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Autophagy Genes as Tumor Suppressors

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Abstract

Autophagy, originally described as a universal lysosome-dependent bulk degradation of cytoplasmic components upon nutrient deprivation, has since been shown to influence diverse aspects of homeostasis and is implicated in a wide variety of pathological conditions, including cancer. The list of autophagy-related (Atg) genes associated with the initiation and progression of human cancer as well as with responses to cancer therapy continues to grow as these genes are being discovered. However, whether Atg genes work through their expected mechanisms of autophagy regulation and/or through as-yet-undefined functions in the development of cancer remains to be further clarified. Here we review recent advances in the knowledge of the molecular basis of autophagy genes and their biological outputs during tumor development. A better understanding of the mechanistic link between cellular autophagy and tumor growth control may ultimately better human cancer treatments.

Introduction

Initially discovered as a physiological response to starvation, autophagy (literally ‘*self-eating*’) has since been proven to be a fundamental homeostatic process that is exhibited by every somatic cell of every eukaryotic organism [1]. Over 30 distinct autophagy-related (Atg) genes have been identified so far in yeast, and more numbers of Atg genes are probably expressed in mammals [2,3]. The physiological importance of autophagy, as one might expect from its homeostatic property, is reflected by the association of autophagy genes with many diseases. In particular, infection, inflammation, neurodegeneration, and cancer can result from pathogen-induced or inherited disruption of the autophagy pathway [4–6]. In this review, we focus on recent findings about the multifaceted roles of autophagy genes in cancer.

Autophagy in brief

Any understanding of the potential role of autophagy in human diseases requires an appreciation of how autophagy is processed and how it normally functions in cells. Autophagy mediates the anterograde transport of intracellular cargoes from currently unknown site(s) to the lysosomal compartment for degradation and recycling, via a centrally important double membrane-bound vesicle, the autophagosome [7,8]. Formed within the cell, the autophagosome serves to surround, sequesters, and finally, seals off extraneous cellular components such as bulk cytoplasm, old and damaged proteins and organelles, from the rest of the inside of the cell. The autophagosome subsequently fuses with the lysosome to form

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autolysosomes, exposing the inner compartment to lysosomal hydrolases. Eventually, the inner membrane of the autophagosome and its sequestered contents are degraded, with the resulting macromolecules recycled [1,7,8]. Thus, autophagy constitutes a scavenging mechanism that maintains quality control of intracellular entities and allows cells to respond appropriately to stress. Notwithstanding extensive studies on the molecular biology of autophagy, relatively little is known about how its rate is controlled and cargo selected. Autophagy can be broadly classified into two distinct modes: selective and non-selective autophagy [3,9]. Non-selective autophagy involves the random sequestration and catabolism of bulk cytosol or other cytoplasmic components, which is necessary for a constant balance of cytoplasmic volume and its contents and probably began congruent with the origins of life. In contrast, selective autophagy is characterized by the highly selective detection and digestion of specific cargoes, including protein aggregates (aggrephagy), mitochondria (mitophagy), ER (reticulophagy), ribosomes (ribophagy), peroxisomes (pexophagy), as well as intracellular bacteria and viruses (xenophagy) [9,10]. It is envisaged that selective autophagy evolved and specialized to match changing demands in different organisms, or even different tissues within the same organism. Thus, autophagy goes far beyond regulating cell's starvation responses, but influences various other aspects of disease progression and everyday life, including stress adaptation, development, lifespan extension, immunity, and protection against neurodegeneration and cancer [1].

Dysregulated autophagy in cancer

The broad involvement of autophagy in metabolic equilibrium and homeostasis makes it an obvious target in human tumors. The first link between autophagy and cancer was made in the seminal work by *Liang et al.* [11] on the basis of observations that ectopic expression of Beclin 1, a yeast homolog of Atg6/Vps30, induces autophagy and concomitantly suppresses breast cancer tumor cell growth. Subsequent studies however, revealed a paradoxical role of autophagy in cancer. On one hand, autophagy was found to be provoked particularly in metabolically stressed solid tumors [12,13]. Since autophagy enables the cell to adapt to stress, it is probably not surprising that in certain circumstances, this pathway is eminently exploited by tumors to ameliorate nutrient emergency and maximize energy production for continued survival and proliferation. Yet, whether such change is simply a by-product of malignant transformation or represents its founding-cause remains to be determined. In fact, recent studies revealed that the activation of oncogenic signals, such as Ras (a common event in malignant progression), can trigger autophagy which in turn leads to cell senescence or autophagic cell death [14,15]. Thus, despite potential advantages for survival that favors tumor growth, an alternative or additional concurrent consequence of autophagy activation in metabolically stressed tumors is its restriction of the proliferative potential of damaged cells.

In keeping with this notion, several lines of evidence based on a range of findings, from the clinical studies of patients to the molecular studies of genetically engineered mice have led to a general acceptance that autophagy is virtually tumor-suppressive [1,16–19]. Mutation of autophagy genes, such as *beclin1*, is common in human cancer [11]. A number of these genes, including *beclin1*, *UVRAG*, and *Bif-1*, function as tumor suppressors in knockout or tumor xenograft mouse models, suggesting a genetic link between autophagy deficiencies and tumor susceptibility [1,20–22]. In addition, many known tumor suppressors, such as PTEN (an AKT inhibitor), tuberous sclerosis 1 (TSC1) and TSC2 (mTOR inhibitors), provide constitutive input signals to activate autophagy, whereas predominant oncogenes, such as mTOR, AKT, and Bcl-2, hinder the autophagic process, demonstrating that elevated autophagy signaling contributes to tumor suppression [1,4,23,24]. The prevailing view of autophagy as a novel mechanism of tumor suppression is further supported by the fact that many anticancer agents act as potent inducers of autophagy (e.g. tamoxifen and rapamycin) [1,25,26]. As mentioned above, autophagy is important in the cells' waste management by removing aged or damaged

proteins and organelles, a potential source of oxidative stress that may lead to genomic damage. Therefore, it is not surprising that deregulation of autophagy can lead to the accumulation of undesirable components, which can fuel inflammation, trigger necrosis, and induce chromosomal instability, ultimately leading to cancer, as have been elegantly demonstrated in the experiments of Karantza-Wadsworth and Mathew *et al.* [12,19,27–29]. However, the precise mechanism that links autophagy and cancer remains elusive.

Taken together, autophagic dysregulation is likely required for the success of cancer cells, but the complexity of cancer and the biology of autophagy do not make it easy to draw a general picture. Understanding how autophagy acts as a tumor suppressor in a tissue-specific and a tumor stage-specific manner may ultimately contribute to the development of new treatments for malignant diseases. It is within this context that we review recently described tumor suppressive activities of autophagy genes.

A multitude of autophagic tumor suppressors

Dysregulated autophagy signaling and trafficking is associated with cancer development; hence the aberrant expression of autophagy genes may initiate or contribute to certain cancer-related pathologies [4,30]. Strikingly, most of the known autophagy effectors or activators are located inside or close to fragile sites, as well as in minimal regions of loss of heterozygosity (LOH), that are associated with cancer [31–33]. For instance, *beclin 1* is located at 17q21, a region commonly deleted in 50–70% of breast cancer and in up to 75% of ovarian cancer patients [31]; the *UVRAG* gene maps to chromosome 11q13, a region that frequently exhibits mutations or deletions in many human cancers, especially in colon malignancies [32,33]; LOH at 1p22, where the *Bif-1* (Bax-interacting factor 1) gene is localized, is observed in many types of tumors [34,35]. A listing of currently identified cancer-related autophagy proteins shows that virtually every stage of autophagy—induction, nucleation, elongation, and maturation is exquisitely controlled by both oncogenic and tumor-suppressive signaling pathways and could be potentially hit in tumor (Table 1). We will for the sake of clarity discuss the multitude—of autophagic tumor suppressors following the linear cascade of autophagy, as also summarized in Figure 1.

Induction

One of the initial events upon autophagy induction as first defined in yeast, is the dephosphorylation of Atg13 which in turn activates the Atg13-associated serine/threonine kinase Atg1 to trigger the autophagic cascade [3]. In a similar vein, the mammalian Atg1 orthologs, including ULK1 and ULK2, as well as ULK3, ULK4, and Fused, regulate autophagy by forming a large molecular weight complex with mAtg13 (mammalian Atg13), FIP200 (the mammalian homologue of Atg17), and the recently identified Atg101 (Figure 1) [36–40]. The Atg1 kinase complex is directly and tightly controlled by the ‘nutrient sensor’, mTOR (mammalian target of rapamycin), which maintains hyperphosphorylation of Atg13 and thereby suppresses the induction of autophagy [41]. While genetic lesions associated with the ULK-mAtg13-FIP200-Atg101 complex have not been detected in human cancers, metabolic signaling relayed to this complex seems to be profoundly altered in cancer cells. Put simply, the oncogenic components in the PI3K-Akt-mTOR-mediated anabolic pathway are constitutively active in most cancers, whereas molecules known to suppress mTOR, including PTEN, AMPK, and TSC1/TSC2, are often mutated [42]. In line with this, the mTOR inhibitor, rapamycin, induces autophagy and is currently actively pursued as a treatment for human malignancies [43]. Moreover, ULK3 was recently found to participate in oncogene-induced cell senescence [14], highlighting the defensive role of autophagy against cancer progression. On the basis of these observations, it is plausible that genetic deficiencies or functional defects of the Atg1 complex may increase cancer susceptibility and facilitate cancer growth, at least in some contexts.

Nucleation

The Beclin1-associated class III PI3K (PI3KC3) complex [44,45] is required for the nucleation of autophagic vesicles and has proven to be crucial in both tumor growth and autophagy activation, thus serving as a key coordinator of both pathways (Figure 1). PI3KC3 activation relies on its association with Beclin1, the first tumor suppressor protein identified in mammalian autophagy [11]. By itself, Beclin1 does not have any enzymatic activity but acts as a platform to recruit other activators including UVRAG, Bif-1 (also called endophilin B1), Ambra-1, and Atg14 [20,22,46–49]. *Beclin1* is probably one of the most frequently mutated tumor suppressors in human cancer. Monoallelic deficiency in this gene initiates and maintains key aspects of the tumor phenotype in several human cancer cell lines as well as in a breast cancer xenograft model [11,12,28]. Additionally, *beclin1* heterozygous mutant mice showed impaired autophagy and an increased propensity for developing spontaneous tumors, further suggesting that the loss of *beclin1* is a causal event in many cancer types [11,31,50]. The importance of Beclin1-mediated autophagy in tumor suppression is further strengthened by data regarding its activator, UVRAG (UV irradiation associated gene). Ectopic expression of UVRAG suppresses the proliferation and tumorigenicity of HCT116 tumor cells and sensitizes these cells to undergo autonomous autophagy even without starvation treatment [20]. Of particular relevance, one copy of the *uvrag* gene, like *beclin1*, is often mutated in human colorectal and gastric cancers [32,51,52], suggesting it is haploinsufficient in tumor suppression. Bif-1 represents another link between autophagic nucleation and tumor suppression. Initially identified as a Bax-binding protein, Bif-1 was recently found to bind and synergize with UVRAG in both autophagy and tumor suppression [22]. Furthermore, the N-terminal BAR domain of Bif-1 confers to it the unique ability to initiate membrane curvature during autophagosome formation [53]. Likewise, Bif-1^{-/-} mice are cancer prone [22]. Thus, different tumor suppressors, irrespective their modes of actions, coordinate this vesicle nucleation program. Layered over this coordination is the pathway that regulates cell survival and apoptosis. The proto-oncogenes Bcl-2 and Bcl-xL bind and hold Beclin1 at bay [54], whereas the pro-death BH3-only molecules, such as Bad, Puma, Bim_{EL}, Noxa, and BNIP3L, as well as BH3 mimetic, such as ABT-737, antagonize Bcl-2 and free Beclin1 from Bcl-2 inhibition [4,23,55–59]. These findings raise the interesting possibility that, under certain conditions in which apoptosis is compromised, autophagy may serve as an alternative or backup mechanism to suppress tumor growth.

Elongation

Autophagosome elongation and completion involves two ubiquitin-like conjugation systems including Atg12, Atg8/LC3, Atg4C (also known as autophagin 3, a cysteine protease that cleaves Atg8), Atg7 (an E1 enzyme), Atg3 and Atg10 (E2 enzymes), and Atg16 (Figure 1; for detail see reference [3]). Among these, Atg4C is more appreciated to have genetic relevance in cancer. Mice with an ablation of *atg4c* showed impaired autophagy and increased susceptibility to carcinogen-induced fibrosarcomas [60]. In addition, frameshift mutations of *atg5* appear to be common in high microsatellite-unstable (MSI-H) tumors and silencing *atg5* in cell culture leads to genomic instability, a hallmark of most cancers [12,28,61]. Most intriguingly, recent genome-wide association analyses have identified *Atg16L1*, an adaptor protein that stabilizes the Atg12-Atg5 conjugates, as a susceptibility gene for Crohn's disease [62,63]. Further, studies employing the *Atg16L1*-hypomorphic mice showed that the Atg16L1-mediated autophagic process plays critical roles in maintaining gut homeostasis and its deficiency predisposes to excessive inflammation, a favorable environment for tumor growth [64–66]. Along this line, it is hence easy to see that this step of autophagy is also engaged by both oncogenic and tumor suppressive signalings. For instance, the anti-apoptotic FLICE-like inhibitory protein (FLIP) that is highly expressed in various primary tumor cells was recently demonstrated to block autophagy by perturbing the Atg3-LC3 interaction [67]. On the basis of this finding, it is postulated that the antiautophagic aspect of FLIP, along with its anti-

apoptotic activity, may contribute to tumor resistance to cytotoxic agents, making it an attractive target for cancer therapy [68]. Unlike FLIP which inhibits LC3 conjugation, the tumor suppressor DAPK (death-associated protein kinase) was shown to activate autophagy by forming a stable complex with the microtubule-associated protein MAP1B, which in turn associates with LC3-II from autophagosomes [69]. Since promoter hypermethylation of the *DAPK* locus is associated with aggressive tumor phenotype and tumor resistance [70], this finding adds a new twist by implicating epigenetic regulation of autophagy in cancer.

Maturation

Autophagosome maturation serves not only as a pivotal checkpoint for the autophagic flux *per se* but is also as the convergence point of receptor signaling and endocytic trafficking. A case in point is Rab7, which organizes the maturation of late endosomes, phagosomes, and autophagosomes, and their eventual fusion with lysosomes [71]. Aberrant Rab7 expression has consistently been linked to various diseases, including cancer, but the underlying mechanism remains to be defined [71,72]. The intimate coordination between the autophagic and endocytic pathways is further strengthened by the observation that UVRAG, which activates Beclin1, also cooperates with the HOPS (homotypic vacuole fusion and protein sorting) tethering complex and the endosome-associated PI3KC3 complex to facilitate autophagosomal maturation and membrane trafficking of multiple growth factor receptors, including the epidermal growth factor (EGF) receptor, probably via distinctly different mechanisms [47, 73]. In contrast, Rubicon (RUN domain protein as Beclin1-interacting and cysteine-rich containing), a newly identified Beclin1 interactor, regulates this process in an opposite manner [73,74]. Since dysregulated receptor trafficking is implicated in cancer, these findings provide another mechanism by which UVRAG can assist autophagy in the suppression of tumors, and it is also tempting to speculate that aberrant expression of Rubicon may be involved in cancer development.

Finally, despite recent advances in our knowledge of the molecular biology of autophagy genes, there is still much to be learned about the relative importance and specificity of each of these genes' functions in the general view of autophagy as a tumor suppressor mechanism *in vivo*. It is also worth noting that the evidence supporting this claim is largely correlative. Future experiments using mouse knockout models of specific autophagy genes may help delineate the complex interplay between autophagy and cancer.

Conclusions

After all is said and done, alteration of the autophagy pathway seems to clearly fuel cancer. Yet, fitting divergent autophagic effects into a simple paradigm of tumor development is less straightforward. For instance, recent studies have shown that in addition to promoting autophagy, p53 inhibits this process [4,75–77]. This unexpected result emphasizes the fact that our knowledge of autophagy function is far from complete. Further studies will be required to clarify the precise mechanism(s) by which autophagy precludes tumor development and by which a tumor evades autophagy. It is also envisioned that different modes of autophagy may be affected in specific contexts of tumors, and understanding such complexity and specificity of the autophagy pathway should enable us translate this knowledge into new therapeutic strategies in the future.

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Figure 1. Schematic representation of the autophagy pathway and its regulation by both oncogenic and tumor suppressive signaling

The induction of autophagy by various stimuli (e.g. starvation, hypoxia, etc) signals through the activation of the TSC1/TSC2 complex that leads to the inhibition of mTOR. Dephosphorylated mAtg13 resulting from mTOR inactivation then associates with and activates the Atg1/ULK kinase. Together, they form the Atg1 kinase complex with FIP200 and Atg101, triggering the autophagic cascade. Vesicle nucleation is primarily driven by the class III phosphatidylinositol (PtdIns) 3-kinase (PI3KC3) complex. The core subunits of this complex include PI3KC3, p150, and Beclin1. Recent data suggest that multiple Beclin1 complexes may be involved in the mammalian autophagy regulation. In particular, the tumor

suppressor, UVRAG, Bif-1 (an UVRAG interactor), and probably Ambra-1 associate with Beclin1 to activate autophagy. This is frequently dysregulated in many human cancers. Additionally, Atg14 was also found to promote autophagy by forming a separate complex with Beclin1. The anti-apoptotic Bcl-2 proteins negatively regulate autophagy by sequestering Beclin1. This is counteracted by the proapoptotic BH3-only molecules which promote autophagy by freeing Beclin1 from Bcl-2 inhibition. Two ubiquitin-like conjugation systems are involved in autophagosomal membrane expansion and completion: one is the Atg8/LC3-phosphatidylethanolamine (PE) conjugation, the other is the Atg12-Atg5-Atg16 conjugation. After proteolytic cleavage of its carboxyterminus by the Atg4C cysteine protease, Atg8 is coupled to the membrane lipid PE after sequential processing by Atg7, an E1-like enzyme and Atg3, an E2-like enzyme. Atg12 is conjugated to Atg5 in a similar manner except Atg10 is used as the E2 enzyme instead of Atg3. During this conjugation process, the interaction of ubiquitin-like Atg8 with Atg3 can be severely disturbed by FLIP, an oncogenic protein. In contrast, proapoptotic DAPK positively regulates this process by associating with an Atg8/LC3-interacting cytoskeleton molecule, MAP1B. The fusion of the autophagosome with the lysosome signifies the maturation stage of the autophagy pathway, a step that is least understood but has been found to functionally converge with the endocytic pathway. Lysosomal fusion is driven by SNAREs, the small GTPase Rab7, PI3KC3, and the HOPS complex which consists of the class C vacuolar protein sorting (C/Vps) complex core (Vps11, Vps16, Vps18, and Vps33) and two additional subunits Vps39 and Vps41 [78–80]. UVRAG forms a complex with HOPS and facilitates HOPS-mediated membrane tethering, which likely involves Rab7 activation. In addition, Rubicon was recently found to associate with the endosome-associated PI3KC3 complex, exerting an inhibitory effect on the PI3KC3 activity and the fusion of the autophagosome with the lysosome. Not only are effector proteins of autophagy implicated in cancer development, but the autophagy pathway is also regulated by both oncogenes and tumor suppressors.

Table 1

Examples of autophagy genes in tumor suppression				
Auto-phagy	Factors	Cancer Relevance	(+) Regulator ONC/TS	(-) Regulator ONC/TS
Induction	ULK1-5	ULK3 is involved in oncogene-induced cell senescence	PTEN (TS)	PI3K (ONC)
	mAtg13			
	FIP200		AMPK (TS)	AKT (ONC)
	Atg101		TSC1/TSC2 (TS)	mTOR (ONC)
Nucleation	Beclin1	Highly mutated in human breast, ovarian and prostate tumors; haploinsufficient TS.	LKB1 (TS)	
	UVRAG	Mutation detected in human colorectal, breast and gastric tumors; haploinsufficient TS candidate.		Antiapoptotic Bcl-2 family proteins (ONC)
	Bif-1	Bif-1 ^{-/-} mice are cancer prone; decreased expression in gastric and prostate cancers		
	Ambra1	Ambra1 ^{-/-} mice have severe neural tube defect		
Elongation	Atg14	?		
	PI3KC3-p150	?		
	Atg12-Atg5	Atg5 frameshift mutations in gastric cancer	DAPK (TS)	
	Atg8/LC3			
Maturation	Atg4C	Atg4C ^{-/-} mice develop fibrosarcomas in response to carcinogen treatment;		
	Atg3, Atg10			FLIP (ONC)
	Atg7			
	Atg16	Mutation detected in Crohn's disease		
Maturation	Rab7	Rab7 aberrant expression in human leukemia	UVRAG (TS)	RUBICON (?)
	HOPS			
	SNARE			
	LAMP1/			
	LAMP2			

(+) Regulator: positive regulator; (-) Regulator: negative regulator; ONC: Oncogene; TS: Tumor suppressor; ULK: unc-51-like kinase; mAtg13: mammalian Atg13; FIP200: focal adhesion kinase family interacting protein of 200kD; PTEN: phosphatase and tensin homolog; AMPK: AMP-activated protein kinase; TSC: tuberous sclerosis; LKB1: liver kinase B1; mTOR: mammalian target of rapamycin; UVRAG: UV irradiation resistance associated gene; DAPK: death-associated protein kinase; FLIP: FLICE-like inhibitory protein. HOPS: homeotypic fusion and vacuolar protein sorting. RUBICON: RUN domain protein as Beclin1 interacting and cysteine-rich containing.