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Development of autophagy inducers in clinical medicine

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Defects in autophagy have been linked to a wide range of medical illnesses, including cancer as well as infectious, neurodegenerative, inflammatory, and metabolic diseases. These observations have led to the hypothesis that autophagy inducers may prevent or treat certain clinical conditions. Lifestyle and nutritional factors, such as exercise and caloric restriction, may exert their known health benefits through the autophagy pathway. Several currently available FDA-approved drugs have been shown to enhance autophagy, and this autophagy-enhancing action may be repurposed for use in novel clinical indications. The development of new drugs that are designed to be more selective inducers of autophagy function in target organs is expected to maximize clinical benefits while minimizing toxicity. This Review summarizes the rationale and current approaches for developing autophagy inducers in medicine, the factors to be considered in defining disease targets for such therapy, and the potential benefits of such treatment for human health.

The past two decades have witnessed an explosion of research on the molecular mechanisms of autophagy and its roles in physiology and disease. Numerous gene products essential for the induction of autophagy, the formation of autophagosomes, the lysosomal clearance of autophagosomes, and the targeting of specific cargo to the autophagosomes have been identified (1–3). The biochemical and structural mechanisms by which these gene products act in an orchestrated manner to execute autophagy are being defined (4). Through loss-of-function studies in mice and other model organisms, we have learned that autophagy plays crucial roles in differentiation and development (5, 6), cellular and tissue homeostasis (7), protein and organelle quality control (8), metabolism (9), immunity (10), and protection against aging (11) and diverse diseases (refs. 12, 13, and Table 1; supplemental references available online with this article; doi:10.1172/JCI73938DS1). Moreover, an increasing number of human diseases are being linked to polymorphisms or mutations in autophagy genes (Table 2) or deficiencies in autophagy function (14, 15). Based on these advances, considerable interest has emerged in developing new (or exploiting old) strategies to induce autophagy — through either pharmacologic or non-pharmacologic approaches. In this Review we provide an overview of the rationale, potential disease targets, and current efforts and future challenges for the development of autophagy inducers in clinical medicine. Other Reviews in this issue discuss the potential use of autophagy inhibitors in clinical medicine (16–18).

Rationale for the development of autophagy inducers

The macroautophagy form of autophagy (herein referred to as autophagy) is an evolutionarily conserved lysosomal degradation pathway that controls cellular bioenergetics (by recycling cytoplasmic constituents) and cytoplasmic quality (by eliminating protein aggregates, damaged organelles, lipid droplets, and intracellular pathogens) (8). In addition, independently of lysosomal degradation, the autophagic machinery can be deployed in the process of phagocytosis, apoptotic corpse clearance, entosis, secretion, exocytosis, antigen presentation, and regulation of inflammatory signaling (7). As a result of the broad range of cellular functions, the autophagy pathway plays a key role in protection against aging and certain cancers, infections, neurodegenerative disorders, metabolic diseases, inflammatory diseases, and muscle diseases (refs. 12, 13, 19–21, and Figure 1).

The recognition that autophagy may prevent the occurrence, delay the progression, and/or decrease the severity of certain diseases provides the primary rationale for the development of pharmacologic agents that induce or enhance autophagy. Several lines of evidence support this approach. First, genetic mutations in autophagy genes in mice (either systemic homozygous or heterozygous deletion, tissue-specific deletion, or knock-in mutations of mutant alleles that are found in human diseases) results in a wide spectrum of disorders (see Table 1) including increased susceptibility to neurodegeneration, cancer, atherosclerosis, diabetes, bone disease, intracellular bacterial infections (e.g., *Mycobacterium tuberculosis*, *Salmonella*), and Paneth cell abnormalities associated with Crohn's disease. Second, mutations or polymorphisms in autophagy genes are associated with susceptibility to human diseases (see Table 2), including Parkinson's disease, inflammatory bowel disease, breast and other malignancies, mycobacterial infections, asthma, chronic obstructive pulmonary disease (COPD), systemic lupus erythematosus, and hereditary neurologic disorders. Third, autophagy gene therapy (via lentiviral or adenovirus-associated viral delivery) in specific target organs results in clinical improvement in rodent models of obesity, α1-antitrypsin deficiency, Parkinson's disease, Alzheimer's disease, Pompe disease, muscular dystrophy, cystic fibrosis, and KRAS-driven lung carcinomas, and systemic transgenic expression

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Table 1. Diseases in mice with mutations in autophagy genes

Gene	Mutation	Disease	Reference
Regulation of phagocytosis and autophagy			
Irgm1	Homozygous deletion	Paneth cell abnormalities and increased susceptibility to DSS-induced intestinal inflammation	S1
Autophagosome formation			
Ambra1	Heterozygous deletion	Increased neuropathic pain mediated by Schwann cell demyelination following peripheral nerve axonal degeneration and autism-like phenotype in females	S2, S3
Atg4b	Homozygous deletion	Inner ear pathology and balance disorders and decreased RGC survival after optic nerve axotomy	S4, S5
Atg4c	Homozygous deletion	Increased susceptibility to chemical carcinogen–induced fibrosarcomas	S6
Atg5	Macrophage-specific deletion	Increased inflammasome activation and atherogenesis and increased severity of pulmonary *M. tuberculosis* infection	S7–S10
	Hepatocyte-specific or mosaic system deletion	Increased liver inflammation, fibrosis, adenomas, and impaired liver regeneration after partial hepatectomy	S11–S13
	Intestinal epithelial cell–specific deletion	Paneth cell abnormalities and increased susceptibility to invasive *Salmonella* infection	S14, S15
	Neuron-specific deletion	Neurodegeneration and increased susceptibility to alphavirus encephalitis	S16, S17
	Dendritic cell–specific deletion	Impaired antigen cross-presentation and increased severity of HSV infection	S18
	Podocyte-specific deletion	Podocyte aging and increased susceptibility to glomerular diseases	S19
	Renal tubular cell–specific deletion	Impaired renal function	S20
	Lens-specific deletion	Age-related cataracts independent of organelle degradation	S21
	Thymic cell–specific deletion	Colitis and multi-organ inflammation	S22
	Myeloid cell–specific deletion	Increased susceptibility to intravenous *C. albicans* infection	S23
	B lymphocyte–specific deletion	Impaired long-lived humoral immunity	S24
	RGC-specific deletion	Decreased RGC survival after optic nerve axotomy	S5
	Inducible cardiac-specific deletion	Heart failure	S25
Atg7	Hepatocyte-specific deletion	Liver adenomas and impaired blood glucose regulation	S12, S26, S27
	Intestinal epithelial cell–specific deletion	Paneth cell abnormalities	S28
	Neuron-specific deletion	Neurodegeneration	S29
	Purkinje cell–specific deletion	Purkinje cell axonal degeneration	S30
	Macrophage/microglia-specific deletion	Increased susceptibility to cerebral and ocular toxoplasmosis	S31
	Hematopoietic cell–specific deletion	Anemia and lymphopenia and atypical myeloproliferation resembling human myelodysplastic syndrome	S32, S33
	Postnatal forebrain–specific conditional deletion	Age-dependent neurodegeneration	S34
	Pancreatic β-cell–specific deletion	Pancreatic β-cell destruction and diabetes	S35
	Skeletal muscle–specific deletion	Muscle atrophy	S36
	B lymphocyte–specific deletion	Impaired virus-specific B cell memory and increased susceptibility to lethal influenza virus challenge	S37
Atg16l1	Hypomorphic deletion	Paneth cell abnormalities and increased susceptibility to lethal chikungunya virus infection	S38, S39
	Null deletion	Enhanced IL-1β production and susceptibility to DSS-induced colitis	S40
	T300A mutation	Defective bacterial clearance and increased inflammatory cytokine production	S41, S42
	Intestinal epithelial cell–specific deletion	Increased susceptibility to invasive *Salmonella* infection	S43
Becn1	Monoallelic deletion	Increased: incidence of spontaneous malignancies, susceptibility to Alzheimer's disease, severity of Desmin-related cardiomyopathy, hypoxia-induced angiogenesis, renal fibrosis following ureteral obstruction, basal renal collagen accumulation, bleeding time, susceptibility to cecal ligation and puncture-induced polymicrobial sepsis, dendritic cell–regulated Th2 cytokine production and lung pathology during respiratory syncytial virus infection, susceptibility to cerebral and ocular toxoplasmosis; reduced/impaired: platelet aggregation, exercise endurance, exercise-induced insulin sensitivity	S31, S44–S54
Bif1	Homozygous deletion	Increased incidence of spontaneous malignancies	S55
FIP200	Neuron-specific deletion	Cerebellar degeneration	S56
LC3b	Homozygous deletion	Increased renal fibrosis following ureteral obstruction, increased susceptibility to hypoxia-induced pulmonary hypertension	S49, S57
Nrbf2	Homozygous deletion	Focal liver necrosis	S58
Vps15	Skeletal muscle-specific deletion	Autophagic vacuolar myopathy	S59
Vps34	Sensory neuron-specific deletion	Neurodegeneration (through impaired endocytosis)	S60
	T lymphocyte–specific deletion	Defective T cell homeostasis and inflammatory wasting syndrome in aged mice	S61
	Liver-specific deletion	Hepatomegaly and hepatic steatosis	S62
	Cardiac-specific deletion	Cardiomegaly and decreased cardiac contractility	S62
	Podocyte-specific deletion	Proteinuria, glomerular scarring, and premature death (impaired autophagy and endocytosis)	S62
	Lens-specific deletion	Congenital cataracts and micropthalmia	S21
Autophagosome maturation and degradation			
Epg5	Homozygous deletion	Neurodegenerative features similar to amyotrophic lateral sclerosis	S63
Lamp2	Homozygous deletion	Vacuolar cardiomyopathy and skeletal myopathy	S64
Sumf1	Homozygous deletion	Lysosomal storage disorder and neurodegeneration	S65
MPS-IIIA	D31N missense mutation	Lysosomal storage disorder and neurodegeneration	S65
Selective autophagy			
Park2/Parkin	Homozygous deletion	Increased susceptibility to *M. tuberculosis* infection	S66
Sqstm1/p62	P394L mutation (equivalent to human P392L)	Paget's-like disease of bone	S67

DSS, dextran sodium sulphate; RGC, retinal ganglion cell.

Table 2. Mutations and polymorphisms in autophagy genes linked to human diseases

Gene	Mutation or polymorphism	Reference
Regulation of phagocytosis and autophagy		
IRGM	Genetic polymorphisms and deletion mutation associated with risk of Crohn's disease, genetic polymorphism associated with protection against *M. tuberculosis*	S68, S69
NOD2	Genetic polymorphisms associated with risk of Crohn's disease and susceptibility to *M. leprae* infection	S68, S70
Autophagosome formation		
ATG2B, ATG9	Frameshift mutations in gastric and colorectal cancers with microsatellite instability	S71
ATG5	Genetic polymorphisms associated with increased risk of asthma and increased risk of systemic lupus erythematosus	S68, S70
ATG7	Genetic polymorphism (V471A) associated with early onset of Huntington's disease	S72, S73
ATG16L1	Genetic polymorphism (T300A) associated with increased risk of Crohn's disease, impaired intestinal dendritic cell antigen sampling and processing and more aggressive clinical course, increased risk of colorectal cancer, and increased susceptibility to *H. pylori* infection, and increased risk of COPD	S68, S70, S74–S78
BECN1	Monoallelic deletion associated with risk of breast, ovarian, and prostate cancer (and decreased expression associated with poor prognosis of multiple cancers)	S79–S89
EI24/PIG8	Mutations and deletions associated with early-onset breast cancers	S90
TECPR2	Frameshift mutation associated with autosomal-recessive form of hereditary spastic paraparesis	S91
WDR45/WIPI4	Heterozygous mutations associated with SENDA	S92, S93
Autophagosome maturation and degradation		
CHMP2B	Mutations that impair autophagosome maturation are associated with frontotemporal dementia and amyotrophic lateral sclerosis	S94
CHMP4B	Mutation that impairs autophagolysosomal degradation of micronuclei causes autosomal-dominant posterior polar cataract	S95, S96
Dynein	Mutations that impair autophagosome movement are associated with motor neuron disease	S97
EPG5	Autosomal-recessive mutations cause the multisystems disorder Vici syndrome	S98
HspB8	Mutations that impair autophagolysosomal fusion are associated with distal hereditary motor neuropathy type II	S99
LAMP2	X-linked deletion associated with Danon's cardiomyopathy	S100
UVRAG	Deletion mutation associated with colorectal cancer	S101
VCP/p97	Mutations that impair autophagosome maturation cause a multisystem disease consisting of inclusion body myopathy, Paget's disease of the bone, and frontotemporal dementia	S102, S103
ZFYVE26 (spastizin)	Autosomal-recessive mutations cause hereditary spastic paraparesis type 15	S104
Selective autophagy		
PARK2/Parkin	Mutations associated with autosomal-recessive or sporadic early-onset Parkinson's disease and with colon, lung, and brain cancers, genetic polymorphisms associated with risk of *M. leprae*, *S. typhi*, and *S. paratyphi* infection	S68, S70, S105–S107
PARK6/PINK1	Mutations associated with autosomal-recessive or sporadic early-onset Parkinson's disease	S68
SQSTM1/p62	Mutations associated with Paget's disease of the bone, amyotrophic lateral sclerosis, and frontotemporal lobar degeneration	S108, S109
SMURF1	Genetic polymorphism associated with increased risk of ulcerative colitis	S110

SENDA, static encephalopathy of childhood with neurodegeneration in adulthood.

of an autophagy gene in mice extends lifespan and improves metabolism (Table 3). Fourth, several commonly used drugs and nutritional supplements induce autophagy (Table 4). Although it is generally unknown whether these agents exert their clinical benefits through autophagy or other pathways, there is considerable overlap between diseases that occur in the setting of autophagy deficiency and diseases that respond to drugs that can induce autophagy. Moreover, some of these agents fail to exert beneficial effects in model organisms lacking autophagy genes. For example, spermidine and resveratrol extend life span in wild-type but not autophagy gene–deficient nematodes (22). Similarly, tyrosine kinase inhibitors do not improve amyloid clearance in the brains of mice with Alzheimer's-like disease when the essential autophagy gene, beclin 1 (*Becn1*), is depleted by shRNA-mediated knockdown (23).

Factors to consider in defining disease targets for autophagy induction

Considerable enthusiasm has emerged for the development of autophagy-inducing agents for the prevention or treatment of diseases in which the upregulation of autophagy is thought to be clinically beneficial. The spectrum of potential disease targets is broad and has been reviewed extensively elsewhere (9, 11, 24, 25); the major focus has been on neurodegenerative disorders, infectious diseases, aging, and metabolic diseases. A common underlying pathophysiologic event in these diseases is the accumulation of harmful contents inside the cell — damaged organelles, protein aggregates, lipid droplets, or pathogens. In these circumstances, the pharmacologic (or non-pharmacologic) enhancement of autophagy-mediated delivery of deleterious structures for lysosomal destruction may be beneficial (Figure 1).

For such manipulations to have therapeutic value, the machinery involved in autophagosome formation, cargo recognition and targeting, or autophagic delivery to lysosomes should not be rate limiting. If polymorphisms in autophagy genes associated with autophagosomal formation were to cause disease via loss of autophagy (which has not yet been determined for the mutations listed in Table 2), it is possible that such mutations could also block the successful upregulation of autophagy by agents that would otherwise induce autophagy in wild-type cells with an intact autophagy machinery. Similarly, it is not known whether patients with mutations in factors involved in autophagic cargo recognition and targeting (Table 2) (such as PARK2 and PINK1 mutations, which

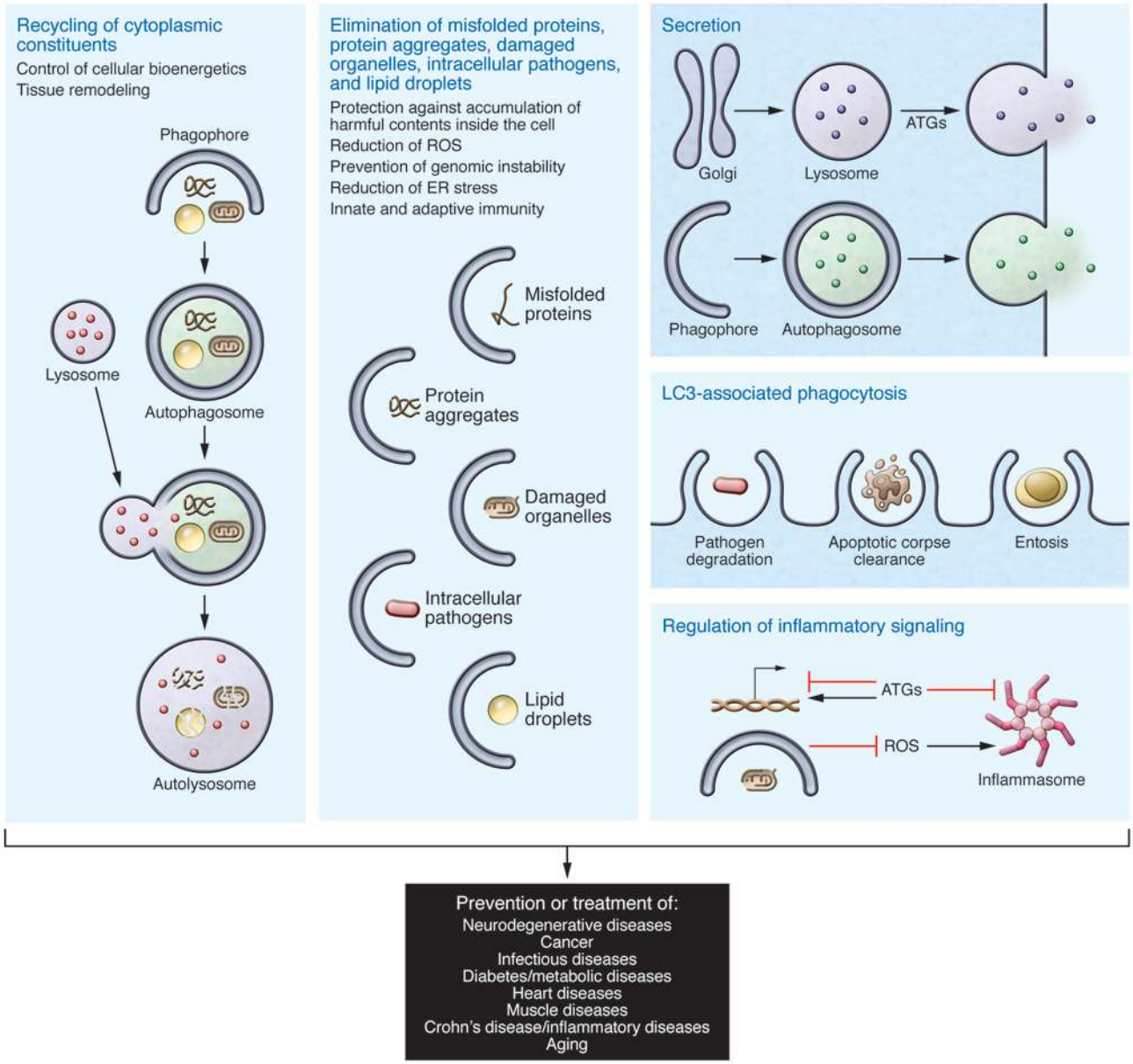

Figure 1. Schematic illustration of potential mechanisms by which autophagy-inducing agents may exert beneficial effects in clinical disease. The precise mechanisms underlying the beneficial effects of autophagy upregulation in preventing or treating different diseases are not fully understood, and multiple different functions of the autophagy pathway or of specific autophagy proteins (acting independently of autophagy) are likely to be contributory. The mechanisms and target diseases shown are representative examples based on animal studies and human genetic data (see Tables 1–3). Other functions of autophagy and autophagy proteins not depicted here may be important, and other diseases not listed here may be targets for autophagy induction.

occur in Parkinson's disease, or SQSTM1/p62 mutation, which occurs in Paget's disease of the bone) will respond to autophagy inducers. Furthermore, several diseases involve an impairment of the delivery of autophagosomes to lysosomes, including human motor neuron disease associated with mutations in the dynein apparatus (26), lysosomal storage diseases (27), and familial Alzheimer's disease caused by presenilin 1 mutations (28). In these cases, increasing autophagosomal membrane formation will not necessarily enhance autophagic substrate degradation and may result in a toxic buildup of cellular membranes, polyubiquitinated aggregates, and dysfunctional mitochondria (29). One way to enhance successful autophagic substrate degradation in the setting of lysosomal dysfunction may be to upregulate transcription factor EB (TFEB), a master regulator of both autophagy gene expression and lysosomal biogenesis (30). As a proof of principle, *Tfeb* gene therapy decreases glycogen storage and excess accumulation of autophagosomes in a murine model of the lysosomal storage disease, Pompe disease (31). However, if the defects in autophagy (at the stage of induction, autophagosome formation, cargo targeting, or autophagolysosomal maturation) are only partial, diseases may still benefit from pharmacologic upregulation of autophagosome formation. Indeed, autophagy-enhancing agents show beneficial effects in induced pluripotent stem cells from patients with the lysosomal storage disorder Niemann-Pick type

Table 3. Rodent studies demonstrating beneficial clinical outcomes with autophagy gene transgenic expression, autophagy gene therapy, or autophagy-inducing peptides

Gene	Outcome	Reference
Transgenic expression		
Atg5 (systemic)	Extended life span, lean phenotype, improved metabolism	S111
Lamp2a (inducible liver)	Prevention of age-related decline in chaperone-mediated autophagy, macroautophagy, and hepatic function	S112
Gene therapy		
Atg7 (liver)	Improvement in hepatic insulin action and systemic glucose tolerance in *ob/ob* mice	S113
Tfeb (liver)	Improvement in liver disease in mice with α1-antitrypsin deficiency	S114
	Improvement in obesity and lipid abnormalities in genetic and dietary obesity models	S115
Tfeb (muscle)	Improvement in the lysosomal storage muscle disease Pompe disease	S116
Tfeb (brain)	Neuroprotective effects in rat model of dopaminergic α-synuclein toxicity	S117
Becn1 (brain)	Neuroprotective effects in rat model of dopaminergic α-synuclein toxicity	S117
	Neuroprotective effects in rat and mouse models of Machado-Joseph (spinocerebellar ataxia type 3) disease	S118
	Neuroprotective effects in α-synuclein mouse models of Parkinson's and Lewy body disease	S119
Becn1 (intranasal)	Decreased lung inflammation in cystic fibrosis (*Cftr*^{F508}del) mouse model	S120
	Reduced tumor progression in lungs of *K-ras*^{LA1} mice	S121
Becn1 (muscle)	Rescue of myofiber degeneration in collagen VI muscular dystrophies	S122
Parkin (brain)	Reduction of Aβ-amyloid levels and brain pathology in Alzheimer's disease transgenic model	S123
Autophagy-inducing peptides		
Tat–beclin 1	Decreased mortality in mice infected with West Nile or chikungunya viruses	S124
Tat-vFLIP	Growth repression of KSHV-associated primary effusion lymphomas	S125

C disease (32) and in NPC1 mutant mouse cells (33). Further studies are warranted to examine the effects of autophagy inducers in other lysosomal storage disorders and diseases associated with impaired cargo delivery to the autophagosome.

An interesting question is whether autophagy induction is warranted in clinical conditions without an apparent defect in the autophagic machinery. Drugs that induce autophagy in autophagy-competent animals have favorable effects in diseases characterized by abnormal accumulation of protein substrates (such as Huntingtin's disease treated with rapamycin [ref. 34] or rilmenidine [ref. 35], and α1-antitryspin deficiency treated with carbamazepine [ref. 36]) or pathogens (such as arboviral infections treated with the autophagy-inducing peptide Tat–beclin 1 [ref. 37] and pulmonary *M. tuberculosis* infection treated with statins [ref. 38]), suggesting that enhancement of autophagy may be beneficial in the absence of an overt autophagy deficiency. Unfortunately, given our current limitations in measuring autophagic flux in patients, we do not know what constitutes a "normal" range of autophagic activity. However, autophagy function declines with aging in humans and other species, and such a decline likely contributes to aging itself as well as age-related increases in susceptibility to neurodegenerative disorders, infectious diseases, and cancer (11). Preliminary studies also indicate that critically ill patients have an autophagy-deficient phenotype, at least in skeletal muscle and liver (39). Thus, many patients, by virtue of advanced age or severe illness, may have a deficiency in autophagy function in the absence of specific mutations in the autophagy pathway.

In defining disease targets appropriate for autophagy induction therapy, it is important to consider whether autophagy acts in a cytoprotective or cytotoxic manner in the specific disease context, and whether such cytoprotective or cytotoxic actions contribute to disease progression. Although autophagy is classically regarded as a means of promoting cell survival during nutrient-limited conditions (5, 40), autophagy can also contribute to cell death (41).

The pro-survival role of autophagy is commonly believed to promote the progression of cancers driven by RAS mutations (42, 43), which has led to intense efforts to inhibit autophagy in this context (discussed in other Reviews in this issue and in refs. 44, 45). However, this postulated pro-survival action is not necessarily sufficient to override the tumor suppressor effects of autophagy in all cancers. For example, enhanced suppression of autophagy in EGFR-driven non–small cell lung adenocarcinoma xenografts increases tumor cell death but also promotes enhanced proliferation, increased tumor growth and tumor dedifferentiation, as well as resistance to EGFR tyrosine kinase inhibitor therapy (46). Even in RAS-driven tumor cells, autophagy inhibition does not have predictable antitumor effects. Specifically, in RAS-driven oncogenesis, autophagy gene knockdown enhances clonogenic survival in human ovarian epithelial cells (47); the presence of a homozygous *p53* mutation transforms the actions of autophagy from a pro-tumorigenic to anti-tumorigenic effect in pancreatic carcinoma (48); autophagy suppresses early oncogenesis in lung adenocarcinoma through effects on regulatory T cells (49); and beclin 1 gene transfer prevents the progression from lung adenomas to adenocarcinomas and enhances tumor cell death (50).

Moreover, autophagy genes are often required for the cytotoxic effects of chemotherapy (51–53), and the combination of antimetabolite and receptor tyrosine kinase inhibitor therapy can increase autophagy-dependent tumor cell death (54). Autophagy also contributes to radiosensitivity in vivo (through immune-dependent responses) even if it contributes to radioresistance in vitro (55). In addition, multiple myeloma cells uti-

Table 4. Select compounds that induce autophagy

Compound	Mechanism of autophagy induction	Reference
FDA approved drugs		
Carbamazepine	Lowers inositol and Ins(1,4,5)P_3 levels	S126, S127
Clonidine	Lowers cAMP levels	S128
Lithium	Lowers inositol and Ins(1,4,5)P_3 levels	S127
Metformin	Upregulates AMPK, which phosphorylates ULK1 and beclin 1	S129–S131
Rapamycin (and rapalogs)	Inhibits mTORC1	S132, S133
Rilmenidine	Lowers cAMP levels	S134
Sodium valproate	Lowers inositol and Ins(1,4,5)P_3 levels	S127, S135
Verapamil	Inhibits L-type Ca^{2+} channel, lowering intracytosolic Ca^{2+}	S128
Trifluoperazine	Unknown	S135
Statins	Depletion of geranylgeranyl diphosphate activates AMPK	S136, S137
Tyrosine kinase inhibitors	Inhibit Akt-mTOR signaling and beclin 1 tyrosine phosphorylation, increase beclin 1/Parkin interaction	S138–S140
Investigational drug		
BH3 mimetics	Disrupt binding between beclin 1 and Bcl-2 family members	S141
Nutritional supplements		
Caffeine	Inhibits mTOR signaling	S142
Omega-3 polyunsaturated fatty acids	Inhibit Akt-mTOR signaling; disrupt beclin 1 and Bcl-2 binding	S143, S144
Resveratrol	Activates sirtuin 1	S145, S146
Spermidine	Acetylase inhibitor	S147
Vitamin D	Calcium signaling, hCAP18/LL37-dependent transcription of autophagy genes	S148, S149
Trehalose	Unknown	S150

lize caspase-10 to restrain autophagy and undergo autophagic cell death following caspase-10 inhibition (56). Interestingly, glucose-starved yeast and mammalian cells do not engage in prosurvival autophagy (56, 57), challenging the notion that glucose deprivation (one of the most common forms of metabolic stress in the tumor microenvironment) induces prosurvival autophagy in vivo. Thus, because the role of autophagy in cancer progression and response to therapy is complex and context dependent, it is possible that — despite the prosurvival effects of autophagy in some tumor cells — the induction of autophagy may still be useful in certain cancers through autophagy-dependent antitumor immunity, autophagy-dependent cytotoxic effects, or other tumor-suppressor effects.

While cytotoxic effects of autophagy or autophagy-dependent anticancer immune responses may be beneficial in certain malignancies, the cytotoxic effects of autophagy may be pathogenic in other diseases. These include mouse models of cigarette smoke–induced COPD (58), acute lung injury caused by avian influenza A H5N1 infection (59), diabetes-induced and pressure overload–induced cardiomyopathies (60–62), pancreatic β-cell death in the setting of *Pdx1* deficiency (63), ischemic brain damage in diabetes (64), and traumatic, ischemic, ischemic/reperfusion, and/or hypoxic injury in the brain, heart, or kidney of nondiabetic subjects (65–69). Such "pro-death" effects of autophagy (whether they are direct through autophagic cell death, indirect through enhanced apoptosis as postulated in lung epithelial cells exposed to cigarette smoke, or a combination of both) have yet to be confirmed in patients. However, if cigarette smoking or diabetes (or other comorbid conditions) increase susceptibility to autophagy-dependent enhancement of organ pathology in the clinical setting, the coexistence of these common comorbid conditions might affect the safety of utilizing autophagy inducers, particularly if they are not organ specific. Even if present, these unwanted effects could occur at doses higher than or durations longer than those needed to enhance the autophagy-mediated delivery of deleterious structures for lysosomal destruction. This would allow for the identification of a useful therapeutic window and the safe development of low doses or short-term or intermittent treatment strategies.

Besides potential unwanted cytoprotective effects or cytotoxic effects, another possible concern regarding autophagy induction relates to the complex roles of autophagy proteins in infectious diseases, immunity, and inflammation. Autophagy plays important roles in protection against several medically important intracellular pathogens through different mechanisms including xenophagy (the selective autophagic degradation of microbes), enhanced adaptive immunity, and the prevention of excessive inflammatory responses (Figure 1), leading to significant optimism that autophagy inducers may represent an important new class of host-direct anti-infective therapy (10, 70). However, the autophagy pathway and/or autophagy pathway–independent functions of autophagy proteins may also enhance the replication of certain viruses and intracellular bacteria. For example, pancreatic cell–specific knockout of *Atg5* dramatically reduces Coxsackie virus replication and virus-induced pathology (71); similarly, liver-specific knockout of *Atg5* reduces HBV DNA replication in mice that transgenically express HBV (72). It is not known whether these phenotypes reflect a role for the autophagy pathway or for autophagy pathway–independent effects of *Atg5*, which can also function in the negative regulation of inflammasomes, recruitment of immunity-related

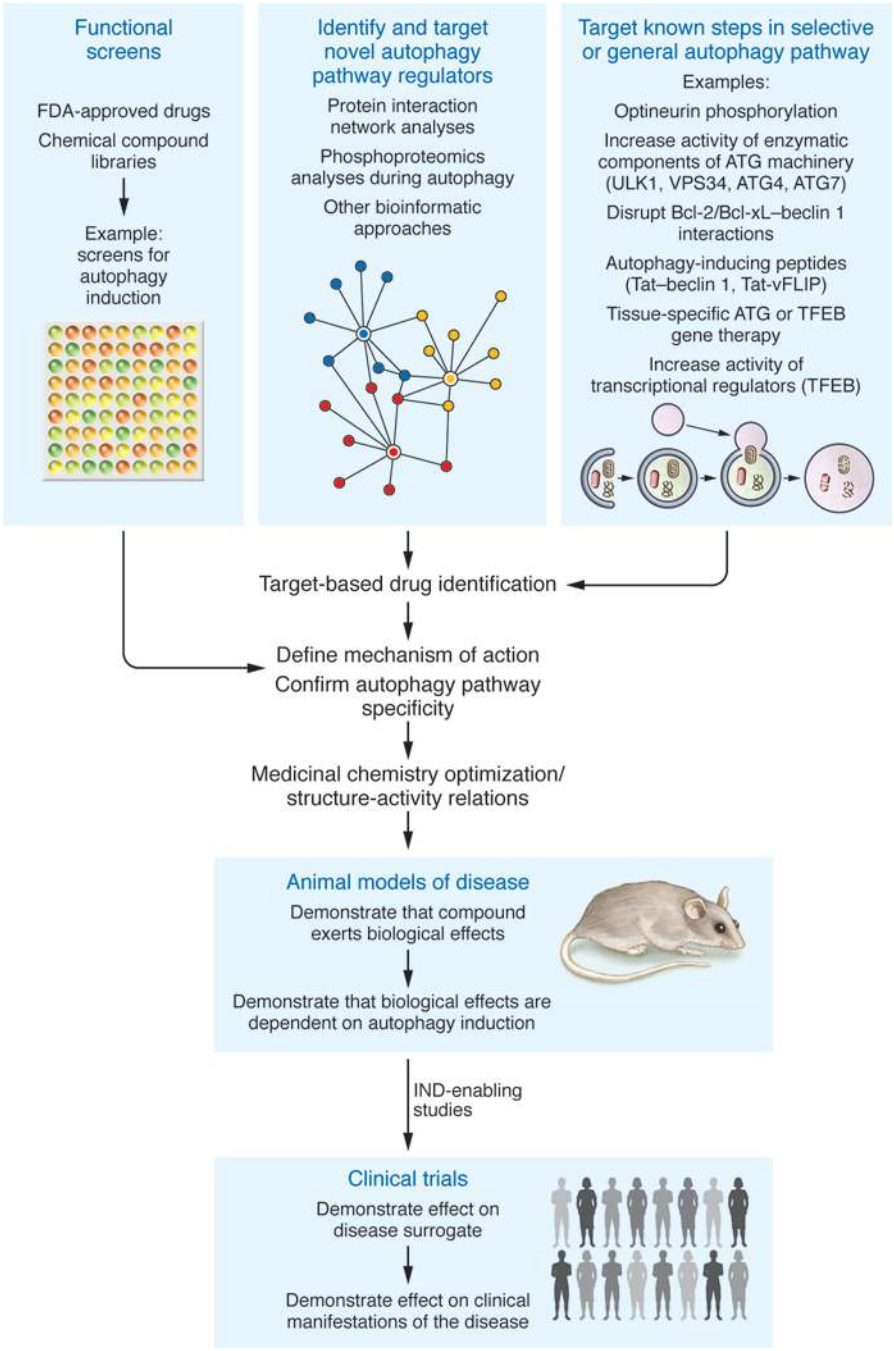

Figure 2. Schematic illustration of different approaches to developing autophagy inducers for the treatment of human diseases. IND, investigational new drug.

Studies of the loss of autophagy gene function in B cells and dendritic cells suggests a crucial role for autophagy in antigen-specific immune responses. For example, B cell–specific deletion of *Atg7* impairs virus-specific B cell memory in mice, leading to lethal influenza virus infection (73). Dendritic cell–specific deletion of *Atg5* results in impaired antigen presentation and increased susceptibility to lethal herpes simplex virus (HSV) infection (74). Moreover, autophagy-competent but not autophagy-deficient tumor cells attract dendritic cells and T cells into tumor beds, leading to enhanced antitumor immunity (75). However, it is unknown whether enhancement of autophagy will augment these antigen-specific immune responses; if so, autophagy inducers may have an important clinical role in enhancing vaccine and antitumor immunity. Of note, rapamycin increases the generation of memory $CD8^+$ T cells in mice following lymphocytic choriomeningitis virus infection or vaccination with a modified vaccinia virus (76), although it is not yet known whether this effect of mTOR inhibition is mediated through autophagy.

Polymorphisms linked to certain genes involved in autophagy regulation or autophagosome formation have been identified in human autoimmune diseases (such as *ATG5* and systemic lupus erythematosus) and inflammatory disorders (such as *IRGM*, *NOD2*, and *ATG16L1* and inflammatory bowel disease) (Table 2), raising the possibility that these disorders may be targets for autophagy induction therapy. However, the effects of the polymorphisms on gene function have not been sufficiently well characterized to allow a prediction of the effects of autophagy induction. For example, the $ATG16L1^{T300A}$ risk allele for Crohn's disease impairs intestinal Paneth cell function, regulation of proinflammatory IL-1β production, and likely, bacterial autophagy (77–79). However, this region of ATG16L1 is not conserved in yeast Atg16 and may not be required for general autophagy (80); thus, it is unknown whether pharmacologic enhancement of autophagy in Crohn's disease will correct the pathophysiologic defects imposed by the $ATG16L1^{T300A}$ mutation. Further studies are required to dissect the molecular function of autophagy risk alleles and their specific roles in the pathogenesis of diseases to determine effective strategies for correcting molecular defects imposed by such genetic variations.

GTPases, secretion, exocytosis, and formation of membranes that serve as scaffolds for viral replication (10, 70). If the mechanism involves autophagy, autophagy inducers might be contraindicated in the setting of certain infections such as HBV. However, if the mechanism involves autophagy-independent functions of *Atg5*, it is unknown whether the upstream upregulation of autophagic flux will also enhance autophagy-independent pro-pathogen functions of individual autophagy proteins. Suppression of inflammatory responses by autophagy induction might also impair the host capacity to clear infectious organisms.

Current approaches to developing autophagy inducers

Non-pharmacologic interventions such as caloric restriction and regular exercise induce autophagy and may improve overall health. Exercise-induced autophagy may be required for exercise-mediated protection against high fat diet–induced diabetes in mice (81); this raises the possibility that autophagy enhancement may also underlie other beneficial health effects of exercise, such as delaying the onset or progression of human cancers and neurodegenerative diseases (82). Similarly, autophagy is required for caloric restriction–induced lifespan extension in *C. elegans* (83) and may underlie the health benefits of caloric restriction in mammals. Intriguingly, the EPaNIC trial found that delayed onset of parenteral nutrition (and hence macronutrient deficiency) in patients in intensive care units led to enhanced autophagy (as measured by light chain 3 class II/light chain 3 class I [LC3-II/LC3-I] ratios and decreased ubiquitin staining) in muscle biopsies and reduced muscle weakness (84). In mice, restriction of food intake decreased the severity of post-infarction heart failure by increasing autophagy in surviving cardiomyocytes (85).

In addition to caloric restriction, other nutritional factors such as the consumption of coffee and vitamin D, may also influence health through autophagy induction. Caffeine-induced autophagy reduces hepatic steatosis in mice with nonalcoholic fatty liver disease (86) and protects against human prion protein–mediated neurotoxicity in cultured cells (87). It is thus tempting to speculate that caffeine-induced autophagy may explain why coffee consumption is associated with dose-dependent decreases in total and cause-specific mortality in middle-aged people (88). Vitamin D is a potent inducer of autophagy (89), and through an autophagy-dependent mechanism it inhibits HIV and *M. tuberculosis* replication in human macrophages and kills human breast tumor cells (90–92). Defects in vitamin D–induced autophagy might therefore underlie the epidemiologic associations between vitamin D deficiency and adverse health outcomes, including susceptibility to certain cancers and infectious diseases (89). Further clinical studies are needed to determine the optimal regimens for caloric restriction, exercise, caffeine consumption, and vitamin D intake that will yield safe and effective autophagy induction and improved clinical outcomes.

Several drugs currently approved by the FDA induce autophagy (Table 4) but generally have pleiotropic actions, making it difficult to parse out the role of autophagy induction in their therapeutic actions in patients. Nonetheless, preclinical studies demonstrate that certain autophagy-inducing agents fail to induce their beneficial effects in host organisms that are deficient in autophagy genes. FDA-approved compounds have been "repurposed" for use in preclinical models of diseases that are believed to respond favorably to autophagy enhancement, e.g., mTOR inhibitors in neurodegenerative diseases (93), EGFR and other tyrosine kinase inhibitors in diabetic nephropathy (94) and neurodegenerative diseases (20, 95–97), carbamazepine in α1-antitrypsin deficiency (36), trifluoperazine in *Salmonella* infection (98), and statins in *M. tuberculosis* infection (38). It is unknown whether autophagy upregulation contributes to the therapeutic effects of these agents for their currently approved clinical indications, but if this proves to be the case it would indicate a broader role than previously appreciated for autophagy in physiology and pathophysiology.

The lack of specificity of known autophagy-inducing agents is not necessarily problematic, provided that the non–autophagy-inducing effects are tolerable for the duration of intended use. For short-term indications such as acute infectious diseases, the non–autophagy-inducing (off-target) actions may lead to unwanted effects that may be tolerable if present for only a few days or weeks. For long-term use, such as the prevention of aging and the treatment of neurodegenerative disorders and metabolic diseases, the adverse effects related to non–autophagy-inducing actions may also be acceptable if they are mild or asymptomatic or are apparent only at doses substantially higher than those needed to enhance autophagy. Nevertheless, the development of highly specific autophagy inducers is strongly desirable (Figure 2), since such agents would be expected to provide the most favorable ratio of benefit to risk and would also provide direct proof-of-principle evidence to support (or refute) a role for autophagy upregulation in a specific disease context.

Beyond optimizing the clinical use of current autophagy-inducing pharmacologic and non-pharmacologic approaches, a key challenge will be to identify agents that can specifically induce autophagy with fewer unwanted side effects than those that accompany currently available drugs. To identify novel autophagy-inducing compounds, chemical screens have been performed using fluorescent measurement of autophagosomes (GFP-LC3–positive puncta) or FACS-based measurement of total levels of LC3 as readouts of autophagy (17, 99–101). For newly identified autophagy-inducing compounds, it is important to (a) determine whether the compound alters autophagy activity at physiologic or clinically meaningful concentrations; (b) demonstrate that the compound exerts its biologic effects through autophagy by showing that it is inactive when autophagy genes are silenced; and (c) identify and confirm its targets by showing that target knockdown or overexpression alters the activity of the compound. Established strategies in medicinal chemistry could then be used to optimize specificity and minimize off-target effects. Although screens based on readouts of autophagy activity have identified new compounds that can induce autophagy, it is not clear whether such approaches will be superior to current repurposing strategies in identifying agents that are specific inducers of autophagy.

To achieve the goal of synthesizing highly specific agents, new approaches will be needed (Figure 2). Strategies most likely to be useful include those that target regulatory steps that are unique to the autophagy pathway or that enhance the activity of specific components of the molecular machinery that are rate-limiting in the process. Since presently known upstream signals that activate autophagy also function in multiple downstream pathways, there is an urgent need to identify new autophagy-specific regulatory molecules or post-translational modification events. In this regard, the use of newer, unbiased proteomic mapping methods in living cells, such as spatially restricted enzymatic tagging (102), may be helpful to uncover such autophagy-specific regulatory steps. In addition, some of the phosphorylation, acetylation/deacetylation, and ubiquitination reactions involved in activation of the Unc-51 like autophagy activating kinase 1 (ULK1) complex (involved in autophagy induction) and the beclin 1/class III PI3K complex (involved in initial formation of the autophagosomal membrane) may become viable pharmacologic targets (103). The

binding between beclin 1 and Bcl-2 anti-apoptotic family members (which inhibit beclin 1 function) can also be pharmacologically disrupted; investigational drugs such as the BH3 mimetics upregulate autophagy via this mechanism (104), but they are not specific for autophagy, as they also upregulate apoptosis by disrupting interactions between Bcl-2 family members and BH3 domains of pro-apoptotic molecules. Based on differences in structural determinants between the BH3 domain of beclin 1 versus those of pro-apoptotic family members, it may be possible to design BH3 mimetics that selectively enhance autophagy. Structure-based design is currently being used to develop inhibitors of class III PI3K, of the E1-activating enzyme, ATG7, and of the ATG4B cysteine protease that cleaves LC3 at its carboxyl terminus (25); these approaches may possibly be used to develop agonists rather than antagonists of these molecules. However, it is unknown whether increased activity of these molecules will increase autophagic flux in specific clinical settings, although in some mouse studies, overexpression of ATG7 (as well as ATG5 or beclin 1) results in increased levels of autophagy (Table 3).

Approaches that may also more specifically induce autophagy include gene therapy (involving the tissue-specific delivery of vectors that express autophagy genes) or the use of cell-penetrating peptides (or drug-like derivatives thereof) (Table 3). In animal models, tissue-specific gene therapy with core autophagy genes, *Atg7* or beclin 1, the selective autophagy factor Parkin, or the transcriptional regulator of autophagy gene expression and lysosomal activity *Tfeb* exerts beneficial effects in a wide ranges of diseases including obesity/diabetes, neurodegenerative diseases, cystic fibrosis, α1-antitrypsin deficiency, Pompe disease, muscular dystrophy, and KRAS-induced lung cancers. The protective effects of *Tfeb* gene therapy in Pompe disease are abolished in the absence of *Atg7*, suggesting the mechanism of action is enhanced autophagy. Similarly, the cell-permeable Tat–beclin 1 peptide, which contains a short amino acid sequence of beclin 1 necessary and sufficient for autophagy induction, reduces the replication of several pathogens in vitro including HIV, enhances the clearance of the exon 1 fragment of mutant huntingtin protein, and decreases mortality in mice infected with West Nile virus or chikungunya virus. These antiviral effects are abolished in mice partially deficient in beclin 1, suggesting that the peptide acts through enhancement of autophagy. Since subcutaneous administration of this peptide induces autophagy in a variety of mouse tissues at doses that lack apparent toxicity, it may exert beneficial effects in other disease models. The possibility of long-term therapy would be enhanced by the development of an orally active peptidomimetic agent with actions similar to those of Tat–beclin 1. One potential advantage of gene therapy approaches and modified peptides that have spatially restricted in vivo activity is the potential to enhance autophagy in an organ-specific manner.

In addition to developing agents that increase autophagosome formation, it may also be possible to increase autophagic substrate recruitment and lysosomal activity. Considerable progress has been made in understanding the molecular machinery of selective autophagy (2, 105), and indeed, the neuroprotective effects of tyrosine kinase inhibitors may be mediated by enhanced functional interaction between the selective autophagy factor Parkin and beclin 1 (23). Innovative new chemical screens are needed to identify compounds that increase autophagic substrate clearance, in parallel with rational drug-based design to increase autophagic targeting through modulating the activity of specific steps in selective autophagy. For example, phosphorylation of the autophagy receptor optineurin promotes the autophagic clearance of ubiquitin-coated bacteria (106) and protein aggregates associated with neurodegenerative disease (107), suggesting that kinases that promote optineurin phosphorylation may enhance certain forms of selective autophagy. With respect to increasing lysosomal activity, modulation of the activity of TFEB, a transcriptional master regulator of lysosomal biogenesis that is regulated by protein phosphorylation (30), is a promising target.

Challenges for translation of preclinical discoveries into human clinical trials

Even if autophagy-inducing agents demonstrate significant activity in animal models of disease, their clinical development into useful drugs will be challenging. Early-phase clinical trials of any new pharmacologic agent are generally designed to identify a dose or range of potentially useful doses based on their ability to produce the expected changes in a biologic or physiologic system in a proof-of-concept study. However, because the magnitude of autophagy induction cannot readily be assessed in humans, no autophagy-specific surrogate endpoint currently exists. Consequently, unless biomarkers capable of assessing the levels of autophagic flux are identified, doses will need to be defined based on disease-specific intermediate markers, but the relationship of such effects to the induction of autophagy will be difficult to determine. Furthermore, for diseases requiring chronic treatment, it is not clear whether the therapeutic effects of autophagy-inducing agents require continuous long-term treatment or can be achieved by only intermittent therapy. The possibility that intermittent enhancement may be sufficient is supported by observations that intermittent fasting or exercise are associated with clinical benefits (82, 108) that may be attributable to autophagy stimulation. Long-term trials will be needed to assess both the effects of autophagy inducers on patient-centered outcomes and the safety and tolerability of these drugs.

Conclusion

The development of autophagy-inducing drugs heralds the potential for highly effective treatments for a wide range of clinical diseases, many of which have responded poorly to current therapy. Studies of the benefits and toxicity of these novel agents are not only likely to affect clinical care, but they will also inform our understanding of the multifaceted roles of autophagy in normal physiology and pathophysiology.

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