Macroautophagy (hereafter referred as “autophagy”) is an eukaryotic subcellular process in which membrane structures engulf cytoplasmic components, including long-lived or malfunctioning proteins and obsolete or damaged organelles, forming double-membraned vesicles called autophagosomes [1, 2]. Autophagosomes fuse with lysosomes to form single-membraned autolysosomes in which the contents are digested by hydrolases and recycled for biosynthesis in the cell [1, 2]. Autophagy is a critical means for cells to maintain homeostasis and help cells survive stressful conditions. Tumor suppressors mostly positively regulate autophagy, whereas oncogene products usually inhibit autophagy. Alterations in key autophagy genes have also been shown to affect cancer development. However, the role of autophagy in cancer depends on the status of the cells and can either suppress or promote tumor growth. In the present review, we report on the current state of knowledge about the reciprocal regulation of autophagy and the potential role of autophagy played in cancer development and therapy.

Abstract: Autophagy is a cellular process to degrade long-lived or malfunctioning proteins and obsolete or damaged organelles. It maintains cellular homeostasis and helps cells survive stressful conditions. Tumor suppressors mostly positively regulate autophagy, whereas oncogene products usually inhibit autophagy. Alterations in key autophagy genes have also been shown to affect cancer development. However, the role of autophagy in cancer depends on the status of the cells and can either suppress or promote tumor growth. In the present review, we report on the current state of knowledge about the reciprocal regulation of autophagy and the potential role of autophagy played in cancer development and therapy.

Keywords: Autophagy, tumor suppressor, oncogene, cancer, cancer therapy, PTEN, p53, RB, E2F1, Bcl-2

Introduction

Macroautophagy (hereafter referred as “autophagy”) is an eukaryotic subcellular process in which membrane structures engulf cytoplasmic components, including long-lived or malfunctioning proteins and obsolete or damaged organelles, forming double-membraned vesicles called autophagosomes [1, 2]. Autophagosomes fuse with lysosomes to form single-membraned autolysosomes in which the contents are digested by hydrolases and recycled for biosynthesis in the cell [1, 2]. Autophagy is a critical means for cells to maintain homeostasis and execute differentiation and development functions [3]. The process also renders cells able to survive stressful conditions such as starvation or pathogen infection [3, 4]. Thus, in contrast to apoptosis, autophagy is mainly a cell survival process. Abnormalities in autophagy function are related to diverse diseases, including neurodegeneration, heart disease, and cancer [3-5].

Regarding to the role played by autophagy in cancer, mounting evidence suggests that autophagy is a tumor suppressor pathway. Tumor suppressor proteins, including phosphatase and tensin homolog (PTEN), p53, and retinoblastoma protein (RB) [3, 6], have been shown to positively regulate autophagy. By contrast, oncogene products, including Bcl-2 and the AKT/TOR pathway, have been found to inhibit autophagy [3]. Further supporting the concept, the inactivation of key autophagy genes, such as Beclin 1 and ATG4C, has been shown to result in the formation of spontaneous tumors [7] or to increase the formation of tumors by carcinogens in mice respectively [8]. However, the relationship between autophagy regulation and tumor is not clear cut; for example, cytoplasmic p53 has been found to inhibit autophagy [9]. In addition, although deficient autophagy has been linked to tumorigenesis [3], autophagy also enables cancer cells to survive stressful conditions such as hypoxia or cytotoxic therapies [3, 10], resulting in resistance to therapies and cancer recurrence.

In this review, we summarize the literature on the role played by tumor suppressors and oncogene products in the regulation of autophagy.
Autophagy regulation and cancer

and the involvement of autophagic genes in cancer. We discuss the relevance of autophagy in cancer development and progression, and the potential role of autophagy in cancer therapy.

**Oncogene products and tumor suppressors in autophagy regulation**

**PI3K-Akt-mTOR pathway**

The phosphatidylinositol 3-kinase (PI3K)–Akt-mTOR pathway is perhaps the most commonly activated signaling pathway in human cancers [11]. The two most widely observed mechanisms of PI3K-Akt-mTOR alteration in human cancers are activation by receptor tyrosine kinases and somatic mutations in specific components of the signaling pathway [12]. This pathway is a key signal transduction system that integrates signals from growth factors, nutrients, and energy to regulate cell growth and proliferation via different cellular processes [11]. mTOR complex 1 (mTORC1) is a master regulator of autophagy that acts in the core autophagy molecular machinery [13]. It links upstream signals that inhibit autophagy through AKT. Under nutrient-rich conditions, mTORC1 phosphorylates ULK1 and ULK2 (two of the five human homologs of Atg1), and mAtg13, inhibiting the initiation of autophagy; however, upon inactivation of mTORC1 by nutrient starvation, mTORC1 dissociates, resulting in activation of ULK1 and ULK2, which initiates the autophagy cascade.

**Bcl-2 family proteins**

Another molecule at the core of autophagy regulation is Beclin 1 [13]. Beclin 1 is the mammalian ortholog of Atg6/Vps30 in yeast [14]. It interacts with class III PI3K (PI3KC3)/Vps34 to form the preliminary autophagic double-membrane, or autophagophore [15]. Beclin 1 is also involved in the recruitment of autophagy proteins—such as UVRAG (UV irradiation resistance–associated gene), Ambra1 (the activating molecule in Beclin 1–regulated autophagy), Bif-1 (Endophillin B1), and Barkor (Beclin 1–associated autophagy-related key regulator)—to the autophagophore for the initiation and maintenance of autophagy [16-19]. Beclin 1 was originally isolated and identified as a B cell lymphoma/leukemia 2 (Bcl-2) interacting protein [20]. It contains a Bcl-2 homology 3 (BH3) domain, conserved in many Bcl-2 family proteins, that is necessary for interaction with Bcl-2 and Bcl-XL [21, 22].

The Bcl-2 protein family plays a dual role in autophagy regulation. Antiapoptotic oncoproteins, such as Bcl-2, Bcl-XL, Bcl-w, and Mcl-1, can inhibit autophagy, whereas proapoptotic BH3-only proteins, such as BNIP3, Bad, Bik, Noxa, Puma, and BimEL, can induce autophagy [23]. In mice and humans, Beclin 1 constitutively interacts with the anti-apoptotic proteins. The interaction of Bcl-2/Bcl-XL with Beclin 1/Vps34 complex decreases PI3KC3/Vps34 activity, whereas the BH3 domain of BH3-only proteins such as Bad may competitively disrupt the inhibitory interaction of Beclin 1 and Bcl-2/Bcl-XL, leading to autophagy.

Recently, c-Jun N-terminal kinase 1 (JNK1), a mitogen–activated protein kinase, has been shown to mediate multisite phosphorylation of Bcl-2, thereby inhibiting its ability to bind Beclin 1 and facilitating autophagy during nutrient deprivation [24]. JNK has also been implicated as a tumor suppressor through its action of limiting Ras-induced transformation in vivo [25]. Furthermore, mutations in the gene for mitogen-activated kinase kinase 4, an upstream activator of JNK, are common in pancreatic, breast, colon, lung, and prostate cancers [26]. Even though JNK involvement in tumor progression remains controversial, the JNK activation of Beclin 1–mediated autophagy may indicate its role in tumor suppression. Another tumor suppressor that positively regulates Beclin 1 activity, DAPK (death-associated protein kinase), physically interacts with Beclin 1 and phosphorylates Beclin 1 on Thr119, a crucial position within the BH3 domain of Beclin 1, and thus promotes the dissociation of Beclin 1 from its inhibitor, Bcl-XL, resulting in autophagy induction [27].

**PTEN**

The tumor suppressor PTEN is the second most frequently mutated tumor suppressor gene in human cancers [28, 29]. PTEN’s primary function is to antagonize PI3K through its intrinsic lipid phosphatase activity by dephosphorylating PtdIns-3,4-P2 and PtdIns-3,4,5-P3 at the D3 position [30, 31]. Ectopic expression of wild-type PTEN has been shown to induce G1 growth arrest, anoikis, apoptosis, or autophagy, depend-
Autophagy regulation and cancer

The molecular mechanisms involved in PTEN-induced autophagy are mainly mediated through its inhibition of the PI3K-Akt-mTOR signaling cascade [35]. FOXO3a, a downstream target of Akt inhibition [36], up-regulates the transcription of a panel of autophagic genes [37, 38], as well as PINK1/PARK6 (PTEN-induced putative kinase 1) [39]. PINK1 is recruited to mitochondria with Parkin and associates with LC3 to induce p62- and VDAC1-dependent mitophagy [40, 41].

However, several mTOR-independent mechanisms have been implicated in autophagy induced by PTEN. For instance, the nuclear accumulation of FOXO3a is also dependent on AMP-activated protein kinase (AMPK) [37], whose activation has also been observed in nuclear-PTEN–expressing cells [42]. Activated AMPK has been shown to induce autophagy through multiple mechanisms, including inhibition of mTOR via TSC2 (tuberous sclerosis 2) phosphorylation/activation [43], activation of p53 [44], and stabilization of p27/kip1 [45]. Whether nuclear PTEN-mediated AMPK activation depends on the Peutz-Jeghers syndrome protein (LKB1) remains to be elucidated, although LKB1 has been found to interact and phosphorylate PTEN [46]. Moreover, PTEN has been shown to mediate the activation of the RNA-dependent protein kinase–eukaryotic translation initiation factor 2α (eIF2α) pathway independently of its phosphatase activity [47]. Phosphorylated eIF2α is known to induce the expression of Atg12 and LC3 conversion from LC3-I to -II, both of which are essential factors for autophagy induction [48]. Alternatively, PTEN can potentially trigger autophagy through cross-talk with other tumor suppressors, such as stabilizing p53 [49] and restoration of RB activity [50].

p53

The TP53 gene is mutated in more than 50% of cases of human cancer, which makes it the most commonly mutated gene in cancers [51]. TP53 encodes for a well-known tumor suppressor protein, p53, which plays a critical role in the cellular responses to several stresses [52]. Since its discovery, p53 has been found to have an ever-increasing number of molecular functions. For that reason, it has been progressively viewed as not simply the “guardian of the genome,” but also as a pleiotropic regulator of multiple cellular responses [53]. Recent studies have revealed a regulation of autophagy by the p53 pathway [54, 55]; however, the role of p53 in autophagy seems to depend on the subcellular localization of the protein. Thus, nuclear p53 seems to facilitate autophagy, whereas cytoplasmic p53 seems to inhibit autophagy [56].

As a transcription factor, p53 can transactivate several targets that stimulate autophagy, mainly by inhibiting the negative regulator of autophagy mTOR [57]. These targets include PTEN, AMPK, TSC2, sestrin1, and sestrin2. AMPK phosphorylates TSC1 and TSC2 complex proteins, which in turn down-regulate mTOR activity [58]. Moreover, sestrin1 and sestrin2 can bind AMPK directly, inducing TSC2 phosphorylation and activation and subsequently inhibiting mTOR activity [59], leading to autophagy [60]. p53 has also been described as inducing autophagy directly through its transcriptional regulation of DRAM (damage-regulated autophagy regulator), a lysosomal protein that induces macroautophagy [61]. Finally, BH3-only proteins Bad, Bax, Bnip3, and Puma, all of which can be transactivated by p53, have been shown to exert proautophagic roles by destabilizing inhibitory interactions between Bcl-2/Bcl-XL and Beclin 1 [57, 62].

As mentioned above, in contrast to the nuclear actions of p53, cytoplasmic p53 seems to inhibit basal autophagy in unstressed cells. Knockout, knockdown, or pharmacological inhibition of p53 has been shown to induce autophagy in human, mouse, and nematode cells, indicating that p53 plays a role in controlling basal autophagy levels [9]. Moreover, the reintroduction of wild-type p53 into p53-null cells suppresses autophagy. This effect persists in enucleated cells, which indicates independence from nuclear translocation. Interestingly, some p53 mutants retain the ability to inhibit autophagy: p53 mutants that have lost their transactivation activity or that cannot bind DNA or Bcl-2 family members are still able to suppress basal autophagy [63]. In the face of chronic starvation, p53 enables reduced, yet sustainable autophagic flux to support cancer cell survival through posttranscriptionally downregulation of LC3 [64].

In summary, p53 exerts a bidirectional control of autophagy depending on its subcellular location. In response to several stresses, nuclear p53 induces autophagy mainly by regulating
direct targets that block mTOR signaling or Bcl-2/Bcl-xL, whereas cytoplasmic p53 inhibits autophagy levels independently of its transcriptional activity.

**RB-E2F1 pathway and CDKIs (cyclin-dependent kinase inhibitors)**

RB was the first identified tumor suppressor. Originally, the tumor suppressor function of RB was mainly attributed to the central role it plays in cell cycle regulation, in which RB arrests cells in the G1 phase through binding and inhibiting E2F transcription factors [65]. However, it is now believed that RB has many cellular functions in addition to serving as a cell cycle brake, including controlling cellular differentiation during embryogenesis and, in adult tissues, regulating apoptotic cell death, maintaining permanent cell cycle arrest, and preserving chromosomal stability [66]. Inactivation of RB is found in many types of cancers [67]. In tumors with wild-type RB gene, RB is frequently inactivated by the lack of expression of activators, such as the p16INK4a (p16) protein, and overexpression of repressors, including cyclin D1 and Cdk4 [67]. RB is also the target of DNA tumor viruses that cause cancer. For instance, in cervical carcinoma and squamous cell carcinoma of the head and neck, the tumors are initiated in part by inactivating RB through expression of the E7 oncoprotein by human papillomavirus [68, 69].

The first clue that RB is linked to autophagy appeared in a report showing that p27 (Kip 1), an RB activator [70], induces autophagy when it is overexpressed in glioma cells [71]. In addition, when cells undergo glucose starvation, p27 is phosphorylated at Thr 198 downstream of the LKB1-AMPK energy-sensing pathway [45]. The phosphorylation of p27 stabilizes p27 and permits cells to survive growth factor withdrawal and metabolic stress through autophagy. p27 and p16, which are both RB activators, have been shown to induce autophagy in an RB-dependent manner [6]. Moreover, restoration of RB expression in RB-null cells causes autophagy. However, when E2F1, the main repression target of RB function, coexpresses with RB in the cells, it antagonizes RB-mediated autophagy, inducing apoptosis; and RB mutants unable to bind E2F cannot induce autophagy. Thus, RB induces autophagy through repressing E2F1 activity. As supporting evidence of this process, Bcl-2, which is transactivated by E2F1 [72] and inhibits autophagy through binding Beclin 1 [73], is down-regulated by RB activity. Therefore, just as these two proteins play opposite roles in the regulation of the cell cycle and apoptosis [74, 75], they act against each other in the regulation of autophagy as well [6, 76].

However, E2F1 transactivates more than 2000 genes, and the role of E2F1 in regulating transcription is highly dependent on context [77]. In addition, RB interacts with more than 200 proteins [67]. Thus, the function of RB-E2F1 in autophagy regulation could be contextual. For example, when cells undergo hypoxia, RB seems to negatively modulate hypoxia-inducible factor–mediated autophagy by attenuating Bnip3 induction by E2F1 [78]. A weak E2F1 transactivation of autophagy-related genes has also been observed in a drug-induced E2F1-expressing system [79]. It has recently been reported that autophagy is activated during senescence and that it may mediate the acquisition of the senescence phenotype [80]. Thus, given the critical role played by CDKIs and RB during cellular senescence [81], there might be a link between the autophagy mediated by these molecules and senescence.

**Autophagy genes as tumor suppressors**

Additional evidence of the connection between autophagy and cancer is that alterations in autophagy genes result in susceptibility to tumorigenesis. A seminal study to identify the role of autophagy genes in cancer development discovered that the beclin 1 locus is located at 17p21 and that this region is deleted in up to 75% of ovarian cancers and 50%–70% of breast cancers [82]. Furthermore, beclin 1 null mice die as early embryos, and although heterozygous mice survive, they have a 4-fold increase in spontaneous tumor formation over wild-type mice [83]. Further studies have indicated that positive regulators of autophagy are located at or near sites common to genetic instability or loss of heterozygosity [84]. For instance, colon and gastric cancers with microsatellite instability frequently have frame-shift mutations in the autophagy genes UVRAG, ATG2B, ATG5, ATG9B, and ATG12 [85, 86]. UVRAG and Bif-1, which enhance the binding of Beclin 1 with PI3KC3 to form the complex required for nucleation of autophagic vesicles, have been shown to have tumor suppression activity [16, 18]. Loss of UVRAG correlates with increased
Autophagy regulation and cancer

tumorigenesis, and the UVRAG⁻/⁻ tumorigenic phenotype can be rescued by the administration of exogenous UVRAG [16]. Bif-1 null mice develop normally, with the exception of an enlarged spleen, and have an increased incidence of spontaneous tumor formation. 82.8% of Bif-1 null mice developed lymphoma, compared with 14.3% of their wild-type counterparts [18]. Moreover, overexpression of ULK3 (Unc 51-like kinase 3), one of the five human homologs of Atg1 (a serine/threonine protein kinase that is an upstream regulator of autophagy under mTORC1 regulation), leads to premature senescence and is up-regulated in response to DNA damage [80]. In Crohn disease, which predisposes patients to cancer [87, 88], genome-wide association scanning has revealed variants of ATG16L1 [89] and IRGM [90]. ATG16L1 encodes Atg16L1 to complex with Atg5-Atg12 for autophagosome elongation [91]. IRGM, which belongs to the p47 immunity-related GTPase family, induces autophagy and thereby control of intracellular Mycobacterium tuberculosis in human macrophages [92]. In addition, Atg4C, a cysteine proteinase involved in cleavage of the C-terminal glycine of LC3B [93], has been shown to increase fibrosarcoma tumorigenesis after chemical carcinogenesis treatment, although ATG4C is not required for normal mouse development [8].

**Autophagy, cancer development and cancer therapy**

In the case of cancer development and cancer therapy, autophagy is a double-edged sword. Tumor suppressors usually positively regulate autophagy, whereas oncogene products inhibit autophagy. Alterations in key autophagy genes have been found to be relevant to cancer development (Figure 1). However, there is a lack of direct evidence showing that autophagy defi-
Autophagy regulation and cancer

Efficiency is required for cancer initiation. Moreover, the mechanisms underlying how autophagy suppresses cancer have not been well established. One hypothesis is that autophagy may function as a housekeeping pathway to exert quality control on organelles, proteins, and DNA. Mathews et al. observed that in autophagy-deficient and apoptosis-incompetent tumor cells, metabolic stress leads to the accumulation of p62, elevated expression of endoplasmic reticulum chaperones, damaged mitochondria, and reactive oxygen species [94], resulting in genome instability and leading to tumorigenesis [95]. Another possibility is that the combined impairment of apoptosis and autophagy promotes necrotic cell death under hypoxia and metabolic stress, both of which have been associated with inflammation and accelerated tumor growth [96]. Generally, chronic tumor inflammation favors protumor immunity, such as infiltration of protumor inflammatory mediators (e.g., macrophages) [97, 98]. Thus, autophagy may suppress cancer by buffering metabolic stress when apoptosis is inhibited, to prevent necrotic cell death [96]. Moreover, as mentioned above, autophagy has been recently identified as a new effector mechanism of senescence [80]. Autophagy is activated during oncogene- and DNA damage-induced senescence; inhibition of autophagy delays the senescence phenotype, including senescence-associated secretion. Thus, autophagy-mediated senescence may limit the proliferation of damaged cells and prevent them from developing into tumors.

However, autophagy can also contribute to tumor survival. The high proliferation rate of cancer cells within a tumor usually causes metabolic stress and hypoxia, such as in poorly vascularized solid tumors. In apoptosis-defective cells, inhibition of autophagy confers sensitivity to metabolic stress [96]. In fact, autophagy is observed in unvascularized, metabolically stressed regions of tumors [96]. In addition, autophagy may promote tumor cell survival during dissemination and metastasis during which the detachment of cells from the extracellular matrix may cause cell death, namely anoikis. In this regard, autophagy has been reported to increase epithelial cell survival during anoikis, including in detached cells harboring antiapoptotic lesions [99].

Increased autophagy is often observed in cancers during chemotherapy or radiation. The majority of the literature has reported a protective role of autophagy, although it has also been shown to facilitate cell death. Specific inhibition of autophagy with RNAi has been shown to accelerate cell death during cancer treatment [100]. Although there may be an off-target effect, a battery of chemical inhibitors of autophagy, such as 3-methyladenine, bafilomycin A1, and chloriquine, enhance cytotoxic cancer therapy [101]. In addition, in cells that have escaped therapy or at metastatic sites, autophagy-mediated senescence may also contribute to tumor dormancy [102]. These cells may recover and reenter the cell cycle to cause a cancer recurrence [103, 104]. Therefore, prompt disruption of autophagy pathway could be an efficient approach to extend the therapeutic benefits and reduce the incidence of cancer recurrence.

Nonetheless, in certain cancer treatments, autophagy is required for inducing robust cell death in tumor cells [101]. Because autophagy is a process to destroy cellular structures in the cytoplasm, depending on the intensity and persistence of the stress, this destruction by autophagy may pass a threshold to cause a catastrophe in the cell, leading to cell death. Moreover, caspase activity has also been reported to contribute to this type of cell death [105-107]. Thus, dissection of the transition of autophagy to autophagic cell death and the cross-talking between autophagy and apoptosis components may shed fresh light on more efficacious interventions in cancer therapy.

Concluding remarks

Autophagy plays an essential role in maintaining cellular homeostasis and protecting cells from stressful conditions. It is not surprising to see the involvement of autophagy in cancer. However, the role of autophagy in tumorigenesis and cancer therapy appears to be contextual. Since autophagy is mainly a survival mechanism to maintain the well-being of the cell, depending on the state of the cell, autophagy could suppress or promote cancer development and growth. In normal cells, autophagy clean up damaged organelles and malfunctioned proteins to prevent the cells from becoming cancerous. In cancer cells, autophagy may help them survive stresses, resulting in enhanced malignancy or recurrence after therapy.

Tumor suppressors regulating autophagy complements their tumor suppression function

367  Am J Cancer Res 2011;1(3):362-372
Autophagy regulation and cancer

through the control of cell cycle, cell growth and apoptosis. Their regulation of autophagy also explains some of the functions contrary to their tumor-suppressing role. For instance, tumor suppressors p53 and RB have been shown to help cancer cells survive stress [64, 75, 108]. Through maintaining better autophagic homeostasis and adjusting the rate of autophagy to changing circumstances, wildtype p53 benefit some cancer cells from the resultant increased fitness under limited nutrient supply [64]. Moreover, loss of RB in cancer cells leads to increased sensitivity to therapy [75, 109, 110] and activation of RB via transfer of p16 increases chemoresistance [111].

Therefore, research into autophagy regulation and its relevance in tumorigenesis and cancer therapy may provide new avenues for cancer prevention and therapeutic strategies. For instance, periodic stimulation of autophagy could be beneficial for cancer prevention. The status of the genes involved in autophagy regulation should be taken into consideration when developing personalized cancer therapy with autophagy-inducing medicines to optimize the therapeutic effect in patients.

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Autophagy regulation and cancer


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Autophagy regulation and cancer


