

Review

Selective Autophagy in Cancer Development and Therapy

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Abstract

Like other cells in the body, tumor cells depend on the evolutionarily conserved autophagy pathway to survive starvation and stress. Simultaneously, autophagy represents an important tumor-suppressive mechanism. Recent studies have shed new light on this apparent discrepancy and revealed mechanisms by which autophagy can modulate different stages of cancer development. The molecular basis of selectivity in autophagy employs specific receptor molecules, such as p62/SQSTM1, which are able to link autophagy targets and autophagosomal membranes. We discuss the emerging principles of selective autophagy in cancer pathogenesis and treatment. *Cancer Res*; 70(9); 3431–4. ©2010 AACR.

Introduction

Cells adapt to stress by catabolizing damaged or unnecessary structures, thus maintaining their homeostasis. Autophagy is the ancient degradative pathway that mediates cell adaptation to a range of stress conditions. In its best-characterized form (macroautophagy; Fig. 1), autophagy manifests itself in the formation of the isolation membrane that gives rise to a double-membrane vesicle, the autophagosome, which sequesters cytosolic substrates and delivers them into the lysosome for degradation (1). Recently, autophagy has been connected to cancer development and progression: (1) it can be stimulated by tumor suppressors PTEN, TSC1, and TSC2; (2) it is inhibited by proto-oncogenes PI3K and Akt; and (3) defects in autophagy can lead to tumor development (2). The controversial view on autophagy as a cell death mechanism (2) has also been supportive of the tumor-suppressing role of this pathway. Yet, recent data indicate that established tumors depend on autophagy to survive starvation and hypoxia (3). Also, anticancer therapies, such as hormone antagonists, DNA-damaging drugs, and irradiation, induce autophagy as a pro-survival response (4), which may underlie adaptation of cancers to treatment seen in the clinic. Because of the apparently dual role of autophagy in cancer, it has been unclear whether this pathway should be inhibited or induced as part of the anticancer strategy.

Selective autophagy is a specialized type of autophagy, which specifically targets protein aggregates, organelles,

and intracellular pathogens (5). By removing dangerous cytosolic components, selective autophagy protects cells from oxidative and genotoxic stresses (6), and may thus constitute a tumor-suppressing mechanism (Fig. 1C and D). This process depends on receptor proteins (e.g., p62/SQSTM1; ref. 5), which link autophagic machinery with the substrates (Fig. 1B). A block in autophagy results in rapid accumulation of both autophagic substrates and receptor molecules, which also regulate cell signaling (6–8). This phenomenon could be exploited to target tumors and monitor their autophagy status.

Selective Autophagy as a Tumor Suppressor Mechanism

The tumor-suppressive role of autophagy was first shown in mice heterozygous for the Beclin-1 autophagy protein (2). These mice showed reduced autophagy and increased cellular proliferation, which translated into increased incidence of spontaneous malignancies, such as lymphomas, lung, and liver cancers (9). Although it has been tempting to explain the tumor-suppressing effect of autophagy on the basis of its proposed pro-death role, this concept is difficult to reconcile with the well-established homeostatic functions of autophagy (2). A more plausible explanation for the tumor-suppressing effect of autophagy can be derived from its role as a selective degradation pathway (Fig. 1C and D).

Experimental ablation of autophagy in mouse tissues, e.g., in *Atg7*^{-/-} mice, leads to profound accumulation of noxious protein aggregates and damaged mitochondria, which are responsible for the demise of nondividing neurons (10). Persistent DNA damage induced by the increased levels of reactive oxygen species (ROS), which are associated with the accumulated mutant proteins and dysfunctional mitochondria found in the autophagy-deficient tissues, is also proposed as a causative link between autophagy inhibition and enhanced tumor formation (3, 6).

Recently, accumulation of the selective autophagy receptor p62 in autophagy-deficient cells was directly linked to tumorigenesis (6). p62 is a member of the growing list of

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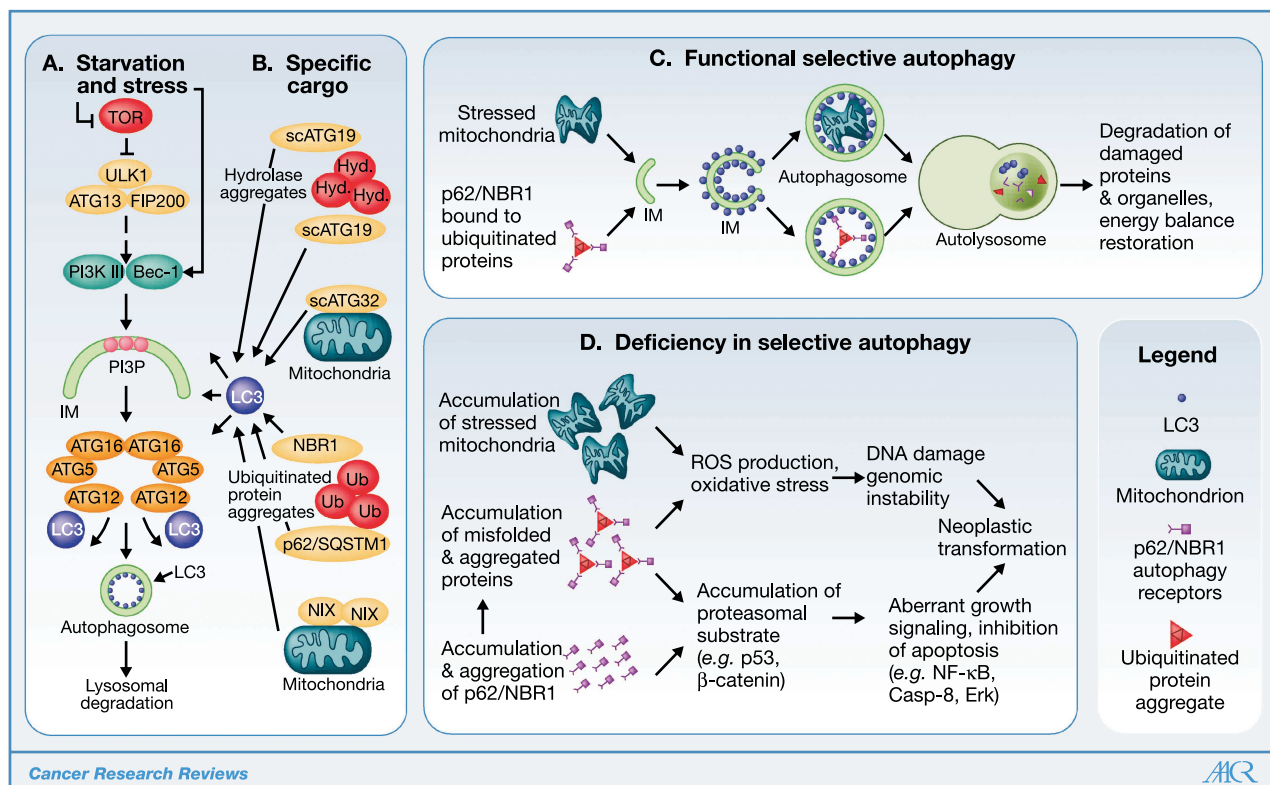


Figure 1. Selective autophagy and its role in cancer development. A, inhibition of target of rapamycin (TOR) kinase during starvation directly inhibits activity of the ULK1-ATG13-FIP200 complex. The class III PI3K (PI3K III)/Beclin-1 complex can be regulated by pro-apoptotic signaling initiated by various stress cues. Phosphatidylinositol-3-phosphate (PI3P; gray circles) is a lipid product of PI3K III activity that promotes recruitment of several proteins, e.g., the ATG16-ATG5-ATG12 complex, to the isolation membrane (IM). ATG12 is covalently conjugated to ATG5 and stimulates conjugation of LC3/GABARAP proteins (LC3) to phosphatidylethanolamine (not shown), a lipid found in the IM. LC3/GABARAP proteins may mediate membrane expansion. Autophagosomes must eventually fuse with lysosomes for their contents to be degraded. B, selective autophagy is induced by a specific cargo, a protein aggregate, or an organelle. Specialized proteins, *Saccharomyces cerevisiae* (sc)ATG19, scATG32, and mammalian p62/SQSTM1, NBR1, and NIX, which bind both to the substrate and to the autophagosome-associated proteins LC3/GABARAP, may direct autophagosome formation. C and D, selective autophagy clears aggregated proteins and stressed mitochondria and simultaneously provides substrates for catabolic reactions, which support cellular energetic balance. Deficiency in selective autophagy leads to accumulation of damaged mitochondria and protein aggregates, which are associated with increased oxidative stress and genomic instability. Accumulation of the selective autophagy receptors p62 and NBR1 may, in turn, lead to aberrant cell signaling with the outcome that cells loosen control over growth and apoptosis programs. The resulting neoplastic transformation may be further supported by concomitant inflammation (not shown), which is associated with necrosis that takes place when apoptotic programs are disabled.

autophagic receptors characterized by the presence of a LC3-interacting region (LIR), which allows interaction of these proteins with the autophagic machinery (via the ATG8 family proteins; Fig. 1B; ref. 5). By binding ubiquitin (Ub) via their C-terminal UBA domains, p62 and the related protein NBR1 mediate degradation of ubiquitinated cargo by selective autophagy (5). Interestingly, the accumulation of p62 in autophagy-deficient cells was shown to inhibit degradation of cancer-relevant proteins whose expression level is primarily regulated by the Ub-proteasomal pathway (i.e., p53 and β-catenin). p62 does so by sequestering ubiquitinated proteins and consequently preventing their accession by the proteasome (Fig. 1D; ref. 7).

Owing to its profound polymerization potential, p62 (also known as sequestosome-1) promotes sequestration of signaling components, such as aPKC, TRAF6, MEK5, and extracellular signal-regulated kinase (ERK), into specific protein

complexes, thereby regulating proliferation, survival, and apoptosis (8, 11–14). Accordingly, perturbation in abundance and/or localization of p62, as a result of autophagy modulation, may have broad consequences for the execution of major signal transduction pathways. For example, p62 knock-out, but also down-regulation by hypoxia-mediated autophagy, is associated with elevated ERK signaling (12, 15). Conversely, nuclear factor kappa-B (NF-κB) activation in Ras-transformed cells is impaired in the p62 knock-out mice (11). In addition, caspase-8 activation and apoptosis in response to tumor necrosis factor-alpha-related apoptosis-inducing ligand (TRAIL) treatment is also impaired in p62-deficient cells (8). The logical conclusion from these findings would be that accumulation of p62 following autophagy inhibition would lead to down-regulation of ERK signaling, whereas activation of NF-κB and caspase-8 in response to appropriate stimuli would be enhanced. Strikingly however,

inhibition of autophagy and the concomitant accumulation of p62 aggregates inhibited expression of NF- κ B-responsive genes in immortalized baby mouse kidney (iBMK) cells (6). Although the reason for this obvious discrepancy is currently unknown, it is possible that large p62 aggregates compromise fine-tuning and functionality of the signal-promoting complexes.

The fact that p62 accumulation as a consequence of autophagy inhibition could enhance tumorigenic properties of the iBMK cells (6) suggests that, in addition to clearing harmful cellular junk, selective autophagy may limit tumor formation by directly affecting cell signaling. Clearly, much more work is required to rigorously test this hypothesis as well as to assess the impact of autophagy modulation on oncogenic signaling in established tumors.

Selective Autophagy in Tumor Stress Response

Hypoxia. Low oxygen concentration (hypoxia) is typical for solid tumors. It has been shown to induce autophagic degradation of mitochondria (mitophagy) in affected cells (16). This measure is thought to protect cells from the increased ROS production under reduced oxygen availability and depends on transcriptional up-regulation of BNIP3 by HIF-1 (17). BNIP3, like the related protein NIX (Fig. 1B), localizes to mitochondria via its C-terminal transmembrane domain, where it might directly induce mitophagy via the interaction with GABARAP and LC3 proteins (18). In addition, BNIP3 and NIX might have a more general role in hypoxia-induced autophagy by disrupting Bcl-2/Beclin-1 interaction and thereby activating the Beclin-1/class III PI3K complex (17). Inhibition of selective autophagy under hypoxic conditions should lead to accumulation of stressed mitochondria and the increase in the oxidative stress burden. In theory, this approach could help limit tumor growth. It was reported, however, that Beclin-1 haploinsufficiency and reduction in autophagy led to increased necrosis in hypoxic tumors, which stimulated the growth of Akt-overexpressing *Beclin-1* \pm tumors (19). More studies using silencing of individual *ATG* genes are mandatory to understand whether autophagy inhibition in hypoxia will be a viable anticancer strategy.

Protein misfolding. Owing to genomic instability and increased mutation rates, cancer cells are predicted to display a high degree of protein misfolding. Locally unfolded polypeptide chains tend to interact nonspecifically via hydrophobic surfaces, which are usually confined to internal spaces of folded proteins. Molecular chaperones of the Heat Shock Protein (HSP) family, such as HSP90, are up-regulated in cancers to promote protein folding and to protect the cells from the toxic effects of protein aggregation, whereas chaperone-associated Ub E3-ligases, such as Parkin, mediate ubiquitination and proteolytic destruction of terminally misfolded proteins (5). However, as the proteasome cannot access bulky substrates, ubiquitinated proteins tend to aggregate and form inclusion bodies. This process may be facilitated by p62 and NBR1, which both cross-link ubiquitinated misfolded proteins and mediate their autophagic degradation (5). Selective autophagy is thus a back-up mechanism for the failed

proteasomal degradation of ubiquitinated misfolded and aggregation-prone proteins. Because ubiquitination has also been implicated in mitophagy (5), modulation of the expression levels of the Ub-binding autophagic receptors p62 and NBR1 by selective autophagy might also play a role in mitophagy.

Inhibition of autophagy in established tumors with high genomic instability could arguably lead to accumulation of misfolded proteins and endoplasmic reticulum and oxidative stresses, which could entail tumor cells' demise either via induction of apoptosis or necrosis. Recent reports have tested this idea in which drugs that lead to accumulation of misfolded proteins were combined with autophagy inhibition. In one study, blocking autophagy (using either lysosomal inhibitor chloroquine or *ATG7* siRNA) strongly enhanced vorinostat-mediated apoptosis in colon cancer cells (20). Vorinostat is a histone deacetylase inhibitor (HDACi) that disrupts HDAC6-mediated formation of the aggresome, a subcellular structure that sequesters protein aggregates and is thought to have a protective function (5). Remarkably, the combined HDAC-autophagy inhibition led to ubiquitinated protein accumulation and ROS generation, which was associated with enhanced cell death (20).

Bortezomib is another drug that affects degradation of ubiquitinated proteins. It blocks the proteasome, and its efficacy in multiple myeloma might be due to a failure of multiple myeloma cells to manage accumulating misfolded immunoglobulins. To test this hypothesis, Hoang and colleagues combined bortezomib with autophagy inhibitors chloroquine, 3-methyladenine, and *Beclin-1* siRNA. Surprisingly, although autophagy in multiple myeloma cells was enhanced by the bortezomib treatment (indicating a stress response induced by the accumulation of ubiquitinated proteins), blocking autophagy did not enhance bortezomib-mediated cytotoxicity and even had an antagonistic effect (21). In the very same report, however, the authors refer to unpublished results from other groups in which a synergy between proteasome and autophagy inhibition in multiple myeloma had been achieved (21). Clearly, more focused dissection of the interplay between the proteasome and autophagy degradation pathways is required to understand whether autophagy inhibition will be beneficial in combination with bortezomib or similar drugs.

Senescence. Protein misfolding rates increase as cells age. Cells can undergo programmed aging (senescence) also as part of the stress reaction regulated by p53 (22). Recently, the co-chaperone BAG3 was described to be up-regulated in aging cells where it localizes to Ub- and p62-positive protein aggregates (23). Senescence is known to depend on autophagy (24) and BAG3 is thus a candidate mediator for senescence-specific autophagic degradation of ubiquitinated proteins. Curiously, the related co-chaperone BAG1 is expressed at higher levels in younger cells, where it plays a role in proteasomal degradation of ubiquitinated proteins. It is suggested that the shift in BAG1-BAG3 expression ratio during cell senescence can activate autophagy in aged cells (23). BNIP3 and NIX have also been shown to be upregulated in senescent cells (24), suggesting that mitophagy might be another feature of aging cells. Because induction of senescence

is a desirable outcome of antitumor therapies, more studies are necessary to understand the role of autophagy in senescence.

Conclusions and Perspectives

Broad conservation of the autophagy pathway from yeast to man (1) underscores the pivotal role for the autophagy in maintaining cellular homeostasis. Yet, in stark contrast to unicellular eukaryotes, starvation is not the issue that a normal cell of a multicellular organism would face on a regular basis. Rather, accumulating evidence suggests that mammalian autophagy is chiefly required for cell cleansing and remodeling. Selective autophagy, mediated by specific receptor proteins, seems to be instrumental in these processes. Failure in selective autophagy is likely to cause accumulation of protein aggregates and damaged organelles that may mediate neoplastic transformation. In contrast, established tumors depend on autophagy for covering their increased metabolic needs. Selective autophagy may ensure tumor survival via degradation of misfolded proteins and damaged organelles that accumulate in genetically unstable tumor cells. Targeting nonspecific autophagy as well as its selective forms may thus prove beneficial in fighting

malignancies. As p62 and NBR1 play a role in signal transduction pathways that promote proliferation and survival, targeting autophagic receptors in the context of cancer could bring a double benefit: inhibition of both tumor cell signaling and garbage disposal.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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