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Review

Anti- and pro-tumor functions of autophagy

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ABSTRACT

Autophagy constitutes one of the major responses to stress in eukaryotic cells, and is regulated by a complex network of signaling cascades. Not surprisingly, autophagy is implicated in multiple pathological processes, including infection by pathogens, inflammatory bowel disease, neurodegeneration and cancer. Both oncogenesis and tumor survival are influenced by perturbations of the molecular machinery that controls autophagy. Numerous oncoproteins, including phosphatidylinositol 3-kinase, Akt1 and anti-apoptotic members of the Bcl-2 family suppress autophagy. Conversely, several tumor suppressor proteins (e.g., Atg4c; beclin 1; Bif-1; BH3-only proteins; death-associated protein kinase 1; LKB1/STK11; PTEN; UVRAG) promote the autophagic pathway. This does not entirely apply to p53, one of the most important tumor suppressor proteins, which regulates autophagy in an ambiguous fashion, depending on its subcellular localization. Irrespective of the controversial role of p53, basal levels of autophagy appear to inhibit tumor development. On the contrary, chemotherapy- and metabolic stress-induced activation of the autophagic pathway reportedly contribute to the survival of formed tumors, thereby favoring resistance. In this context, autophagy inhibition would represent a major therapeutic target for chemosensitization. Here, we will review the current knowledge on the dual role of autophagy as an anti- and pro-tumor mechanism.

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1. Introduction

“Autophagy” is a term of Greek derivation that indicates several intracellular pathways converging on a common phase of lysosomal degradation, including chaperone-mediated autophagy, micro- and macroautophagy. During macroautophagy (to which we refer as “autophagy”), intact organelles and/or parts of the cytoplasm are enclosed in double-membraned vacuoles that are known as autophagosomes. Autophagosomes (formerly also termed “early

autophagic vacuoles”) mature by fusing with lysosomes, thereby forming the so-called autophagolysosomes/autolysosomes (formerly dubbed “late autophagic vacuoles”), in which lysosomal hydrolases are activated and degrade the luminal content [1]. Usually, the cytoplasmic accumulation of autophagosomes (which can be observed by electron microscopy) coincide with an upregulation of the autophagic pathway. Nonetheless, autophagolysosomes can also amass when lysosomal degradation is inhibited, for instance upon small interfering RNA (siRNA)-mediated downregulation of the lysosome-associated membrane protein 2 (LAMP-2) or in the presence of hydroxychloroquine [2]. Thus, at least another parameter (such as the cleavage and/or the redistribution of the autophagosome marker LC3 or the destruction of the autophagic substrate p65) should be monitored to quantify autophagic flow in an unambiguous fashion [3,4].

Autophagic vacuolization has been observed in numerous pathophysiological scenarios [5,6], and the term “autophagic cell death” has been extensively employed to indicate a cell death subroutine lacking the morphological features of classical apoptosis and rather manifesting with the cytoplasmic accumulation of autophagosomes [7]. In the vast majority of these settings, however, pharmacological and/or genetic inhibition of autophagy does not prevent cell death, and often accelerates it. Thus, autophagy is presumably activated by dying cells as part of an (inconclusive) attempt to cope with stress, yet mainly

Abbreviations: AMPK, AMP-activated protein kinase; atg, autophagy related; Bec-1, Beclin 1; BH3, Bcl-2 homology domain 3; CRTC, CREB-regulated transcription coactivator; DAPK-1, death-associated protein kinase 1; DRAM, damage-regulated autophagy regulator; LAMP-2, lysosome-associated membrane protein 2; mTOR, mammalian target of rapamycin; NFI, neurofibromin 1; PDPK1, 3-phosphoinositide dependent protein kinase 1; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; ROS, reactive oxygen species; RTKs, receptor tyrosine kinases; shRNA, short hairpin RNA; siRNA, small interfering RNA; smARF, small mitochondrial ARF; STK11, serine threonine kinase 11; TORC, TOR complex; TRAIL, tumor necrosis factor-related apoptosis inducing ligand; TSC, tuberous sclerosis complex; UPR, unfolded protein response; UVRAG, UV radiation resistance associated gene; WT, wild type

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represents a pro-survival mechanism [8]. For these reasons, the expression “autophagic cell death” has been recently recognized as a misnomer, and the Nomenclature Committee on Cell Death has strongly discouraged its use in scientific reports [9,10].

Baseline levels of autophagy contribute to the physiological elimination of old/damaged organelles as well as to the turnover of long-lived proteins. Moreover, the autophagic flow can be dramatically augmented in response to numerous conditions of extracellular and/or intracellular stress [8,11]. Among others, these include nutrient and/or growth factor deprivation, hypoxia, protein misfolding and infection by intracellular pathogens. In starvation conditions, enhanced autophagy provides the cells with metabolic intermediates to meet their bioenergetic demands [12]. During hypoxia, the selective removal of reactive oxygen species (ROS)-overproducing mitochondria through the autophagic pathway (a process known as mitophagy) may limit oxidative damage [13]. The autophagic elimination of protein aggregates contributes to the cytoprotective mechanism known as unfolded protein response (UPR) [14], in which the endoplasmic reticulum plays a prominent role [15,16]. Finally, upon invasion, autophagy favors the clearance of intracellular pathogens, thereby representing the most ancient effector mechanism of innate immunity [17].

These few examples substantiate the notion that autophagy occupies a central position in the response of eukaryotic cells to intrinsic and environmental stress. Intriguingly, several genetic and epigenetic modifications that have been associated with oncogenesis (including activation and/or amplification of oncoproteins and mutation/inactivation of tumor suppressor proteins) also negatively regulate the cellular machinery for autophagy, which suggests that basal levels of autophagy may function as a tumor suppressor mechanism [18]. Conversely, multiple lines of evidence indicate that autophagy may contribute to the survival of established tumors, for instance by allowing adaptation to adverse metabolic conditions or by favoring chemoresistance [18]. In this review, we will discuss the molecular mechanisms underlying the anti-tumor and pro-tumor roles of autophagy.

2. Anti-tumor roles of autophagy in oncogenesis

The mammalian target of rapamycin (mTOR) kinase represents the major negative regulator of autophagy in human cells. In all higher eukaryotes, mTOR is part of at least two distinct multiprotein complexes (known as TOR complexes, TORCs, or CREB-regulated transcription coactivators, CRTC), which exhibit partially overlapping functional profiles. In physiological conditions, mTOR prevents autophagy by maintaining the hyperphosphorylation of proteins required for the initiation of the autophagic cascade. Conversely, mTOR activity is rapidly shut down in conditions of stress, which allows for the rapid upregulation of autophagy [8]. mTOR takes part in multiple signaling cascades that regulate cell growth, and especially in those emanating from receptor tyrosine kinases (RTKs). In cancer cells, constitutively active RTKs and/or the persistent production of autocrine growth factors lead to the hyperphosphorylation of mTOR substrates. Moreover, several tumors are characterized by activating mutations of the key signal transducers connecting RTKs to mTOR, including the small GTPase Ras, phosphatidylinositol 3-kinase (PI3K) as well as the kinases Akt1 and 3-phosphoinositide dependent protein kinase 1 (PDK1) [19,20]. Finally, mTOR function can be augmented in tumor cells as a result of mutations that inactivate negative regulators of the RTK–mTOR signaling axis [20]. These include neurofibromin 1 (NF1, an antagonist of Ras), tuberous sclerosis complex 1 and 2 (TSC1 and 2, which form a functional complex inhibiting mTOR-mediated phosphorylation of translation regulators) [21], LKB1/STK11 (a kinase that positively regulates the AMP-activated protein kinase, AMPK, and hence TSC2) [22,23], as well as the Ras-related protein ARHI/DIRAS3 [24] and the phosphatase PTEN (both of which function as direct antagonists of PI3K) [19]. Interestingly, germline mutations of the

nearly all these proteins have been associated with familial cancer syndromes (e.g., neurofibromatosis type 1, tuberous sclerosis, Peutz–Jegher's syndrome, Cowden's disease), which share clinical features characterized by hamartomatous tumors [20]. In line with these observations, it has been demonstrated that constitutively active Akt1 functions as an autophagy inhibitor *in vitro* and *in vivo*, while promoting tumor growth *in vivo*, in particular in cells that fail to express the pro-apoptotic proteins Bax and Bak (Table 1) [25].

Reduced levels (or the complete absence) of multiple proteins that are required for autophagy have also been associated with facilitated tumor progression, both in animal models and in humans (Table 1). The essential autophagy regulator beclin 1 (Bec-1, the human ortholog of murine Atg6) interacts with several cofactors (including Ambra1, Bif-1 and UVRAG) to activate the lipid kinase Vps34, which is required for the initiation of autophagic pathway [26–28]. Immortalized kidney and mammary epithelial cells that harbor a monoallelic deletion of *bec-1* are characterized by increased growth rates – as compared to their wild type (WT) counterparts – upon the inoculation into immunodeficient recipient mice [25,29,30]. *atg6*^{+/-} heterozygous mice display an increased propensity to develop age-related tumors (including mammary precancerous lesions, lymphomas as well as lung and hepatocellular carcinomas), and are more susceptible to hepatitis B virus-induced carcinogenesis than their WT littermates [31,32]. One copy of the *bec-1* gene is deleted in a relevant fraction of human breast, ovarian and prostate tumors [33]. Moreover, a large proportion of brain cancers are characterized by reduced levels of Bec-1 protein, which inversely correlate with clinical malignancy [34]. Altogether, these observations point to Bec-1 as a *bona fide* tumor suppressor protein [32], a property that might be shared with other components of the Bec-1-containing Vps34-activating complex. One copy of the *uvrag* gene is often deleted in human colon cancers, and UVRAG overexpression has been shown to suppress the tumorigenicity of human colon cancer cells [27]. Moreover, *bif-1*^{-/-} mice spontaneously develop lymphomas, sarcomas and carcinomas at higher frequency than WT animals [28]. Since the *ambra1* gene knockout results in profound neurological defects and early embryonic lethality [26], data on the propensity of *ambra1*^{-/-} mice to develop spontaneous tumors are lacking. However, some of the proteins that constitute the molecular machinery for autophagy downstream of Bec-1 have also been associated with tumor suppressing functions. As an example, in mice, the biallelic deletion of *atg4c* (which codes for a cysteine protease involved in the processing of LC3/Atg8) results in tissue-specific defects of the autophagic pathway, and favors the development of chemically-induced fibrosarcomas [35].

Bec-1 has been first identified as a Bcl-2-interacting protein [33]. Bcl-2 and its anti-apoptotic homologues (e.g., Bcl-X_L, Bcl-w and Mcl-1) are prominent oncoproteins [36], while their pro-apoptotic counterparts (e.g., Bax, Bak and BH3-only proteins) exert tumor suppressing roles [37–39]. Whereas the implication of the Bcl-2 protein family in tumorigenesis was initially ascribed only to their ability to modulate cell death, it has now become evident that these factors control multiple aspects of metabolism, including autophagy (Table 1) [40]. In particular, anti-apoptotic factors like Bcl-2 [41], Bcl-X_L [42], Bcl-w [43] and Mcl-1 [42] suppress autophagy, mainly by interacting with and therefore inhibiting Bec-1. Accordingly, inhibition of Bcl-2 by RNA interference (RNAi) [44] or via specific antisense oligonucleotides [45] has been shown to induce autophagy in multiple tumor cell lines. Conversely, pro-apoptotic BH3-only proteins including Bad [42], Bik [46], BNIP3L [47,48], Noxa, Puma and Bim_{EL} [49] promote the autophagic flow, (at least in part) by freeing Bec-1 from inhibitory interactions with anti-apoptotic Bcl-2-like proteins. Such interactions involve the BH3 domain of Bec-1 [50] and the so-called “BH3 receptor” domain of anti-apoptotic Bcl-2 family members, and can be disrupted by small BH3-mimetic chemicals like ABT-737 [42,51]. In apoptosis-resistant cells, ABT-737 efficiently disrupts Bec-1/Bcl-2 and Bec-1/Bcl-X_L complexes, thereby inducing massive autophagy [52]. This effect

Table 1
Examples of autophagy regulation by oncoproteins and tumor suppressor proteins.

Name	Link(s) to tumorigenesis	Link(s) to autophagy	Ref.
<i>Oncogenes</i>			
Akt1	Gain-of-function mutations or amplifications found in a high fraction of human cancers	Constitutively active Akt1 functions as an autophagy inhibitor <i>in vitro</i> and <i>in vivo</i>	[20]
ARHI/DIRAS3	Downregulated in more than 60% of ovarian cancers	Induces autophagy by blocking PI3K signaling, thereby inhibiting mTOR. Upregulates Atg4C Colocalizes with cleaved LC3 in autophagosomes	[24]
Bcl-2 Bcl-X _L	Overexpressed in a relevant proportion of human cancers, and notably in hematological malignancies	Negatively regulate autophagy by sequestering Bec-1	[40,111]
LKB1/STK11	Germline mutations cause Peutz-Jegher's syndrome. Somatic mutations observed in NSCLC.	Activates AMPK and hence TSC2 May also stimulate autophagy by stabilizing p27 ^{KIP1}	[22,23]
NF1	Loss-of-function mutations cause type I neurofibromatosis	Ras antagonist, negatively regulates the RTK–mTOR pathway	[20]
PDPK1	Gain-of-function or amplifications of PDPK1 characterize several human tumors	Positive signal transducer of the RTK–mTOR pathway	[20]
PI3K	Gain-of-function mutations or amplifications of PI3K are common to many human cancers	mTOR activating kinase	[19]
PTEN	Germline mutations cause Cowden's disease. Mutated or silenced in several tumors	Phosphatase that antagonizes the activity of PI3K, thereby inhibiting mTOR	[19,20]
Ras	Hyperactivated in most human cancers	Signal transducer of the RTK–mTOR pathway	[20]
TSC-1 TSC-2	Germline mutations cause tuberous sclerosis	TSC-1 and -2 form a functional complex inhibiting mTOR-mediated phosphorylation	[21]
<i>Oncosuppressors</i>			
Atg4c	Implicated in the development of chemically-induced fibrosarcomas	Cysteine protease required for a proper autophagic response under stressful conditions	[35]
Bec-1	Deleted in a relevant fraction of human breast, ovarian and prostate tumors. Brain tumors are characterized by reduced expression of Bec-1	Essential modulator of autophagy	[33,65]
BH3-only proteins	Loss of expression due to inactivating mutations has been recorded in multiple human tumors (e.g., melanoma, renal cell carcinoma)	BH3-only proteins promote autophagy by freeing Bec-1 from inhibitory interactions with anti-apoptotic members of the Bcl-2 protein family	[42,46–49]
Bif-1	Knockout of <i>bif-1</i> significantly enhances the development of spontaneous tumors in mice	Interacts with Bec-1 through UVRAG and functions as a positive regulator of the class III PI3K	[28]
DAPK-1	Frequently silenced in human tumors by epigenetic mechanisms	Induces autophagy by interaction with the microtubule-associated factor MAP1B. May promote autophagy via activation of p53	[54]
p14 ^{ARF}	Mutated or lost in multiple types of human cancer including leukemia, lymphoma, breast carcinoma and NSCLC	Full length p14 ^{ARF} promotes autophagy by interacting with MDM2, thereby de-inhibiting the transcriptional functions of p53	[61]
p150 (?)	Overexpressed in a large fraction of gastric and esophageal carcinomas. Expression inversely correlates with clinical grade	Favors autophagy by interacting with Bec-1 and hVps34, forming a functional PI3K complex	[63,64]
p53	Mutated in >50% of all human tumors	Nuclear p53 transactivates autophagy-promoting factors (e.g., DAPK-1, DRAM). Cytoplasmic p53 exerts a tonic inhibition of autophagy	[69,84]
Rab7	Rearranged in several types of leukemia	Participates in the maturation of autophagosomes	[65,67]
smARF	Mutated or lost in multiple types of human cancer including leukemia, lymphoma, breast carcinoma and NSCLC	Promotes autophagy independently of p53, presumably by releasing Bec-1 by Bcl-X _L -mediated inhibition	[58–60]
UVRAG	Monoallelically deleted at high frequency in human colon cancers	Interacts with Bec-1 to form a class III PI3K signaling complex	[65]

Abbreviations: AMP-activated protein kinase; atg, autophagy related; Bec-1, beclin 1; BH3, Bcl-2 homology domain 3; DAPK-1, death-associated protein kinase 1; DRAM, damage-regulated autophagy modulator; NF1, neurofibromin 1; mTOR, mammalian target of rapamycin; NSCLC, non-small cell lung cancer; PDPK1, 3-phosphoinositide dependent protein kinase 1; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; RTK, receptor tyrosine kinase; STK11, serine threonine kinase 11; TSC, tuberous sclerosis; UVRAG, UV radiation resistance associated gene.

cannot be counteracted by Bcl-2 and/or Bcl-X_L overexpression, yet can be efficiently prevented by transfection with a Mcl-1-encoding plasmid or by small interfering RNAs (siRNAs) targeting Bec-1, in line with the notions that ABT-737 fails to occupy the BH3 receptor domain of Mcl-1 [51] and that its pro-autophagic effects depend on Bec-1 [52]. The fact that oncoproteins and tumor suppressors proteins from the Bcl-2 family also control autophagy further substantiates the notion that tumor development may be favored by autophagy inhibition.

Additional lines of evidence corroborate this idea (Table 1). For instance, the expression of the death-associated protein kinase 1 (DAPK-1) is commonly shut down in human tumors by epigenetic mechanisms [53]. Similarly to BH3-only proteins, DAPK-1 can induce apoptosis and autophagy by independent mechanisms, thereby displaying pronounced tumor and metastasis suppressing functions. On one hand, DAPK-1 contributes to cell death by catalyzing the

phosphorylation of myosin light chain, which mediates apoptotic membrane blebbing [54]. On the other hand, DAPK-1 promotes autophagy by binding to the microtubule-associated protein MAP1B [54], which is an LC3 interactor with anti-autophagic functions [55]. Since DAPK-1 exerts a wide range of functions and can also activate the p53 system (which represents one of the most prominent tumor suppressor mechanisms in humans, see below) [53], it remains formally possible that DAPK-1-activated transcriptional programs might also stimulate autophagy.

The expression of another positive regulator of p53, p14^{ARF}, is lost in a large number of human tumors (including lung carcinoma, melanoma and prostate cancer), due either to inactivating mutations or heterochromatinization-dependent gene repression [56]. In the nucleolus, p14^{ARF} binds to the product of the *mdm2* gene, thereby preventing the ubiquitination-dependent destruction of p53 [57]. A

short mitochondrial form of p14^{ARF} (smARF) reportedly induces massive autophagy independently of p53 [58], possibly by interacting with Bcl-X_L, thereby freeing Bec-1 [59]. Thus, the tumor suppressing functions of smARF may not be limited to its pro-apoptotic potential. Only upon extreme overexpression, full length p14^{ARF} localizes to nuclear and extranuclear compartments, thereby activating autophagy via both p53-dependent and p53-independent mechanisms [60,61]. Conversely, nucleolus-restricted expression p14^{ARF} fails to induce autophagy when p53 is inactive [60], indicating that the pro-autophagic action of p14^{ARF} from within the nucleolus completely relies on p53-dependent transcriptional programs.

p150 positively regulates autophagy by interacting with Bec-1 and hVps34, thereby forming a functional PI3K complex [62], and its expression is augmented in a large fraction of gastric and esophageal carcinomas [63,64]. These apparently counterintuitive data may point to an autophagy-unrelated function of p150 that may be important for the development of gastric/esophageal cancers. Nonetheless, it should be noted that high p150 expression characterized most low-grade lesions, and that p150 was progressively lost in invasive and metastatic tumors [63].

In mammalian cells, the small GTPase Rab7 also participates in the PI3K-dependent maturation of autophagic vacuoles [65]. Interestingly, the *rab7* gene maps to a locus of chromosome 3 that is frequently rearranged in different types of leukemia [66]. Moreover, Rab7 can limit growth-factor independent survival by preventing the cell-autonomous expression of nutrient transporters [67].

Taken together, these observations strongly support the idea that autophagy inhibition favors tumorigenesis, in turn suggesting that autophagy may represent a *bona fide* tumor suppressor mechanism (Fig. 1). At least three hypotheses can be put forward to explain how reduced autophagy may stimulate tumorigenesis [68]. First, autophagy inhibition reportedly augments necrotic cell death within tumors, and may therefore promote tumor growth by aggravating local inflamma-

tion [25]. Second, compromised autophagy has been shown to promote chromosomal instability, in particular in cells subjected to metabolic stress, which may lead to oncogene activation and tumor progression [29,30]. However, the mechanisms through which autophagy preserves genome integrity remain elusive. An intriguing possibility is that autophagy may contribute to the regulation of the cell cycle, for instance by degrading proteins and/or organelles involved in specific cell cycle checkpoints [69]. Alternatively, autophagy might function at a more general level to ensure the minimal supply of ATP and other metabolites required for DNA repair [70]. Third, deficient autophagy may promote tumorigenesis by allowing for the accumulation of old and/or damaged organelles, which in turn may act as sources of intrinsic genotoxic/tumorigenic chemical species (e.g., ROS from uncoupled mitochondria) [13].

3. Controversial regulation of autophagy by p53

The general rule that tumor suppressor proteins facilitate autophagy does not entirely apply to the most important tumor suppressor protein of all, p53 (Fig. 2) [71]. The p53 system is blocked in more than 50% of human cancers, for instance due to inactivating mutations of p53 or of genes coding for p53-phosphorylating kinases (e.g., ATM, Chk1), following the amplification-dependent overexpression of MDM2, the E3 ubiquitin ligase that directs p53 to proteasome-mediated degradation, or as a result of the loss of p14^{ARF} function [72,73]. p53 was initially described as a transcription factor that transactivates pro-apoptotic and cell cycle arresting programs [72], therefore inducing apoptosis and/or senescence of cancer cells characterized by irreparable genomic alterations, either in the context of tumor progression or following chemo- and/or radiotherapy [73]. Moreover, p53 is able to transactivate the gene coding for the lysosomal protein damage-regulated autophagy modulator (DRAM), which can trigger autophagy but is also required for p53-mediated

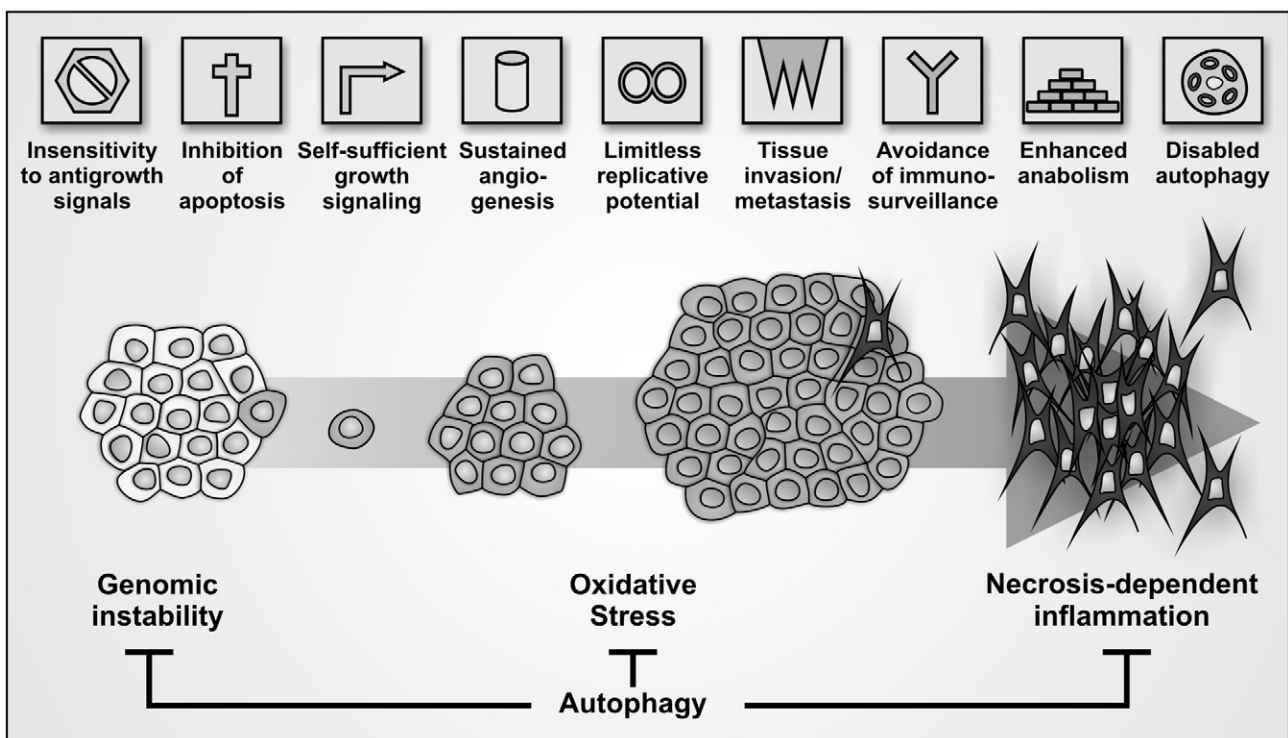


Fig. 1. Tumor suppressing functions of autophagy. In 2000, Hanahan and Weinberg [108] enumerated six cell-intrinsic hallmarks of cancer, and in particular: provision of autonomous growth signals, insensitivity to antiproliferative signals, unlimited replicative potential, production of angiogenic factors, disabled apoptosis and tissue invasion with metastasis. Subsequently, escape from immune surveillance and enhanced anabolism have been proposed as additional features that characterize cancer cells. Autophagy acts as a tumor suppressor mechanism by (at least) three distinct means, namely by preserving genome stability, by limiting oxidative stress, and by reducing intratumoral necrosis-dependent inflammation. Thus, autophagy inhibition may represent yet another hallmark of tumors.

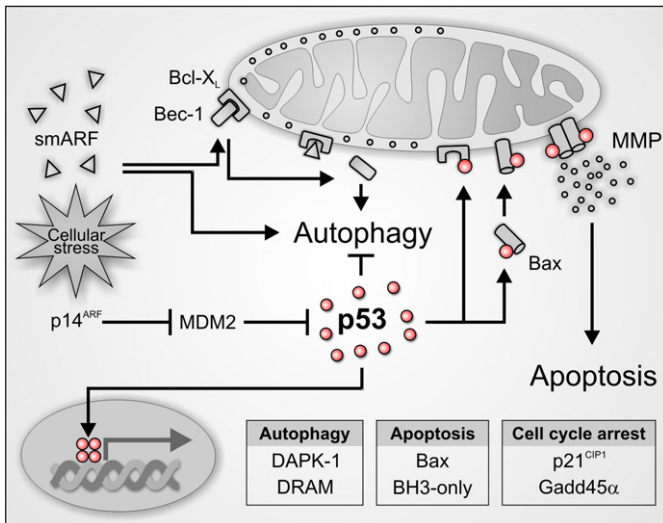


Fig. 2. The p53 network and autophagy. The p53 system can be activated by DNA damage and other stress signals through the p14^{ARF}-dependent sequestration of MDM2, the E3 ubiquitin ligase that normally targets p53 to proteasomal degradation. In the nucleus, active p53 tetramers bind DNA and can transactivate pro-apoptotic (e.g., Bax, BH3-only proteins), cell cycle-arresting (e.g., p21^{CIP1}, Gadd45 α) or autophagy-promoting (e.g., DAPK-1, DRAM) factors. Moreover, p53 can translocate to mitochondria and directly promote cell death, by favoring mitochondrial membrane permeabilization (MMP). Presumably, p53-induced MMP results from the binding of p53 to both pro- (e.g., Bax) and anti-apoptotic (e.g., Bcl-2, Bcl-X_L) members of the Bcl-2 family. Cytoplasmic p53 has also been shown to suppress autophagy, through yet undetermined protein interactors or via hitherto undetermined metabolic circuitries. Both full-length p14^{ARF} and a small mitochondrial isoform of this protein (smARF) have been shown to promote autophagy. In particular, smARF may promote the autophagic flow by releasing Bec-1 from Bcl-X_L-dependent inhibitory interactions. Endogenous levels of p14^{ARF} result in a near-to-complete localization within the nucleolar compartment, where the pro-autophagic functions of p14^{ARF} completely rely on p53. Conversely, mitochondrial smARF also activates autophagy in p53-independent fashion.

damage-induced cell death [74]. Accordingly, several groups have documented the induction of p53-dependent autophagic responses, for instance upon DNA damage [75], ARF activation [61], or following the reintroduction of exogenous p53 in p53^{-/-} cancer cells [76].

Recently, great attention has been paid to the transcription-independent functions of cytosolic p53 (Fig. 2). First, it has been demonstrated that active p53 can accumulate in the cytosol and initiate mitochondrial cell death [77–80], either by directly activating Bax [81] and/or by inhibiting anti-apoptotic members of the Bcl-2 protein family [82]. In this context, the p53-dependent transactivation of Puma (a BH3-only protein that promotes the disruption of inhibitory complexes engaging Bax and p53 with Bcl-2/Bcl-X_L) would couple nuclear and extranuclear functions of p53 [83]. Moreover, cytosolic p53 has been shown to exert a tonic inhibition of the autophagic flow [69]. Thus, human, mouse and nematode cells subjected to knockout, knockdown or pharmacological inhibition of p53 manifest several signs of enhanced autophagy, including LC3 maturation/redistribution and appearance of autophagic vacuoles [4], both *in vitro* and *in vivo* [69]. p53 inhibition also promotes autophagy in enucleated cells, suggesting that the nuclear pool of p53 is not directly implicated in the suppression of autophagy [69]. In line with this notion, upon reintroduction in p53^{-/-} human colon cancer cells, several cancer-associated p53 mutants were shown to inhibit autophagy to an extent that correlated with their propensity to redistribute to the cytoplasm [84]. In particular, maximal autophagy inhibition was observed upon the expression of a p53 mutant that is actively excluded from the nuclear compartment due to the deletion of its nuclear export signal (which results in the accumulation of p53 within the nucleus) totally abolished p53 autophagy-inhibitory functions [69,84].

Intriguingly, large deletions affecting the DNA-binding domain of p53 had no effects on the suppression of autophagy by cytoplasmic p53 [84]. Some amino acids of the DNA-binding domain are required for the interaction between p53 and proteins from the Bcl-2 family [85,86], yet their mutation/absence fails to modify the p53 autophagy-inhibitory potential [84]. Thus, the molecular features that account for autophagy suppression by p53 remain to be discovered. It is also elusive whether this effect relies on the direct interaction between p53 and hitherto unidentified factors or rather is the result of more indirect metabolic circuitries.

Not surprisingly, p53 inhibition (as well as many other pro-autophagic triggers) has been reported to promote autophagy only in the G₁ and early S phases of the cell cycle [87,88]. It would have appeared paradoxical, indeed, that p53-deficient cells (which are characterized by a growth rate similar to that of their WT counterparts) continuously upregulate autophagy throughout the cell cycle (including the late S and G₂/M phases), because a simultaneous increase in catabolism and anabolism would dramatically augment bioenergetic needs of the cells, thereby limiting their rate of proliferation [87].

While p53-dependent transcriptional programs may stimulate autophagy, several lines of evidence indicate that cytoplasmic (but not nuclear) p53 inhibits the autophagic pathway. Short deletions and single amino acids substitutions in p53 are found in more than 50% of human tumors. Mutant p53 proteins constitute a multifaceted family of several hundred members with heterogeneous properties [89]. Still, most of the oncogenic mutations of p53 map to its DNA-binding domain [90], which is required for the activity of p53 as a transcription factor and for its interaction with Bcl-2 [85], but not for its autophagy-suppressing functions [69,84]. In this scenario, it is tempting to speculate that some (but probably not all) cancer-associated mutations may “hit” p53 activity in a double (or even triple) fashion. At least theoretically, such mutations may indeed promote tumorigenesis by inactivating the mitochondrial (pro-apoptotic) and nuclear (pro-apoptotic, cell cycle-arresting and pro-autophagic) functions of p53, while leaving unaffected (or even potentiating) p53-mediated suppression of autophagy [84]. Conclusive experimental data to confirm/disconfirm this hypothesis are urgently awaited.

4. Cytoprotective roles of autophagy during anticancer therapy

Numerous conventional and experimental antitumor strategies are able to induce a massive accumulation of autophagosomes, both *in vitro* (in cancer cell lines) [7] and *in vivo* (in tumors xenografted to immunodeficient mice) [76,91,92]. Frequently, such an extensive vacuolization of the cytoplasm has been considered as (one of) the most prominent manifestation(s) of the so-called “autophagic cell death” [8,93]. According to this perspective, which nowadays is rather discredited [9,10], autophagy would represent an additional mechanism of cell death, and its inhibition would afford cytoprotective effects. On the contrary, while the inhibition of autophagy often shifts the cell death morphotype to a non-autophagic one, it rarely prevents the radio- and chemotherapy-induced demise of cancer cells, and often accelerates it [7,8].

Thus, both pharmacological inhibitors of autophagy (e.g., 3-methyladenine, hydroxychloroquine, bafilomycin A1, monensin) and siRNAs that target essential modulators of the autophagic machinery (e.g., Atg3, Atg4b, Atg4c, Bec-1/Atg6, Atg10 and Atg12) have been shown to sensitize cancer cells to a wide spectrum of stress conditions, including (but not limited to): glucose and amino acid deprivation [70,94]; growth factor withdrawal [12]; detachment from the extracellular matrix (i.e., anoikis) [95,96]; estrogen receptor antagonism with tamoxifen [97]; androgen deprivation [98]; radiation therapy [99]; DNA alkylation damage with cyclophosphamide [76] or temozolomide [100]; inhibition of TKRs with imatinib [101]; inhibition of cyclooxygenases with sulindac sulfide [102]; as well as N-(4-hydroxyphenyl) retinamide- [103], anthocyanin- [104] and TRAIL-induced cell death

[105] (Table 2). Similarly, in a murine model of Myc-induced lymphoma, inhibition of autophagy with either chloroquine or Atg5-targeted short hairpin RNAs (shRNAs) facilitated tumor regression

induced by p53 reactivation and DNA damage [76]. Altogether, these examples substantiate the idea that autophagy is activated by tumor cells as a pro-survival mechanism against cytotoxic agents and may

Table 2

Examples of autophagy inhibition as a tumor cell sensitizing strategy.

Cell type*	Autophagy inhibition	Stress condition	Ref.
Breast adenocarcinoma cells <i>MCF7</i> <i>T47D</i>	siRNA-mediated downregulation Atg5 Bec-1	Estrogen receptor antagonism with tamoxifen	[97]
Cervical cancer cells <i>HeLa</i>	Pharmacological inhibitors 3-MA HCQ siRNA-mediated downregulation Atg5 Atg12	Nutrient depletion	[70]
Colorectal cancer cells <i>HT-29</i>	Pharmacological inhibitors 3-MA	Cyclooxygenase inhibition with sulindac sulfide	[102]
Colorectal cancer cells <i>bax</i> ^{-/-} <i>HCT-116</i> <i>HCT-116-FLIP</i> WT <i>HCT-116</i>	Pharmacological inhibitors 3-MA PepA siRNA-mediated downregulation Bec-1	TRAIL-induced cell death	[105]
Colorectal cancer cell lines <i>DLD-1</i> <i>SW480</i> <i>WiDr</i>	Pharmacological inhibitors 3-MA PepA Atg7-targeted siRNA	Amino acid and glucose deprivation	[94]
Glioma cell lines <i>U87-MG</i>	Pharmacological inhibitors 3-MA	4-HPR-induced cell death	[103]
Glioma cell lines <i>LN229</i> <i>U87-MG</i>	Pharmacological inhibitors 3-MA RTA 203 Atg5-targeted siRNA	Inhibition of TKRs with imatinib	[101]
Glioma cell lines <i>A172</i> <i>U87-MG</i>	Pharmacological inhibitors 3-MA	TMZ-induced cell death	[100]
HCC cells <i>PLC/PRF/5</i>	Pharmacological inhibitors 3-MA	Anthocyanin-induced cell death	[104]
Rat HCC cells <i>McArdle</i>	Atg5-targeted siRNA		
Prostate cancer cells <i>LNCaP</i>	Pharmacological inhibitors 3-MA Bec-1-targeted siRNA	Androgen deprivation	[98]
Murine primary cells <i>bax</i> ^{-/-} <i>bak</i> ^{-/-} bone marrow cells	Pharmacological inhibitors 3-MA siRNA-mediated downregulation Atg5	Interleukin-3 withdrawal	[12]
Murine primary cells <i>Myc/p53^{ERTAM}</i> bone marrow cells	Pharmacological inhibitors CQ Atg7-targeted shRNA	DNA alkylation damage with cyclophosphamide	[76]
Primary MECs <i>1^oHMECs</i> Canine kidney cell line <i>MDCK-2</i> Murine fibroblasts <i>atg5</i> ^{-/-} MEFs	siRNA-mediated downregulation Atg5	ECM detachment (anoikis) Starvation	[95]
Various tumor cell lines <i>A549</i> <i>HTB43</i> <i>Sw707</i>	siRNA-mediated downregulation Atg3 Atg4c Atg12	γ -irradiation	[99]

* Unless otherwise indicated, cells were of human origin. Abbreviations: 3-MA, 3-methyladenine; 4-HPR, N-(4-hydroxyphenyl)retinamide; Atg, autophagy related; Baf-A1, bafilomycin A1; Bec-1, beclin-1; CQ, chloroquine, HCC, hepatocellular carcinoma; HCQ, hydroxychloroquine; ECM, extracellular matrix; MDCK, Madin-Darby canine kidney; MECs, mammary epithelial cells; MEFs, mouse embryonic fibroblasts; PepA, pepstatin A; shRNA, short hairpin RNA; siRNA, small interfering RNA; TKRs, tyrosine kinase receptors; TMZ, temozolomide; TRAIL, tumor necrosis factor-related apoptosis inducing ligand; WT, wild type.

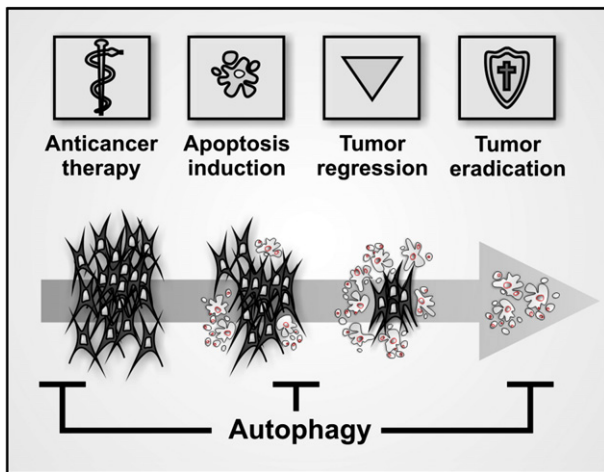


Fig. 3. Pro-survival roles of autophagy. Upon hypoxic, metabolic, detachment-induced and therapeutic stress, tumor cells activate a general pro-survival response centered on autophagy. In multiple experimental settings, pharmacological and/or genetic inhibition of autophagy has been shown to sensitize cancer cells to the lethal effect of different types of stress. Thus, suppression of the autophagic pathway may represent a valuable therapeutic strategy for radio- and chemosensitization.

therefore favor radio- and chemoresistance (Fig. 3). Thus, suppression of the autophagic flow may represent a general strategy to sensitize cancer cells to radio- and chemotherapy-induced cell death. Upon surgical resection, the combination of chloroquine (a lysosomotropic amine that has been widely employed against malaria) with conventional antitumor therapies has shown encouraging results for the therapy of glioblastoma multiforme [106]. Only future will tell whether autophagy inhibition will become a widespread clinical approach for radio- and chemosensitization of tumors.

5. Concluding remarks

Autophagy occupies the center of a complex network of cellular responses to stress. For this reason, the relationship between autophagy and cancer cannot be recapitulated by a simple and uniform principle [107]. As we have discussed here, on one hand autophagy may exert tumor suppressing functions by: (1) limiting chromosomal instability and therefore preventing the accumulation of oncogenic mutations; (2) restricting oxidative stress, which is also an oncogenic stimulus; and (3) reducing intratumoral necrosis and local inflammation. Thus, disabled autophagy might constitute a common feature of cancer that adds to the hallmarks originally formulated in 2000 by Hanahan and Weinberg [108] and more recently integrated by other authors [109,110], namely provision of autonomous growth stimuli, insensitivity to antiproliferative signals, disabled apoptosis, limitless replication, production of angiogenic factors, tissue invasion with metastasis [108], avoidance of the immune response [110], and enhanced anabolic metabolism [109]. On the other hand, enhanced autophagy likewise represents a prominent mechanism used by tumor cells to escape hypoxic, metabolic, detachment-induced and therapeutic stress. Moreover, p53, which has a crucial role in suppressing tumorigenesis, regulates autophagy in an apparently paradoxical fashion, depending on its subcellular localization. The pharmacological modulation of autophagy might therefore represent a valuable tool for anticancer therapy. Autophagy induction might be employed for tumor chemoprevention. Conversely, suppression of the autophagic pathway may be combined with conventional or experimental antitumor regimens to achieve increased efficacy, thereby allowing for lower dosage and limited side effects. Still, autophagy inhibition might have an intrinsic oncogenic potential and it may be applicable only in selected clinical settings. As it

stands, only some of the links between autophagy and cancer have been elucidated. Results of future studies are urgently awaited to shed additional light on this therapeutically promising relationship.

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