBella KA, Harper JW, Goessling W (2021) Quantitative intravaltral imaging in zebrafish reveals in vivo dynamics of physiological-stress-induced mitophagy. J Cell Sci 134: 1–16

Xia L, Xu Z, Zhou H, Bergmann F, Grabe N, Buchler MW, Neoptolemos JP, Hackert T, Kroemer G, Fortunato F (2020) Impaired autophagy increases susceptibility to endotoxin-induced chronic pancreatitis. Cell Death Dis 11: 889

Xie X, Koh JY, Price S, White E, Mehret M (2015) Atg7 overcomes senescence and promotes growth of BrafV600E-driven melanoma. Cancer Discov 5: 410–423

Xiong J (2015) Atg7 in development and disease: pancrea or Pandora’s Box? Protein Cell 6: 722–734

Yamada T, Murata D, Adachi Y, Ittoh K, Kameoka S, Igarashi A, Tati K, Araki Y, Huganir RL, Dawson TM et al (2018) Mitochondrial stasis reveals p62-mediated ubiquitination in parkin-independent mitophagy and mitigates nonalcoholic fatty liver disease. Cell Metab 28: 588–604 e5

Yamaguchi H, Honda S, Tori S, Shimizu K, Kato K, Miyake K, Miyake N, Fujikake N, Sakurai HT, Arakawa S et al (2020) Wipi3 is essential for alternative autophagy and its loss causes neurodegeneration. Nat Commun 11: 5311

Yamaguchi M, Noda NN, Yamamoto H, Shirai T, Kumeta H, Kobashigawa Y, Akada R, Ohsumi Y, Inagaki F (2012) Structural insights into Atg10-mediated formation of the autophagy-essential Atg12-Atg5 conjugate. Structure 20: 1244–1254

Yang S, Wang X, Centino G, Liesa M, Sahin E, Ying H, Bause A, Li Y, Stommel JM, Dell’Antonio G et al (2011) Pancreatic cancers require autophagy for tumor growth. Genes Dev 25: 717–729

Yang W, Liu Y, Tu Z, Xiao C, Yan S, Ma X, Guo X, Chen X, Yin P, Yang Z et al (2019) CRISPR/Cas9-mediated PINK1 deletion leads to neurodegeneration in rhesus monkeys. Cell Res 29: 334–336

Yang Y, Karsli-Uzunbas G, Poillet-Perez L, Sawant A, Hu ZS, Zhao Y, Moore D, Hu W, White E (2020) Autophagy promotes mamalian survival by suppressing oxidative stress and p53. Genes Dev 34: 688–700

Yorimitsu T, Klionsky DJ (2005) Autophagy: molecular machinery for self-eating. Cell Death Differ 12(Suppl 2): 1542–1552

Yoshii S, Kuma A, Akashi T, Hara T, Yamamoto A, Kurikawa Y, Itakura E, Tsukamoto S, Shirata H, Eishi Y et al (2016) Systemic Analysis of Atg5-null mice rescued from neonatal lethality by transgenic ATG5 expression in neurons. Dev Cell 39: 116–130
Yu Z, Ma J, Li X, Liu Y, Li M, Wang LU, Zhao M, He H, Zhang Y, Rao Q et al (2018) Autophagy defects and related genetic variations in renal cell carcinoma with eosinophilic cytoplasmic inclusions. Sci Rep 8: 9972.

Yue W, Hamai A, Tonelli G, Bauvy C, Nicolas V, Tharinger H, Codogno P, Mehrpour M (2013) Inhibition of the autophagic flux by salinomycin in breast cancer stem-like/progenitor cells interferes with their maintenance. Autophagy 9: 714 – 729.

Zeng Y, Huo G, Mo Y, Wang W, Chen H (2015) MiR137 regulates starvation-induced autophagy by targeting ATG7. J Mol Neurosci 56: 815 – 821.

Zhang J, Randall MS, Loyd MR, Dorsey FC, Kundu M, Cleveland JL, Ney PA (2009a) Mitochondrial clearance is regulated by Atg7-dependent and -independent mechanisms during reticulocyte maturation. Blood 114: 157 – 164.

Zhang Y, Cross SD, Stanton JB, Marmorstein AD, Le YZ, Marmorstein LY (2017) Early AMD-like defects in the RPE and retinal degeneration in aged mice with RPE-specific deletion of Atg5 or Atg7. Mol Vis 23: 228 – 241.

Zhang Y, Goldman S, Mo Y, Wang W, Chen H (2017b) Adipose-specific deletion of autophagy-related gene 7 (atg7) in mice reveals a role in adipogenesis. Proc Natl Acad Sci USA 106: 19860 – 19865.

Zhao Y, Yang J, Liao W, Liu X, Zhang H, Wang S, Wang D, Feng J, Yu L, Zhu WG (2010) Cytosolic FoxO1 is essential for the induction of autophagy and tumour suppressor activity. Nat Cell Biol 12: 665 – 675.

Zhong Z, Sanchez-Lopez E, Karim M (2016) Autophagy, Inflammation, and immunity: a troika governing cancer and its treatment. Cell 166: 288 – 298.

Zhou H, Qian X, Xu N, Zhang S, Zhu G, Zhang Y, Liu D, Cheng C, Zhu X, Liu Y et al (2020) Disruption of Atg7-dependent autophagy causes electromotility disturbances, outer hair cell loss, and deafness in mice. Cell Death Dis 11: 913.

Zhong Z, Sanchez-Lopez E, Karin M (2016) Autophagy, Inflammation, and immunity: a troika governing cancer and its treatment. Cell 166: 288 – 298.

Zhou X, Xie L, Xia L, Bergmann F, Buchler MW, Kroemer G, Hackert T, Fortunato F (2017) RIP3 attenuates the pancreatic damage induced by deletion of ATG7. Cell Death Dis 8: e2918.

Zhu J, Li Y, Tian Z, Hua X, Gu J, Li J, Liu C, Jin H, Wang Y, Jiang G et al (2017) ATG7 overexpression is crucial for tumorigenic growth of bladder cancer in vitro and in vivo by targeting the ETS2/miRNA196b/FOXO1/p27 Axis. Mol Ther Nucleic Acids 7: 299 – 313.

Zhu K, Dunner Jr K, McConkey DJ (2010) Proteasome inhibitors activate autophagy as a cytoprotective response in human prostate cancer cells. Oncogene 29: 451 – 462.

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