REVIEW ARTICLE

MECHANISMS OF DISEASE

Autophagy in Human Health and Disease

Augustine M.K. Choi, M.D., Stefan W. Ryter, Ph.D., and Beth Levine, M.D.

THE COMPLEX INTEGRATION OF BIOLOGIC AND PHYSIOLOGICAL PROCESSES such as inflammation, apoptosis, cell proliferation, differentiation, and metabolism can influence the pathogenesis of human diseases. Understanding the cellular and molecular bases of these processes is crucial for identifying new diagnostic and therapeutic targets. During the past decade, interest in defining the basic cellular mechanism of autophagy ("self-eating"; see the Glossary) and its roles in human health and disease has become widespread.[1-3] Macroautophagy (hereafter referred to as autophagy) is a homeostatic process that takes place in all eukaryotic cells and involves the sequestration of cytoplasmic components in double-membraned autophagosomes. These structures subsequently fuse with lysosomes, where their cargoes are delivered for degradation and recycling (Fig. 1). Autophagy is now widely implicated in pathophysiological processes (e.g., cancer, metabolic and neurodegenerative disorders, and cardiovascular and pulmonary diseases) and in physiological responses to exercise and aging.[1-3]

From the Department of Medicine, Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston (A.M.K.C., S.W.R.); and the Howard Hughes Medical Institute and Department of Internal Medicine, Center for Autophagy Research, University of Texas Southwestern Medical Center, Dallas (B.L.). Address reprint requests to Dr. Choi at the Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, 75 Francis St., Boston, MA 02115, or at amchoi@rics.bwh.harvard.edu.

MOLECULAR REGULATION OF AUTOPHAGY

Autophagy responds to environmental cues through regulatory factors that signal to the autophagic machinery (Fig. 2), which consists of homologues of products of the autophagy-related genes (*Atg*) originally identified in yeast (Table 1).[2,3] Starvation, a potent physiological regulator of autophagy,[4] induces this process through inhibition of the mammalian (or mechanistic) target of rapamycin (mTOR), which resides in a macromolecular complex, mTORC1. In response to growth factors that stimulate the class I phosphatidylinositol 3-kinase–AKT pathway, or other nutrient-related signals (e.g., leucine), mTORC1 negatively regulates a complex consisting of UNC-51–like kinase 1 (ULK1), ATG13, ATG101, and FIP200. Conversely, nutrient depletion or energy exhaustion inhibits mTORC1, which permits activation of ULK1 — a crucial initiating step in autophagy.[3,5]

The Beclin 1–interacting complex, a regulatory platform, consists of Beclin 1, BCL-2 family proteins (which inhibit autophagy), the class III phosphatidylinositol 3-kinase (VPS34), and ATG14L (required for autophagy).[6] Stimulation of this complex generates phosphatidylinositol-3-phosphate, which promotes autophagosomal membrane nucleation.[6] Autophagosomal elongation requires two ubiquitin-like conjugation systems: the ATG5–ATG12 conjugation system and the microtubule-associated protein light chain 3 (LC3–ATG8) conjugation system.[3] The conversion of a cytosolic truncated form of LC3 (LC3-I) to its autophagosomal membrane–associated, phosphatidylethanolamine-conjugated form (LC3-II), visible as discrete puncta on immunofluorescence analysis, indicates autophagosome formation.[3] Impaired autophagosome–lysosome fusion may result in increases in the number of autophagosomes, as observed in several diseases.[3]

Glossary

Aggrephagy: A selective pathway in which denatured and polyubiquitinated proteins are assembled into organized structures called aggresomes and then targeted to autophagosomes for degradation by autophagy.

AMPK (adenosine 5′-monophosphate–activated protein kinase): A positive regulator of autophagy that responds to energy depletion (AMP accumulation). AMPK activation negatively regulates mammalian target of rapamycin (mTOR) complex 1.

Antigen presentation: A process by which immune cells (e.g., macrophages and dendritic cells) capture foreign antigens to facilitate their recognition by T cells. Major-histocompatibility-complex (MHC) class I molecules present internal antigens to CD8+ cytotoxic T cells. MHC class II molecules on dendritic cells present peptides originating from external antigens to CD4+ helper T cells. Autophagy may facilitate the latter process as a source of antigenic peptides.

Apoptosis: A type of genetically regulated programmed cell death that involves the activation of proteases (e.g., caspases) and nucleases within an intact plasma membrane. The major morphologic characteristics of apoptosis include chromatin condensation, chromosomal DNA fragmentation, cell-surface blebbing and detachment, cell shrinkage, and cellular decomposition into membrane-bound apoptotic bodies that are substrates for phagocytosis.

Autophagic flux: Autophagic activity as represented by completion of the entire autophagy pathway: from the fusion of cargo-laden autophagosomes to lysosomes to cargo degradation within the lysosome. Also used to distinguish autophagic activity from the initiation of autophagy, in which autophagosome numbers and LC3B-II expression increase but do not necessarily signify completion of the pathway. Autophagic flux is determined by assessing the steady-state levels of autophagic substrates in the absence and presence of inhibitors of autophagosome–lysosome fusion or lysosomal degradation.

Autophagosome: A double-membrane vesicle that sequesters cytosolic cargo for ultimate delivery to the lysosome.

Autophagy: A catabolic pathway involving the degradation of cellular components through the lysosomal machinery, the major subtype of which is macroautophagy (see definition below).

Autophagy proteins: A family of proteins originally identified in yeast as being critical for the regulation of autophagy. Mammalian homologues of many of these autophagy-related (Atg) proteins have now been identified (see Table 1).

Beclin 1–interacting complex: A macromolecular complex that is important in the initiation of autophagy. The Beclin 1–interacting complex consists of Beclin 1, the class III phosphatidylinositol-3-kinase (VPS34), and ATG14L. A number of additional proteins, including BCL-2 family proteins (which inhibit autophagy), may interact with this complex. Activation of this complex generates phosphatidylinositol-3-phosphate, which promotes autophagosomal membrane nucleation.

Chloroquine: An antimalarial drug that inhibits lysosomal acidification. Chloroquine is used in autophagy-related studies as an inhibitor, since it impairs autophagosome–lysosome fusion and lysosomal degradative activity. Chloroquine and its derivative, hydroxychloroquine, are currently being assessed in clinical trials for therapeutic effectiveness in combination with cancer chemotherapy.

Efferocytosis: A process by which apoptotic or necrotic cells are removed by phagocytosis. This process may be performed by phagocytes (e.g., macrophages and dendritic cells) as well as other cell types (e.g., epithelial cells and fibroblasts).

Histone deacetylases: A class of enzymes that catalyze the removal of an acetyl functional group from histones and other protein substrates.

LC3B (microtubule-associated protein light chain 3): A major regulator of autophagosome formation, which remains associated with the mature autophagosomal membrane. Conversion of a cytosolic truncated form of LC3 (LC3-I) to its autophagosomal membrane–associated, phosphatidylethanolamine-conjugated form (LC3-II) is an indicator of autophagosome formation. LC3B is the mammalian homologue of yeast Atg8.

LC3B puncta: Discrete punctate structures (puncta), visible on immunofluorescence staining, that are indicative of autophagosome formation.

Lipophagy: The selective targeting of lipids to autophagosomes for degradation through the autophagy pathway.

Macroautophagy: A cellular process that involves the sequestration of cytoplasmic components into double-membrane autophagosomes that fuse with lysosomes, where their cargoes are delivered for degradation and recycling. Macroautophagy is distinguished from several autophagy subtypes, including microautophagy and chaperone-mediated autophagy. Microautophagy involves the direct assimilation of cargo into lysosomes by invagination of the membrane without the intermediacy of autophagosomes. In chaperone-mediated autophagy, molecular chaperones (i.e., heat-shock cognate proteins) facilitate the transfer of proteins to the lysosomes.

Major-histocompatibility-complex (MHC) class II loading compartments: Lysosome-like, multivesicular, antigen-processing compartments in which MHC class II molecules bind with antigenic peptides. These structures may receive cargo from autophagosomes.

Mammalian (or mechanistic) target of rapamycin (mTOR): A Ser/Thr signaling kinase that exerts pleiotropic cellular effects, including the regulation of cell growth and proliferation and of autophagy.

Mitophagy: The selective targeting of damaged or dysfunctional mitochondria to autophagosomes for degradation through the autophagy pathway. Critical regulators of mitophagy include phosphatase and tensin homologue (PTEN)–induced putative kinase 1 (PINK1) and Parkinson protein 2 (parkin).

mTOR complex 1 (mTORC1): A macromolecular complex composed of mTOR, the regulatory associated protein of mTOR (Raptor), G protein beta subunit–like protein (GβL), and proline-rich AKT/PKB substrate 40 kDA (PRAS40). This multiprotein complex is activated by nutrient-associated signals, including amino acids and growth factors, and negatively regulates autophagy.

Sirolimus (formerly called rapamycin): A macrolide compound that has been widely used as an immunosuppressive agent and anticancer drug. Sirolimus selectively binds to and inhibits mTOR and thereby acts as a potent inducer of autophagy.

ULK1: The mammalian uncoordinated-51-like protein kinase, which acts as an important regulator of autophagy. ULK1 resides in a macromolecular complex consisting of ATG13, ATG101, and FIP200. The ULK1 complex is negatively regulated by mTORC1. Changes in ULK1 and ATG13 phosphorylation are critical signals for the initiation of autophagy.

Xenophagy: A selective form of autophagy in which intracellular pathogens, including bacteria and viruses, are degraded through the macroautophagic pathway.
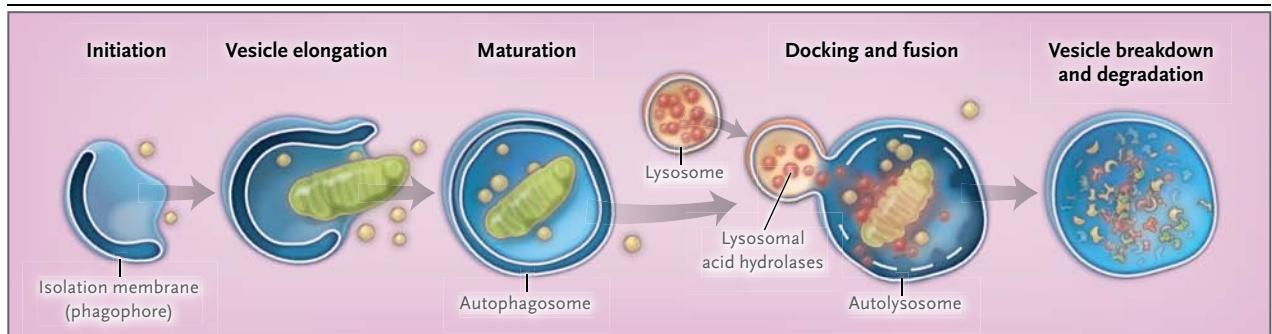
Figure 1. Phases of the Autophagic Pathway.

The autophagic pathway proceeds through several phases, including initiation (formation of a preautophagosomal structure leading to an isolation membrane, or phagophore), vesicle elongation, autophagosome maturation and cargo sequestration, and autophagosome–lysosome fusion. In the final stage, autophagosomal contents are degraded by lysosomal acid hydrolases and the contents of the autolysosome are released for metabolic recycling.

FUNCTIONS OF AUTOPHAGY

Autophagy acts as a survival mechanism under conditions of stress, maintaining cellular integrity by regenerating metabolic precursors and clearing subcellular debris.[1-3] This process contributes to basal cellular and tissue homeostasis, as well as developmental regulation in higher organisms, and can affect pathogenesis (Fig. 3 and Table 2). Recent investigations have identified selectivity in the recognition of autophagy substrates (previously considered to occur through nonspecific sequestration of cytosol), which is orchestrated by cargo-specific factors.[27] Autophagy participates in the turnover of mitochondria (through the selective process of mitophagy) and other organelles (e.g., endoplasmic reticulum and peroxisomes).[27,28] Furthermore, autophagy is involved in the clearance of polyubiquitinated protein aggregates (i.e., aggrephagy), which accumulate during stress, aging, and disease owing to perturbations in protein structure or folding.[29] Autophagy has also been implicated as a regulator of lipid metabolism (i.e., lipophagy).[30]

Autophagy primarily acts as a protective mechanism that may prevent cell death. Interaction between regulatory elements of both autophagy and apoptosis (e.g., the inhibitory interaction between BCL-2 and Beclin 1 and the interaction between LC3B and Fas)[31,32] suggests, however, complex cross-talk between these two processes. Although neither excessive autophagy nor impaired autophagy has been proved to be a direct cause of cell death, both may be associated with apoptosis in some model systems.[3]

During infection, autophagy assists in the immune response by degrading intracellular bacteria and viruses (i.e., xenophagy).[7,14,20] Autophagy contributes to the suppression of inflammation, including the down-regulation of both interferon responses to viral infection and proinflammatory cytokine responses to invading pathogens and the inhibition of inflammasome-dependent maturation and secretion of proinflammatory cytokines (e.g., interleukin-1β and interleukin-18), through the preservation of mitochondrial function.[33-35] Autophagic proteins may also regulate an unconventional pathway for secretion of cytokines (e.g., interleukin-1β).[36]

Autophagy can also play crucial roles in adaptive immune responses such as antigen presentation and lymphocyte development.[14,20] Autophagosomes can fuse with major-histocompatibility-complex (MHC) class II loading compartments.[37] In addition, autophagy can facilitate the generation of a self-tolerant T-cell repertoire. High constitutive expression of autophagy in thymic epithelial cells delivers endogenous proteins to MHC class II molecules and contributes to CD4+ T-cell selection.[38] These examples suggest that autophagy can affect the regulation of inflammation and immune-system function.

AUTOPHAGY IN DISEASE

CANCER

Autophagy may exert a multifactorial influence on the initiation and progression of cancer, as well as on the effectiveness of therapeutic inter-
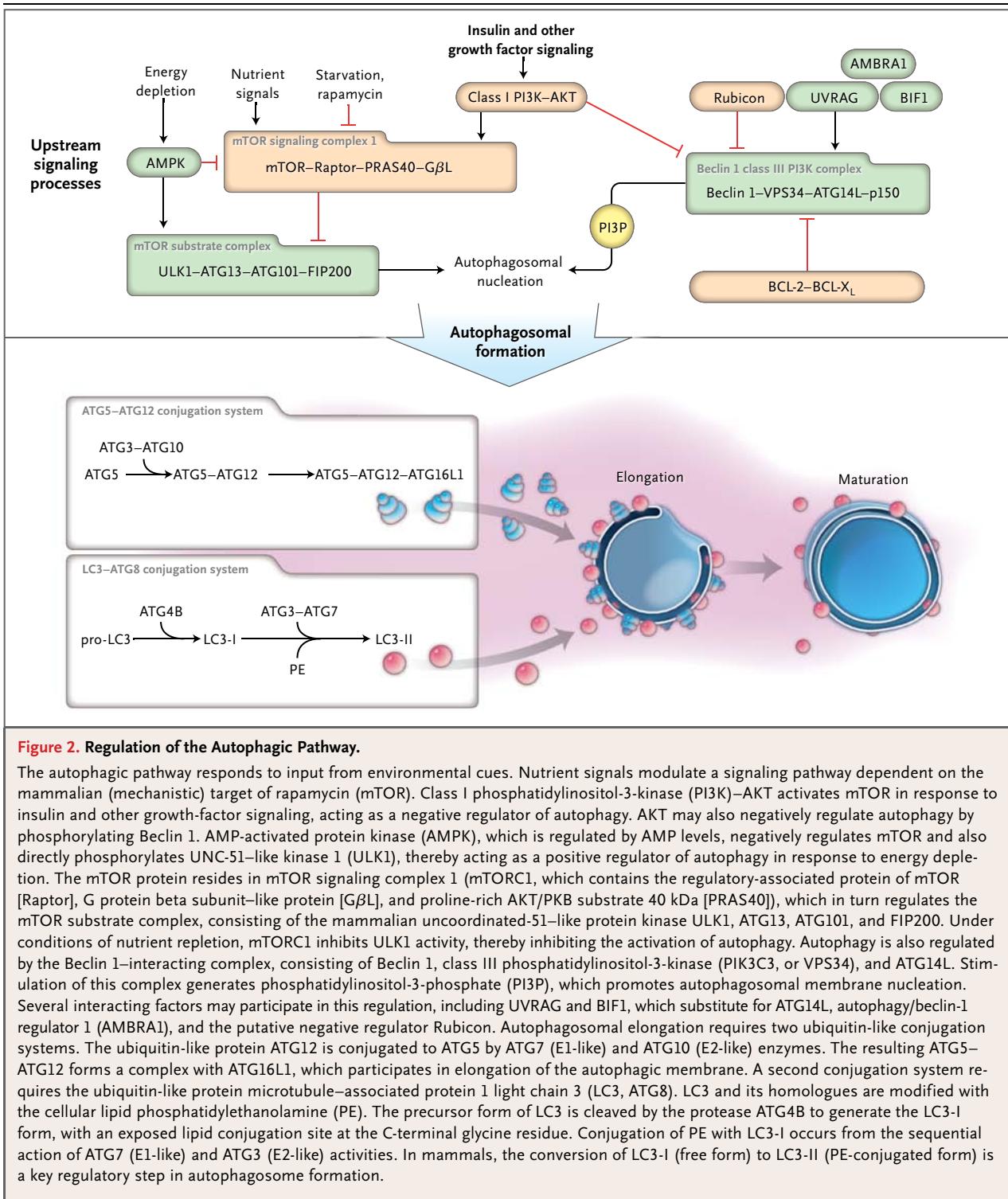
Figure 2. Regulation of the Autophagic Pathway.

The autophagic pathway responds to input from environmental cues. Nutrient signals modulate a signaling pathway dependent on the mammalian (mechanistic) target of rapamycin (mTOR). Class I phosphatidylinositol-3-kinase (PI3K)–AKT activates mTOR in response to insulin and other growth-factor signaling, acting as a negative regulator of autophagy. AKT may also negatively regulate autophagy by phosphorylating Beclin 1. AMP-activated protein kinase (AMPK), which is regulated by AMP levels, negatively regulates mTOR and also directly phosphorylates UNC-51–like kinase 1 (ULK1), thereby acting as a positive regulator of autophagy in response to energy depletion. The mTOR protein resides in mTOR signaling complex 1 (mTORC1, which contains the regulatory-associated protein of mTOR [Raptor], G protein beta subunit–like protein [GβL], and proline-rich AKT/PKB substrate 40 kDa [PRAS40]), which in turn regulates the mTOR substrate complex, consisting of the mammalian uncoordinated-51–like protein kinase ULK1, ATG13, ATG101, and FIP200. Under conditions of nutrient repletion, mTORC1 inhibits ULK1 activity, thereby inhibiting the activation of autophagy. Autophagy is also regulated by the Beclin 1–interacting complex, consisting of Beclin 1, class III phosphatidylinositol-3-kinase (PIK3C3, or VPS34), and ATG14L. Stimulation of this complex generates phosphatidylinositol-3-phosphate (PI3P), which promotes autophagosomal membrane nucleation. Several interacting factors may participate in this regulation, including UVRAG and BIF1, which substitute for ATG14L, autophagy/beclin-1 regulator 1 (AMBRA1), and the putative negative regulator Rubicon. Autophagosomal elongation requires two ubiquitin-like conjugation systems. The ubiquitin-like protein ATG12 is conjugated to ATG5 by ATG7 (E1-like) and ATG10 (E2-like) enzymes. The resulting ATG5–ATG12 forms a complex with ATG16L1, which participates in elongation of the autophagic membrane. A second conjugation system requires the ubiquitin-like protein microtubule–associated protein 1 light chain 3 (LC3, ATG8). LC3 and its homologues are modified with the cellular lipid phosphatidylethanolamine (PE). The precursor form of LC3 is cleaved by the protease ATG4B to generate the LC3-I form, with an exposed lipid conjugation site at the C-terminal glycine residue. Conjugation of PE with LC3-I occurs from the sequential action of ATG7 (E1-like) and ATG3 (E2-like) activities. In mammals, the conversion of LC3-I (free form) to LC3-II (PE-conjugated form) is a key regulatory step in autophagosome formation.

ventions in this disease. Monoallelic disruption of *BECN1* on chromosome 17q21 occurs in 40 to 75% of human breast, ovarian, and prostate tumors.[3,7-10] Clinical studies have associated poor prognosis and aggressive tumor phenotypes with

aberrant expression of Beclin 1 in tumor tissue.[7,11,12]

In mice, homozygous deletion of *Becn1* results in embryonic lethality, whereas monoallelic loss of *Becn1* (*Becn1*^{+/−}) results in spontaneous tumori-

Table 1. Major Mammalian Autophagic Proteins.

| Autophagic Protein	Gene	Yeast Homologue	Function in Autophagic Regulation
Unc-51–like kinase 1	ULK1	Atg1	Interfaces with mTORC1; major initiator of the regulation of autophagy
ATG3	ATG3	Atg3	Ubiquitin (E2)-like enzyme; acts as ligase for ATG8 and ATG12, catalyzes the conjugation of ATG8-like proteins to phosphatidylethanolamine (PE)
ATG4B	ATG4B	Atg4b	ATG8 cysteine peptidase; converts pro-LC3 (ATG8) to LC3-I, delipidates autophagosomal LC3-II
ATG5	ATG5	Atg5	Forms a complex with ATG12; assists in autophagosomal elongation
Beclin 1	BECN1	Atg6/Vps30	BCL-2–binding protein; forms a regulatory complex with class III phosphatidylinositol-3-kinase (VPS34)
ATG7	ATG7	Atg7	E1 ubiquitin conjugase–like enzyme; facilitates conjugation of ATG8 proteins to PE, acts as an E1 enzyme for ATG12 conjugation to ATG5 and ATG3
Microtubule-associated protein 1 light chain 3B	MAP1LC3B	Atg8	Ubiquitin-like modifier; stably associates with autophagosomal membrane
ATG9A	ATG9A	Atg9	Associates with preautophagosomal structure in yeast, assists in autophagosomal assembly
ATG10	ATG10	Atg10	E2 ubiquitin ligase-like enzyme; catalyzes the conjugation of ATG5 and ATG12
ATG12	ATG12	Atg12	Forms a complex with ATG5; assists in autophagosomal elongation
ATG14L	ATG14	Atg14	Autophagy-specific subunit of Beclin 1–class III phosphatidylinositol complex
ATG16L1	ATG16	Atg16	Associates with isolation membrane in complex with ATG5–ATG12; assists in autophagosomal elongation

genesis, identifying Beclin 1 as a haploinsufficient tumor-suppressor protein.[39,40] Studies in mouse models have assigned tumor-suppressor function to additional autophagy-associated proteins (i.e., Uvrag and Bif1)[13,41] and core autophagy proteins (i.e., Atg4C, Atg5, and Atg7).[42,43] Antioncogenic signaling pathways triggered by known tumor-suppressor proteins (i.e., PTEN, TSC1/2, LKB1, and p53) may stimulate autophagy.[7] Furthermore, Beclin 1–dependent autophagic functions may be suppressed in human cancer through activation of AKT.[44]

Altered expression of other autophagy proteins has been observed in human cancer specimens (e.g., ATG5 expression was up-regulated in prostate carcinomas).[45] Furthermore, chromosomal aberrations of several autophagy genes occur frequently in human cancers.[1] LC3 puncta formation is elevated in human tumors and is responsive to systemic chemotherapy.[46]

According to an emerging hypothesis, autophagy provides an anticarcinogenic function in primary cells by safeguarding against metabolic stress through the homeostatic turnover of mitochondria and the clearance of protein aggregates. Genetic deletion of autophagy proteins causes mitochondrial dysfunction, increased oxidative stress, and susceptibility to proinflammatory stimuli — conditions that permit DNA damage and thus lead to genetic instability.[47]

In established tumors, however, autophagy may confer a survival advantage on tumor cells that are under metabolic stress, as a result of a high proliferation rate and exposure to hypoxia from insufficient vascularization, and that are under selective pressure from therapeutic interventions. Chemical or genetic inhibition of autophagy inhibits the growth of pancreatic cancer cells.[48] Autophagy may contribute to acquired resistance of tumor cells to chemotherapeutic agents, since the genetic knockdown of autophagy proteins (i.e., ATG5 and ATG7) increases therapeutic cell killing.[47] Although a prosurvival role for tumor autophagy during chemotherapy is generally accepted, contrasting findings suggest that autophagy may facilitate chemotherapeutic or radiation-induced cytotoxicity in apoptosis-resistant tumor cells through autophagy-associated cell-death pathways.[47] More studies are needed to unravel the complex relationships between autophagy proteins and acquired tumor resistance to treatment.
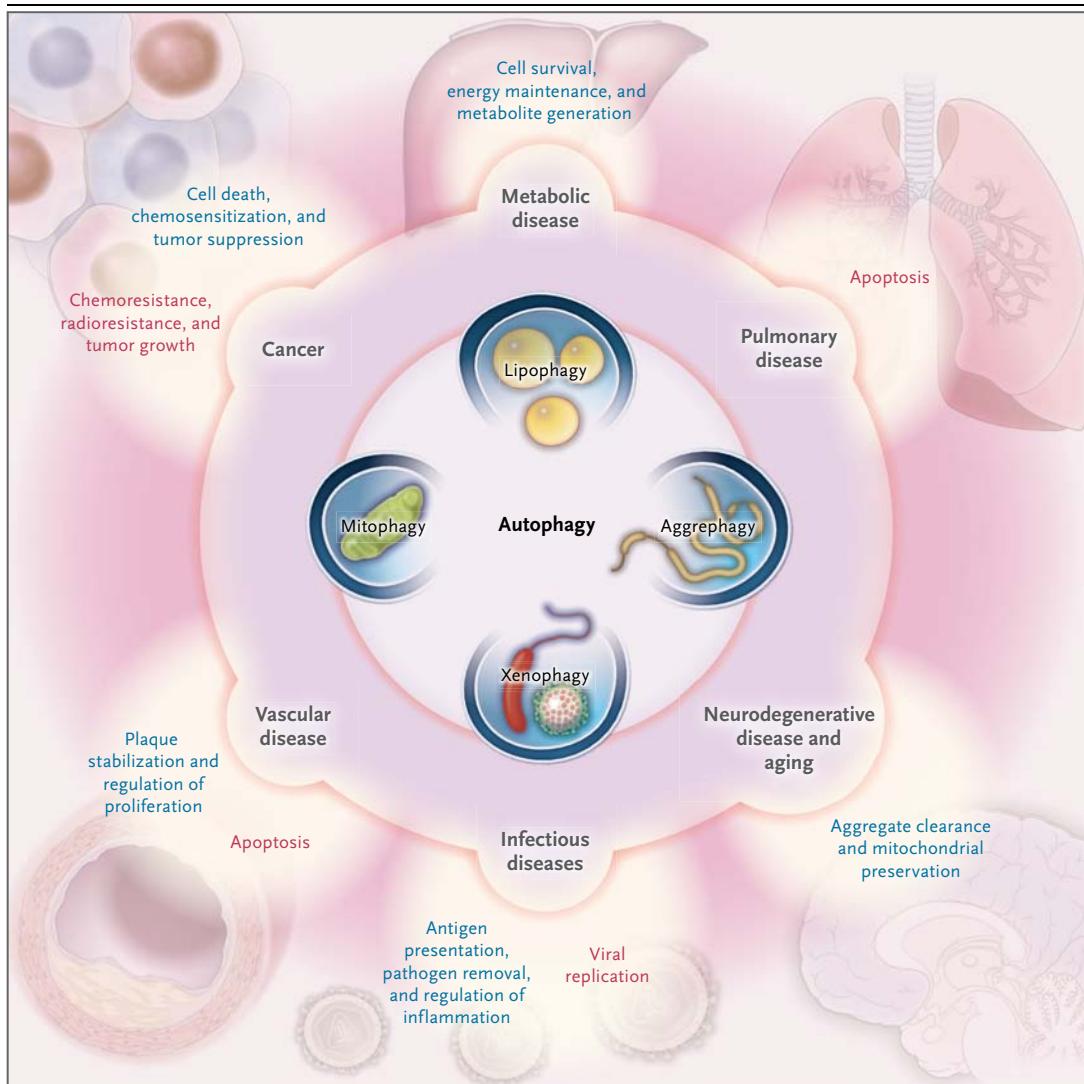
Figure 3. Effects of Autophagy on Disease Progression.

Autophagy influences a number of processes that have various effects on disease progression. Processes that are beneficial (i.e., that inhibit disease progression) are shown in blue, and those that are putatively detrimental (i.e., that promote disease progression) are shown in red. In many diseases, autophagy plays a common role that involves clearance of dysfunctional mitochondria and protein aggregates. The disease mechanisms discussed in this review may be related to the failure of autophagy to perform these functions. Additional mechanisms may include a role for autophagy in the regulation of cell death and proliferation, as shown in models of cardiac and lung diseases. In cancer, autophagy may prevent tumorigenesis but may also promote tumor-cell survival and tumor growth, thus variably affecting the efficacy of anticancer therapies. In infectious disease, autophagy plays a direct role in clearing intracellular pathogens (i.e., xenophagy) and is also involved in regulating inflammatory and immune responses. Regulation of lipid metabolism (lipophagy) is a newly identified function of autophagy that may be important in liver and metabolic diseases.

NEURODEGENERATIVE DISEASES

The neurodegenerative diseases are age-dependent hereditary or sporadic disorders that are manifested by progressive loss of neural function. Common features in their pathogenesis are mitochondrial dysfunction and the accumulation of protein aggregates as a result of mutation and impaired clearance mechanisms.[49,50] Autophagy is dysregulated in neurodegenerative disorders.[51] For example, in the brains of persons with Alz-

heimer's disease, the accumulation of autophagosomes is accelerated.[52,53] By providing a selective pathway for the clearance of aggregate-prone proteins, autophagy may represent an adaptive process in neurodegenerative diseases. In mouse models, genetic deficiencies of autophagy proteins promote age-dependent neurodegeneration associated with the accumulation of protein aggregates.[54-56] Pharmacologic stimulation of autophagy can alleviate symptoms related to neurodegeneration in mouse models.[57,58]

Huntington's disease and associated polyglutamine disorders involve the accumulation of mutant proteins with polyglutamine-rich extensions.[59] Mutant huntingtin (mhtt), which forms perinuclear cytoplasmic aggregates and intranuclear inclusions in the neurons of patients with Huntington's disease, can be degraded through the autophagic pathway.[60] A recent study suggests that mhtt interferes directly with autophagosomal cargo recognition, thus rendering the autophagic pathway inefficient.[61] Another proposed mechanism for impaired autophagy in Huntington's disease is the sequestration of essential autophagy proteins in mhtt aggregates.[62]

Alzheimer's disease involves the aberrant accumulation of hyperphosphorylated forms of microtubule-associated protein tau, leading to the formation of neurofibrillary tangles and the accumulation of beta amyloid peptide (Aβ) in neural plaques.[49] Aβ may impair lysosomal function and autophagic clearance of this protein. Furthermore, the autophagosome, which contains γ-secretase and related enzymes involved in the generation of Aβ from precursor forms, may provide a source of Aβ under conditions of impaired autophagosome–lysosome fusion.[63]

The involvement of mitochondrial dysfunction in neurodegeneration is exemplified by Parkinson's disease.[64] The mobilization of dysfunctional mitochondria to the autophagosome for turnover (i.e., mitophagy) is regulated by distinct proteins, including phosphatase and tensin homologue (PTEN)–induced putative kinase 1 (PINK1) and Parkinson protein 2 (parkin).[65] Mutations in PINK1 and PARK2 (the genes that encode PINK1 and parkin, respectively) result in recessive familial forms of human Parkinson's disease and correlate with mitochondrial dysfunction in mouse models.[24,66] However, parkin deficiency does not abolish mitophagy or uniquely account for neurodegeneration in mouse mod-

els.[67] Sporadic Parkinson's disease involves the accumulation of α-synuclein aggregates in neural cytoplasmic inclusions (Lewy bodies), which in turn promotes mitochondrial dysfunction.[68] Although α-synuclein is an autophagic substrate, the accumulation of this protein impairs autophagy, thereby interfering with its own clearance.[69] Thus, autophagy appears to represent an initial adaptive response in neurodegeneration, subject to inhibition by pathologic accumulation of substrates, which may represent a failed repair mechanism that also contributes to disease progression.[51]

INFECTIOUS DISEASES

Autophagy contributes to the regulation and function of innate and adaptive immune responses.[7,14,20] Several medically important human pathogens are degraded in vitro by xenophagy, including bacteria (e.g., group A streptococcus, *Mycobacterium tuberculosis*, *Shigella flexneri*, *Salmonella enterica*, *Listeria monocytogenes*, and *Francisella tularensis*), viruses such as herpes simplex virus type 1 (HSV-1) and chikungunya virus, and parasites such as *Toxoplasma gondii*. Autophagy genes have been shown to play a protective role in vivo against many of these pathogens.[7,14,20] Recent

Table 2. Association of Autophagy-Related Factors and Human Disease.*

Gene	Association
BECN1	Monoallelically deleted at high frequency in human breast, ovarian, and prostate cancers[5,7-10]
	Altered expression found in many human tumors[11,12]
UVRAG	Deleted at high frequency in human colon cancers[13]
ATG5	SNPs associated with risk of systemic lupus erythematosus[14]
	SNPs associated with risk of childhood and adult asthma and decline in lung function[15,16]
ATG16L1	SNPs associated with increased risk of Crohn's disease[17-19]
NOD2	SNPs associated with increased risk of Crohn's disease and susceptibility to *Mycobacterium leprae* infection[14,19,20]
IRGM	SNPs associated with increased risk of Crohn's disease[19,21]; one SNP associated with increased resistance to *M. tuberculosis* infection[22]
LAMP2	X-linked deletion associated with Danon's cardiomyopathy[23]
PARK2	Mutations associated with Parkinson's disease and colon, lung, and brain cancers[7,24]
p62/SQSTM1	Mutations associated with Paget's disease[25]
SMURF1	SNP associated with increased risk of ulcerative colitis[26]

* SNP denotes single-nucleotide polymorphism.

studies have elucidated the mechanisms by which intracellular bacteria and viruses are targeted to autophagosomes for degradation,[27,70,71] as well as the mechanisms by which successful intracellular pathogens evade or co-opt the autophagy pathway.[14,20] Macrophage-specific deletion of *Atg5* in mice increases their susceptibility to *M. tuberculosis* infection. DNA sensing pathways in the host may be used to target the bacteria for ubiquitination and subsequent removal by means of autophagy.[71]

Thus, pharmacologic up-regulation of autophagy, enhancement of strategies to target intracellular pathogens to autophagosomes, and inhibition of microbial virulence factors that block host autophagy defenses may represent novel strategies for the treatment of certain infectious diseases.[7] Notably, vitamin D and sirolimus (formerly called rapamycin) inhibit replication of the human immunodeficiency virus and of *M. tuberculosis* in human macrophages through an autophagy-dependent mechanism. Furthermore, the antimycobacterial action of standard antituberculosis agents is associated with induction of autophagy.[72-74]

The importance of autophagy as a regulator of adaptive immunity is illustrated by experiments showing that in Epstein–Barr virus infection and herpes simplex virus infection, MHC class II antigen presentation to CD4+ T cells is inhibited by knockdown of ATG12 and ATG5, respectively.[75,76] The targeting of an influenza virus matrix protein (MP1) to autophagosomes enhanced anti-MP1 CD4 T-cell responses, and in mice immunized with sirolimus-treated dendritic cells infected with mycobacterial vaccine strains, CD4+ T-cell–mediated protection was increased on challenge with virulent *M. tuberculosis*.[77,78] Thus, the augmentation of autophagy-dependent adaptive immune responses may be beneficial in vaccine development.

An increasing number of genetic links have been identified between autophagy genes and susceptibility to infectious and inflammatory diseases. Human genomewide association studies have revealed associations between single-nucleotide polymorphisms (SNPs) in genes that regulate autophagy (e.g., *ATG16L1*, nucleotide-binding oligomerization domain–containing protein 2 [*NOD2*], and immunity-related p47 guanosine triphosphatase M protein [*IRGM*]) and an increased risk of Crohn's disease,[17-19,21] as well as between a SNP in *SMURF1* (which encodes a se-

lective autophagy factor) and ulcerative colitis.[26] SNPs in *IRGM* are associated with resistance to *M. tuberculosis* infection in humans.[22] Furthermore, genomewide association studies have linked several SNPs in *ATG5* to susceptibility to the autoimmune disease systemic lupus erythematosus.[14] Collectively, the data from mouse models and human genomewide association studies suggest that further investigation of the role of autophagy deregulation in human inflammatory and infectious diseases is warranted.

CARDIOVASCULAR DISEASES

Modulations in autophagy have been associated with diseases of the heart, including cardiomyopathies, cardiac hypertrophy, ischemic heart disease, heart failure, and ischemia–reperfusion injury.[79] Genetic X-linked deficiency in lysosome-associated membrane protein 2 (LAMP2), which assists in autophagosome–lysosome fusion, causes cardiomyopathy known as Danon's disease.[23] In patients with this disease, cardiomyocytes with evidence of mitochondrial dysfunction have an increased number of autophagosomes,[23] as does cardiac tissue from patients with heart failure.[80,81] In a mouse model of desmin-related cardiomyopathy, autophagic activity was shown to provide cardioprotection.[82]

Experimental ischemia–reperfusion injury also causes morphologic indicators of autophagy to increase in response to stress signals, including depleted ATP, hypoxia, and altered Ca^{2+} balance and may play various roles, depending on the phase of the injury.[83] Increased numbers of autophagosomes are evident in macrophages from atherosclerotic plaques.[84] Autophagy may stabilize atherosclerotic plaques by preventing macrophage apoptosis and plaque necrosis and by preserving efferocytosis.[85]

METABOLIC DISEASES

Autophagy regenerates and releases amino acids, lipids, and other metabolic precursors, which may have a profound effect on tissue metabolism.[7] Genetic deletion of autophagy proteins promotes the storage of triglycerides in lipid droplets in the liver, suggesting that autophagy acts as a regulator of lipid metabolism and storage.[30] Mutations of *p62/SQSTM1* have been linked to Paget's disease, a disorder of bone metabolism.[25]

During exercise, autophagy is increased in cardiac and skeletal muscle, adipose tissue, and pancreatic beta cells. In mice, exercise-induced

autophagy provides protection against glucose intolerance associated with a high-fat diet.[86] Hepatic autophagy is down-regulated in the liver in mouse models of obesity and insulin resistance.[87] In contrast, adipose-specific deletion of autophagy proteins leads to altered homeostasis and differentiation of adipose tissue and promotes insulin sensitivity.[88] Genetic deletion of autophagy proteins in the brain can lead to dysregulation of food intake.[89] Further studies are needed to understand how metabolic changes incurred by autophagic stimulation affect the progression of human diseases such as diabetes.

PULMONARY DISEASES

Divergent roles of autophagy have been reported in pulmonary diseases associated with declining lung function. Notably, increased autophagy was associated with a propathogenic and proapoptotic phenotype in chronic obstructive pulmonary disease (COPD), which results from chronic exposure to cigarette smoke. The expression of LC3B-II and autophagosome formation are increased in lung tissue from patients with COPD. In animals subjected to long-term inhalation of cigarette smoke, a genetic deficiency in LC3B was associated with resistance to emphysema.[90] Mechanistic studies have linked increased autophagic activity with enhanced epithelial-cell apoptosis during exposure to cigarette smoke.[90] However, alveolar macrophages isolated from human smokers without COPD showed evidence of impaired autophagic activity and the accumulation of substrates.[91] Genetic deficiency of α1-antitrypsin causes pulmonary emphysema and hepatic dysfunction, which are associated with pathologic accumulation of mutant α1-antitrypsin. Like other disorders of protein aggregation, autophagy may act as a clearance mechanism in this disease.[90,92]

Lung tissue isolated from patients with pulmonary hypertension, including those with idiopathic pulmonary arterial hypertension, have increased LC3B activation and autophagosome formation, as compared with lung tissue from patients without pulmonary vascular disease.[93] A recent preclinical study suggests that impaired clearance of aggregated protein (aggrephagy) is the pathogenic mechanism in cystic fibrosis, a genetic disorder caused by mutation in the cystic fibrosis transmembrane conductance regulator (CFTR).[94] Airway epithelial cells and nasal-biopsy specimens from patients with cystic fibrosis are characterized by dysfunctional aggrephagy, as evidenced by the accumulation of polyubiquitinated proteins and decreased clearance of aggresomes that accumulated mutant CFTR.[94] Impaired autophagy was also noted recently in human idiopathic pulmonary fibrosis.[95] Thus, therapeutic interventions aimed at correcting deficiencies in autophagy may be useful in these diseases.

Relatively little is known about the role of autophagy in asthma, which involves abnormal inflammatory responses of the airways to allergens. Increased autophagosomes have been noted in bronchial-biopsy specimens from patients with asthma,[15] and ATG5 expression is elevated in nasal-biopsy specimens from children with asthma.[16] Intronic SNPs of *ATG5* have been associated with an increased incidence of asthma and a decline in lung function in cohort studies of adults[15] and with deterioration of lung function in children with asthma.[16]

AGING

The homeostatic functions of autophagy with respect to turnover of long-lived proteins and removal of damaged organelles and cellular debris are believed to constitute an antiaging process.[96] Aging is similar to neurodegeneration in that the accumulation of unprocessed material (i.e., lipofuscin pigments and ubiquitinated protein aggregates) accelerates while the function of compensatory mechanisms (i.e., autophagy) declines. Evidence from studies of mice treated with sirolimus[96] and from studies of the roundworm *Caenorhabditis elegans*[97] suggests that modulation of autophagy can influence lifespan. Furthermore, analysis of gene expression in the brain in old persons as compared with young persons revealed down-regulation of autophagy genes (i.e., *ATG5*, *ATG7*, and *BECN1*) with age and, conversely, up-regulation in persons with Alzheimer's disease.[62,98] Caloric restriction may represent a mechanism for reversing the age-dependent decline in autophagy.[99]

AUTOPHAGY IN CLINICAL APPLICATIONS

Current therapeutic targeting of autophagy in human disease is limited by an incomplete understanding of how the process contributes to pathogenesis, the lack of specificity of compounds that can influence autophagy, and the

limited availability of candidate therapeutics with clinical efficacy. Pharmacologic enhancement of autophagy (i.e., with vitamin D or adenosine 5′-monophosphate–activated protein kinase [AMPK] activators) promises to benefit certain diseases (i.e., infectious or neurodegenerative diseases). Sirolimus, a clinically approved immunosuppressive and anticancer drug that inhibits mTOR and thereby exerts pleiotropic effects, including the activation of autophagy, has been used to enhance autophagy in experimental models. Cytosolic or histone deacetylases (i.e., sirtuin-1, HDAC1, HDAC2, and HDAC6) may act as regulators of autophagic initiation and of autophagic flux.[100] Thus, HDAC inhibitors, or inhibitors of lysosomal acidification (e.g., chloroquine and hydroxychloroquine), may represent useful pharmacologic strategies for modulating autophagy. Current clinical trials are examining the usefulness of autophagy as a target in disease. Chloroquine and its derivative, hydroxychloroquine, are being tested for enhancement of chemotherapeutic efficacy (e.g., in the Preventing Invasive Breast Neoplasia with Chloroquine trial [ClinicalTrials.gov number, NCT01023477]

and the Phase 1 Trial of MK-2206 and Hydroxychloroquine in Solid Tumors and Prostate Cancer [NCT01480154]), including therapies for ductal carcinoma (chloroquine) and for pancreatic adenocarcinoma, breast cancer, and non–small-cell lung cancer (hydroxychloroquine). The design of therapeutic agents is complicated by the fact that many autophagy proteins, as well as pharmacologic inhibitors (e.g., chloroquine), may also affect biologic processes independently of autophagy activation.

An improved understanding of the mechanism (or mechanisms) by which autophagy can prevent pathogenesis may lead to the identification of new targets for both diagnostic and therapeutic approaches. Drug screening for agonists or antagonists of autophagic activity, including upstream regulators and downstream targets of autophagy, may yield additional therapeutic targets. If the advances in autophagy continue at an accelerated pace, agents acting on autophagy may eventually provide useful therapies for human diseases.

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

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