

## REVIEW ARTICLE

Dan L. Longo, M.D., *Editor*

## Autophagy in Human Diseases

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**A**UTOPHAGY (“SELF-EATING”) IS THE PROCESS THROUGH WHICH PARTS OF the cell are degraded in the lysosome. Elucidation of the key genes essential for autophagy — originally identified in yeast — has led to a new era in our understanding of mammalian physiology and the pathophysiology of human diseases. Mutations in autophagy-related genes have been linked to numerous human diseases, shedding light on new therapeutic targets in the autophagy pathway.

#### MEMBRANE DYNAMICS AND MOLECULAR MECHANISMS OF AUTOPHAGY

In autophagy, cytoplasmic materials are degraded in the lysosome.<sup>1-3</sup> Because a lysosome has a limiting membrane that serves as a safety mechanism, blocking leakage of its degradative enzymes, the process of autophagy involves complex membrane dynamics. Three types of autophagy involving different modes of cargo delivery to the lysosome have been noted: macroautophagy, microautophagy, and chaperone-mediated autophagy (Fig. 1). Macroautophagy is the major regulated form of autophagy that responds to environmental and physiological cues. Microautophagy involves the direct engulfment of cytoplasmic contents by lysosomes,<sup>8</sup> whereas chaperone-mediated autophagy involves chaperone-assisted translocation of substrate proteins (and possibly DNA and RNA) across the lysosomal membrane.<sup>9,10</sup> In this review, we focus specifically on the process of macroautophagy.

In macroautophagy, a portion of the cytoplasm is engulfed by a thin membrane cistern termed the isolation membrane, or phagophore, which results in the formation of a double-membrane organelle called the autophagosome (Fig. 1). On fusion of the outer autophagosomal and lysosomal membranes, lysosomal enzymes degrade the inner autophagosomal membrane and the enclosed material. Macroautophagy was once considered a nonselective process, but it is now known to degrade selective cargoes, such as damaged mitochondria (mitophagy), ruptured lysosomes (lysophagy), and intracellular microbes (xenophagy) (Fig. 1).<sup>3,6,7</sup> Although macroautophagy degrades various macromolecules and organelles en bloc, the proteasome degrades ubiquitinated proteins one by one. These two major degradation pathways are connected functionally and even share key molecules; for example, ubiquitin serves as a signal not only for the proteasome but also for macroautophagy (Fig. 1).<sup>7</sup>

The process of macroautophagy (hereafter referred to as autophagy) involves the orchestrated action of multiple complexes of proteins encoded by evolutionarily conserved, autophagy-related (ATG) genes, which were originally identified in yeast.<sup>4,5</sup> Of the more than 40 ATG genes identified in yeast, 15 are called core ATG genes (ATG1 through ATG10, ATG12, ATG13, ATG14, ATG16, and ATG18) because they are required for both nonselective and selective autophagy and are evolutionarily

conserved. *ATG11* (also known as *RB1CC1*) and *ATG101* could also be considered core ATG genes in many other organisms (but not in yeast). The products of these 15 or 17 ATG genes, together with other membrane traffic factors, regulate autophagosome formation at distinct steps, including induction (typically driven by metabolic stresses such as starvation), membrane nucleation and elongation on the endoplasmic reticulum, closure, and tethering and fusion with lysosomes (Fig. 1). Selective cargoes can also initiate autophagosome formation by recruiting specific ATG proteins and are recognized by the autophagosomal membrane at the nucleation–elongation step (Fig. 1). Identification of ATG proteins and other autophagy-related factors has not only facilitated our understanding of the mechanism of autophagy but also provided valuable research tools such as molecular markers to label autophagic structures and genes for knock-out studies in organisms.

Although these autophagy-related factors are well conserved and required for autophagy, recent evidence suggests that many or possibly all these factors are not strictly specific to canonical autophagy. For example, autophagy genes are required for certain types of unconventional secretion of cytosolic leaderless proteins (e.g., interleukin-1 $\beta$  and interleukin-18)<sup>11</sup> and for phagosome and endosome maturation, termed LC3-associated phagocytosis (LAP).<sup>12</sup> (Leader sequences are characterized by hydrophobic amino acids that facilitate insertion of a protein into the lipid bilayer of the endoplasmic reticulum to guide the protein's secretion; leaderless proteins require an alternative mechanism for secretion.) Noncanonical functions of ATG genes are important factors in understanding the pathophysiological roles of autophagy.

#### PHYSIOLOGICAL ROLES OF AUTOPHAGY

The physiological functions of autophagy have been defined primarily by the phenotypes of organisms (or tissues) with genetic deletion of autophagy genes and the occurrence of autophagy visualized with ATG proteins, particularly LC3 family proteins (homologues of yeast Atg8).<sup>1,3</sup> Table 1 and Table S1 in the Supplementary Appendix (available with the full text of this article

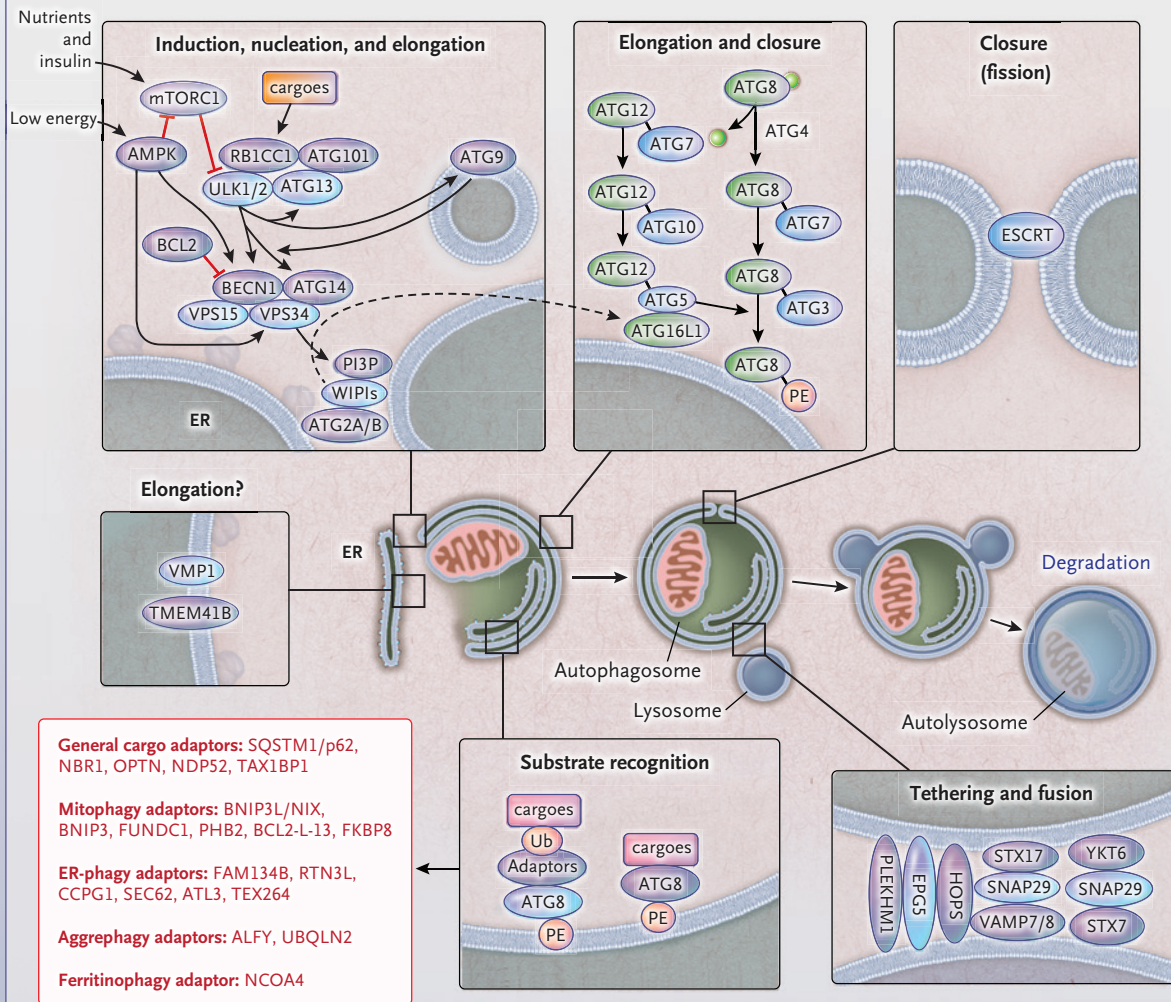
at NEJM.org) summarize the major functions of autophagy in mammals. Basically, autophagy mediates several biologic functions in the cell, such as elimination of cytoplasmic material, generation of degradation products, and cytoplasm-to-lysosome transport. Each physiological function at the organismal level can be attributed to at least one of these biologic processes and in many cases to a combination of them.

The most fundamental and evolutionarily conserved role of autophagy is adaptation to metabolic demands (Table 1 and Table S1). For example, autophagy is up-regulated during starvation and aerobic exercise, degrading macromolecules to produce the nutrients that are required as building blocks or energy sources. In addition, autophagy is necessary for several crucial steps in mammalian development, such as nutrient supply during preimplantation embryogenesis and, presumably, elimination of paternal mitochondria (at least in the nematode *Caenorhabditis elegans*). Autophagy is also important for the development and differentiation of various tissues.

Autophagy plays homeostatic roles, particularly in long-lived populations of cells, in which obsolete material cannot be diluted by cell proliferation (Table 1 and Table S1). For instance, deletion of *Atg* genes in neuronal cells causes neurodegeneration and the accumulation of ubiquitin-positive aggregates,<sup>13</sup> whereas deletion in the liver leads to hepatomegaly and hepatic dysfunction.<sup>14</sup> Similar homeostatic roles have been observed in many other organs and tissues.<sup>1,3</sup> These phenotypes could be caused not only by an impairment of constitutive bulk turnover of cytoplasmic contents but also by a defect in selective mechanisms against harmful organelles (e.g., ruptured lysosomes and mitochondria that produce reactive oxygen species) and protein condensates (e.g., misfolded protein aggregates and membraneless organelles containing proteins, nucleic acids, or both that are produced by the liquid–liquid phase separation) (Fig. 1).<sup>3,6,7,15</sup>

Autophagy is also important for fine-tuning of the levels of certain proteins and lipids (Table 1). For instance, the autophagy substrate SQSTM1 (also known as p62) should be kept at low levels to inhibit aggregate formation and hyperactivation of the oxidative stress-responsive NRF2 pathway, which can cause hepatic dysfunction and tumorigenesis.<sup>14</sup> Autophagy can

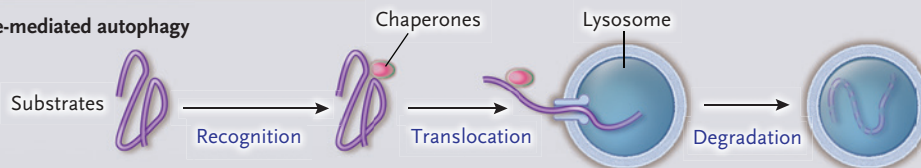
## A Macroautophagy



## B Microautophagy



## C Chaperone-mediated autophagy





**Figure 1 (facing page). Types of Autophagy.**

Macroautophagy (Panel A) is mediated by the autophagosome.<sup>1,5</sup> A portion of the cytoplasm is enclosed by a thin membrane cisterna, termed the isolation membrane, or phagophore, to form an autophagosome. On fusion with lysosomes, the enclosed material is degraded by lysosomal enzymes in autolysosomes. Formation of the autophagosome in mammalian cells is initiated by a protein kinase complex comprising UNC-51–like kinase 1 or 2 (ULK1 or ULK2), ATG13, RB1CC1 (also called ATG11 or FIP200), and ATG101. This initiation complex receives two classes of signals. The first class includes various nutrient and stress signals that mainly converge on mTORC1 (mechanistic target of rapamycin complex 1); mTORC1 is activated by amino acids and growth factors such as insulin and inhibits ULK1/2. In a state of starvation, this inhibition is released, leading to translocation of the initiation complex to the site of autophagosome formation, which is on the endoplasmic reticulum (ER) or closely related membranes. In addition, a low-energy status activates ULK1/2 through AMP-activated protein kinase (AMPK). The second signal class involves autophagy cargoes, such as damaged mitochondria, which can also activate the initiation complex by direct interaction with RB1CC1. To nucleate autophagosomal membranes, ULK1/2 phosphorylates components of the class III phosphatidylinositol 3-kinase (PI3K) complex, which comprises ATG14, Beclin 1, VPS34, and VPS15 and generates phosphatidylinositol 3-phosphate (PI3P) on autophagosomal precursor membranes. Also involved are ATG9 vesicles. Next, PI3P-interacting WIPI family proteins (WIPIs) and the lipid transfer protein ATG2A/B are recruited.

On the ER, the multispanning membrane proteins VMP1 and TMEM41B are also required for autophagosome formation, probably at the elongation step. WIPI2 recruits a complex consisting of ATG12–ATG5 and ATG16L1, which promotes the conjugation between ATG8 family proteins (LC3 and GABARAP subfamilies) and phosphatidylethanolamine (PE). ATG8 family proteins are considered to be important for membrane elongation and autophagosome closure.

Once the edges of the autophagosome are sealed through ESCRT (endosomal sorting complex required for transport) machinery, autophagosomes acquire SNAP receptor (SNARE) proteins such as syntaxin 17 (STX17) and YKT6, which interact with SNAP29 and lysosomal SNARE proteins (e.g., VAMP7, VAMP8, and STX7) to promote fusion with lysosomes. The fusion step is also regulated by tethering machinery (e.g., the HOPS complex, EPG5, and PLEKHM1). ATG8 family proteins on the inner autophagosomal membrane recognize selective cargoes such as mitochondria (mitophagy), ER fragments (ER-phagy), lysosomes (lysophagy), protein aggregates (aggrephagy), and ferritin (ferritinophagy).<sup>3,6,7</sup> ATG8 proteins either directly recognize substrate proteins that have LIRs (LC3-interacting regions) or indirectly recognize them through LIR-containing adaptor proteins that can be cargo-specific (e.g., mitophagy and ER-phagy adaptors) or cargo-nonspecific (soluble adaptors). Soluble adaptors often recognize ubiquitinated cargoes.

Microautophagy (Panel B) is mediated by direct engulfment of a portion of the cytoplasm by the lysosomal membrane.<sup>8</sup> In chaperone-mediated autophagy (Panel C), cytosolic chaperones and lysosomal membrane translocators deliver unfolded proteins into the lumen of lysosomes.<sup>9</sup> Cytosolic RNA and DNA can be degraded by a similar mechanism (termed RNautophagy–DNautophagy) (not shown).<sup>10</sup> Ub denotes ubiquitin.

degrade intracellular membranes and lipid droplets.<sup>16</sup> It also controls lipid metabolism by positively regulating the function of peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ), a major transcription factor for many lipid-metabolizing enzymes (Table 1).

Autophagy and related pathways (e.g., LAP and unconventional secretion) are central homeostatic mechanisms in immunity and inflammation.<sup>2,3</sup> Indeed, whole-body deletion of *Atg7* in adult mice leads to their death within 2 or 3 months as a result of neurodegeneration or infection.<sup>17</sup> Besides xenophagy, the autophagy pathway also intersects in multiple complex ways with diverse aspects of innate and adaptive immunity. Generally, autophagy helps the host to activate immunity to control infection while limiting detrimental, uncontrolled inflammation.<sup>18</sup>

Autophagic activity is reduced during aging, and autophagy helps to extend the mammalian life span and “health span” (Table 1 and Table S1).<sup>19</sup> Genetically engineered mice with increased autophagy (e.g., *Becn1*<sup>F121A/F121A</sup> knock-in mice and *Rubcn* knockout mice) have improvement in age-related phenotypes, such as cardiac and renal fibrosis and spontaneous tumorigenesis, and can live longer than normal mice.<sup>20,21</sup> Studies in *C. elegans* prove that the autophagy pathway is essential for most longevity states (e.g., caloric restriction and reduced insulin signaling).<sup>19</sup> Autophagy may promote longevity by improving protein and organellar quality control, maintaining “stemness,” promoting genomic stability, or a combination of these factors. Basal autophagy is also necessary to maintain the stem-cell quiescent state in mice.<sup>22</sup> This function has been observed in muscle satellite cells, hematopoietic stem cells, intestinal stem cells, and neural stem cells.

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HUMAN DISEASES INVOLVING  
AUTOPHAGY DYSREGULATION

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Given that autophagic failure has been shown to promote cellular degeneration, age-related changes, tumor formation, and detrimental infection in mice, it might also play key roles in human diseases. Indeed, autophagy (both basal and

**Table 1. Physiological Functions of Autophagy in Mammals.\***

Function	Mechanism
<b>Adaptive metabolic response to starvation and exercise</b>	Enhanced degradation to maintain protein synthesis and energy production
<b>Development</b>	
Embryonic development	Degradation of maternal proteins to produce zygotic proteins, degradation of paternal mitochondria
Differentiation and tissue development	Adipose tissues, lymphocytes, erythrocytes, heart, intestine, and other organs (e.g., testis and ovary)
<b>Homeostasis†</b>	
Basal turnover	Continuous bulk degradation of cytoplasmic contents (e.g., proteins, nucleic acids, and glycogen)
Protein quality control	Active degradation of misfolded proteins or condensates and aggregates
Organelle homeostasis	Elimination of excess, damaged, harmful, or ruptured organelles
Lipid homeostasis	Degradation of membrane lipids and lipid droplets (lipophagy) and regulation of PPAR $\alpha$
Redox homeostasis	Degradation of damaged mitochondria (mitophagy)
Nrf2 regulation	Degradation of the KEAP1-binding protein SQSTM1/p62
Iron homeostasis	Degradation of ferritin
<b>Immunity or inflammation</b>	
Control of pathogen replication	Selective elimination of pathogens (xenophagy)
Regulation of innate immunity	Regulation of inflammasome activation, innate immune signaling, and cytokine secretion
Regulation of B- and T-cell responses	Lymphocyte differentiation and antigen presentation
<b>Other functions</b>	
Antiaging	Homeostatic roles of autophagy
Stem-cell maintenance	Homeostatic roles of autophagy
Genomic integrity	Homeostatic roles of autophagy
Conventional secretion	Enhancement of regulated or constitutive secretion
Unconventional secretion	Fusion of autophagosomes (or related structures) with the plasma membrane
Cell death	Various mechanisms, including autosis

\* A reference list for the information in this table and in Table S1 is provided in the Supplementary Appendix, available with the full text of this article at NEJM.org. Nrf2 denotes nuclear factor erythroid 2–related factor 2, and PPAR $\alpha$  peroxisome proliferator–activated receptor  $\alpha$ .

† The following types of autophagic degradation are named according to their specific substrate: aggrephagy (protein aggregates), ER-phagy or reticulophagy (endoplasmic reticulum), ferritinophagy (ferritin), glycophagy (glycogen), lipophagy (lipid droplets), lysophagy (lysosomes), mitophagy (mitochondria), nucleophagy (nucleus), pexophagy (peroxisomes), ribophagy (ribosomes), and xenophagy (microbes).

regulated types) has long been thought to be closely linked to human diseases, but because autophagic activity in humans cannot be precisely measured, little evidence has been generated to suggest that it is elevated or reduced in specific conditions. During the past decade, however, human genetic studies have increasingly pointed to the involvement of autophagy, particularly in neurodegenerative diseases, can-

cers, inflammatory diseases, and autoimmune disorders. Mutations in autophagy-related genes are now known to cause mendelian disorders, and autophagy gene polymorphisms have been found to be associated with susceptibility to some diseases (Table 2 and Table S2). We discuss some examples of genetic links between autophagy-related genes and diseases. The question that remains to be answered, however, is

**Table 2. Diseases Associated with Autophagy-Related Gene Mutations.\***

Category	Examples of Diseases (Related Genes)
Adult neurodegenerative disorders	Parkinson's disease ( <b>PRKN/PARK2</b> [AR], <b>PINK1/PARK6</b> [AR], <b>LRRK2/PARK8</b> [AD], <b>ATP13A2/PARK9</b> [AR], <b>GBA</b> , <b>TMEM175</b> ), amyotrophic lateral sclerosis ( <b>OPTN</b> [AD?], <b>VCP</b> [AD], <b>SQSTM1/p62</b> [AD], <b>TBK1</b> [AD], <b>UBQLN2</b> [XLD], <b>CHMP2B</b> [AD], <b>SPG11</b> [AR], <b>VAPB</b> [AD], <b>C9orf72</b> ), frontotemporal dementia ( <b>OPTN</b> [AD?], <b>VCP</b> [AD], <b>SQSTM1/p62</b> [AD], <b>TBK1</b> [AD], <b>UBQLN2</b> [XLD], <b>CHMP2B</b> [AD], <b>GRN</b> [AD], <b>C9orf72</b> ), neuronal ceroid lipofuscinosis ( <b>GRN</b> [AD]), fulminant neurodegeneration ( <b>ATP6AP2</b> [XLR]), dementia with Lewy bodies ( <b>C9orf72</b> )
Pediatric neurodevelopmental disorders	Spinocerebellar ataxia ( <b>ATG5</b> [AR], <b>RUBCN</b> [AR]), cortical atrophy and epilepsy ( <b>PIK3R4/VPS15</b> [AR]), childhood-onset neurodegeneration ( <b>SQSTM1/p62</b> [AR]), BPAN ( <b>WDR45/WIPI4</b> [XLD]), spastic quadriplegia and brain abnormalities ( <b>WDR45B/WIPI3</b> [AR]), primary microcephaly ( <b>WDFY3/ALFY</b> [AD]), hereditary spastic paraplegia ( <b>SPG49/TECPR2</b> [AR], <b>SPG11</b> [AR], <b>SPG15/ZFYVE26</b> [AR], <b>ATP13A2</b> [AR]), ataxia with spasticity ( <b>VPS13D</b> [AR]), Rett's syndrome ( <b>WDR45/WIPI4</b> [XLD], <b>MECP2</b> [XLD]), Joubert's syndrome ( <b>INPP5E</b> [AR]), leukoencephalopathy ( <b>VPS11</b> [AR]), adolescence-onset dystonia ( <b>VPS16</b> [AR]), CEDNIK syndrome ( <b>SNX14</b> [AR]), Pelizaeus–Merzbacher–like disorder ( <b>SNAP29</b> [AR]), West's syndrome ( <b>WDR45/WIPI4</b> [XLD], <b>SNAP29</b> [AR])
Hereditary neuropathies	Sensory and autonomic neuropathy type II ( <b>FAM134B</b> [AR]), Charcot–Marie–Tooth disease ( <b>RAB7A</b> [AD], <b>LRSAM1</b> [AD,AR], <b>VCP</b> [AD], <b>SPG11</b> [AR], <b>HSPB8</b> [AD]), sensory and autonomic neuropathy type IF ( <b>ATL3</b> [AD]), distal hereditary motor neuronopathy ( <b>HSPB8</b> [AD])
Ophthalmologic diseases	Primary open-angle glaucoma ( <b>OPTN1</b> [AD]), cataracts ( <b>CHMP4B</b> [AD])
Cardiac and skeletal myopathies	Danon's cardiomyopathy ( <b>LAMP2</b> [XLD]), distal myopathy with rimmed vacuole ( <b>SQSTM1/p62</b> [AD]), dilated cardiomyopathy ( <b>PLEKHM2</b> [AR]), sporadic inclusion-body myositis ( <b>VCP</b> [AD]), X-linked myopathy with excessive autophagy ( <b>VMA21</b> [XLR])
Inflammatory disorders	Crohn's disease ( <b>ATG16L1</b> , <b>ULK1</b> , <b>CALCO/NDP52</b> , <b>IRGM</b> , <b>LRRK2</b> , <b>ATG9A</b> ), ulcerative colitis ( <b>LRRK2</b> , <b>ATG9A</b> , <b>MTMR3</b> , <b>SMURF1</b> , <b>GPR65</b> ), childhood asthma ( <b>ATG5</b> )
Autoimmune diseases	Systemic lupus erythematosus ( <b>ATG16L2</b> , <b>ATG5</b> , <b>DRAM1</b> , <b>CLEC16A</b> ), diabetes ( <b>CLEC16A</b> ), other autoimmune diseases ( <b>CLEC16A</b> )
Infectious diseases	<i>Mycobacterium tuberculosis</i> ( <b>IRGM</b> , <b>LRRK2</b> ), <i>M. leprae</i> ( <b>PRKN/PARK2</b> , <b>LRRK2</b> )
Skeletal disorders	Osteopetrosis ( <b>TCIRG1/ATP6V0A3</b> [AR], <b>PLEKHM1</b> [AD,AR]), Paget's disease of the bone ( <b>SQSTM1/p62</b> [AD], <b>VCP</b> [AD], <b>OPTN</b> ), Kashin–Beck disease ( <b>ATG4C</b> )
Congenital multisystem disorders	Global developmental abnormalities ( <b>WIPI2</b> [AR]), Vici's syndrome ( <b>EPG5</b> [AR]), Zellweger syndrome ( <b>PEX13</b> [AR]), glycosylation disorder with autophagy defects ( <b>ATP6AP2</b> [XLR]), Zimmerman–Laband syndrome ( <b>ATP6V1B2</b> [AD]), Hermansky–Pudlak syndrome ( <b>VPS33A</b> [AR]), multisystem proteinopathy ( <b>VCP</b> [AD], <b>SQSTM1/p62</b> [AD])
Cancer (frequently mutated genes)	Breast and ovarian cancer (somatic: <b>BECN1</b> , <b>RB1CC1</b> , <b>PRKN/PARK2</b> , Fanconi anemia pathway genes, <b>FAM134B</b> , <b>EI24</b> ), colorectal cancer (somatic: <b>ULK1</b> , <b>ULK2</b> , <b>UVRAG</b> , <b>PRKN/PARK2</b> , <b>FAM134B</b> , <b>EI24</b> ), HBV-related hepatocellular carcinoma (germline: <b>ATG5</b> ), other solid tumors (somatic: <b>PRKN/PARK2</b> , Fanconi anemia pathway genes, <b>FAM134B</b> , <b>EI24</b> ), hematopoietic cancers (germline: <b>ATG2B</b> ; somatic: Fanconi anemia pathway genes)

\* Boldface type indicates causative mutations in mendelian diseases; regular type indicates risk variants or predisposing mutations (identified by genomewide association studies or large-scale analyses). AD denotes autosomal dominant, AR autosomal recessive, BPAN beta-propeller protein–associated neurodegeneration, CEDNIK cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoma, HBV hepatitis B virus, XLD X-linked dominant, and XLR X-linked recessive. A reference list for the information in this table and in Table S2 is provided in the Supplementary Appendix.

whether the phenotype is produced through a defect in autophagy or through nonautophagic functions of these genes.

#### NEURODEGENERATIVE DISEASES

Because deletion of autophagy genes in mice causes neurodegeneration, an unevaluated hypothesis proposes that defects in autophagy may cause neurodegenerative diseases in humans. Indeed, many neurodegenerative diseases are

characterized by the accumulation of abnormal protein condensates or aggregates (e.g., tau, TDP-43, SOD1,  $\alpha$ -synuclein, and polyglutamine proteins), which could be cleared by autophagy.<sup>13</sup> Although these condensates are in some cases generated by specific mutations in accumulated proteins, the precise mechanisms, particularly in sporadic cases, generally remain unknown. Thus far, defects in the autophagy pathway have been suggested for several major neurodegenera-

tive diseases. For example, in neurons in Alzheimer's disease, factors promoting amyloidogenesis (amyloid precursor protein and presenilins) can affect lysosomal function and autophagosome clearance.<sup>23</sup>

Moreover, recent genetic studies have provided direct evidence linking autophagy with human diseases, with mutations in core ATG genes causing a number of degenerative diseases (Fig. 1, Table 2, and Table S2). Three of the four WIPI proteins (mammalian homologues of yeast Atg18, Atg21, and Hsv2) are linked to neurodegenerative diseases with different clinical features. Patients with a homozygous mutation (V249M replacement) in *WIPI2* have skeletal abnormalities and neurologic symptoms, including intellectual disability and speech and language impairment, with subclinical hypothyroidism.<sup>24</sup> A disease with homozygous *WDR45B/WIPI3* mutations is characterized by intellectual disability, spastic quadriplegia, and epilepsy accompanied by cerebral hypoplasia.<sup>25</sup> Heterozygous mutations (in females) and hemizygous mutations (in males) in *WDR45/WIPI4* in the X chromosome cause beta-propeller protein-associated neurodegeneration (BPAN; originally called static encephalopathy of childhood with neurodegeneration in adulthood [SENDA]), which is associated with infancy-onset psychomotor retardation, epilepsy, and autism, as well as adolescence-onset dystonia, parkinsonism, and dementia with iron accumulation in the globus pallidus and substantia nigra.<sup>26,27</sup> The neurologic phenotype of BPAN is partially recapitulated in *Wdr45*-deletion mice.<sup>28</sup> A pathogenic mutation in *ATG5* that impairs ATG12–ATG5 covalent conjugation was also identified in a disease involving cerebellar ataxia and intellectual disability.<sup>29</sup> Some degree of defective autophagy is observed in these diseases with mutations in core ATG genes. The clinical symptoms and histopathological features differ, however, possibly because of differences in the tissue distribution of paralog expression, the remaining activity of mutant proteins, the autophagy-independent functions of these proteins, or a combination of these factors.

Mutations in genes involved in selective autophagy have also been identified in neurodegenerative diseases (Fig. 1, Table 2, and Table S2). Mutations in *PRKN/PARK2* (encoding parkin) and *PINK1/PARK6* cause familial Parkinson's disease.

The ubiquitin ligase parkin is recruited to damaged mitochondria in a PINK1-dependent manner, which induces autophagic degradation of mitochondria (mitophagy).<sup>30</sup> Although *Prkn* or *Pink1* knockout mice have no obvious Parkinson's disease–like phenotype, the mice have increased levels of inflammatory cytokines after exhaustive exercise.<sup>31</sup> In addition, on a genetic background with a high level of mitochondrial DNA mutations, aged *Prkn* knockout mice have a Parkinson's disease–like phenotype that is dependent on innate immunity signaling. These findings suggest that parkin-dependent and PINK1-dependent mitophagy mitigates inflammation caused by mitochondrial stress and prevents Parkinson's disease. The adaptors required for selective autophagy are also linked to neurodegenerative diseases (Fig. 1 and Table 2). For instance, mutations in *FAM134B*, which mediates autophagic degradation of the endoplasmic reticulum (ER-phagy), are found in patients with hereditary sensory and autonomic neuropathy type II.<sup>32</sup> Loss of SQSTM1, a soluble cargo adaptor, causes childhood-onset neurodegeneration manifested as ataxia, dystonia, and gaze palsy.<sup>33</sup>

The aforementioned diseases are characterized by recessive inheritance, suggesting that the genetic defects are loss-of-function mutations. However, some diseases caused by mutations in autophagy-related genes have an autosomal dominant pattern of inheritance (Table 2 and Table S2). Amyotrophic lateral sclerosis (ALS), a motor neuron disease, is often associated with frontotemporal dementia (FTD), which shares susceptibility genes with ALS.<sup>34</sup> Genes linked to ALS include many autophagy-related genes, such as those encoding the selective autophagy adaptors SQSTM1 and OPTN (optineurin), and autophagy regulators, such as ubiquilin 2, TBK1, VAPB, and VCP. Mutations in these genes all show dominant inheritance. Although partial loss of autophagic activity (due to dysfunction of one allele) could be involved, toxic gain-of-function mechanisms are likely to account for many of these diseases.<sup>34</sup> Because both autophagy and ALS are related to the liquid–liquid phase separation,<sup>15,35</sup> this mechanism may link autophagy gene mutations to the pathogenesis of ALS. In addition, it is notable that mutations in SQSTM1, VCP, and OPTN cause a wide spectrum of diseases (termed multi-system proteinopathies), including not only ALS–

**Table 3. Roles of Autophagy in Tumors.\***

Role	Cell-Autonomous Effects	Cell-Nonautonomous Effects
Antitumorigenic	Increased chromosome or genome stability Decreased metabolic stress Decreased oxidative stress (e.g., through mitophagy) Decreased NRF2 activity (through p62 degradation) Increased cellular senescence Increased anticancer immunogenicity Decreased metastasis	Decreased cell death–induced inflammation Increased anticancer immunity
Protumorigenic	Increased metabolic, energy, and redox homeostasis Decreased p53 Decreased surface MHC I Granzyme degradation Decreased recruitment of NK cells Decreased endoplasmic reticulum stress Increased metastatic dormancy	Increased nutrient supply from nontumor cells in the microenvironment Increased systemic arginine levels (decreased degradation by arginase) Decreased anticancer T-cell immunity

\* A reference list for the information in this table and in Table S3 is provided in the Supplementary Appendix. MHC I denotes major histocompatibility complex class I, and NK natural killer.

FTD but also Paget's disease of the bone and myopathies (Table 2). Although this may be explained by differences in gain-of-function properties, the phenotypes of SQSTM1-associated diseases partly depend on the coinheritance of the N357S variant of TIA1, a 3' untranslated region (UTR) messenger RNA-binding protein, which enhances the liquid-liquid phase separation and impairs the clearance of SQSTM1-containing stress granules.<sup>36</sup>

### CANCER

The association between cancer and autophagy is complex (Table 3 and Table S3). Most of the evidence has been inferred from studies in mice or cultured cancer cells. The first corroboration of the association was derived from studies of Beclin 1. Monoallelic deletion of *BECN1* is often seen in breast, ovarian, and prostate cancers, and cancers develop spontaneously at a high rate in *Becn1*<sup>+/-</sup> mice.<sup>3</sup> Enhanced tumorigenesis has also been observed in other *Atg*-gene-deficient mice,<sup>37</sup> suggesting that autophagy exerts anti-tumorigenic effects in normal cells. These effects have been attributed to various roles of autophagy, including maintenance of genomic stability, suppression of oxidative stress, and inhibition of NRF2 activation (Table 3). Autophagy also plays a protective role by suppressing metastasis.<sup>38</sup> Although the effects are primarily cell-autonomous, cell-nonautonomous antitumor mechanisms also exist. Important effects are exerted through innate and adaptive anticancer immunity (Table 3).<sup>3,39</sup>

In addition, autophagy has protumorigenic roles, which can be cell-autonomous or cell-nonautonomous (Table 3 and Table S3). The role of autophagy in metabolic homeostasis might be more important in cancer cells than in normal cells.<sup>17,40-42</sup> Autophagy also keeps the levels of p53 low<sup>40</sup> and inhibits the surface expression of major histocompatibility complex (MHC) class I in cancer cells.<sup>43</sup> As a cell-nonautonomous, protumorigenic function, autophagy in nontumor cells supplies nutrients to tumor cells.<sup>44-46</sup> Another protumorigenic function of autophagy is maintenance of the blood arginine level through reduction of the level of arginase secreted from the liver.<sup>47</sup> Autophagy can suppress antitumor immunity mediated by CD8+ T cells<sup>48</sup> and can also promote the survival of dormant cancer cells and metastasis.<sup>49</sup>

These conflicting functions may depend on phase and context. In *Atg* gene knockout mice, only benign tumors develop, such as liver adenomas in wild-type mice and pancreatic intraepithelial neoplasia in *Kras*<sup>G12D/+</sup> mice, but these tumors are not fully malignant,<sup>37,50-52</sup> suggesting that autophagy initially suppresses tumorigenesis but later promotes tumor growth.<sup>37</sup> The divergent roles of autophagy may also depend on other factors, such as the mutational state of the p53 gene<sup>51</sup>; however, evidence is conflicting on this point.<sup>41</sup> Furthermore, although autophagy gene mutations have been reported in human cancers (Table 2 and Table S2), extensive genomic analysis has not revealed that mutations



in *Atg* genes are recurrent or are driver mutations in human cancer. Thus, autophagy could be one of the multiple factors regulating tumorigenesis, tumor growth, or an adaptive response in tumor cells.

#### INFLAMMATORY AND AUTOIMMUNE DISEASES

The antiinflammatory function of autophagy may partly explain the growing number of inflammatory and autoimmune human disorders that are associated with mutations in core autophagy genes and selective autophagy molecules (Table 2 and Table S2). *ATG16L1* is a risk allele for Crohn's disease, an inflammatory bowel disease.<sup>53,54</sup> The T300A mutation, which is located in the middle of *ATG16L1*, between the N-terminal region (conserved in yeast *Atg16*) and the C-terminal WD-repeat domain (absent in yeast *Atg16*), increases the risk of Crohn's disease. *Atg16l1*-deficient mice or *Atg16L1*<sup>T300A</sup> knock-in mice have various abnormalities, such as enhanced release of proinflammatory cytokines from macrophages, reduced granule secretion from Paneth cells, increased susceptibility to salmonella infection, and dysregulated T-cell immunity, which could all be consistent with Crohn's disease.<sup>55</sup> However, the T300A mutation might not substantially affect the activity of canonical autophagy and LAP.<sup>56,57</sup> More studies are needed to confirm that the T300A variant is associated with Crohn's disease through autophagy.

Genomewide association studies have also identified several autophagy genes associated with susceptibility to autoimmune disorders, particularly systemic lupus erythematosus (SLE) (Table 2).<sup>58</sup> The products of SLE-associated genes are enriched in the ATG conjugation systems and lysosomal proteins, which are also required for LAP. An SLE-like phenotype consistently develops in mice that are deficient in genes required for both LAP and canonical autophagy but not in autophagy-specific genes (*Rb1cc1* and *Ulk1*). The phenotype involves increased levels of serum inflammatory cytokines and anti-DNA antibodies, as well as glomerulonephritis, suggesting that a defect in LAP rather than canonical autophagy contributes to the pathogenesis of SLE.<sup>59</sup> This hypothesis is consistent with a role proposed for LAP in the delivery of large DNA-containing immune complexes to TLR9 in macrophages.<sup>60</sup>

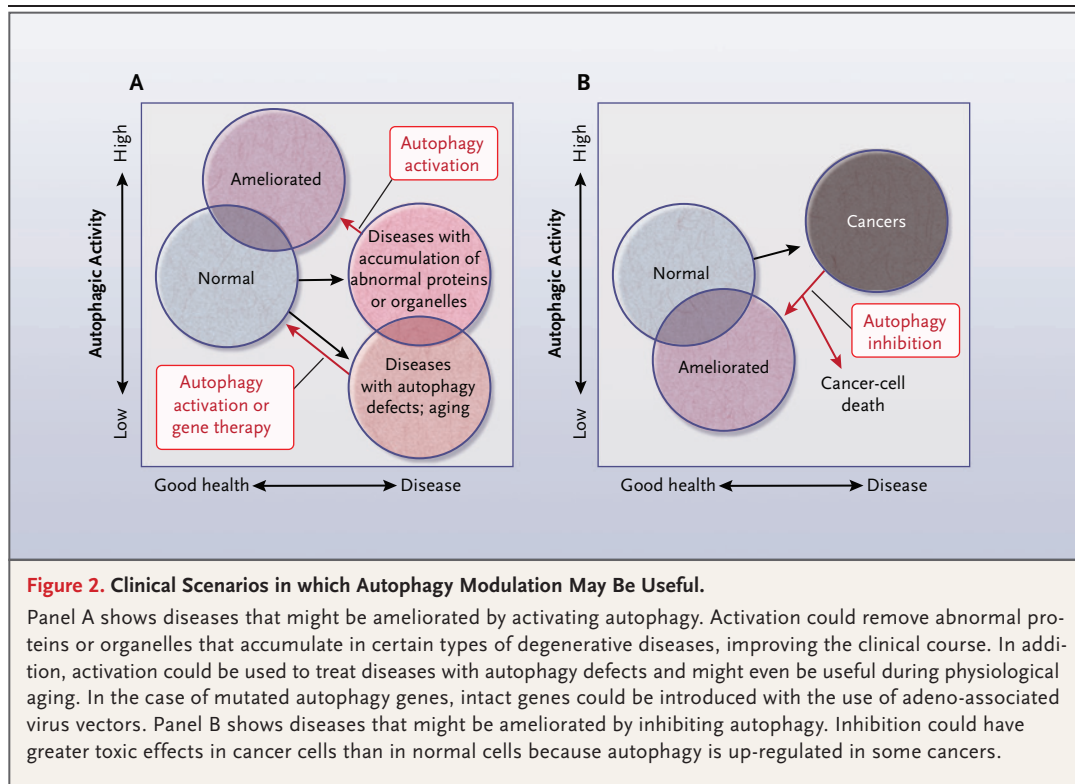
The involvement of T cells, B cells, and dendritic cells has also been proposed.<sup>58</sup>

#### DISEASE-SPECIFIC TREATMENTS TARGETING AUTOPHAGY

Because autophagic activity may be altered in several human diseases, regardless of whether they are specifically caused by mutations in autophagy-related genes, it may be worth trying to restore autophagic activity in these diseases (Fig. 2). Recent advances in gene therapy with the use of adeno-associated virus vectors have received considerable attention for this purpose.<sup>61</sup> However, these diseases are not necessarily the only targets of autophagy-modulating treatments. It is reasonable to hypothesize that abnormal or toxic proteins, their condensates, or both can be eliminated by autophagy activation even when autophagic activity is normal (Fig. 2). On the other hand, autophagy inhibition could be useful for cancer therapy. An analogy is the use of proteasome inhibitors in the treatment of multiple myeloma. Although this disease is not caused by abnormalities in the ubiquitin–proteasome system, multiple myeloma cells that produce excessive amounts of immunoglobulins are overly dependent on proteasomal degradation and are therefore sensitive to proteasome inhibitors.<sup>62</sup>

#### DEGENERATIVE DISEASES

In the past decade, many preclinical studies have investigated autophagy-inducing drugs for degenerative diseases of the nervous system and the liver (alpha<sub>1</sub>-antitrypsin deficiency).<sup>13,63,64</sup> Autophagy induction can also be achieved with the use of an autophagy-inducing peptide.<sup>65</sup> Several clinical trials of autophagy-modulating drugs for neurodegenerative diseases, including ALS, Alzheimer's disease, and Huntington's disease, have been reported or registered in the National Institutes of Health clinical trial registry or the European Union Clinical Trials Register.<sup>66–68</sup> Most of the autophagy-enhancing drugs used in these trials aim to inhibit mTORC1 (mechanistic target of rapamycin complex 1) and include rapamycin,<sup>68</sup> idalopirdine,<sup>69</sup> and SB-742457.<sup>70</sup> Other, mTORC1-independent drugs are also being tested, such as spermidine (in relation to memory performance in older adults) and lithium (in relation to SCA2).<sup>71,72</sup> Although some improvements



in neurologic symptoms have been observed,<sup>69</sup> larger studies are needed to obtain conclusive results.

Therapeutic modulation of autophagy could involve not only bulk autophagy but also selective autophagy. Two recent preclinical studies showed that chemicals linking autophagy substrates such as mitochondria and mutant huntingtin proteins to the autophagosomal membrane induce selective degradation of these substrates (one such molecule is AUTAC).<sup>73,74</sup> This is analogous to the recently expanded strategy for proteasomal degradation, PROTACS (E3-guided proteolysis-targeting chimeras).<sup>75</sup>

Activating autophagy is a promising strategy for treating neurodegenerative diseases, but autophagy-inducing drugs rely on lysosomal activity. A concern is that lysosomes may be dysfunctional in neurodegenerative diseases such as Alzheimer's disease.<sup>13,76</sup> The same may be true for ALS; for example, rapamycin treatment accelerates motor neuron degeneration in SOD1(G93A) mice.<sup>77</sup> Thus, we need to choose target diseases and stages carefully for autophagy-modulating therapies to be highly effective.

One of the major caveats with autophagy-modulating treatments is that although many promising drugs have been identified, they are not strictly specific to autophagy.<sup>78,79</sup> For example, mTORC1 inhibitors also inhibit multiple metabolic pathways, including protein synthesis. Some drugs may have both autophagy-inducing and autophagy-inhibiting effects. For instance, trehalose is thought to enhance autophagy in an mTORC1-independent manner and has beneficial effects in animal models of neurodegenerative diseases (e.g., ALS models), but it can also block lysosomal function and autophagic flux.<sup>80</sup> Thus, the therapeutic effect of trehalose might partly involve an autophagy-independent function.

#### CANCER

Conversely, inhibition of autophagy is thought to be beneficial in the treatment of cancer (Fig. 2). This theory is based on the rationale that cancer cells have a greater reliance on autophagy than do normal cells. In most cases, hydroxychloroquine and chloroquine are used<sup>41,81</sup> to inhibit general lysosomal functions, including the final degradation step of autophagy. Clinical trials of

these drugs have been conducted for various cancers, including glioblastoma, multiple myeloma, melanoma, and other solid tumors, mostly in combination with other chemotherapeutic agents or radiation therapy. As of June 2020, more than 50 clinical studies using hydroxychloroquine or chloroquine were registered at ClinicalTrials.gov. Although partial responses were reported in some patients, the effects of these agents have been mixed.<sup>41,82</sup>

Contradictory findings have also been reported, which is not entirely surprising, given the multifaceted functions of autophagy in cancer (Table 3).<sup>51</sup> Another caveat is that autophagy inhibition activated metastatically dormant cancer cells and induced recurrence in a mouse model of breast cancer.<sup>83</sup> Therefore, just as proteasome inhibitors have been shown to be particularly effective in multiple myeloma, it would be important to identify cancer types with specific gene mutations for which autophagy inhibition is effective. *BRAF*, *KRAS*, *EGFRvIII*, and *LKB* (but not *p53*) mutations may be indicators of autophagic dependence.<sup>41,81,82</sup> Alternatively, cancers that overproduce abnormal proteins or organelles and that can be eliminated by autophagy might be good targets. In addition, determining acceptable periods of autophagy inhibition would be important because long-term suppression would lead to degeneration of nervous and other tissue.

Again, drug specificity could be an issue. The

lysosomal inhibitors hydroxychloroquine and chloroquine are not specific to autophagy; they inhibit all lysosome-related functions, including endocytosis. Some reports suggest that the anticancer effect of chloroquine may be independent of autophagy.<sup>82,84,85</sup> To more specifically inhibit autophagy, inhibitors targeting upstream autophagy factors such as *ULK1* and class III phosphatidylinositol 3-kinase (*VPS34*) have been developed and used in preclinical studies.<sup>82</sup> Further investigation of these drugs should clarify whether inhibiting autophagy itself, rather than other lysosomal functions, has an anticancer effect.

## CONCLUSIONS

Genetic studies have provided concrete evidence that mutations in autophagy genes cause a variety of diseases in humans, suggesting the importance of autophagy and related cellular functions in pathogenesis. In the future, because autophagy has a waste disposal function, its activation and inhibition could be a novel therapeutic strategy for neurodegenerative diseases and cancers. Progress in assessing the role of autophagy in human diseases and their treatment relies heavily on the development of methods for monitoring autophagic activity in humans.<sup>86</sup>

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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