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Oncosuppressive Functions of Autophagy

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Abstract

Macroautophagy (herein referred to as autophagy) constitutes a phylogenetically old mechanism leading to the lysosomal degradation of cytoplasmic structures. At baseline levels, autophagy exerts homeostatic functions by ensuring the turnover of potentially harmful organelles and long-lived aggregate-prone proteins. Moreover, the autophagic flow can be dramatically upregulated in response to a plethora of stressful conditions, including glucose, amino acid, oxygen, or growth factor deprivation, accumulation of unfolded proteins in the endoplasmic reticulum, and invasion by intracellular pathogens. In some experimental settings, stress-induced autophagy has been shown to contribute to programmed cell death. Nevertheless, autophagy most often confers cytoprotection by providing cells with new metabolic substrates or by ridding them of noxious intracellular entities including protein aggregates and invading organisms. Thus, autophagy has been implicated in an ever-increasing number of human diseases including cancer. Autophagy inhibition accelerates the demise of tumor cells that are subjected to chemo- or radiotherapy, thereby constituting an interesting target for the development of anticancer strategies. However, several oncosuppressor proteins and oncoproteins have been recently shown to stimulate and inhibit the autophagic flow, respectively, suggesting that autophagy exerts *bona fide* tumor-suppressive functions. In this review, we will discuss the mechanisms by which autophagy may prevent oncogenesis. *Antioxid. Redox Signal.* 14, 2251–2269.

Introduction

Coined in 1963 by the British biochemist Christian de Duve [who was awarded the Nobel Prize in Physiology and Medicine in 1974, for his pioneering work on lysosomes (46)], the term "autophagy"—which has been derived from a Greek word and means self- ("auto") eating ("phagy")—was introduced upon the electron microscopic observation of double-membrane vacuoles containing disintegrating cytoplasmic organelles (8, 35, 47, 186). During the next two decades, the autophagic pathway was dissected at the ultrastructural level, leading to the identification of prominent intermediate structures including the phagophore (from which autophagosomes are generated, see below) (78) and the amphysome (which mediates the convergence between the autophagic and the endocytic pathways) (80). In 1992, it was demonstrated that yeast cells possess an autophagic machinery that is highly similar to that of their mammalian counterparts (226), a breakthrough discovery that laid the basis for two decades of feverish autophagy research in this model organism. In 1993, the first genetic screen aimed at identifying autophagy-defective mutants in *Saccharomyces cerevisiae* was launched, leading to the identification of 15 autophagy-related (*atg*) genes that are involved in autophagy in yeast (235). During this period, several research groups approached the same objective (*i.e.*, the isolation of autophagy-deficient yeast stains) by different techniques, resulting in the characterization of ~40 *bona fide atg* genes (95, 119, 234, 235). At the beginning of the 21st century, autophagy research has literally exploded, as witnessed by the logarithmic increase in the number of scientific reports that have been published on this topic starting from 1998 (116). In 1998, the molecular characterization of mammalian *ATG* genes began with the cloning of *ATG5* and *ATG12* and with the

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demonstration that the yeast conjugation system relying on Atg5 and Atg12 proteins is evolutionarily conserved (174). One year later, Beth Levine's group discovered that Beclin 1, the human ortholog of yeast Atg6, physically interacts with the oncoprotein BCL-2 and behaves as a (haploinsufficient) tumor suppressor (140, 258), unraveling for the first time an intimate link between autophagy and tumorigenesis. During the last decade, the effector machinery of autophagy and its upstream regulators have been elucidated in ever-increasing detail, and it has become clear that autophagy contributes to normal development, cellular differentiation, and tissue remodeling (27, 135). Moreover, it has been discovered that deregulated autophagy plays a major role in a wide array of human diseases (136), including inflammatory disorders (238), neurodegeneration (117, 152, 209), infections by intracellular pathogens (134), and cancer (133).

Until now, three forms of autophagy have been described, that is, chaperone-mediated autophagy, microautophagy, and macroautophagy, which differ with respect to their physiological functions and their mode of cargo delivery to the lysosome (70). During chaperone-mediated autophagy, cytosolic proteins containing peptide sequences biochemically related to the consensus KFERQ motif are unfolded and directly translocated across the lysosomal membrane by cytosolic and lysosomal chaperones (50, 136). Conversely, both micro- and macroautophagy are initiated by the isolation of organelles and/or portions of the cytoplasm by a sequestering membrane. However, microautophagy proceeds *via* the direct engulfment of the cargo by the lysosomal membrane (through phenomena of septation/invagination), whereas a membranous organelle other than the lysosome, the autophagosome, intervenes during macroautophagy (70, 157, 218). This review will focus on macroautophagy (hereafter referred to as autophagy), owing to its peculiar features (which place it among the most impressive cytoprotective mechanisms for eukaryotic cells) as well as to its profound physiopathological and therapeutic implications (70, 136).

Autophagosomes, the double-membraned vesicles that sequester cytoplasmic components and deliver them to lysosomes for degradation, constitute the major morphological hallmark of autophagy. The intracellular prevalence of autophagosomes is one of the parameters currently employed to monitor the autophagic flow (118, 229). Autophagosomes originate from phagophores (also known as isolation membranes), vesicular organelles whose subcellular source has been the subject of an intense debate (70, 250). Isolation membranes have been indeed suggested to constitute microvesicular derivations of the Golgi apparatus (143, 251) or to originate from mitochondria (149). However, recent evidence strongly supports the notion [first suggested by Locke in 1969 (142)] that phagophores develop from the endoplasmic reticulum (190, 256). Driven by an autophagic stimulus, the phagophore (which in yeast is generated at the so-called phagophore assembly site) progressively expands while enwrapping the material to be degraded and eventually closes up to generate a double-membraned autophagosome (249, 254, 257). At this stage, autophagosomes (which in the past were also called "early autophagic vacuoles") contain virtually intact cytoplasmic components that can be easily observed by transmission electron microscopy owing to the relatively electron-transparent nature of the autophagosomal lumen (70, 118, 229).

Autophagosomes mature by fusing with lysosomes, coincident with the acidification of the luminal microenvironment and the activation of lysosomal hydrolases (249, 254, 257). According to current nomenclature, the resulting organelles (which in the past were also known as "late autophagic vacuoles") are referred to as autophagolysosomes or autolysosomes. The auto(phago)lysosome is characterized by a single-membraned and electron-dense morphology, owing to the fact that both its luminal content and its inner membrane (which altogether, in yeast, are known as autophagic body) are degraded by lysosomal enzymes shortly after the lysosomal–autophagosomal fusion (70, 118, 229). Finally, permeases of the auto(phago)lysosomal membrane transfer the substrates generated by the degradation of the autophagic body to the cytosol, where they can reenter bioenergetic or biosynthetic metabolic circuitries (249, 257) (Fig. 1).

Cellular Roles of Autophagy

Autophagy is crucial for the maintenance of intracellular homeostasis and mediates an adaptive response to distinct types of cellular stress (70, 175). In virtually all healthy cells, baseline levels of autophagy exert homeostatic functions by ensuring the disposal of peptidic aggregates, long-lived proteins, and aging/damaged (and hence potentially harmful) organelles, thereby assuring the quality control of all essential cytoplasmic components (70, 135). The flow through the autophagic pathway can be dramatically upregulated in

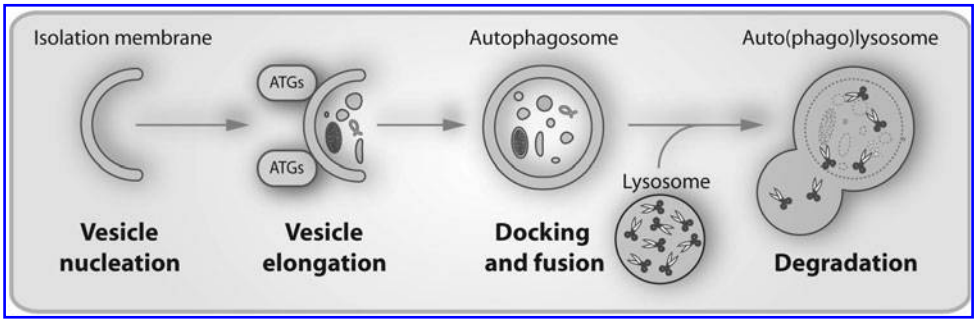

FIG. 1. The autophagic flow. Autophagy is initiated by the generation of the so-called phagophore, an isolation membrane that likewise derives from the endoplasmic reticulum. Sustained by the activity of multiple autophagy-related (ATG) proteins, the phagophore expands, surrounds the material destined to degradation, and eventually forms a double-membraned vesicle known as autophagosome. Autophagosomes mature by fusing with lysosomes or late endosomes and hence generate auto(phago)lysosomes. Finally, the luminal content of the auto(phago)lysosome is catabolized by acidic hydrolases, resulting in the generation of metabolic substrates that are reexported into the cytosol *via* permeases of the auto(phago)lysosomal membrane.

response to a variety of stressful conditions and chemical triggers, including (but not limited to) the following conditions (70): glucose and/or amino acid deprivation (105); growth factor withdrawal, a condition that mimics nutrient deprivation (because growth factor-deprived cells are unable to take up sufficient nutrients to maintain cellular bioenergetics) (9, 148); hypoxia, which activates a cell-wide stress response that is orchestrated by the transcription factor hypoxia-inducible factor 1 (167); invasion by intracellular pathogens including viruses, bacteria, and eukaryotic parasites (134); accumulation of unfolded proteins within the endoplasmic reticulum lumen (127, 208); increased cytosolic Ca^{2+}concentrations (72); exposure to radio- and chemotherapy (28, 145, 177); pharmacological (with xestospongin B) or genetic inhibition of the inositol 1,4,5-trisphosphate (IP_3) receptor (41, 44, 237); pharmacological (with pifithrin-α) or genetic inhibition of the cytosolic pool of the tumor suppressor protein p53 (154, 230, 231); activation of the IκB kinase complex, which is also required for the stress response coordinated by the transcription factor NF-κB (42, 43); and administration of the polyamine spermidine (52), the polyphenol resveratrol (179, 180), or the immunosuppressant macrolide rapamycin (4), all of which exert autophagy-dependent antiaging effects (176).

In many circumstances, cells that have been stressed beyond a certain threshold die by manifesting the cytoplasmic accumulation of autophagosomes and auto(phago)lysosomes, an observation that has often been (mis)interpreted as if the autophagic machinery would actively contribute to cellular suicide (125). Thus, hundreds of scientific publications have described "autophagic cell death" (also dubbed "type 2 cell death") as a lethal process that is accompanied by massive autophagic vacuolization and hence is morphologically distinct from apoptosis ("type 1 cell death") and necrosis ("type 3 cell death") (66, 122, 124). Although "autophagic cell death" undeniably exists as a morphological entity (124), mammalian cells rarely (if ever) succumb to the activation of the autophagy machinery, and the cellular demise occurs together with (rather than through) autophagy (125). In this setting, autophagy most likely represents a desperate attempt of cells to cope with stress that eventually turns out to be futile and hence precedes/accompanies cell death. Accordingly, pharmacological or genetic inhibition of autophagy accelerates the apoptotic or necrotic demise of cells that otherwise would endure nutrient and growth factor deprivation, hypoxia, ionizing irradiation, and chemotherapeutic agents (10, 18, 79, 125, 163). It should be noted that in selected (and most often non-mammalian) experimental scenarios (for instance, in developing *Drosophila melanogaster* midgut and salivary glands), autophagy has been formally demonstrated to contribute to cell death (11, 12, 49). Nevertheless, in mammalian cells, autophagy constitutes a prominent cytoprotective mechanism (175).

Molecular Machinery for Autophagy

The molecular characterization of the "core" machinery for autophagy (*i.e.*, the subset of proteins that is absolutely required for the generation, maturation, and disposal of autophagosomes) has lagged until the end of the 20th century, when *ATG* genes were first discovered in yeast, followed by the identification of their orthologs in higher eukaryotes (253, 254). Several ATG proteins function in a non-redundant manner, and the inhibition of just one ATG protein usually suffices to block the autophagic pathway (257). This can be achieved by genetic or pharmacological means and can lead to (i) a global arrest of the autophagic cascade, when autophagy-initiating factors are targeted; (ii) the accumulation of immature phagophores, if proteins that mediate phagophore elongation are inhibited; (iii) the accumulation of autophagosomes, if the autophagosomal–lysosomal fusion is blocked; or (iv) the accumulation of auto(phago)lysosomes, when the pH-dependent activation of lysosomal hydrolases is prevented (70, 157).

In mammalian cells, phagophore nucleation is regulated by the balance between class I and class III phosphatidylinositol 3-kinase (PI3K) enzymatic activities (198). The class III PI3K enzyme hVPS34 (the ortholog of yeast Vps34) catalyzes the phosphorylation of phosphatidylinositol to phosphatidylinositol-3-phosphate, a molecular signal that promotes the recruitment of essential ATG proteins (*e.g.*, ATG7 and ATG10) (185, 252). hVPS34 has been found in at least three distinct supramolecular complexes, whose precise composition affects the catalytic activity of hVPS34 (254) (Fig. 2A). These complexes invariably contain Beclin 1 and the myristylated kinase p150 (the orthologs of yeast Atg6 and Vps15, respectively) (157, 254) and can interact with a number of additional factors including the hVPS34 stimulators UV irradiation resistance-associated tumor suppressor gene (UVRAG) (137), BAX-interacting factor 1 (also known as endophilin B1) (224), AMBRA1 (59), and ATG14L (the mammalian ortholog of yeast Atg14) (165, 264) as well as the hVPS34 inhibitor RUBICON (165, 264). In this context, antiapoptotic members of the BCL-2 protein family (including BCL-2 itself and BCL-X_L) reportedly exert antiautophagic functions by sequestrating Beclin 1 [which contains a *bona fide* BCL-2 homology 3 (BH3), domain (19)] into inactive complexes (155, 195). Accordingly, BH3-only proteins, pharmacological BH3 mimetics (146, 153), as well as posttranslational modifications that decrease the affinity of Beclin 1 for BCL-2–like proteins (including Beclin1 phosphorylation by death-associated protein kinase 1 [DAPK1] and by c-Jun N-terminal kinase 1) (244, 259, 260) have all been shown to stimulate autophagy by displacing Beclin 1 from BCL-2/X_L–mediated inhibition.

Of note, the role of some hVPS34-containing complexes in the autophagic pathway is not limited to the early stage of phagophore nucleation but extends to the maturation of autophagosomes (254). Knockdown of RUBICON by RNA interference has been shown to enhance the maturation of autophagosomes (and to stimulate the endocytic pathway) (165), whereas its ectopic overexpression reportedly results in aberrant late endosomal/lysosomal structures and impaired autophagosome maturation (264). Moreover, Beclin 1-bound UVRAG can interact with the class C VPS complex (a key component of the endosomal fusion machinery), thereby stimulating the fusion between autophagosomes and lysosomes (138). Thus, multiple hVPS34-containing complexes exist, which participate in distinct steps of the autophagic cascade. Although class I PI3Ks also generate phosphatidylinositol-3-phosphate, their activation does not stimulate autophagy (198). Rather, class I PI3Ks exert autophagy-inhibitory functions by synthesizing other phosphoinositides, particularly phosphatidylinositol-3,4,5-trisphosphate (PIP_3), which indirectly activates the master inhibitor of autophagy in eukaryotic organisms, the mammalian target of rapamycin (mTOR) (194, 257).

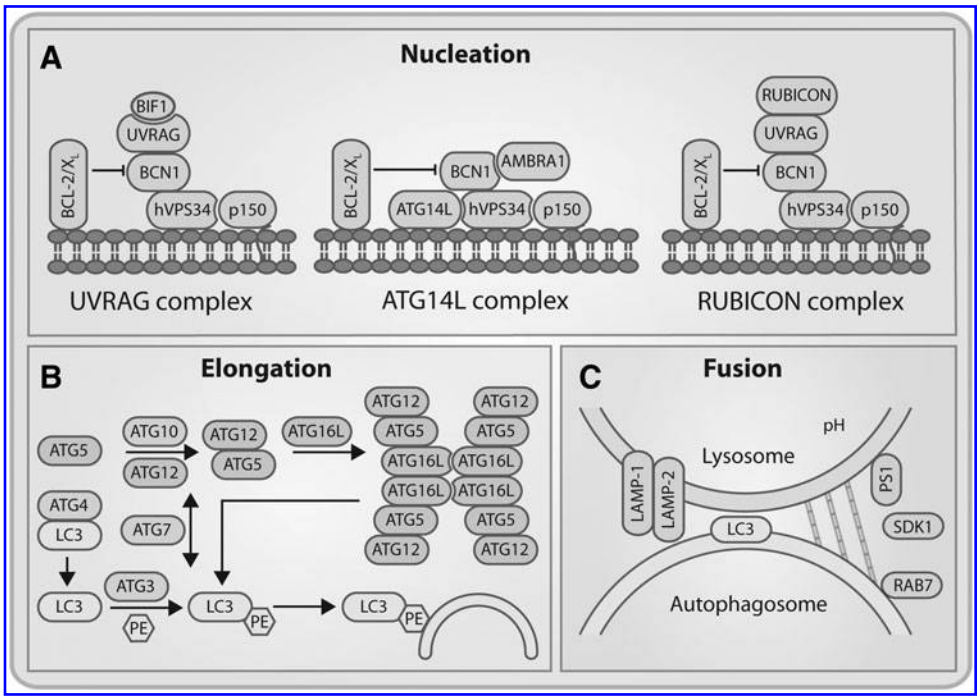

FIG. 2. The autophagic core machinery. The biochemical cascade for the execution of autophagy can be subdivided into three distinct (but intimately interconnected) modules. **(A)** Phagophore nucleation is sustained by the products of the class III phosphatidylinositol 3-kinase (PI3K) enzyme hVPS34. This kinase can interact with several proteins with pro- or anti-autophagic functions, including Beclin 1 (BCN1), p150, UV irradiation resistance-associated tumor suppressor gene (UVRAG), BAX-interacting factor 1 (BIF1), AMBRA1, ATG14L, and RUBICON. **(B)** The elongation of the phagophore is mediated by two ubiquitin-like conjugation systems that altogether promote the assembly of the ATG16L complex and the processing of LC3. PE, phosphatidylethanolamine. **(C)** Several factors affect the fusion between autophagosomes and lysosomes, including LC3, the lysosomal membrane proteins LAMP-1 and LAMP-2, the GTP-binding protein RAB7, the ATPase SKD1, the microtubular network, the pH of lysosomes, and possibly presenilin 1 (PS1). See the main text for further details.

In mammalian cells, the elongation and expansion of phagophores reportedly involves two distinct (though similar and functionally interconnected) ubiquitin-like conjugation systems (76, 189) (Fig. 2B). Notably, these conjugation systems are highly conserved and are also required for autophagy in lower eukaryotes including yeast (85, 114, 166, 173, 219). First, ATG12 is conjugated to ATG5 by the sequential activity of ATG7 and ATG10 (functioning as an E1-like and an E2-like enzyme, respectively). The resulting ATG5-ATG12 noncovalently interacts with ATG16L, which oligomerizes to assemble a supramolecular structure of ~800 kDa known as the ATG16L complex (62). Second, LC3 (the mammalian ortholog of yeast Atg8) undergoes ATG4-mediated proteolytic cleavage and is then conjugated to the phospholipid phosphatidylethanolamine by the activity of ATG7 and ATG3 (an additional E2-like enzyme) (227, 228). The immature form of LC3 (also known as LC3-I) is cytosolic, whereas lipidated LC3 (LC3-II) binds to forming autophagosomes, thereby providing a specific marker of autophagy that can be monitored by immunofluorescence or immunoblotting (118, 229). Of note, LC3-II initially binds to both faces of the phagophore membrane and it is then removed from the outer autophagosomal membrane. This occurs after the fusion between autophagosomes and lysosomes or late endosomes (253, 254). Recent reports suggest the existence of a bidirectional interconnection between the ATG5-ATG12-ATG16L and the LC3 conjugation systems. On one hand, the ATG16L complex would indeed specify the site of LC3 lipidation (62) (also) by functioning as an E3-like enzyme (84). On the other hand, the LC3 conjugation machinery can influence the formation of the ATG16L complex. Thus, *atg3*$^{-/-}$ mice fail to convert LC3-I into LC3-II (as expected), but also exhibit impaired ATG5-ATG12 conjugation, delayed dissociation of the ATG16L complex from phagophores, and structural alterations of autophagosomes (220). Accordingly, overexpression of an inactive ATG4 mutant results in inhibited processing of pro-LC3, impaired LC3-I → LC3-II conversion, and appearance of a relevant fraction of nearly complete but open autophagosomes (61).

The fusion between autophagosomes and lysosomes or late endosomes is well characterized in yeast (98, 240) but poorly studied in mammalian cells (150). Regulators of the autophagosomal–lysosomal fusion process include LC3 itself (183), the lysosomal membrane proteins LAMP-1 and LAMP-2 (210), the small GTP-binding protein RAB7 (99), the AAA-type ATPase SKD1 (184), the microtubular network (120), the luminal pH within lysosomes (which is also critical for the proper activation of acidic hydrolases) (110), and possibly presenilin 1 (which has also been linked to Alzheimer's disease) (54) (Fig. 2C).

Autophagy Regulation

mTOR (ortholog of yeast TOR1 and TOR2) is an evolutionarily conserved serine/threonine kinase that continuatively adjusts several aspects of cellular metabolism (*e.g.*, protein translation, ribosome biogenesis, autophagy, cell cycle progression, cell death) to the availability of nutrients, growth factors, and energy (ATP) (13, 26, 60). In higher eukaryotes, TOR can be found in at least two distinct multiprotein complexes, which are referred to as TOR complex 1 (TORC1, also known as CREB-regulated transcription coactivator 1) and TORC2 (CREB-regulated transcription coactivator 2) (144). Among these complexes, TORC1 exerts the most prominent autophagy-regulatory function (36, 254).

Nevertheless, TORC2 also can influence autophagy by catalyzing the activating-phosphorylation of AKT1 (also known as protein kinase B) (213), a serine/threonine kinase (which is also regulated by the plasma membrane levels of PIP_3) that promotes the autophagy-inhibitory functions of TORC1 (see below) (14, 70, 254). TORC2 exhibits a lower sensitivity to acute pharmacological inhibition with rapamycin than TORC1 (14, 144). Still, the sensitivity of TORC2 to rapamycin can be enhanced (at least in some cell types) by prolonged exposure or by the use of derivatives of this agent (212).

The lipophilic macrolide rapamycin is (one of) the most prominent and most effective pharmacological agent(s) currently employed to stimulate autophagy in laboratory conditions, an effect that—so far—has been entirely ascribed to its TORC1-inhibitory functions (253, 254). Indeed, rapamycin and its derivatives (the so-called "rapalogs") are known to bind to the immunophilin FK506-binding protein 12, thereby stimulating its ability to sequestrate TOR within an inactive molecular complex and allosterically inhibiting TORC1 (21, 94). Recent reports challenge this notion. First, both rapamycin and RNA interference-mediated knockdown of one of the key downstream effectors of mTORC1 (the 70-kDa ribosomal S6 kinase 1) inhibit (rather than stimulate) DNA damage-induced autophagy in cancer cells (261). Second, TORC1 also exerts rapamycin-resistant autophagy-suppressive functions, which can be blocked by competitive inhibition of its kinase activity (233). Thus, the precise molecular mechanisms underlying the autophagy-modulatory effects of rapamycin needs to be reevaluated.

When nutrient and growth factors are available, mTORC1 transduces upstream antiautophagic signals by binding to a multiprotein complex including unc-51-like kinases 1 and 2 (ULK1 and ULK2), mATG13, and FIP200 (orthologs if yeast Atg1, Atg13, and Atg17) (63, 106), thereby maintaining ULKs and mATG13 in a hyperphosphorylated (inactive) state (71, 104). In autophagy-inducing conditions, mTORC1 rapidly dissociates from the ULK-mATG13-FIP200 complex, allowing for partial dephosphorylation of ULKs and mATG13, ULK activation, and ULK-mediated phosphorylation of mATG13 and FIP200 (71, 104) (Fig. 3). Recently, another mATG13- and ULK1-interacting protein, ATG101, has been discovered (90, 169), although its exact contribution to autophagy initiation remains to be elucidated.

Several signaling pathways that emanate from both the extracellular and the intracellular microenvironment are linked to mTOR (36). The availability of growth factors is mostly monitored by receptor tyrosine kinases (RTKs) at the plasma membrane. Upon ligand binding, RTKs oligomerize, autophosphorylate, and acquire catalytic activity, thereby stimulating two crucial signal transducers, class I PI3Ks and the small GTPase RAS. By producing PIP_3, class I PI3Ks induce the plasma membrane recruitment of AKT1 and of its activator phosphoinositide-dependent protein kinase 1, resulting in the generation of an antiautophagic signal that is transduced by the tuberous sclerosis complex 1/2 (TSC1/TSC2) (97). In the presence of growth factors and nutrients, TSC1-bound TSC2 functions indeed as an inhibitor of autophagy by activating the GTPase activity of RHEB, a small GTP-binding protein that (in its GTP-bound form) interacts with (thereby activating) mTORC1 (73, 96, 263). The implication of RAS in autophagy regulation is controversial. Indeed, RAS has been shown to inhibit autophagy *via* the PIP_3-AKT1-mTORC1 pathway (64), but also to stimulate a signal transduction cascade involving extracellular signal-regulated kinases (193), which are known to stimulate autophagy (also) by catalyzing the phosphorylation-dependent inactivation of TSC2 (151, 241, 254) (Fig. 3).

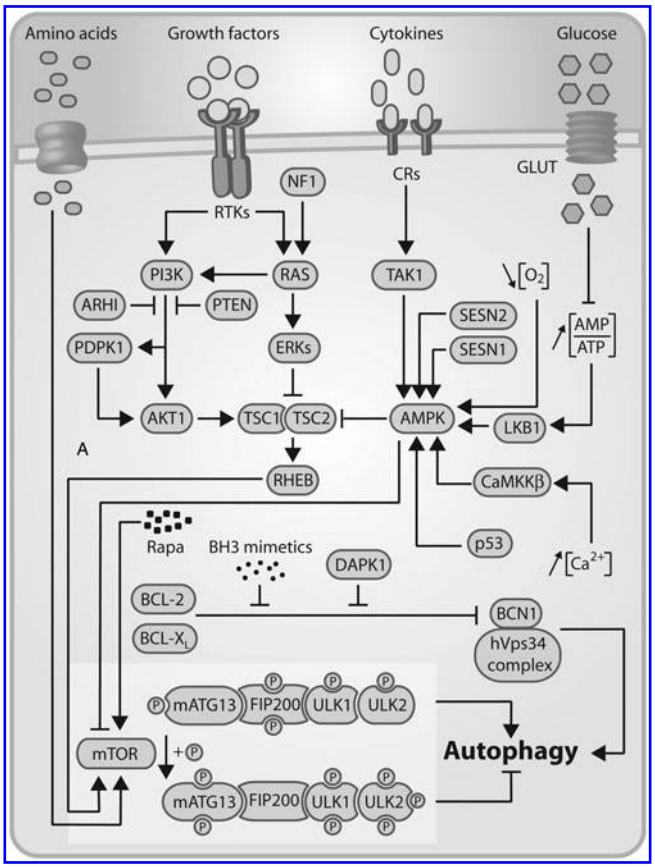

FIG. 3. Autophagy regulation. The mammalian target of rapamycin (mTOR) kinase is a master regulator of autophagy. The abundance of glucose, amino acids, and growth factors is detected by dedicated molecular sensors that relay the information to mTOR, which actively suppresses autophagy in physiological circumstances. Under conditions of stress (*e.g.*, nutrient deprivation, growth factor withdrawal, cytokine stimulation, hypoxia, Ca^{2+} overload), the kinase activity of mTOR is shut down, allowing for the partial dephosphorylation of a supramolecular complex composed of unc-51–like kinases 1 and 2 (ULK1 and ULK2), mATG13, and FIP200. Several oncoproteins and tumor suppressor proteins influence the regulation of autophagy. See the main text for further details. AMPK, AMP-activated protein kinase; ARHI, aplasia Ras homolog member I; CaMKKβ, calmodulin-dependent kinase kinase β; CR, cytokine receptor; DAPK1, death-associated protein kinase 1; ERK extracellular signal-regulated kinase; GLUT, glucose transporter; NF1, neurofibromin 1; PDPK1, phosphoinositide-dependent protein kinase 1; PTEN, phosphatase and tensin homologue deleted on chromosome 10; Rapa, rapamycin; RTK, receptor tyrosine kinase; SESN, sestrin; TAK1, transforming growth factor β-activating kinase 1; TSC, tuberous sclerosis complex.

By responding to decreased ATP/AMP ratios, the AMP-activated protein kinase (AMPK) functions as an indirect sensor of nutrient and energy levels (92). In this case, AMPK activation is mediated by the upstream kinase LKB1 (141, 216,

248) and transduces a multifaceted proautophagic signal by phosphorylating TSC2 (38), the mTORC1 component regulatory-associated protein of mTOR (83, 86, 112), as well as the cell cycle inhibitor $p27^{Kip1}$ (which has also been attributed with autophagy-stimulatory functions) (139). Besides sensing low ATP levels, AMPK also responds to increased cytosolic Ca^{2+} concentrations (resulting in the activation of the calmodulin-dependent kinase kinase β) (91), cytokine administration (which activates the transforming growth factor β-activating kinase 1) (89), hypoxia (191, 214), and stabilization of p53 [which has been shown to transactivate the β1 and β2 subunits of AMPK (57, 58) as well as its activators sestrin 1 and 2 (22, 23, 156)] (Fig. 3).

Oncosuppressor Proteins with Autophagy-Stimulatory Properties

Since the discovery that Beclin 1 behaves as a (haploinsufficient) tumor suppressor (140), the molecular machineries for autophagy regulation and execution have been functionally linked to an ever-growing number of oncoproteins and tumor suppressor proteins (177). Multiple proteins with established oncosuppressive functions have been shown to positively modulate autophagy (Fig. 3), suggesting that autophagy inhibition may favor (or at least be permissive for) tumorigenesis (Table 1) (69).

Both the phosphatase and tensin homolog deleted on chromosome 10 (PTEN) (45) and the RAS-related protein aplasia Ras homolog member I (also known as DIRAS3) (5, 147) promote autophagy by antagonizing PI3K-mediated signaling (7). Germline mutations of *PTEN* cause Cowden's and Bannayan-Riley-Ruvalcaba's syndromes, two hamartoma syndromes with an increased risk of breast, thyroid, and endometrial cancers. In addition, *PTEN* is affected by somatic mutations or silenced in multiple types of cancers (45). Aplasia Ras homolog member I is downregulated in a high proportion of ovarian cancers because of promoter hypermethylation or loss of heterozygosity (56).

Loss-of-functions mutations of *neurofibromin 1* (*NF1*) lead to type I neurofibromatosis, a condition characterized by a high incidence of benign and malignant tumors (33, 34, 215). Neurofibromin 1 functions as a GTPase-activating protein for RAS, and its absence has been associated with constitutive mTOR signaling owing to AKT1-mediated TSC2 inactivation (33, 103). TSC, an autosomal dominant disorder characterized by the formation of hamartomas in a wide range of human tissues, is caused by mutations in either *TSC1* or *TSC2* (97, 236). Germline mutations in the gene coding for the LKB1 kinase (an AMPK activator, see above) provoke the hereditary intestinal polyposis disease known as Peutz-Jeghers's syndrome (88). Moreover, inactivation of LKB1 has been recorded in a significant fraction of lung adenocarcinomas and squamous cell carcinomas (100).

Deletions of *beclin 1* are frequently found in breast, ovarian, and prostate cancers (137), and the downregulation of Beclin 1 has been observed in distinct tumor types including cerebral, gastric, and colorectal cancer (3, 171, 217). *Beclin 1* haploinsufficiency has been associated with increased tumorigenicity in mice (202), and a number of functional studies have corroborated the implication of Beclin 1 in oncogenesis (25, 140, 258). Some proautophagic Beclin 1 interactors also exert *bona fide* oncosuppressive functions. This applies to BAX-interacting factor 1, which is downregulated in human colorectal adenocarcinoma (37) and whose knockout enhances the development of spontaneous tumors in mice (224, 225); UVRAG, which is mutated at high frequency in human colon and gastric cancers (113, 137); as well as to BH3-only proteins such as BAD (155), BIK (204), BIM (1, 77), BNIP3L (10), NOXA (1), and PUMA (255), whose expression is lost or severely impaired in an array of human malignancies (131, 246). Notably, the role of another component of the hVPS34-containing class III PI3K complex, p150, in oncogenesis remains controversial. Indeed, p150 is overexpressed in cervical carcinomas, presumably owing to large-scale genomic alterations that involve the locus 3q21.1–23 (247).

The tumor suppressor $p19^{ARF}$ is mutated or lost in several solid and hematological malignancies (55, 74, 129). $p19^{ARF}$ exerts prominent oncosuppressive functions by activating p53 in response to a number of potentially tumorigenic stimuli (168), but also mediates p53-independent proapoptotic and proautophagic effects (2, 207). A short mitochondrial isoform of $p19^{ARF}$ can stimulate autophagy by displacing Beclin 1 from BCL-X_L–mediated inhibition (200).

The expression of the multidomain protein kinase DAPK1 is reduced in several types of solid tumors owing to epigenetic mechanisms, particularly promoter hypermethylation (29–32). DAPK1 mediates oncosuppressive functions by activating a $p19^{ARF}$/p53-dependent apoptotic checkpoint (162, 205) as well as by stimulating autophagy (87, 260). This latter effect presumably results from the ability of DAPK1 to phosphorylate Beclin 1, thereby displacing it from inhibitory interactions with antiapoptotic BCL-2 family members (259, 260).

Other proautophagic factors that may have tumor-suppressive functions are ATG4C and RAB7A. The knockout of *Atg4c* increases the propensity of mice to develop chemical-induced fibrosarcomas (160). RAB7A has been shown to prevent growth factor-independent survival by inhibiting cell-autonomous nutrient transporter expression (51) and the *RAB7A* gene is frequently rearranged in different types of leukemia (109).

Oncoproteins with Autophagy-Inhibitory Functions

The notion that autophagy constitutes a *bona fide* tumor suppressor mechanism is further corroborated by the observations that a number of oncoproteins have autophagy-inhibitory effects (Fig. 3) (Table 2).

More than one third of human cancers all confounded are characterized by activating mutations of RTKs or downstream signal transducers, including AKT1, class I PI3K, RAS, and phosphoinositide-dependent protein kinase 1 (158, 170, 192, 197, 211). Constitutive activation of the RTK-mTOR axis emancipates the proliferation of cancer cells from negative microenvironmental signals such as the deprivation of growth factors (158) and, at the same time, suppresses autophagy. Intriguingly, the tumorigenic hyperactivation of AKT1, class I PI3K, and PDK1 has clear antiautophagic effects, whereas RAS regulates autophagy in a more controversial fashion (see above) (64, 193).

Another prominent feature of cancer cells is their intrinsic resistance to cell death (69). In various types of solid and hematological tumors, this results from the overexpression of antiapoptotic proteins of the BCL-2 family, including BCL-2 itself, BCL-X_L, BCL-W, MCL-1, and A1 (107). Some BCL-2–like proteins have been shown to sequestrate Beclin 1 into inactive complexes (155, 195), and this property may actively contribute to their tumorigenic activity (69, 177).

Table 1. Oncosuppressor Proteins with Autophagy-Stimulatory Properties

Protein(s)	*Link(s) to oncogenesis*	*Link(s) to autophagy*	*References*
ATG4C	ATG4C-deficient mice exhibit an increased sensitivity to chemically induced tumors	Cysteine protease that participates in the conversion of LC3-I into LC3-II	(160, 228)
Beclin 1	Deleted in a consistent fraction of mammary, ovarian, and prostate cancers; downregulated at the protein level in brain, gastric, and colorectal cancer	Essential component of the hVPS34-containing class III PI3K complex	(3, 140, 171, 217, 258)
BH3-only proteins	Mutated or underexpressed in multiple human tumors (*e.g.*, melanoma, renal carcinoma)	Stimulate autophagy by releasing Beclin 1 from BCL-2/BCL-X_L–mediated inhibition	(131, 146, 153, 246, 255, 262)
DIRAS (ARHI)	RAS-related tumor suppressor downregulated in >60% ovarian cancers	Negatively regulates mTOR *via* PI3K signaling and upregulates ATG4	(5, 56, 147)
SH3GLB1 (BIF1)	Downregulated in colorectal adenocarcinoma, knockout of SH3GLB1 enhances the development of spontaneous tumors in mice	Positive modulator of the hVPS34-containing class III PI3K complex	(37, 224)
DAPK1	Often silenced in human tumors by epigenetic mechanisms (promoter hypermethylation)	Interacts with MAP1B, may induce the activation of p53, favors the release of Beclin 1 from BCL-2/X_L	(29–32, 87, 162, 259, 260)
NF1	Loss-of-function mutations lead to type I neurofibromatosis	Negative regulator of RAS signaling with indirect proautophagic effects	(33, 34, 103, 215)
$p19^{ARF}$	Mutated or lost in multiple types of human cancer (*e.g.*, lymphoma, NSLC, breast cancer)	Inhibits MDM2 in the nucleolus, thereby allowing for stabilization of nuclear p53	(2, 200, 201, 207)
p150 (?)	Overexpressed in cervical carcinoma	Essential component of the hVPS34-containing class III PI3K complex	(157, 247, 254)
p53	Mutated in >50% human cancers	Controversially regulates autophagy, depending on its subcellular localization	(67, 68, 154, 178, 221)
PTEN	Germline mutations cause Cowden's and Bannayan-Riley-Ruvalcaba's diseases; somatically mutated/silenced in many cancers	Phosphatase that antagonizes the activity of PI3K, thereby inhibiting mTOR	(7, 45)
RAB7A	Rearranged in several types of leukemia	Small GTP-binding protein that plays a role in autophagosome maturation	(51, 54, 99, 109)
smARF	Mutated or lost in multiple types of human cancer (*e.g.*, lymphoma, NSLC, breast cancer)	Acts at mitochondria by releasing Beclin 1 from BCL-2/BCL-X_L–mediated inhibition	(2, 200, 201, 207)
STK11 (LKB1)	Germline mutations cause Peutz-Jeghers's syndrome; somatic ones are found in NSCLC	AMPK activator; may also promote autophagy by stabilizing $p27^{KIP1}$	(38, 88, 100, 141, 216, 248)
TSC1 TSC2	Germline mutations cause TSC	TSC1/TSC2 stimulates the GTPase activity of RHEB, thus inhibiting mTOR	(73, 96, 97, 236, 263)
UVRAG	Often monoallelically deleted in colon cancer; affected by frameshift mutations in gastric carcinomas with microsatellite instability	Positive modulator of the hVPS34-containing class III PI3K complex	(113, 137, 138)

AMPK, AMP-activated protein kinase; BH3, BCL-2 homology domain 3; BIF1, BAX-interacting factor 1; DAPK1, death-associated protein kinase 1; mTOR, mammalian target of rapamycin; NF1, neurofibromin 1; NSCLC, nonsmall cell lung cancer; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; SH3GLB1, SH3-domain GRB2-like endophilin B1; smARF, small mitochondrial ARF; STK11, serine/threonine kinase 11; TSC, tuberous sclerosis complex; UVRAG, UV irradiation resistance-associated gene.

Autophagy Regulation by p53

The transcription factor p53 [which is inactivated in >50% human cancers (221)] coordinates a cell-wide response to potentially tumorigenic stimuli, including DNA damage and oncogene activation, by (i) transactivating cell cycle-arresting and/or proapoptotic genes, (ii) regulating the expression of enzymes involved in energy metabolism (239), (iii) exerting extranuclear proapoptotic functions (68, 178), and (iv) regulating autophagy (Table 3) (67, 154).

Table 2. Oncoproteins with Autophagy-Inhibitory Functions

Protein(s)	*Link(s) to oncogenesis*	*Link(s) to autophagy*	*Reference*
AKT1 (PKB)	Gain-of-function mutations or amplifications characterize a high fraction of human cancers	Constitutively active AKT1 functions as an autophagy inhibitor *in vitro* and *in vivo*	(14, 158, 254)
BCL-2 BCL-X_L	Overexpressed in a plethora of human cancers, notably in hematological malignancies	Inhibit autophagy by sequestering Beclin 1 into inactive complexes	(107, 155, 195)
PDPK1	Gain-of-function mutations or amplifications are found in a high fraction of human tumors	PIP_3-responsive kinase that inhibits the TSC1/TSC2	(197, 213)
PI3K (class I)	Gain-of-function mutations or amplifications are common to many human cancers	Generates PIP_3, which indirectly activates the antiautophagic functions of mTOR	(192, 194, 211, 257)
RAS (?)	Hyperactivated in a relevant proportion of human cancers all confounded	Signal transducer of the RTK-mTOR pathway with dual roles in autophagy regulation	(64, 170, 193)

PDPK1, phosphoinositide-dependent protein kinase 1; PKB, protein kinase B; PIP_3, phosphatidylinositol-3,4,5-trisphosphate; RTK, receptor tyrosine kinase.

p53 has a controversial influence on autophagy, depending on its subcellular localization (Fig. 4). On one hand, stress-induced p53 has been shown to transactivate proautophagic factors including the β1 and β2 subunits of AMPK (57), proapoptotic BCL-2 family members such as BAX and several BH3-only proteins (146, 155, 255), DAPK1 (87, 259, 260), the lysosomal protein damage-regulated autophagy modulator (39, 40), sestrin 1 and 2 (22), and TSC2 (57). Interestingly, tumor protein p53-induced nuclear protein 2 has been recently discovered as an essential modulator of autophagy in mammalian cells (187), although it remains elusive whether *TP53INP2* represents a *bona fide* p53 target gene like its close relative TP53INP1 (24). Finally, nuclear p53 can favor autophagy by repressing the transcription of Beclin 1-inhibiting BCL-2–like proteins including BCL-2 itself, BCL-X_L, and MCL-1 (172, 199, 223).

Pharmacological and genetic inhibition of p53 reportedly stimulate autophagy (in a cell cycle-dependent fashion, principally in the G_0/G_1 phase), suggesting that p53 constitutively suppresses self-cannibalism (230, 231). Pharmacolo-

Table 3. Autophagy-Modulatory Functions of the p53 System

Protein(s)	*Link(s) to p53*	*Link(s) to autophagy*	*References*
AMPK β1 AMPK β2	p53 target genes (activator RE) p53-phosphorylating kinase	Positive regulators of autophagy in response to decreased energy levels and other stress stimuli	(38, 57, 58, 83, 86, 89, 91, 112, 139, 191, 214)
ARF	p53 stabilized (MDM2 inhibitor)	Stimulates autophagy *via* both p53-dependent and p53-independent signaling pathways	(2, 55, 74, 129, 168, 207)
BAD	p53 target gene (activator RE)	BH3-only protein with proautophagic functions	(101, 153)
BAX	p53 target gene (activator RE)	Proapoptotic and proautophagic BCL-2 family member	(172, 255)
BCL-2 BCL-X_L	p53 target gene (inhibitor RE)	Antiapoptotic BCL-2 family members with antiautophagic functions	(155, 172, 195, 223)
BNIP3L	p53 target gene (activator RE)	BH3-only protein involved in mitophagy	(82, 262)
DAPK1	p53 target gene (activator RE)	Beclin 1-phosphorylating kinase	(87, 162, 259, 260)
DRAM	p53 target gene (activator RE)	Proautophagic and proapoptotic lysosomal protein	(39, 40)
MCL-1	p53 target gene (inhibitor RE)	Autophagy-inhibitory, antiapoptotic BCL-2 family member	(53, 199)
PUMA	p53 target gene (activator RE)	BH3-only protein with proautophagic functions	(146, 182, 255)
SESN1 (PA26) SESN2 (HI95)	p53 target genes (activator RE)	Proautophagic factors (AMPK activators)	(22, 23, 156)
TP53INP2	p53 target gene? (activator RE)	Essential modulator of autophagy in mammalian cells	(187)
TSC2	p53 target gene (activator RE)	Proautophagic signal transducer	(57, 97)

DRAM, damage-regulated autophagy modulator; PUMA, p53-upregulated modulator of apoptosis; RE, responsive element; SESN, sestrin; TP53INP2, tumor protein p53-induced nuclear protein 2.

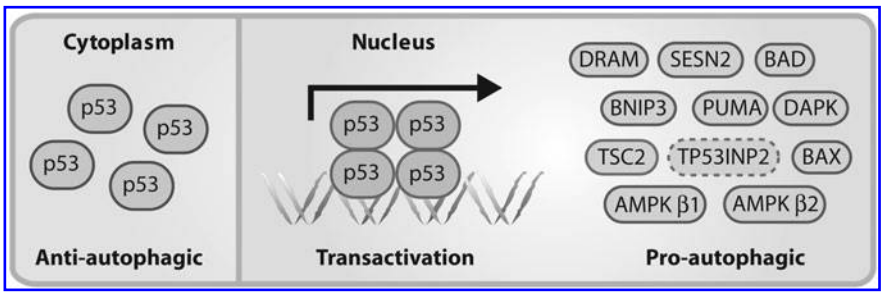

FIG. 4. p53 and autophagy. p53 regulates autophagy depending on its subcellular localization. Nuclear p53 can transactivate a number of (direct or indirect) proautophagic factors and also can repress the transcription of genes that mediate autophagy inhibition. Conversely, extranuclear p53 exerts a tonic suppression on autophagy, through hitherto poorly characterized mechanisms. DRAM, damage-regulated autophagy modulator; PUMA, p53-upregulated modulator of apoptosis; TP53INP2, tumor protein p53-induced nuclear protein 2.

gical inhibition of p53 effectively induced autophagy in cytoplasts (230). Moreover, p53 deletion mutants lacking the DNA-binding domain retained their autophagy-suppressive activity, which positively correlated to their cytoplasmic (as opposed to nuclear) localization (181, 230). Taken together, these data demonstrate that the autophagy-inhibitory functions of p53 are independent of transcription and are mediated by the cytoplasmic pool of the protein. In line with these observations, $p53^{-/-}$ colon carcinoma cells are characterized by higher levels of basal autophagy than their wild-type (WT) counterparts (230), which can be normalized by reintroduction of WT p53 but not of a nucleus-restricted mutant (owing to the deletion of its nuclear export signal) (181, 230).

Mechanistic Links Between Autophagy and Tumor Suppression

The biochemical and epidemiological data discussed above strongly suggest that autophagy acts (at least in some scenarios) as a *bona fide* tumor suppressor mechanism (20, 28, 245). One single function of the autophagic machinery that would account alone for its oncosuppressive activity has not been identified yet and presumably does not even exist. Rather, autophagy-mediated tumor suppression is likely to be mediated by multiple molecular mechanisms, reflecting the crucial and multifaceted role that baseline levels of autophagy exert in the maintenance of homeostasis and in the management of constitutive stress (70, 177).

Autophagy, Oxidative Stress, and Genomic Stability

One of the functional links between autophagy and tumor suppression is provided by reactive oxygen species (ROS), highly genotoxic/mutagenic chemicals that are constantly generated as side products by mitochondrial respiration (196). Physiological levels of ROS are held in check by a multilayered system of enzymatic and non-enzymatic antioxidant defenses (75, 159), whereas ROS overgeneration (for instance, as it occurs in uncoupled mitochondria) may have dramatic consequences for the cell, ranging from increased oxidative damage of macromolecules (including proteins, lipids, and DNA) (161, 222) to activation of the intrinsic pathway of apoptosis and cell death (65, 123). ROS can directly damage DNA by attacking the nucleobases of the deoxyribosyl backbone, but can also mediate an indirect oxidative injury, resulting from the activity of reactive intermediates that originate from ROS-modified cellular components such as lipids (161). Irrespective of these molecular details, ROS are well known to accelerate mutagenesis, thereby favoring oncogene activation (or tumor suppressor inactivation) and stimulating carcinogenesis (161). In this context, constitutive levels of autophagy and, in particular, of mitophagy may exert a multifaceted oncosuppressive activity by operating a quality control on the mitochondrial network (102, 132) and by removing protein aggregates (163, 206). Mitochondria represent the most prominent source of intracellular ROS, and this is considerably aggravated in old or (partially) damaged organelles, which are highly prone to uncoupling (69). Mitochondrial DNA mutations (which accumulate in old organelles) reportedly contribute to the development of a malignant phenotype by affecting the efficiency of the respiratory chain (thereby indirectly favoring uncoupling and ROS overgeneration) as well as by sustaining the metabolic modifications that characterize most (if not all) cancers (69, 126, 265). Thus, the selective removal of potentially harmful mitochondria through mitophagy may mitigate ROS overgeneration and limit ROS-dependent tumorigenesis (102), but also impede cancer-associated metabolic reprogramming (126, 265). Accordingly, damaged mitochondria have been shown to accumulate *in vivo* upon whole-body or tissue-specific inhibition of autophagy (121, 128, 220).

In autophagy-deficient tumor cells, the accumulation of protein aggregates containing the ubiquitin moieties and the specific autophagic substrates $p62^{SQSTM1}$ (15, 16) and neighbor of *BRCA1* gene 1 (115) has been recently associated with ROS overgeneration and genomic instability, pointing to yet another mechanism (*i.e.*, the selective removal of aggregated proteins, also known as "aggrephagy") by which the autophagic machinery may counteract the oncogenic potential of ROS (163). Compromised autophagy had been previously shown to increase the propensity of cancer cells to accumulate genomic alterations (108, 164). Now, Mathew and colleagues have demonstrated that ROS mediate at least part of the genotoxic effects of deficient autophagy (163), but the existence of additional mechanisms through which autophagy may prevent genomic instability has not been formally excluded. As an intriguing possibility, autophagy may be involved in the regulation of some (DNA damage-related) cell cycle checkpoint (231, 232). In such a scenario, defective autophagy may favor tumorigenesis by allowing for a deregulated, DNA damage-independent progression of cells through the cell cycle. Alternatively, autophagy might favor genomic stability by acting at a more general level and, in

particular, by ensuring the minimal supply of ATP and other metabolites required for DNA repair (18, 177).

Autophagy and Inflammation

Cancer cells tend to be more resistant to metabolic stress than their WT counterparts, for at least two reasons. First, most (if not all) tumors are characterized by an extensive metabolic reprogramming and exhibit the so-called Warburg effect (a high rate of glycolysis despite normoxic conditions), providing them with the possibility to mobilize energy stores in a rapid manner (which is required to sustain the massive biosynthesis of intracellular structures that is typical of highly proliferating cells) as well as with a limited sensitivity to varying oxygen tension (69, 126, 242, 243). Second, cancer cells often manifest defects in the molecular machinery that executes mitochondrial apoptosis, which render them intrinsically more resistant to metabolic stress-induced cell death (69, 126). Nevertheless, a fraction of cancer cells is destined to succumb to metabolic stress throughout tumorigenesis and can do so *via* specific cell death subroutines that differ from each other at the morphological, biochemical, and immunological level (122, 124). Apoptosis most frequently [but not always (111, 188)] occurs in an immunologically silent fashion, whereas necrosis is often associated with a prominent inflammatory reaction, which represents a strong stimulus for oncogenesis (17, 81, 130). In multiple experimental settings, particularly in apoptosis-deficient cancer cells, the inhibition of autophagy has been shown to favor the rapid demise of stressed cells through a necrotic cell death subroutine (48, 70, 177), suggesting that autophagy might exert oncosuppression also by restricting necrosis and inflammation. Intriguingly, it has been proposed that part of the anti-inflammatory effects of autophagy derives from its involvement in the removal of cell corpses (245). *ATG5*$^{-/-}$ embryonic stem cells are characterized by a defect in apoptotic corpse engulfment during embryonic development (203) and autophagy-deficient immortalized mammary epithelial cells also accumulate corpses when grown as three-dimensional mammospheres (108). Although this hypothesis remains to be validated, the persistence of such corpses may constitute a chronic proinflammatory and tumorigenic stimulus, and their clearance (as a result of functional autophagy) would therefore turn out to be oncosuppressive. Finally, as the autophagic machinery has been linked to many other aspects of innate and adaptive immunity (*e.g.*, pathogen resistance, production of type I interferon, antigen presentation, tolerance and lymphocyte development, negative regulation of cytokine signaling) (238), it is tempting to speculate that autophagy might function as an antitumor mechanism by (indirectly) favoring immunosurveillance (266).

Concluding Remarks

Several authors have made a parallel between the role of autophagy in oncology and a "double-edged sword" (6, 245). Indeed, although autophagy acts as a tumor-suppressive mechanism during early oncogenesis, it also exerts critical cytoprotective functions (and hence behaves as a protumor mechanism) for formed cancers (and established cancer cell lines) (20, 177, 245), as demonstrated by the fact that pharmacological or genetic inhibition of autophagy sensitizes tumor cells to the lethal effect of stressful events including chemo- and radiotherapy, both *in vitro* and *in vivo* (177). At least on theoretical grounds, the pharmacological modulation of autophagy might therefore represent a doubly valuable tool for anticancer therapy (93). However, although inhibitors of autophagy, such as chloroquine and hydroxychloroquine, are currently being evaluated as chemosensitizers in multiple clinical settings (245), chemopreventive approaches based on proautophagic agents have not yet been attempted. Anyway, great caution should be used in the design and development of autophagy-targeting therapies, because—on one hand—autophagy inhibitors may stimulate tumorigenesis and—on the other hand—autophagy-stimulatory agents might favor the survival of formed (possibly undiagnosed) tumors. Given the dual nature of autophagy, it is not surprising that modulators of the autophagic machinery are intrinsically endorsed with both pro- and antitumor properties. Further investigation is therefore urgently awaited to better elucidate the molecular liaisons that bridge autophagy and cancer, hopefully leading to the development of novel, efficient, and safe antitumor regimens.

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Abbreviations Used

AMPK = AMP-activated protein kinase
ARHI = aplasia Ras homolog member I
atg = autophagy-related (genes)
BCN1 = Beclin 1
BH3 = BCL-2 homology domain 3
BIF1 = BAX-interacting factor 1
CaMKKβ = calmodulin-dependent kinase kinase β
CR = cytokine receptor
DAPK1 = death-associated protein kinase 1
DRAM = damage-regulated autophagy modulator
ERK = extracellular signal-regulated kinase
GLUT = glucose transporter
IP_3 = inositol 1,4,5-trisphosphate
mTOR = mammalian target of rapamycin
NF1 = neurofibromin 1
NSCLC = nonsmall cell lung cancer
PDPK1 = phosphoinositide-dependent protein kinase 1
PE = phosphatidylethanolamine
PI3K = phosphatidylinositol 3-kinase
PIP_3 = phosphatidylinositol-3,4,5-trisphosphate
PKB = protein kinase B
PS1 = presenilin 1
PTEN = phosphatase and tensin homologue deleted on chromosome 10
PUMA = p53-upregulated modulator of apoptosis
Rapa = rapamycin
RE = responsive element
ROS = reactive oxygen species
RTK = receptor tyrosine kinase
SESN = sestrin
SH3GLB1 = SH3-domain GRB2-like endophilin B1
smARF = small mitochondrial ARF
STK11 = serine/threonine kinase 11
TAK1 = transforming growth factor β-activating kinase 1
TORC = TOR complex
TP53INP2 = tumor protein p53-induced nuclear protein 2
TSC = tuberous sclerosis complex
ULK = unc-51-like kinase
UVRAG = UV irradiation resistance-associated tumor suppressor gene
WT = wild type

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