

Role of Autophagy in Cancer Prevention

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Abstract

Macroautophagy (autophagy hereafter) is a catabolic process by which cells degrade intracellular components in lysosomes. This cellular garbage disposal and intracellular recycling system maintains cellular homeostasis by eliminating superfluous or damaged proteins and organelles and invading microbes and by providing substrates for energy generation and biosynthesis in stress. Autophagy thus promotes the health of cells and animals and is critical for the development, differentiation, and maintenance of cell function and for the host defense against pathogens. Deregulation of autophagy is linked to susceptibility to various disorders including degenerative diseases, metabolic syndrome, aging, infectious diseases, and cancer. Autophagic activity emerges as a critical factor in the development and progression of diseases that are associated with increased cancer risk as well as in different stages of cancer. Given that cancer is a complex process and autophagy exerts its effects in multiple ways, the role of autophagy in tumorigenesis is context-dependent. As a cytoprotective survival pathway, autophagy prevents chronic tissue damage that can lead to cancer initiation and progression. In this setting, stimulation or restoration of autophagy may prevent cancer. In contrast, once cancer occurs, many cancer cells upregulate basal autophagy and utilize autophagy to enhance fitness and survive in the hostile tumor microenvironment. These findings revealed the concept that aggressive cancers can be addicted to autophagy for survival. In this setting, autophagy inhibition is a therapeutic strategy for established cancers. *Cancer Prev Res*; 4(7); 973–83. ©2011 AACR.

Introduction

Tumorigenesis is a complex, multistage process. It includes tumor initiation, promotion, progression to malignancy, and metastasis. This process involves profound alteration of cells in terms of metabolism, growth, proliferation, stress tolerance, and survival, as well as interaction with the microenvironment where they grow. Genetic and epigenetic changes initiate cancer and facilitate progression of cells toward malignancy. Chronic tissue damage and inflammation provide a promutagenic environment to accelerate this process by creating a cancer-promoting environment to support survival and proliferation of abnormal cells.

Autophagy is the cell's garbage disposal and intracellular recycling system—a catabolic process by which cells degrade intracellular components in lysosomes. Thus, autophagy maintains cellular homeostasis by eliminating superfluous or damaged proteins and organelles and invading

microbes, as well as provides substrates for energy generation and biosynthesis. Autophagy plays an important role not only in different stages of tumorigenesis but also in the disease states that give rise to a microenvironment that promotes tumorigenesis in the first place. The role of autophagy in disease states associated with a higher risk of cancer, such as chronic liver disease, obesity, and inflammatory bowel disease (IBD), is becoming increasingly clear. Pharmacologic manipulation of autophagy with the intention of preventing a microenvironment rife for tumor initiation in these disease states may require an opposite approach wherein stimulation of autophagy limits progression of premalignant lesions. In this review, we highlight the regulation of autophagy machinery with specific emphasis on drugable targets, the role of autophagy in physiology and in the aforementioned cancer risk-associated disease states, and the role of autophagy within the cells destined to become cancer. We also discuss how induction of autophagy may limit the promotion of a hospitable microenvironment for tumors whereas inhibition of autophagy may limit tumor growth and metastases in established tumors.

Regulation of the Autophagy Machinery

Autophagy produces the engulfment of intracellular components including proteins and organelles (cargo) into double-membrane vesicles, called autophagosomes, which fuse with lysosomes to form autolysosomes, where the

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autophagic cargos are degraded. The availability of nutrients, growth factors, and hormones and stress regulate autophagy. The mTOR complex 1 (mTORC1) is a major negative regulator of autophagy. It promotes protein synthesis, cell division, and metabolism in response to nutrient, growth factor, and hormone availability while suppressing autophagy. Tumor cells frequently activate mTOR and its growth-promoting functions by acquiring mutations upstream. As a result of mTOR activation, suppression of autophagy may occur in some tumors. Stressors such as amino acid depletion or hypoxia suppress mTORC1 and induce autophagy (1, 2). Autophagy is also regulated independent of mTORC1, such as by hypoxia-inducible factors, LKB1-AMP-activated protein kinase (AMPK), and protein kinase C (3–10).

The central machinery of autophagy includes a series of complexes composed of autophagy-related (Atg) proteins that assembles autophagosomes. The Unc-51-like kinases 1 and 2 (Ulk1 and Ulk2; mammalian Atg1) complex receives signals from mTORC1 and AMPK. AMPK regulates the Ulk1 complex by phosphorylating Ulk1 or the mTORC1 component raptor (9–12). Several beclin1/Vps34 (class III phosphatidylinositol-3 kinase)-containing complexes dictate the sequential steps of autophagosome formation and promote fusion of autophagosomes with lysosomes. Two interconnected ubiquitin-like conjugation systems act in the expansion of autophagosome membranes; the detailed molecular mechanism is described elsewhere (5). Proteins that can be targeted to modulate autophagy are highlighted in Figure 1. Rapamycin and its analogues, as well as metformin, resveratrol, lithium, and carbamazepine (CBZ), stimulate autophagy (13–15). Vps34 inhibitors can potentially suppress autophagy induction, whereas chloroquine (CQ) and its analogue hydroxychloroquine (HCQ) prevent lysosomal acidification and impede the degradation of autophagic cargo and turnover of autolysosomes (16).

Distinct from proteasomal degradation, which only proteolyzes individual, soluble proteins inside proteasomal barrels, autophagy degrades large cellular components such as protein aggregates and entire organelles, and is the only cellular mechanism that does so. Autophagic substrates may include cytoplasm, organelles, proteins, and protein aggregates as well as the autophagy components that associate with the autophagosomal inner membrane (Fig. 1).

Autophagy can function in the nonselective bulk degradation of cytoplasmic contents or in the selective turnover and elimination of specific cellular components. To selectively target substrates to autophagosomes, protein and organelle substrates are conjugated with ubiquitin, whereas selective autophagic degradation of lipid droplets may use other signals (17, 18). Autophagy cargo receptors, such as p62 and neighbor of BRCA1 (NBR1), interact with both the ubiquitin on cargo and the autophagy machinery through light chain 3 (LC3)-interacting (LIR) domains. This enables the cargo receptors to recognize and deliver cargo to autophagosomes (19–22; Fig. 2). The Bcl-2 family BH3-only proteins BNIP3 (BCL2/adenovirus E1B 19 kDa-interacting protein) and BNIP3L (or NIX) are also critical for

selective elimination of mitochondria (23). Evidence suggests that NIX, which localizes to the mitochondrial outer membrane, interacts with LC3 family proteins such as γ -aminobutyric acid receptor-associated protein (24, 25) and likely serves as a cargo receptor for mitophagy in erythroid maturation (26). NIX may also promote mitochondrial loss of membrane potential (27), facilitating selective autophagy. Whether BNIP3 acts as a cargo receptor is still unknown. Accumulation of cargo receptors and their cargo, often in large aggregates in the case of p62, is symptomatic of autophagy inhibition.

Autophagy in Cellular Refreshment

Autophagy-mediated protein quality control

Autophagy constitutively degrades excess or damaged proteins and organelles through its basal activity, which is critical to the maintenance of cellular homeostasis and function. This service is especially important for postmitotic cells, which cannot dilute cellular waste products through cell division. Impaired autophagy in mice causes quiescent cells such as neurons and hepatocytes to accumulate ubiquitin- and p62-positive protein inclusions, aberrant membranous structures, and deformed mitochondria, accompanied by neuronal degeneration and liver injury (28, 29). p62- and ubiquitin-containing inclusions (Mallory–Denk bodies) have been associated with a variety of liver diseases including hepatitis and hepatocellular carcinoma (30). This accumulated p62 causes hepatotoxicity in the liver, shown by the partial suppression of protein inclusions and liver injury through genetic ablation of p62 in autophagy-deficient mice (19). It will be of interest to see whether genetic impairment of autophagy is the cause of some liver disease in humans and whether stimulation of autophagy mitigates disease progression.

The accumulation of p62 does not account for the degeneration of neuronal cells observed in autophagy-deficient mice, as the ablation of p62 does not prevent neurodegenerative disease, indicating that the role of autophagy in disease development is context-dependent. The failure of autophagy to remove dysfunctional mitochondria (mitophagy) has been mechanistically linked to the neurodegenerative Parkinson's disease (20). Furthermore, autophagy deficiency accelerates the progression of neurodegenerative diseases precipitated by expression of aggregation-prone mutant proteins, which rely on autophagy for clearance. Enhancement of autophagy has been suggested as an approach to mitigate neurodegeneration caused by pathogenic proteins such as mutant Huntingtin in Huntington's disease (13, 31).

An autophagy deficit may also contribute to Alzheimer's disease (AD). It was reported recently that presenilin1 (PS1), which is commonly mutated in early-onset familial AD, is required for proteolytic activity of lysosomes/autolysosomes and the clearance of autophagosomes and cargo (32). This raises the possibility that defective autophagy causes AD and that restoration of autophagic degradation of intracellular components may slow the progression of AD.

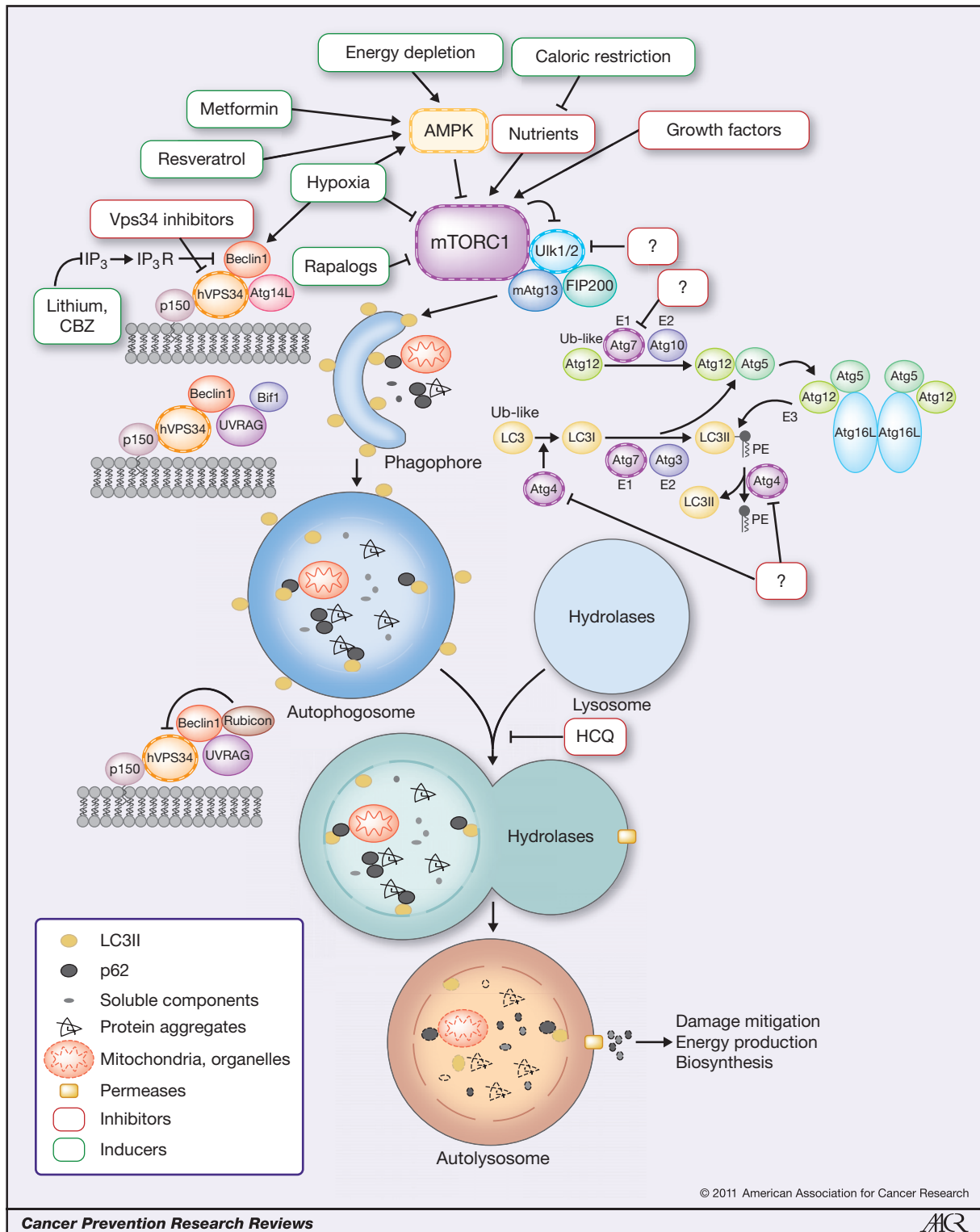


Figure 1. Machinery and small-molecule modulators of autophagy. The events of autophagosome formation—nucleation, expansion, and maturation—are depicted along with molecular machinery that regulates this process. The major negative regulator of autophagy, mTORC1, which integrates stimuli including availability of nutrients or growth factors, energy depletion, or hypoxia, is also shown. Drugable protein targets are highlighted with striated outlines. Autophagy stimulators are indicated by green boxes, autophagy inhibitors by red boxes. Question marks represent inhibitors aiming at potential targets, the kinase Ulk1, the cysteine protease Atg4, and the E1-like ubiquitination enzyme Atg7. PE, phosphatidylethanolamine; IP₃, inositol-1,4,5-triphosphate; IP₃R, IP₃ receptor.

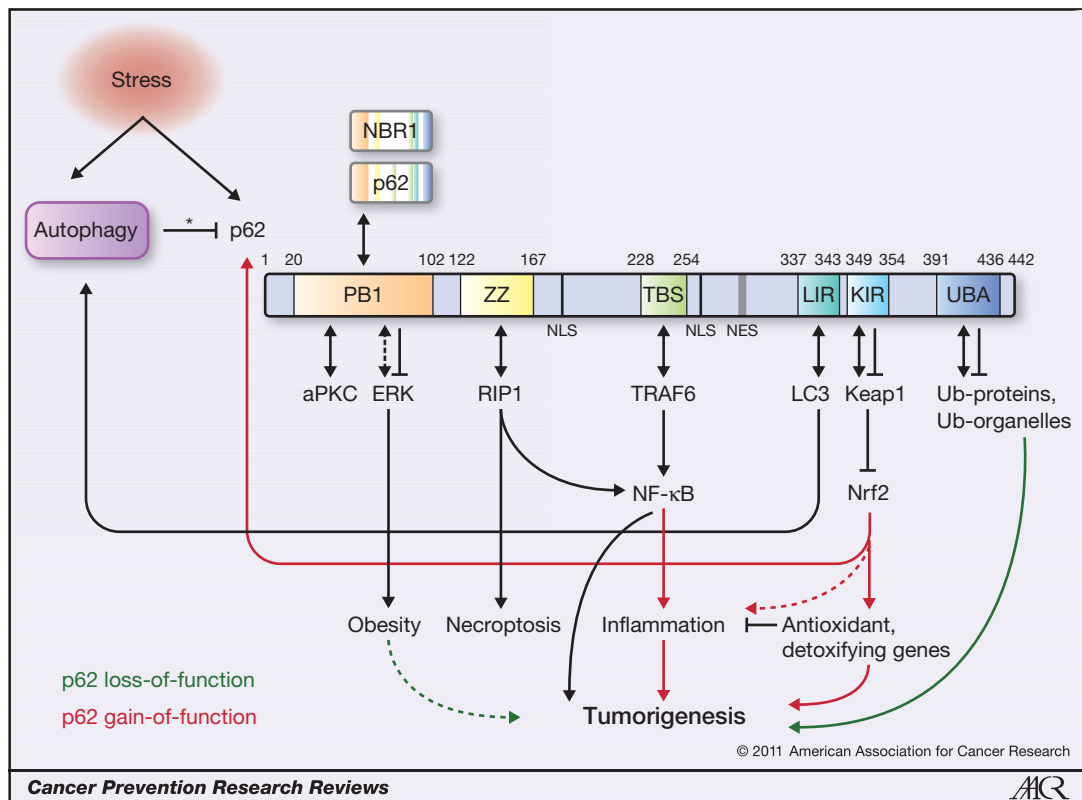


Figure 2. Role of autophagic regulation of p62 in cell signaling. Stress upregulates autophagy and induces the accumulation of the autophagic cargo receptor protein p62. Autophagy regulates p62 turnover and its protein levels in cells (denoted by an asterisk, upper left). Domains of p62 are diagrammed. Numbers across the top of the p62-domain diagram represent the corresponding amino acid positions in mouse p62. Brown arrows indicate p62-protein interactions. The dashed brown arrow (toward upper left) indicates a putative interaction between p62 and ERK (80). Accumulation of p62 activates Nrf2, whereas p62 itself is a transcriptional target of Nrf2, creating a positive feedback loop (45). Signals occurring with p62 loss of function are indicated by solid green arrows, when p62 gain of function by solid red arrows. The dashed green arrow (lower left) shows a putative role of obesity in tumorigenesis (also see Fig. 3). The dashed red arrow (lower right) shows a putative consequence of hyperactive Nrf2. PB1, Phox, and Bem1p-1 oligomerization domain; ZZ, zinc finger; TBS, TRAF6-binding sequence; KIR, Keap1-interacting region; UBA, ubiquitin-associated domain; NLS, nuclear localization signal; NES, nuclear export signal (40, 43, 81).

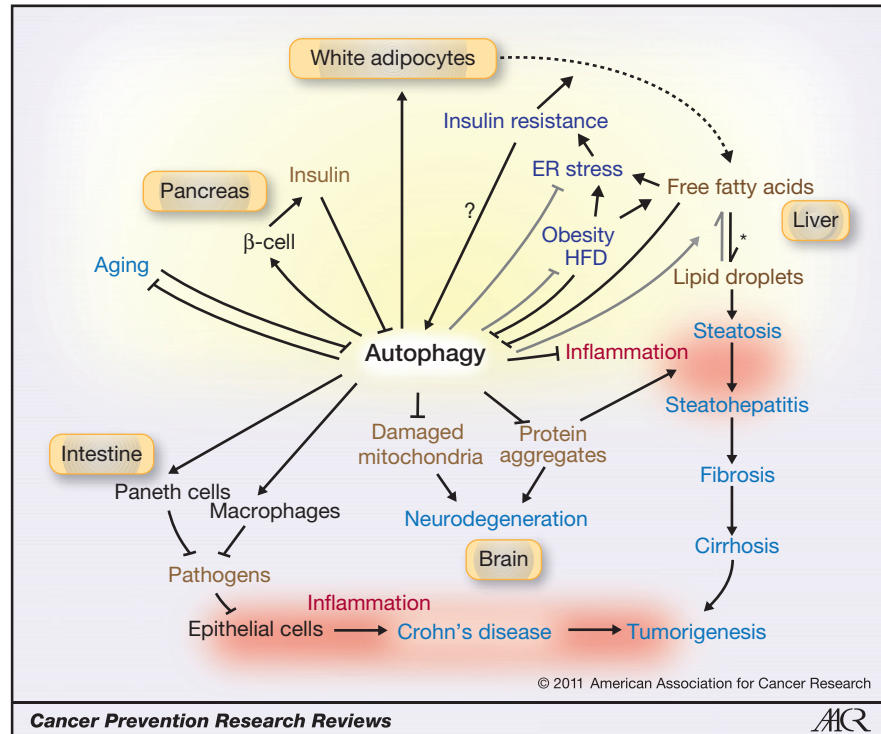
Development of liver disease in α 1-antitrypsin (AT)-deficient patients is another case exemplifying the significance of autophagy in cellular and organismal well-being. In this setting, autophagy degrades an aggregate-prone toxic mutant protein and links the autophagy-mediated garbage disposal with chronic liver disease that is associated with an increased risk of cancer. A point mutation in the liver-derived secretory AT protein (ATZ) renders it aggregate prone, causing its accumulation in the endoplasmic reticulum (ER). ATZ aggregates cause hepatotoxicity, liver injury, inflammation, and carcinogenesis. However, only a subgroup of afflicted homozygous patients develop liver fibrosis and eventually hepatocellular carcinoma (33). This discrepancy has been attributed to the interaction of ATZ with genetic or environment determinants. The autophagy-stimulating drug CBZ reduces the ATZ load and alleviates the associated symptoms in mice. This suggests that autophagy impairment may predispose ATZ homozygous patients to the ATZ-associated progressive liver disease and that enhancement of autophagy may be beneficial for disease prevention and therapy (ref. 34; Fig. 3).

Autophagy-mediated organelle quality control

Autophagy eliminates damaged organelles and ensures their quality control, thereby maintaining organelle function and preventing the harmful consequences of the accumulation of damaged organelles. Autophagy may sustain cellular metabolism through maintenance of mitochondrial quality control, whereas impaired autophagy may lead to compromised or altered metabolism in part through the accumulation of dysfunctional mitochondria. Skeletal muscle-specific Atg7 deficiency in mice causes reduced mitochondrial function, revealing the significance of autophagy for preservation of the functional mitochondrial pool (35). This mitochondrial function preserved by autophagy is particularly important for cells that need mitochondrial β -oxidation and oxidative phosphorylation for efficient energy production. Moreover, autophagy-mediated turnover of peroxisomes, which also function in fatty acid β -oxidation, may additionally contribute to sustaining normal metabolism.

The autophagic elimination of damaged proteins and organelles, particularly mitochondria and peroxisomes,

Figure 3. Role of autophagy in health or disease progression. Autophagy is required for cellular and organismal health and is involved in the pathogenesis of a variety of diseases. Disorders are labeled in blue. Arrows represent positive regulation of the pathway—lines with a block mark at the end represent negative regulation of the pathway. Pathways upregulated during lipid homeostasis collapse are represented in bold, pathways downregulated during this process in gray. The dashed arrow (top right) depicts flux of fatty acids from adipocytes to liver. The asterisk (far right) indicates the abnormal deposit of fatty acids in liver when lipid homeostasis is disrupted. The question mark (middle, toward top) denotes a putative regulation. Yellow shading shows where autophagy is involved in the regulation of lipid homeostasis. Red shading shows inflammation, which promotes tumorigenesis and is suppressed by autophagy. HFD, high-fat diet.



also removes potential sources of reactive oxygen species (ROS). ROS production in autophagy-defective mice is associated with tissue damage, chronic cell death, and liver inflammation (19, 36). In mouse skeletal muscle, autophagy deficiency causes accumulation of abnormal mitochondria and elevated oxidative stress in myofibers accompanied by muscle degeneration with age (37). Studies in yeast suggest that the peroxisomal autophagy also restricts intracellular ROS. In methylotrophic yeast, autophagy constitutively degrades peroxisomes. Mutations in *Atg1*, the yeast homologue of mammalian *ULK1*, cause peroxisome accumulation and decreased activity of the peroxisomal enzyme catalase, which functions in detoxification by converting H_2O_2 to H_2O and decreasing ROS levels. Senescent human cells also show accumulation of peroxisomes with reduced capacity to import catalase, associated with an increased load of ROS (38). The autophagy status was not assessed in these aging cells; nevertheless, it is possible that autophagy also preserves the functional integrity of peroxisomes and limits ROS production from peroxisomes in mammals (38). Thus, failure of organelle quality control by autophagy can lead to toxic ROS production and disease.

The Importance of Autophagy in Controlling p62 Levels

The ability of autophagy to selectively eliminate specific proteins has an important role in cellular function. The autophagic cargo receptor p62, which is induced by stress

to facilitate selective autophagic degradation, itself is a substrate of autophagy (39). p62 contains oligomerization and protein interaction domains and facilitates cargo degradation, and when autophagy is defective, protein aggregate formation (refs. 19, 40; Fig. 2). Failure to clear p62 due to impaired autophagy causes liver damage in mice and promotes tumorigenesis of allografts (19, 36). Thus, autophagic degradation of a specific protein, p62, has a role in preventing disease development and progression.

p62 regulates NF- κ B signaling

p62 is required for oncogenic *Ras*-driven NF- κ B activation and lung adenocarcinoma growth in mice (41). In contrast, aberrant p62 accumulation in autophagy-defective cells abrogates NF- κ B signaling that may promote tumorigenesis in the liver (36). NF- κ B activates pro-survival and pro-inflammatory gene transcription. The role of NF- κ B in tumorigenesis in the liver is becoming clear. Inhibition of NF- κ B activation in hepatocytes in the liver causes hepatocyte cell death. Dying cells activate and recruit immune cells (such as Kupffer cells), causing cytokine and growth factor production and inflammation, which lead to compensatory proliferation and tumorigenesis. Furthermore, inhibition of NF- κ B activation in Kupffer cells prevents expression of tumor-promoting cytokines and inflammation and suppresses tumor development (42). Therefore, the role of NF- κ B in tumorigenesis is context-dependent. It will be interesting to delineate the interplay between autophagy deficiency-dependent p62 accumulation and NF- κ B signaling in tumorigenesis.

p62 accumulation activates Nrf2-mediated transcription

Recent studies indicate that the autophagy deficiency-dependent p62 accumulation alters the regulation of another signaling pathway, transcription by NF-E2-related factor 2 (Nrf2). Nrf2 is a transcription factor mediating transcription of antioxidant and detoxifying genes. p62 interacts with the Nrf2-binding site on the Nrf2 inhibitor Keap1, an adaptor for the Cullin-based E3-ligase that promotes Nrf2 degradation. By binding and sequestering Keap1, p62 accumulation activates Nrf2 and Nrf2-target gene expression (43–45). The enzymes encoded by these genes metabolize and detoxify environmental carcinogens or endogenous mutagens such as ROS, protecting cells from damage and carcinogenesis. Counterintuitively, simultaneous ablation of Nrf2, and the cytoprotective antioxidant/detoxifying pathway, in mouse liver partially alleviates autophagy deficiency-dependent liver injury (43). Whether this is directly related to the function of Nrf2-targeted genes remains to be investigated. Constitutive activation of Nrf2 is associated with increased cancer incidence (46). It will be of interest to see whether the autophagy-dependent accumulation of p62 promotes tumorigenesis through activating both Nrf2 and NF- κ B.

Autophagy in Metabolic Homeostasis

Recent findings have indicated multiple roles of autophagy in lipid homeostasis. Epidemiologic studies have linked disruption of lipid homeostasis manifested as obesity to increased risk for developing several types of cancer (47), suggesting the relevance of autophagy in cancer developed under this condition. Fatty liver disease and systemic metabolic disorders are associated with disturbed lipid homeostasis. Fatty liver ranges from simple steatosis to steatohepatitis and cirrhosis that can ultimately lead to hepatocellular carcinoma. Dysregulation of lipid metabolism or hepatic lipotoxicity is thought to trigger inflammation and fibrogenesis, which are associated with development of aggressive disease (48). Accumulation of fatty acids and the resulting fatty acid metabolites in liver renders hepatocytes more susceptible to injury, leading to cell death and activation of the inflammatory responses (49). This is similar to the phenotypes of autophagy-deficient mice; allelic loss of *beclin1* causes steatosis, steatohepatitis, and spontaneous hepatocellular carcinoma (50). Although it is still unclear how exactly autophagy deficiency contributes to initiation or progression of disease, failure of lipid homeostasis leading to lipotoxicity and chronic inflammation, in addition to p62 accumulation, is a likely possibility.

Autophagy is important for accessing lipid stores through lipophagy, and by promoting lipid breakdown, prevents lipid accumulation in liver (18). In liver, lipid droplets continually undergo hydrolysis and the resulting free fatty acids are used for β -oxidation or synthesis of very low-density lipoprotein for export, or are re-esterified back to triglycerides. Autophagy constitutively degrades lipid

droplets (lipophagy), and inhibition of autophagy causes accumulation of lipid droplets both in cultured hepatocytes and in mouse liver (18). Thus, autophagy facilitates the metabolism of fatty acids and prevents fatty acid accumulation that causes lipotoxicity in liver.

Lipid homeostasis also involves the whole body storage and mobilization of fatty acids, and autophagy contributes to this systemic aspect. Recent data show that autophagy is required for differentiation of white adipose tissue, where most lipids in the body are stored. Adipocyte-specific Atg7 deficiency in mice causes decreased numbers of white adipocytes, lower fatty acid levels in plasma, an imbalanced adipokine secretion profile, and higher insulin responsiveness. These mice are lean even when subjected to a high-fat diet, indicative of altered lipid metabolism (51, 52). Moreover, autophagy is also important for the integrity and function of the pancreatic β -cells, which secrete insulin, a hormone regulating the storage and utilization of glucose and fat. β -Cell mass augmentation and insulin secretion are also reciprocally regulated by fatty acids. Autophagy is again indispensable for the compensatory β -cell mass augmentation in response to a high-fat diet (53, 54). Therefore, autophagy is involved in local hepatocyte lipid metabolism as well as the systemic regulation of lipid homeostasis. To delineate the impact of autophagy defects in each aspect on pathology of lipid imbalance, inducible conditional knock-out mouse models will be useful.

Autophagy is critical for lipid homeostasis. Unfortunately, factors that disrupt lipid homeostasis commonly suppress autophagy, leading to a vicious cycle. To achieve homeostasis, lipophagy should be upregulated during fasting to facilitate the mobilization of fatty acids and β -oxidation in hepatocytes in response to fluctuation in nutrient availability. However, a long-term high-fat diet impairs the selectivity of autophagic degradation toward lipid droplets in liver when mice fed with high-fat diet were starved (18). Obesity also suppresses autophagy. In liver, inhibition of autophagy is likely responsible for the obesity-induced ER stress and insulin resistance (55). Insulin resistance, which mimics starvation, causes flux of fatty acids from white adipose tissue to liver, where it is deposited. Impaired lipid autophagy impedes access of lipid. Autophagy inhibition-induced insulin resistance further augments the accumulation of lipids in liver. Inhibition of autophagy by a high-fat diet or obesity, therefore, exacerbates the imbalanced homeostasis (Fig. 3). In this regard, enhancement of autophagy would help restore lipid homeostasis.

Autophagy declines with age (56), correlating with altered metabolism manifested as ectopic fat deposition and intracellular garbage accumulation. This suggests that the imbalance of lipid homeostasis as well as waste accumulation and cellular functional degeneration due to suppressed autophagy may accelerate aging. In worms, autophagy is required for life span extension provided by dietary restriction (56). Resveratrol, which mimics starvation and induces autophagy, increases survival of mice fed with a high-fat diet and reverses age-related syndromes (57). Pharmacologic inhibition of mTOR by rapamycin

also extends life span in mice (58), providing a possibility that autophagy improves fitness and mediates longevity in mammals. Rapamycin prolongs the life span of mice, even late in their life (58), suggesting that enhancement of autophagy may possibly interrupt aging and related syndromes including increased cancer incidence (Fig. 3).

Autophagy Confronts Stress and Environmental Insults

Autophagy is upregulated in response to stress, including growth factor and nutrient limitation, energy depletion, and hypoxia. In yeast, starvation induces autophagy, which recycles intracellular constituents to support metabolism, leading to adaptation and survival. In mammals, this self-cannibalistic function is conserved, and autophagy-deficient mice cannot survive the neonatal starvation period and their tissues display indications of energy depletion (59).

The capability of autophagy to degrade proteins, lipids, sugars, glycogen, and nucleic acids provides cells the flexibility to utilize intracellular components or access-dedicated nutrient stores for energy production and biomass production under stress (4, 60). Autophagy generates substrates such as nucleosides, amino acids, fatty acids, and sugars from the breakdown of intracellular components. Metabolism of different substrates can produce unequal redox equivalents such as NADPH, which can support lipid biosynthesis and maintain cytosolic redox equilibrium (61, 62). Therefore, through selective autophagy, central metabolism may be supported by different substrates to restore metabolic and energy homeostasis, redox balance, and biomass production. In this way, autophagy can enable stress adaptation, maintenance of cellular fitness, and survival. Considering the nature of autophagy, inducible, selective, or nonselective bulk degradation within a defined, membrane-enclosed area, autophagy is an efficient way to reallocate intracellular "macromolecular stores" to support bioenergetics and for use as building blocks for biosynthetic pathways under stress conditions.

Autophagy and Host Defense

Autophagy can also prevent disease by degrading microbial invaders (xenophagy). The autophagic endomembrane system also delivers viral nucleic acids or microbial antigens to endosomes/lysosomes for induction of interferon or antigen presentation, activating immune responses (56, 63, 64). Recent findings indicate that impairment of the host defense function of autophagy cooperates with environmental factors such as infection to affect development of the chronic IBD Crohn's disease. IBD increases the risk of developing cancer (65).

The etiology of Crohn's disease has been attributed to the interaction of pathogens and genetic factors. A genome-wide study in patients of Crohn's disease identified the association of variants of autophagy genes *ATG16L1* and *IRGM* with disease susceptibility (64). *ATG16L1* is recruited

to the bacterial entry site, triggering autophagic degradation of pathogens and antigen presentation. This prevents persistence of pathogens and chronic inflammation (66, 67). Moreover, mice engineered to have a hypomorphic mutation in *ATG16L1* develop disease that mimics Crohn's disease. Hypomorphic *ATG16L1* causes an abnormal response to murine norovirus infection in the intestine. In the mutant mice, Paneth cells, the epithelial cells in the small intestine that secrete antimicrobial peptides to protect the intestine, show abnormal granule packaging and secretion and altered transcription profiles upon infection (Fig. 3). If additionally challenged by chemical-induced injury, these mice manifest aberrant cytokine production and inflammation in intestine. These phenotypes resemble what has been seen in patients with Crohn's disease (68). It will be interesting to see whether these *Atg16L* hypomorphic mutant mice are also tumor prone and whether mice engineered to express the human Crohn's-specific *ATG16L1* variant have a similar phenotype. Role of autophagy or autophagy-related process in this type of disease, therefore, is multifaceted (63, 64).

Autophagy and Cancer Initiation

Autophagy suppresses tumor initiation by limiting genome mutation

The housekeeping function of autophagy maintains turnover of proteins and organelles and ensures homeostasis and cellular health, preventing disease conditions. In response to stress, autophagy eliminates damaged proteins and organelles. This damage mitigation by autophagy can be important for survival of tumor cells, which are commonly subjected to metabolic stress due to insufficient vascularization. Autophagy-deficient murine cells, although more susceptible to metabolic stress, have evident DNA damage response activation and an increased frequency of chromosome gains and losses (69). Autophagy-deficient mice are tumor prone, and liver tumors therefrom, as well as human liver tumors, accumulate p62-containing protein aggregates, ER chaperones, and activate the DNA damage response (36). Thus, by taking out the garbage, autophagy may suppress genomic instability to limit tumor initiation and progression.

Autophagy may also hinder proliferation of cells with cancer mutations, in addition to limiting genomic mutations in cells. Inhibition of autophagy delays oncogene-induced senescence (70). Senescence is believed to be a tumor-suppressive mechanism attributed to the induction of permanent cell-cycle exit. By facilitating senescence, autophagy can limit propagation of oncogenic mutations, thereby suppressing tumorigenesis.

Autophagy suppresses tumor initiation and progression by limiting chronic inflammation

Autophagy maintains homeostasis by removing excess or damaged intracellular components and microbial invaders as well as by regulating lipid metabolism. This not only restrains damage, including genome instability, but also

the subsequent inflammation. In autophagy-suppressive conditions, persistence of unresolved damage leads to chronic cell death and inflammation. In liver and other tissues, this can provide a cancer-promoting microenvironment.

Autophagy provides internal resource to support metabolism and mitigates damage, allowing cells to survive stress. Autophagy-deficient murine tumor cells in a background of defective apoptosis, which commonly occurs in tumors, undergo necrosis when subjected to metabolic stress (71). Necrosis also occurs in tumor allografts when apoptosis and autophagy are inhibited concurrently and is associated with inflammation. Therefore, autophagy limits genetic instability and inflammation that can predispose to tumor initiation, promotion, and progression. In this regard, by suppressing the formation of a mutation- or inflammation-prone microenvironment, autophagy stimulation may prevent initiation of tumorigenesis.

Pharmacologic Manipulation of Autophagy for Cancer Prevention

Autophagy induction with rapamycin or metformin may reduce tumor initiation

Epidemiologic studies have linked metformin treatment with a reduced cancer risk in type 2 diabetic patients. Findings from *in vitro* and animal models also indicate that metformin suppresses tumorigenesis of various types of cancer. This anticancer activity of metformin has been attributed to activation of AMPK and the subsequent metabolic responses including reduced insulin resistance, insulin/insulin-like growth factor levels, and mTOR signaling (15). It is therefore reasonable to speculate that autophagy activation with metformin, which is involved in these altered metabolic responses, will lower cancer risk. Retrospective analysis in renal transplant recipients reveals that rapamycin suppresses tumorigenesis. Animal models also suggest the delayed tumor development with rapalog treatment (72). Given the role of autophagy in physiology discussed above, stimulation of autophagy with metformin or a rapalog may benefit patients with metabolic syndrome, chronic hepatitis, or IBD.

Ras-Driven Cancers Are Addicted to Autophagy

Oncogene activation induces high basal autophagy that promotes cancer survival

Because autophagy supports metabolism and confers a survival advantage in stress, cancer cells might be inherently more reliant on autophagy. Normal mammalian cells *in vitro* and *in vivo* have low basal autophagy and dramatically upregulate autophagy in stress such as starvation. Expression of an oncogenic *Ras* gene induces transformation *in vitro* or tumorigenesis *in vivo* and dramatically upregulates basal autophagy that is required to survive stress and starvation (73–75). Human cancer cell lines with *Ras* mutations also have high basal autophagy and are exquisitely sensitive to genetic ablation of

autophagy whereupon they either growth arrest or die (73, 75). Deficiency in the essential autophagy genes *Atg5* or *Atg7*, or the cargo receptor p62, impairs *Ras*-mediated tumorigenesis in mouse tumor allografts, and short hairpin RNA (shRNA) knockdown of *Atg5* or *Atg7* expression suppresses tumorigenesis of human cancer cell lines with activated *Ras* (73, 75). Loss of tumorigenesis with autophagy impairment is associated with reduced mitochondrial respiration, depletion of tricarboxylic cycle (TCA) metabolites citrate, aconitate, and isocitrate, and failure to maintain cellular energy homeostasis (73, 75). Thus, *Ras*-driven cancers are addicted to autophagy for survival, suggesting that targeting the autophagy pathway in this setting may be a novel approach to treat these aggressive cancers. How autophagy maintains mitochondrial metabolism in *Ras*-driven cancers is not yet known, but it may be required to provide mitochondrial substrates for TCA cycle function (4). Whether other cancers are addicted to autophagy remains to be investigated.

Pharmacologic autophagy inhibition sensitizes cancer cells to cell death

Because autophagy is a stress survival pathway, aggressive (e.g., metastatic) cancer cells may be more sensitive to pharmacologic as well as genetic autophagy inhibition. The autophagy inhibitor CQ, which induces lysosomal stress and prevents autophagic cargo degradation, preferentially kills *Myc*-expressing mouse cells *in vitro* in a p53-dependent manner. CQ impairs spontaneous lymphomagenesis in both *Myc*-transgenic mice that are a model for Burkitt's lymphoma, and in *atm*-deficient mice that are a model for ataxia telangiectasia (76). Thus, lysosomal stress and/or autophagy inhibition may enhance tumor suppression pathways and promote cancer cell death.

In a p53-deficient, *Myc*-induced mouse model of lymphoma, inhibition of autophagy promotes cell death and delays the recurrence of tumors following p53 reactivation or alkylating agent treatment (77). This suggests that once cancer occurs, tumor cells may rely on the function of autophagy to survive not only the metabolic demand resulting from proliferation, reprogramming of metabolism, and environmental stress (73, 75) but also the therapeutic stress. Taking advantage of the dependency of tumor cells on autophagy to survive, many clinical trials testing the utility of HCQ as an autophagy inhibitor are underway for advanced disease (78, 79).

Conclusions

As a cell-refreshing and metabolism-supporting pathway, autophagy is required for normal operation of cellular and organismal physiology. Autophagy-deficient mouse models have revealed the role of autophagy in preventing initiation and progression of chronic diseases. This context underscores the potential of autophagy stimulation/restoration as a strategy to prevent disease initiation and progression by sustaining normal physiology and handling

stress properly. Of note, as autophagy has multiple effects, the consequence of autophagy modulation is highly context-dependent. In addition to benefits, the risk of autophagy stimulation should be taken into account (79). For example, the magnitude and duration of autophagy activation may lead to different consequences. Overinduction may lead to perturbation of homeostasis, an autophagy traffic jam, or autophagy-induced cell death. Moreover, in contrast to efforts to exploit the cytoprotective nature of autophagy to prevent tumor initiation, inhibition of autophagy may promote cell death of existing cancers. This approach may be particularly effective against cancers with high basal autophagy and autophagy addiction (73, 75).

Our understanding of the role of autophagy in physiology and pathology is still at an early stage. Further investigation of the role of autophagy in different contexts could lead to the development of tractable regimens for modulating autophagy for cancer prevention.

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