Mechanisms of Autophagy at The Cellular and Molecular Levels: A Review

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ABSTRACT: ***Autophagy is a self-degradative process that is crucial for balancing energy sources throughout development and in response to nutritional stress. Autophagy also cleans up after itself, removing misfolded or aggregated proteins, cleaning damaged organelles including mitochondria, endoplasmic reticulum, and peroxisomes, and removing intracellular infections. Autophagy is therefore often regarded as a survival process, despite the fact that its dysregulation has been related to non-apoptotic cell death. Although the processes governing parts of selective autophagy are not completely understood, autophagy may be either non-selective or selective in the removal of particular organelles, ribosomes, and protein aggregates. Autophagy promotes cellular senescence and cell surface antigen presentation, protects against genome instability, and prevents necrosis, making it important in the prevention of diseases such as cancer, neurodegeneration, cardiomyopathy, diabetes, liver disease, autoimmune diseases, and infections, in addition to eliminating intracellular aggregates and damaged organelles. This review highlights the most recent research on how autophagy is carried out and controlled at the molecular level, as well as how its disturbance may result in illness.***

KEYWORDS: ***Autophagy, Apoptosis, Cancer, Disease, Energy, Infection, Mechanisms, Neurodegeneration, Stress.***

1. INTRODUCTION

The term 'autophagy,' which comes from the Greek for 'self-eating,' was coined by Christian de Duve over 40 years ago, and was largely based on the observation of mitochondrial and other intracellular structures being degraded within the lysosomes of rat liver perfused with the pancreatic hormone glucagon. The molecular mechanism of glucagon-induced autophagy in the liver is currently unknown, apart from the fact that it needs cyclic AMP-induced protein kinase-A activation and is tissue-specific. In recent years, the scientific community has rediscovered autophagy, with many labs making significant advances to our molecular knowledge and awareness of the physiological importance of this process. Although autophagy's significance in mammalian systems is widely understood, many of the fundamental advances in understanding how autophagy is controlled and performed at the molecular level have been achieved in yeast (Saccharomyces cerevisiae). Currently, genetic screening has discovered 32 distinct autophagy-related genes (Atg) in yeast, and many of these genes are conserved in slime mold, plants, worms, flies, and mammals, highlighting the significance of the autophagic process in hunger responses throughout phylogeny. Macro-autophagy, micro-autophagy, and chaperone-mediated autophagy are the three forms of autophagy that all induce proteolytic destruction of cytosolic components at the lysosome. Macro-autophagy transports cytoplasmic cargo to the lysosome through a double membrane-bound vesicle called an autophagosome, which merges with the lysosome to create an autolysosome.

By contrast, cytosolic components are directly taken up by the lysosome via invagination of the lysosomal membrane in micro-autophagy. Autophagy, both macro and micro, may swallow massive structures via both selective and non-selective processes. Targeted proteins are translocated across the lysosomal membrane in a compound with chaperone proteins (such as Hsc-70) that are recognized by the lysosomal membrane receptor LAMP-2A, resulting in their unfolding and destruction. This review focuses on the molecular and cellular components of macroautophagy (henceforth referred to as ‘autophagy') and how it is controlled under both healthy and pathological circumstances due to recent and increasing interest in macroautophagy and its involvement in illness [1].

1.1 The Basic Autophagy Machinery:

Autophagy starts with an isolating membrane, also known as a phagophore, that is likely formed from a lipid bilayer supplied by the endoplasmic reticulum (ER) and/or the trans-Golgi and endosomes, but the precise origin of the phagophore in mammalian cells is debated. This phagophore extends to engulf intracellular cargo including protein aggregates, organelles, and ribosomes, encasing them in a double-membraned autophagosome. The loaded autophagosome develops by fusing with the lysosome, allowing lysosomal acid proteases to degrade autophagosomal contents. Amino acids and other breakdown by-products are exported back to the cytoplasm via lysosomal permeases and transporters, where they may be

re-used to construct macromolecules and for metabolism. As a result, autophagy may be viewed of as a cellular "recycling factory" that also improves energy efficiency via ATP production and damage management through the removal of non-functional proteins and organelles. What role does the molecular level play in this complicated process? Figure 1 depicts the five critical stages: (a) phagophore nucleation; (b) Atg5–Atg12 conjugation, interaction with Atg16L, and multimerization at the phagophore; (c) LC3 processing and insertion into the extending phagophore membrane; (d) capture of random or selective targets for degradation; and (e) fusion of the autophagosome with the lysosome, followed by proteolytic degradation of engulfed molecules by lysosome [2].

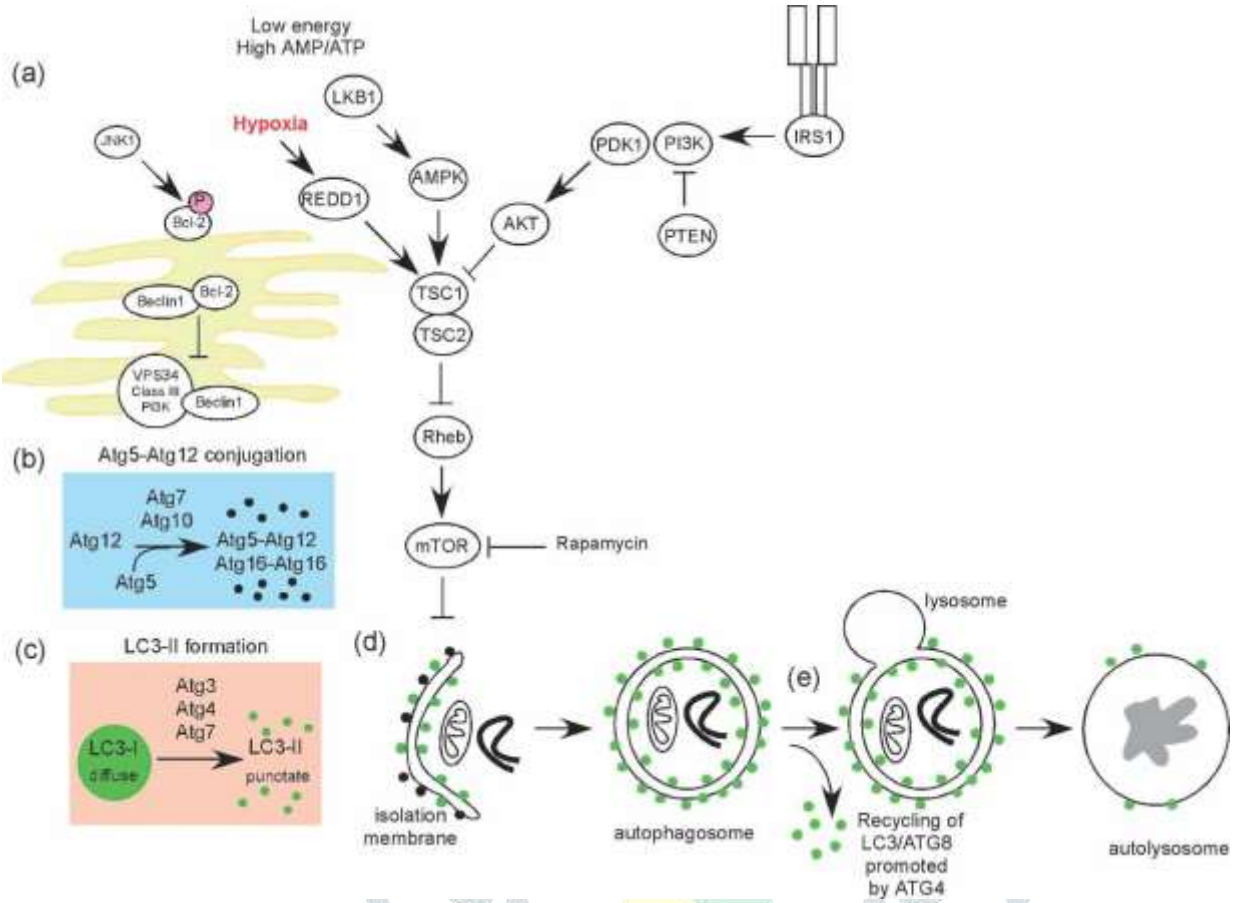

Figure 1: Molecular circuitry and signalling pathways regulating autophagy. Autophagy is a complex self-degradative process that involves the following key steps: (a) control of phagophore formation by Beclin-1/VPS34 at the ER and other membranes in response to stress signalling pathways; (b) Atg5–Atg12 conjugation, interaction with Atg16L and multimerization at the phagophore; (c) LC3 processing and insertion into the extending phagophore membrane; (d) capture of random or selective targets for degradation, completion of the autophagosome accompanied by recycling of some LC3-II/ATG8 by ATG4, followed by; (e) fusion of the autophagosome with the lysosome and proteolytic degradation by lysosomal proteases of engulfed molecules. Autophagy is regulated by important signalling pathways in the cell, including stress-signalling kinases such as JNK-1, which promotes autophagy by phosphorylating Bcl-2, thereby promoting the interaction of Beclin-1 with VPS34. Perhaps the central signalling molecule in determining the levels of autophagy in cells is the mTOR kinase that likely mediates its effects on autophagy through inhibition of ATG1/Ulk-1/-2 complexes at the earliest stages in phagophore formation from lipid bilayers. mTOR is key to integrating metabolic, growth factor and energy signalling into levels of both autophagy, on the one hand, which is inhibited by mTOR when nutrients are plentiful and, on the other hand, to growth-promoting activities, including protein translation, that are stimulated by mTOR signalling. Autophagy is induced by hypoxia and low cytosolic ATP levels that feed through REDD1 and AMP-kinase to inhibit mTOR activity through reduced Rheb GTPase activity. Conversely, autophagy is inhibited by increased growth factor signalling through the insulin receptor and its adaptor, IRS1, as well as other growth factor receptors that activate the Class I group of PI3-kinases and Akt, to promote mTOR activity through inhibition of TSC1/TSC2 and increased Rheb GTPase activity[3].

2. REVIEW OF LITERATURE

R L Deter in his study discuses about the Studies on the mechanical fragility, osmotic sensitivity, and sedimentation characteristics of rat liver lysosomes that were used to assess their reaction to an intraperitoneal dose of glucagon. Hepatic lysosomes showed a rapid increase in sensitivity to mechanical stimuli and reduced osmotic pressure approximately (1/2) hour after glucagon administration. Simultaneously, their sedimentation characteristics alter dramatically, with a substantial rise in the sedimentation coefficient of a major percentage of the total particles. Furthermore, glucagon induces an increase in the percentage of slowly sedimenting particles, causing the sedimentation coefficient distribution within the overall population to become bimodal. The latter alteration is more noticeable in acid phosphatase, less noticeable in cathepsin D, and hardly discernible in acid deoxyribonuclease. All of these changes peak between 45 and 90 minutes after injection and return to normal within 4 hours. They are consistent with the hypothesis that glucagon causes an increase in lysosomal size, and may be related to the autophagic-vacuole formation known to occur after glucagon administration, with the exception of the increase in the slow component, for which no explanation can be offered at this time [4].

Pierre-Emmanuel Rautou in his study focuses on autophagy that is engaged in key areas of hepatology, according to new research. Autophagy mostly has a prosurvival function in liver ischemia reperfusion damage, enabling the cell to cope with nutrition deprivation and anoxia. Autophagy is enhanced with hepatitis B or C infection; however it is hijacked by viruses for their own advantage. The level of autophagy is reduced in hepatocellular cancer. Autophagy has an anti-tumor function in this setting, and treatment methods that increase autophagy, such as rapamycin, benefit patients. Furthermore, Beclin-1, an autophagy protein, has a predictive relevance in hepatocellular carcinoma. The aggregation-prone ATZ protein accumulates in the endoplasmic reticulum in -1-antitrypsin deficiency. The autophagic response is triggered, with the goal of degrading mutant ATZ. In -1-antitrypsin deficiency, certain FDA-approved medicines that promote autophagy and the disposal of aggregation-prone proteins may be helpful. Autophagy is reduced in liver cells after alcohol intake, most likely due to a reduction in intracellular 5'-AMP-activated protein kinase (AMPk) and a change in vesicle transport in hepatocytes. The development of Mallory-Denk bodies and the demise of liver cells are both aided by a reduction in autophagy. In obese people, hepatic autophagy is impaired, and increasing it improves insulin sensitivity [5].

Hitoshi Nakatogawa in his study talks about the creation of double membrane-bound compartments that sequester materials to be destroyed in lytic compartments, a process that seems to be mechanistically different from normal membrane traffic, is the most striking aspect of autophagy. The discovery of autophagy in yeast, as well as the organism's genetic tractability, enabled us to identify genes involved for this process, resulting in the current explosion of this study area. Analyses of autophagy-related (Atg) proteins have revealed dynamic and varied features of the processes that underpin autophagy membrane formation [6].

3. DISCUSSION

3.1 Phagophore formation is under the control of multiple signalling events:

In yeast, phagophore membrane development occurs at or around a cytosolic structure known as the pre-autophagosomal structure (PAS), but no evidence of a PAS has been found in mammals. Phagophore membranes in mammalian cells seem to originate mainly from the ER in dynamic equilibrium with other cytosolic membrane structures such as the trans-Golgi and late endosomes, and may even derive membrane from the nuclear envelope under certain circumstances. However, due to the absence of transmembrane proteins in autophagosomal membranes, de novo membrane synthesis from cytosolic lipids in mammalian cells cannot be entirely ruled out. The Atg1 kinase is needed for phagophore development in yeast, perhaps through controlling the recruitment of the transmembrane protein Atg9, which may promote lipid recruitment to the growing phagophore. The energy-sensing TOR kinase regulates this phase by phosphorylating Atg13 and preventing it from engaging with Atg1, making autophagy initiation sensitive to growth factor and food availability. Ulk-1, a mammalian homologue of Atg1, is required for autophagy in developing reticulocytes, but it is unknown if Ulk-1, or indeed Ulk-2 (another Atg1 homologue), promotes autophagy in mammalian systems in the same way. Given that these activities are closely controlled in yeast and serve as a hub for signaling input in higher systems, these early stages in

phagophore production in mammalian systems need further study and are expected to provide many significant discoveries.

In mammalian systems, the function of class III PI-3 kinases in phagophore formation and autophagy is reasonably well known, particularly Vps34 (vesicular protein sorting 34) and its binding partner Atg6/Beclin-1. Vps34 is engaged in a variety of membrane-sorting activities in the cell, but when complexed with Beclin-1 and other regulatory proteins, it is specifically implicated in autophagy. Vps34 is unusual among PI3-kinases in that it exclusively uses phosphatidylinositol (PI) as a substrate to produce phosphatidyl inositol triphosphate (PI3P), which is required for phagophore elongation and recruitment of other Atg proteins. Beclin-1's association with Vps34 enhances its catalytic activity and raises PI3P levels, although it's unclear how this is controlled in response to hunger signaling. Because Beclin-1 is monoallelically deleted in human breast, ovarian, and prostate cancer, many cancer biologists have suggested that autophagy possesses tumor-suppressor characteristics. Beclin-1 heterozygous mice are susceptible to lymphoma, hepatocellular carcinoma, and various malignancies, while Beclin-1 null animals are embryonic fatal. Limiting necrosis and inflammation, causing cell cycle arrest, and avoiding genomic instability are all thought to be ways for autophagy to inhibit cancer. Autophagy has also recently been demonstrated to be necessary for important features of the anti-tumourigenic senescent cell phenotype. Others, however, believe that autophagy promotes drug resistance and tumor cell adaptability to stress as a cell survival strategy. Finally, the function of autophagy in cancer may differ depending on the cell type and/or stage [7].

UVRAG, BIF-1, Atg14L, and Ambra, as well as Rubicon and Bcl-2, form complexes with Vps34 and Beclin-1 at the ER and nucleated phagophore to either promote or inhibit autophagy. UVRAG has been found to be mono-allelically deleted in human cancer, similar to Beclin-1. Signaling processes in the cell that control the exact component composition of complexes including Vps34 and Beclin-1 at the ER are still being understood, although they are often sensitive to food availability in the microenvironment. The interaction of Beclin-1 with Bcl-2, which impairs Beclin-1's connection with Vps34, is one well-studied regulating event. Thus, contact with Bcl-2 (and Bcl-XL) in the ER inhibits Beclin-1 activity in autophagy. The BH3 domain of Beclin-1 mediates this connection, which is broken by Jnk1-mediated phosphorylation of Bcl-2 in response to starvation-induced signaling, enabling autophagy to occur. Thus, Bcl-2 has a dual role in determining cell viability, which may be dependent on its subcellular localization: (a) a pro-survival function at mitochondria, inhibiting cytochrome c release and thus preventing apoptosis; and (b) an autophagy-inhibitory activity at the ER, mediated by interaction with Beclin1, which can result in non-apoptotic cell death. Beyond the regulation of Beclin1 and Bcl-2, there is an interaction between autophagy and apoptosis. Calpain-mediated cleavage of Atg5 inhibited its autophagy function and led it to translocate to the mitochondria, where it interacted with Bcl-XL to induce cytochrome c release, caspase activation, and apoptosis. The balance between autophagy and apoptosis in the cellular response to particular stressors is a study topic of great interest because of its importance in disease development and therapy, but it is still an open question [8].

3.2 Signalling pathway that regulate autophagy:

In most cell types, autophagy is active at low levels, and it is thought to serve a housekeeping function in preserving the integrity of intracellular organelles and proteins. Autophagy, on the other hand, is a crucial component of cells' and organisms' adaptive responses to food restriction that supports survival until nutrients become available again. In response to hunger cues, how is autophagy induced? The target of rapamycin (TOR) kinase, which is a signaling control point downstream of growth factor receptor signaling, hypoxia, ATP levels, and insulin signaling, is a key role in nutrition sensing and regulating cell growth and autophagy. TOR kinase is triggered by Akt kinase, PI3-kinase, and the growth factor receptor. It signals when nutrients are available and promotes development by inducing ribosomal protein production and increasing protein translation. Importantly, under such growth-promoting circumstances, TOR inhibits autophagy, and although this is done in yeast and Drosophila by inhibiting Atg1 kinase activity, it is unclear how this is accomplished in human cells. Signals that detect nutritional shortage, like as hypoxia, suppress TOR kinase. The inhibitory action of the Tsc1/Tsc2 tumour suppressor proteins on Rheb, a small GTase essential for mTOR function, is promoted by activation of adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) in response to low ATP levels upstream of TOR. Reduced Akt activity in response to decreased growth factor receptor activity also inhibits TOR kinase

through Tsc1 and Tsc2, and TOR may be suppressed artificially by rapamycin treatment of cells. As a result, decreased TOR activity triggers autophagy, guaranteeing that the cell adjusts to its changing environment by reducing growth and increasing catabolism. Based on these findings, as well as the fact that TOR is downstream of oncogenes like Akt, rapamycin has been tried in cancer treatment clinical trials, where it is thought to suppress tumor development by inhibiting protein translation and triggering autophagy. TOR, on the other hand, may serve as a catalytic component of two different complexes, TORC1 and TORC2, and rapamycin seems to have higher inhibitory effect against TORC1, prompting the hunt for so-called "rapalogs" that target both TORC1 and TORC2 [9].

As previously stated, hypoxia promotes autophagy via both HIF-dependent and HIF-independent actions, which are likely mediated by TOR suppression downstream of AMPK, REDD1, and Tsc1/Tsc2. Given that hypoxia causes ER stress via the unfolded protein response, and that mitochondrial function in oxidative phosphorylation is reduced under hypoxia, autophagy induction may allow the cell to eliminate compacted ER and reduce mitochondrial mass at a time when oxygen is not available to accept free electrons from the respiratory chain. This hypoxia-adaptive response would avoid excessive ATP use in the ER and reduce reactive oxygen species formation in the mitochondria. Increased autophagy would also enable the cell to produce ATP through catabolism at a time when oxidative phosphorylation ATP generation is restricted. BNIP3 and BNIP3L, non-canonical members of the Bcl-2 class of cell death regulators, are specific HIF targets in autophagy. Despite their connection to cell death, these proteins seem to have a normal role in mitophagy. As previously mentioned, BNIP3L/NIX has a physiological function in the clearance of mitochondria from developing reticulocytes, while BNIP3 serves a similar role in cardiac and skeletal muscle in response to oxidative stress. The degree to which BNIP3 and BNIP3L are functionally redundant is unknown, and differences in their expression regulation may explain some of their non-redundancy in vivo. BNIP3/BNIP3L function in mitophagy has been explained using a variety of theories, including BNIP3's involvement in derepressing Beclin-1 via disrupting its connection with Bcl-2. However, BNIP3L has been shown to have a more direct function in mitochondrial clearance by interacting with the LC3-related protein GABARAP, whereas BNIP3 interacts with Rheb, suggesting an extra indirect involvement in hypoxia-induced autophagy. While it appears that nutrient deprivation-induced inhibition of TOR activity and downstream effects on translation of key cell cycle genes like cyclin D1 are largely responsible for autophagy-induced cell cycle arrest, it is unclear whether autophagy can cause cell cycle arrest without TOR signaling. Given the significance of knowing how and at what stages autophagy functions in tumor development, this is an area of study that will certainly get more attention in the future [10].

3.3 Selective Autophagy:

We concentrate on selective autophagy in this paper because of its importance in neuropathies, cancer, and heart disease. p62/SQSTM1 interacts with polyubiquitinated proteins and aggregates via its ubiquitin-binding domain (UBD) , with LC3B-II through its LC3-interacting Region (LIR), and controls NF-B signaling through contact with Traf-6, as briefly stated before. When autophagy is impaired, such as in mice with targeted Atg7 deletion, p62-associated poly-ubiquitinated aggregates develop in cells, and simultaneous ablation of Atg7 and p62 was shown to ‘rescue' the buildup of these aberrant cytosolic inclusions. Recent research shows that increased p62 levels play an active role in deregulating NF-B signaling and promoting inflammation-associated carcinogenesis in human hepatocellular carcinoma. In the aetiology of neurodegenerative disorders such as dementia, Alzheimer's, Huntington's, Parkinson's, and Creutzfeldt–Jakob/prion diseases, intracellular aggregate buildup is especially important. Polyglutamine-expansion repeats, such as those seen in mutant huntingtin (Huntington's disease), mutant forms of -synuclein (familial Parkinson's disease), and various forms of tau (Alzheimer's disease), are all autophagy-dependent. Inactivation of the essential autophagy genes Atg5 or Atg7 in neurons consistently leads in intracellular aggregate buildup and neurodegeneration in mice. The discovery of a connection between autophagy and neuropathies has piqued researchers' interest in developing autophagy-inducing medicines to treat these debilitating illnesses. Mitophagy, or autophagy-dependent mitochondrial breakdown, is essential for preserving mitochondrial integrity and reducing the generation of reactive oxygen species. Uth1p, a yeast protein needed for mitochondrial clearance by autophagy, was the first protein implicated in mitophagy. However, it is unclear how Uth1 interacts with the autophagosome and controls mitophagy, and there are no known human homologues. Atg32, a mitochondria-anchored protein, was recently shown to be needed for mitophagy in yeast, where it interacts with Atg8 and Atg11, suggesting that it serves as a mitophagy mitochondrial receptor. Atg32, like Uth1, has no known mammalian homologues, although it

does include the WXXI amino acid motif, which is essential for interaction with Atg8 and Atg11 and is preserved in the LIR of p62. BNIP3L, which is involved in mitochondrial clearance in developing red blood cells, Ulk-1, which is the mammalian counterpart of Atg1, and Parkin, which is encoded by a gene related to Parkinson's disease, are other molecules implicated in mitophagy. Parkin is an E3 ubiquitin ligase found at the outer mitochondrial membrane, indicating that important mitochondrial components must be ubiquitinated in order for autophagosomes to pick up mitochondria [11].

In yeast, autophagy selectively eliminates both peroxisomes and ribosomes. Micropexophagy (direct engulfment by the vacuole) and macropexophagy (autophagosome-mediated delivery to the vacuole) are used by methylotrophic yeasts to remove peroxisomes during adaptation to an alternative energy source, and Atg30 is required as an adaptor, interacting with peroxisome proteins (Pex3 and Pex14) and autophagosome proteins (Atg11 and Atg17). During famine, ribosomes are preferentially destroyed (ribophagy), a process that relies on the catalytic activity of the Ubp3p/Bre5p ubiquitin protease. These specific types of autophagy in mammalian systems are understudied in contrast to yeast [12].

4. CONCLUSION

Our knowledge of autophagy in mammalian cells is still limited, including questions about how the phagophore forms in the first place, how particular cargo is targeted for destruction, and how alternative Atg5/Atg7-independent autophagy processes are controlled. However, increasing data connecting mutations or loss of function of essential autophagy genes to cancer, neuropathies, heart disease, auto-immune illness, and other diseases demonstrates the importance of autophagy abnormalities for disease and aging. Autophagy is either tumor suppressive (through cell cycle arrest, promoting genome and organelle integrity, or inhibiting necrosis and inflammation) or oncogenic (through cell cycle arrest, promoting genome and organelle integrity, or inhibiting necrosis and inflammation), according to cancer biologists (by promoting cell survival in the face of spontaneous or induced nutrient stress). Autophagy is more generally recognized as helpful in other illnesses, including as neuropathies (Huntington's, Alzheimer's, and Parkinson's diseases) and ischemic heart disease, because of its function in removing "toxic assets" and increasing cell viability. As a result, autophagy has emerged as a novel and powerful regulator of disease development that is both scientifically and therapeutically interesting.

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