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Autophagy as a Possible Target for Cancer Therapy

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Abstract

Autophagy is an evolutionarily conserved catabolic process that targets cellular organelles and cytoplasmic constituents to the lysosomes for degradation. According to the type of cargo delivery, there are three main types of autophagy systems in mammals: macroautophagy, microautophagy, and chaperone-mediated autophagy.

Macroautophagy, often simply (and hereafter) referred to as autophagy, is the best studied autophagic process and focus of this review. Autophagy has been recognized to be a pro-survival mechanism at times of cellular stress including starvation. Besides, increasing evidence indicated the importance of autophagy in in the pathogenesis of several diseases including cancer. But, its role in cancer is more complex and still controversial; it appears to be tumor suppressive during tumorigenesis, but contributes to tumor cell survival during cancer progression. Besides, autophagic capacity was shown to significantly affect responses of cancer cells to anticancer agents and radiation. Even though there is still a gap about how autophagy is regulated in cancer, it appears to provide a promising target for cancer treatment. This review aimed at examining the multiple roles of autophagy as a novel target for cancer therapy..

Keywords: Authophagy; Novel targets; Tumorogenesis; Cancer progression

Introduction

Autophagic responses can be relatively non-selective or, on the contrary, highly specific. In this regard, non-selective autophagy executed at times of nutrient deprivation and ones activated several intracellular entities could be degraded to meet metabolic demand of cells by generating metabolic precursor for cell survival [1]. Whereas, selective autophagy is triggered in response to specific cellular homeostatic needs [2]. For instance, ribophagy elicited in order to selectively degrade defective ribosomes, while mitophagy make sure the selective degradation of defective mitochondria, and there are also other selective autophagic process targeted against other cellular components and cellular aggregates and intracellular bacteria and virus [3].

The molecular basis of autophagy has been well studied, mainly in yeasts, through examination of autophagy-defective mutants to identify the responsible genes (designated as AutophaGy; ATG), and according to current reports about 35 ATG genes have been discovered in yeast. More than half of these genes have obvious mammalian counterparts, and many of the core aspects of the process are conserved [1]. During autophagic process ATG proteins are sequentially activated to regulate different stages of autophagy. These proteins can be grouped into various complexes based on their functions: the uncoordinated-51-like kinase (ULK) complex, the ATG9-ATG2-WIPI1/ATG18 complex, class III phosphatidylinositol 3-kinase (PI3KIII) complex, and 2 ubiquitin-like conjugation systems, which include the ATG12–ATG5 and microtubule-associated protein I light chain 3 (LC3)/γ amino butyric acid A receptor-associated protein-like 1 (GABARAP) proteins. The fundamental sequential steps of process (Figure 1) includes induction, nucleation of membrane, elongation and maturation, fusion and, degradation of cargo contents by lysosome, recycling and autophagic lysosome reformation [4-8]. Furthermore, several signalling pathways regulate autophagy in mammalian cells (Figure 2), including phosphoinositide 3-kinase/v-akt murine thymoma viral oncogene homolog/mTOR-C1 (PI3K/AKT/mTOR-C1), adenosine monophosphate activated protein kinase (AMPK), mitogen-activated protein kinase (MAPK) pathways [9-17]. To this end this review aimed at scrutinizing the multiple roles of autophagy as a novel target for cancer therapy.

Role in Tumor Development and Progression

There is now increasing evidence that autophagy has complex and paradoxical roles in tumorigenesis, tumor progression and cancer therapeutics. Currently, there is a consensus that tumor suppressive functions of autophagy act during tumor initiation, and as a survival strategy by established tumors to cope with diverse stresses of the microenvironment that are encountered during tumor progression and metastasis [4,18-20].

Tumor Suppression

Early studies indicated that malignant or transformed cells often display lower basal autophagic activity than their normal counterparts [21,22]. This finding in differential expression suggests a link between tumorigenesis and decreased levels of autophagy. Furthermore, the identification of the genes required for the process offers opportunity to use genetic approaches to explore the role of autophagy in cancer development. From early studies, it was observed that *Becn1* is monallelically deleted in around 50% of breast, ovarian and prostate cancers [23,24]. Moreover, one genetic study revealed that mice homozygously deleted for Beclin1 die during embryogenesis [25]. However, subsequent studies using mice with heterozygous deletion of Beclin1 showed that increased frequency of spontaneous cancers including lung adenocarcinomas, hepatocellular carcinomas (HCC), and lymphomas [25,26]. Thus, these studies provided the first direct genetic evidence that Beclin1 functions as a haplo insufficient tumor

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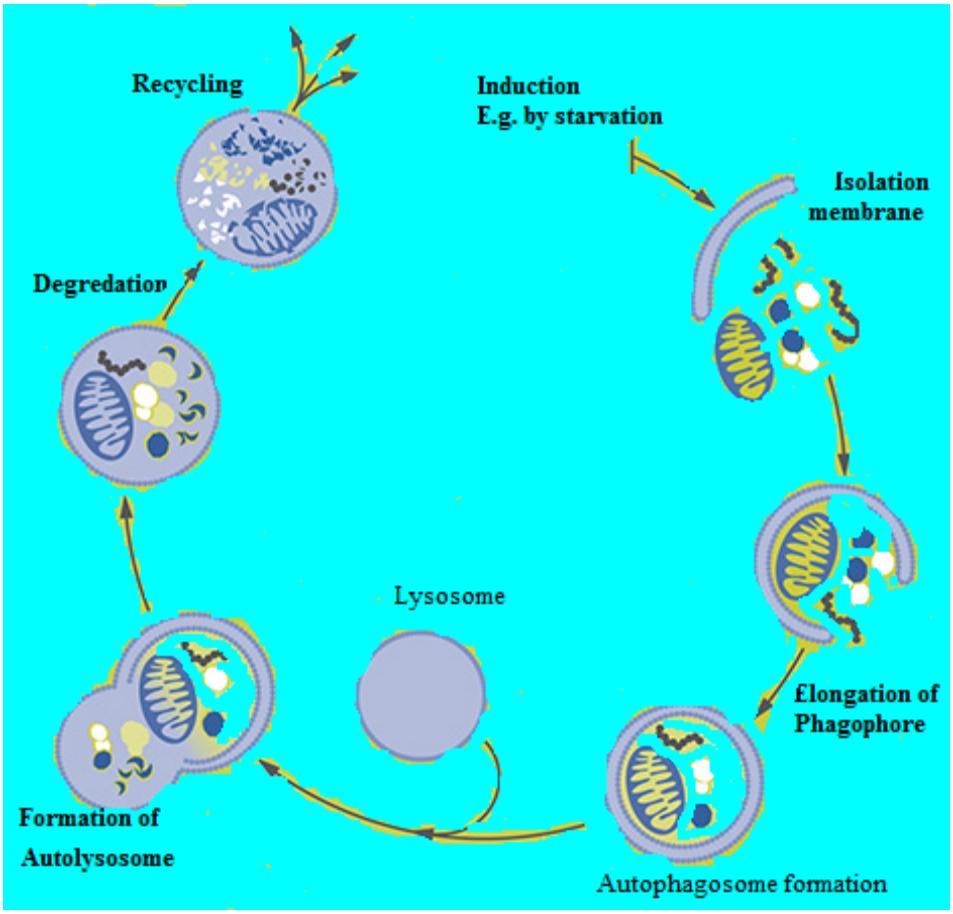

Figure 1: The process of autophagy.

suppressor. In addition, other study findings also suggested the role of autophagy regulator, Beclin 1, in these *in vitro* and *in vivo* studies ectopic over expression of Beclin1 in Beclin 1 deficient mammary carcinoma cells result in reduction in tumor cell proliferation and also reduced tumorigenic potential *in vivo*, further suggesting a role for this autophagy regulator in tumor suppression [27,28]. Like Beclin1, UVRAG is mono-allelically deleted in human colon carcinoma [29]. Whereas, in human gastric carcinomas a frame-shift mutations in poly (A) tail of *UVRAG* gene has been documented in a study done by Kim et al. [30] that resulted in reduction in autophagy activity in these tumor cells. In addition to the above, tumor-associated deletions or mutations have been found in a number of other autophagy regulators as shown in Table 1.

Not only do mutations of the autophagy gene promote tumorigenesis, but autophagy is also positively regulated by the tumor suppressor genes and negatively regulated by the oncogenic pathways. Oncogenes like Akt and Ras inhibit autophagy primarily by activating the mTOR signaling pathway. Conversely, tumor suppressor PTEN which inhibit PI3K/Akt/mTOR-C1 pathway can activate autophagy [11,31]. Therefore, mutations in *PTEN* result in constitutive activation of the pathway, suppression of autophagy, and may contribute to tumor formation [6]. Other tumor suppressors such as TSC1, TSC2, p53, and liver kinase B1 (LKB1) stimulate autophagy through their inhibitory effects on mTOR-C1 [10]. To this end, number mechanisms could clarify the tumor suppressive roles of autophagy, including prevention of oxidative stress and genomic instability, inhibition of necrosis and inflammation, promotion of cancer cell death, modulation of antitumor immune response, maintenance of normal stem cells and degradation of oncogenic proteins [4].

Prevention of Oxidative Stress and Genomic Instability

It has been known that, during carcinogenesis there is a reduction or inactivation of apoptosis, which regarded as the primary mechanism involved in clearing defective cells. In this case cells greatly relay on autophagy to maintain cellular fitness. A study done by Mathew et al. using baby mouse kidney epithelial cells immortalized from mutant mice (iBMK cells) reported that defects in autophagy, associated with either allelic loss of *beclin1* or lack of ATG5, result in diminished survival during metabolic stress and a heightened DNA damage response. Moreover, in the same study loss of *Beclin1* function promotes gene amplification and chromosomal instability that result in aneuploidy, which are typical features of tumor. The author's suggested that autophagy serves an important role in the preservation of genomic integrity. Furthermore, Karantza-Wadsworth et al., investigated the mechanism by which allelic loss beclin1 promotes breast tumorigenesis using mouse mammary epithelial cells (MMECs) and the author's reported that allelic loss of beclin1 that resulted in defective autophagy sensitized mammary epithelial cells to metabolic stress and accelerated lumen formation in mammary acini. The author's also reported that defective autophagy resulted in activation of DNA damage response

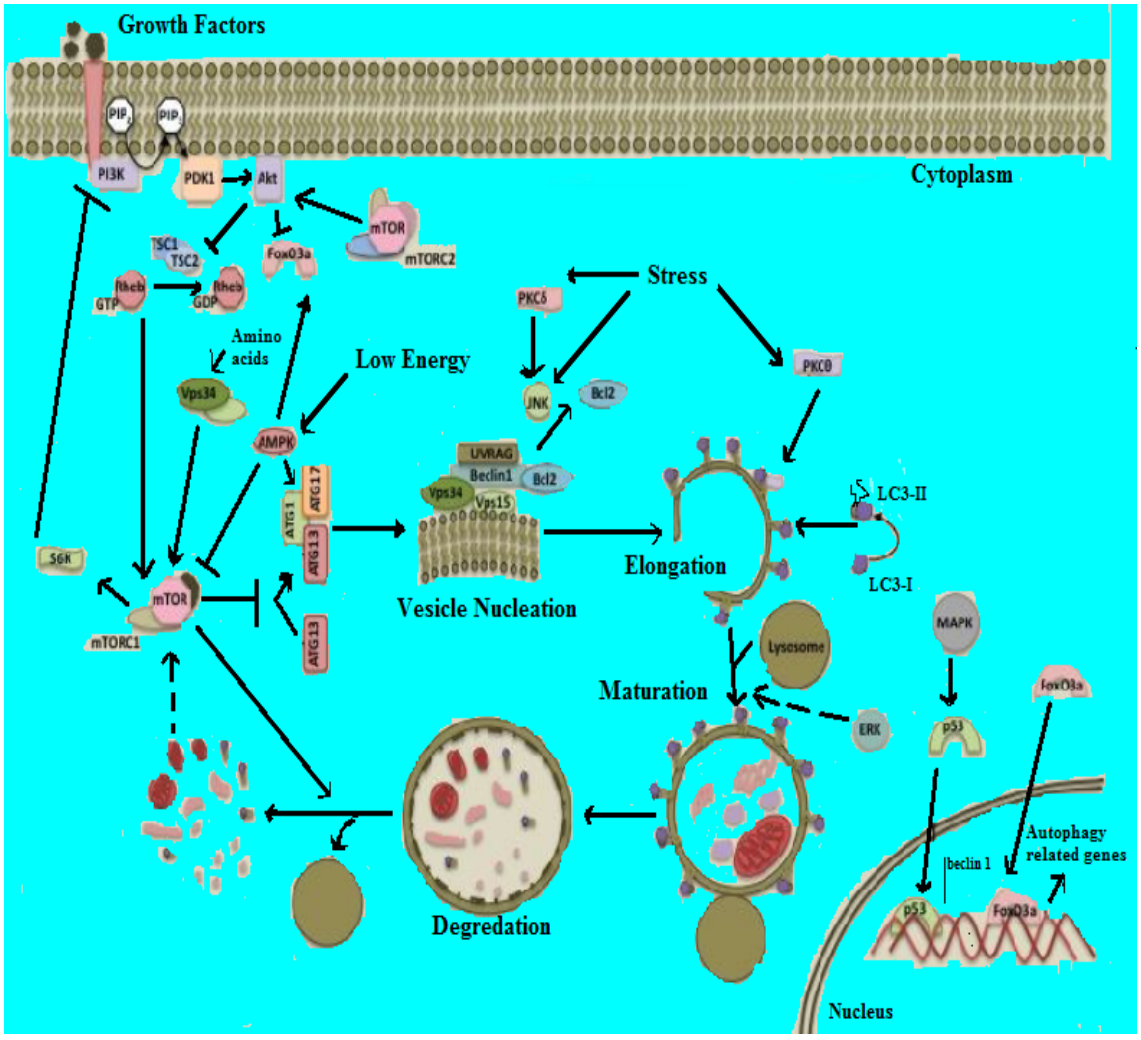

Figure 2: Signalling pathways regulating autophagy in mammalian cells. PI3K: phosphoinositide 3-kinases; PDK1: phosphoinositide-dependent kinase-1; mTOR: mammalian target of rapamycin; TSC: tuberous sclerosis complex; AMPK: AMP-dependent protein kinase; ERK: extracellular signal-regulated kinase; PKC: protein kinase C [10].

in vitro and in mammary tumors *in vivo* that heightened gene amplification, and synergized with disabled apoptosis to accelerate mammary carcinogenesis.

Moreover, p62, a scaffolding protein involved in signal transduction events, as well as directing poly-ubiquitinated proteins and aggregates to autophagosomal degradation, serves as a critical link between defective autophagy and tumorigenesis. In this regard, in autophagy-compromised cells, abnormal buildup of defective mitochondri a and protein aggregates could lead to accumulation in reactive oxygen species (ROS) that result in DNA damage along with p62 accumulation. Again p62 accumulation upon metabolic stress leads to ROS generation, thereby creating a positive feedback loop. It is therefore, autophagy contribute to oncosuppressive function by fighting against ROS accumulation and associated DNA damage.

Inhibition of Necrosis and Inflammation

Necrosis is a form of cellular demise characterized by several features such as ATP depletion, the loss of cellular osmolality, and release of various factors such as high mobility group box 1 protein (HMGB1) and breakdown of cell, which all lead to a strong inflammatory response. In this regard, tumor cells can evade this ATP-limiting demise by activating the energy sensor LKB1/AMPK complex, which, in turn, inhibits mTOR-C1, leading to activation of autophagy [14]. In this regard, activation of autophagy promotes cancer cells development in the face of inhibiting necrosis-associated inflammatory responses, because necrosis can promote the release of pro-metastatic immune-modulatory factors such as HMBG1, and this may lead to increased metastasis. Additionally, necrosis has been shown to be activated by AKT and Ras oncogenic signaling and is up-regulated when autophagy is compromised by mono-allelic deletion of BECN1. Thus, autophagy is perceived to avert necrosis in order to limit further cellular damage that may promote tumorigenesis and metastasis.

Promotion of Cancer Cells Death

In addition to promoting tumor cell survival at times of stress,

Gene/protein	Cancer-related changes
ULK1	↑ed expression in esophageal squamous cell & HCC; ↓ed expression in breast cancers
ATG2B	Frameshift mutations in gastric & colorectal cancers
ATG3	↓ed expression in myelodysplastic syndrome patients with leukemic evolution
ATG4B	↑ed expression in CD34(+) chronic myeloid leukemia cells
ATG5	Low-frequency frameshift mutations in gastric & colorectal cancers; Genetic variations may correlate with thyroid carcinoma susceptibility; ↓ed expression in natural killer cells & in gastric, colorectal, & HCC; ↑ed expression in CD34(+) chronic myeloid leukemia cells
LC3	↑ed expression in triple-negative breast cancer, colorectal & pancreatic cancers, & non-Hodgkin lymphomas; ↓ed expression in lung cancers, melanomas, & glioblastomas
ATG9B	Frameshift mutations in gastric & colorectal cancers
ATG10	↑ed expression in colorectal cancers is associated with metastasis; Genetic variations may correlate with breast cancer susceptibility
ATG12	Frameshift mutations in gastric & colorectal cancers
ATG16L1	Genetic variations may correlate with colorectal & thyroid cancers susceptibility; ↑ed expression in oral squamous cell carcinomas
BIF1	↓ed expression in pancreatic ductal adenocarcinomas, colorectal, gastric, urinary bladder, gallbladder, & prostate cancers
BECN1	↓ed expression in breast cancers, non-small cell lung cancers, renal clear cell carcinomas, brain tumors, cervical squamous cell, HCC, ovarian cancers, osteosarcomas, melanomas, & glioblastomas; Mutations in ovarian, human breast, prostate cancers; ↓ed expression due to gene methylation in breast cancers; ↑ed expression in CD34(+) chronic myeloid leukemia cells, colon cancers (stage IIIB), non-Hodgkin lymphomas, cholangiocarcinomas
UVRAG	Mutations in colorectal & gastric cancers
PIK3C/hVPS34	Differentially expressed b/n the TEL/AML1-positive & negative acute lymphocytic leukemia groups; SNP associated with esophageal squamous cell carcinomas
GABARAP	↑ed expression in colorectal carcinomas & benign & malignant thyroid tumors; ↓ed expression in neuroblastomas & breast cancers
GABARAPL1	↓ed expression in lymph node-positive high-grade breast cancers, acute myelocytic leukemias, & HCC
GABARAPL2	↓ed expression in acute myelocytic leukemias

Table 1: Cancer-related changes in mammalian core autophagy genes and proteins.

Agent	Class	Tumor type	Role of autophagy
Rapamycin	mTORC1 inhibitors	Malignant glioma	pro-death
Everolimus		Acute lymphoblastic leukemia, prostate cancer	pro-death
Temsirolimus		Mantle cell lymphoma	pro-death
AZD8055		Lung cancer	pro-death
Imatinib	Tyrosine kinase inhibitors	Chronic myeloid leukemia	pro-death
INNO-406		Gastrointestinal stromal tumor	pro-survival
		Chronic myeloid leukemia	pro-survival
Dasatinib		Ovarian cancer	pro-death
		Chronic lymphocytic leukemia	pro-survival
Perifosine	Akt inhibitors	Chronic myelogenous leukemia	pro-survival
Triciribine		Acute lymphoblastic leukemia	pro-survival
Metformin	AMPK activators	Cervical cancer	unknown
		Melanoma	pro-death
Bortezomib	Proteasome inhibitors	Cervical cancer	pro-death
		Prostate cancer	pro-survival
NPI-0052		Prostate cancer	pro-survival
Vorinostat	Histone deacetylase (HDAC) inhibitors	Gastric cancer	pro-survival
SAHA		Chondrosarcoma, endometrial stromal sarcoma, HCC	pro-death
MHY218		Breast cancer	pro-death
S1	Bcl-2 inhibitors	Malignant glioma	pro-survival
Z18		Breast cancer	pro-death
(–)-gossypol		prostate cancer	pro-death
Tamoxifen	Estrogen receptor	Breast cancer	pro-survival
	modulator		
Nelfinavir	HIV-protease inhibitor	Multiple cancer	pro-death
Etoposide	Topoisomerase II inhibitor	HCC	pro-death
3-MA	PI3K inhibitor	Colon cancer, Prostate cancer	pro-survival
CQ	Lysosomotropic agents	Glioblastoma, colon cancer	pro-survival
HCQ		Breast cancer	pro-survival
Bafilomycin A1	Vacuolar ATPase inhibitor	Breast cancer, colon cancer	pro-survival

Table 2: Representative autophagy-modulating agents in cancer therapy.

autophagy could play in eliciting a type-II programmed cell death, known as autophagic cell death (ACD) [12]. ACD refers to cell death caused by autophagy rather than cell death with autophagy. Thus, the ultimate cell death process of ACD is executed by over-activated autophagic flux rather than apoptosis or necroptosis. The genetic or drug-based inhibitors of autophagy, but not apoptosis or necroptosis inhibitors rescue this type of cell death. Moreover, several studies have suggested a potential relationship between ACD and cancer progression. For example, one study showed that blocking the CXCR4/mTOR signalling pathway induced ACD and the anti-metastatic properties of peritoneally disseminated gastric cancer cells [12]. The exact mechanism(s) connecting autophagy and apoptosis as partners in cell killing is still uncertain, however, apoptotic regulators antiapoptotic Bcl-2 family members Bcl-X_L and Bcl-2 have been shown to control autophagy, and a cleaved form of Atg5 known to be autophagy regulating protein has been shown to induce apoptosis directly in the mitochondria [11,14].

Furthermore, co-activation of autophagy and apoptosis has also been shown in tumor cells. For instance, DRAM-1 was found to have both proautophagic and proapoptotic roles downstream from p53. However, a study done by Yee et al. reported that, p53 up-regulated modulator of apoptosis (PUMA) is the main mediator of p53-dependent apoptosis, and further the study revealed that, PUMA orchestrates this apoptosis by activating Bax and mitochondrial outer membrane permeabilization and release of cytochrome *c*. Moreover, the same study reported the capacity of PUMA to prompt autophagy that results in mitophagy. But, inhibition of PUMA or Bax-induced autophagy reduces the apoptotic response, suggesting the existence of synergy between autophagy and apoptosis in this apoptosis response. Although apoptosis and autophagy can occur in a cooperative manner to elicit cell death, these processes are sometimes mutually antagonistic [12]. For example, caspases (the effectors of the apoptotic cell death) have been shown to cleave and inactivate Atg6/Beclin 1, and the suppression of Atg6 function increases apoptotic cell death. Thus, apoptosis is either inhibited or delayed when autophagy is present, with the probable conclusion that autophagy is activated to protect cells from dying.

Other contrasting behaviors between apoptosis and autophagy have been attributed to the health status of the cell, or stage of transformation. Dominant-negative FADD invokes a death stimulus involving autophagy in healthy cells but not in cancer cells and induces varying amplitudes of death responses at different stages of cancer progression. In addition, oncogenic Ras has also recently been shown to cause ACD in the absence of apoptosis, even though other reports have indicated that autophagy is required for Ras-driven tumor growth. It is therefore, the decision to activate, repress, or simply not to manipulate autophagy, may be governed by the nature of the stimuli or the upstream regulator of an autophagic and/or apoptotic protein, as well as the health status of cells, and the net outcome of these three possibilities is either to promote or repress tumor survival, or both.

Maintenance of Normal Stem Cells

Autophagy appears to ensure maintenance of normal stem cells; this is particularly relevant for hematological malignancies, which are normally characterized by changes in proliferation or differentiation potential that alter the delicate equilibrium between toti-, pluri- and unipotent precursors in the bone marrow [4]. For instance, ablation of Atg7 in murine hematopoietic stem cells (HSCs) has been shown to disrupt tissue architecture, eventually resulting in the expansion of a population of bone marrow progenitor cells with neoplastic features. Similarly, tissue-specific deletion of the gene coding for FIP200 alters the fetal HSC compartment in mice, resulting in severe anemia and perinatal lethality. Interestingly, FIP200 gene knocked out murine HSCs do not exhibit increased rates of apoptosis, but an increased proliferative capacity. Moreover, Becn1$^{+/-}$ mice display an expansion of progenitor-like mammary epithelial cells. Noteworthy, in human, autophagy has also known to be vital for conservation of normal stem cell compartments. Certainly, hematopoietic, dermal, and epidermal stem cells transfected with a short-hairpin RNA (shRNA) against *ATG5* gene resulted in loss of self-renew ability while differentiating into neutrophils, fibroblasts, and keratinocytes, respectively.

Degradation of Oncogenic Proteins

Studies reported that autophagy is involved in the degradation of oncogenic proteins, including mutant (but not wild-type) TP53, p62, PML-RARA, and BCR-ABL1 [4,19]. It has been known that accumulation of mutant TP53 in tumor cells that functions as a principal-negative factor, has been recognized to abolish the antitumor role of the wild-type p53 [15]. Moreover, in a study done by Choudhury et al. reported that increased accumulation of mutant TP53 in tumor cells having low level of autophagy regulators ULK1, BECN1 or ATG5. But, the same study demonstrated that depletion of mutant TP53 in case of transgene-driven overexpression of Becn1 or ATG5. It is therefore, autophagy-dependent removal of mutant TP53 could reinstate the wild-type TP53 capacity of halting malignant transformation.

Moreover, p62 protein levels are frequently found to be up-regulated in human cancers and thought to promote tumorigenesis. According to studies, the accumulation of p62 leads to increased ER stress and DNA damage as well as it contributes to the deregulation of the nuclear factor kappa B (NF-kB) and antioxidant nuclear factor erythroid 2-related factor 2 (NRF2) pathways in cancer. Meanwhile, in the context of autophagy, p62 acts as an adaptor protein that links LC3 with ubiquitin moieties on misfolded proteins and degradation of p62 together with ubiquitylated proteins will be mediates by autophagy. At this juncture, defect in autophagic activity would cause accumulation of p62 and plays a role in carcinogenesis. In support of this notion, Takamura et al. reported that, mouse models with either a mosaic deletion of ATG5 or a liver-targeted deletion of ATG7 results in development of benign tumors with accumulation of p62. Furthermore, in the same study, deletion of p62 suppresses tumor growth, which indicates a causative link between p62 accumulation and adenoma formation.

Modulation of Anti-tumor Immune Response

It has been known that, antigens derived from outside the cell are subjected to degradation inside the lysosomes. In this regard, autophagy know to be involved in trafficking antigens meant for degradation, plus trafficking components from degraded antigen back to cell surface for presentation on the class II major histocompatibility complex (MHC). As reported by studies, most tumor antigens are derived from inside the cell, and there breakdown is mainly via proteasomes, leading to presentation on class I MHC. However, according to studies, autophagy stills plays a major role regarding to presentation of tumor antigens on antigen-presenting cells (APCs) directly on class II MHC or via a process termed "cross-presentation" on class I MHC. A study reported by Li et al. demonstrated that induction of autophagy by rapamycin in melanocytes significantly enhance the priming of APCs CD8+ T cells in presenting the melanocyte-derived tumor antigen gp100. However, in the same study, this effect has been reversed when autophagy is blocked using 3-methyladenine (3-MA, a PI3K III inhibitor). Subsequent study done by Lee et al. has established the the requirement of autophagy to enhance class I antigen presentation by dendritic cells

(DCs). In this study the author's reported that Atg5−/− DCs present antigen on class II molecules but has shown a reduced capacity to stimulate CD4+ lymphocytes via boosting antigen breakdown in autolysosomes. The same study also revealed that autophagy triggered by starvation and pharmacological treatment with rapamycin reduces class II presentation. As a result, both cancer cell-intrinsic and systemic defects in autophagy may prevent the host immune system to properly recognize and eliminate pre-malignant and malignant cells.

Contributes to Oncogene-induced Senescence

It has been established that irreversible form of cell cycle arrest could arise in response to stress signals, including telomere shortening and oncogene activation or oncogene-induced senescence (OIS) and it is known as cellular senescence [4]. Several studies have demonstrated that this phenomenon, which was initially identified and characterized in vitro, acts as a robust tumor suppressor mechanism in vivo. In this regard, it has been suggested that autophagy contributes to OIS. In support of this, Young et al. reported that several ATG genes, including ULK3, Atg7, and LC3 are up-regulated during oncogene-induced senescence. Moreover, both pharmacological inhibitors of autophagy and small-interfering RNAs targeting ATG5, ATG7 or Becn1 prevented spontaneous senescence in primary human melanocytes or diploid fibroblasts while preventing the degradation of an endogenous, dominant-negative TP53 variant [4]. Meanwhile, much thought has been put into the precise role of autophagy in OIS. White and Lowe reported that autophagy is evoked to break down specific cellular components to enable physical remodeling associated with senescence.

Antiviral and Antibacterial Effects

Autophagy may suppress carcinogenesis owing to its key role in the first line of defense against viral and bacterial infection. Actually, autophagy has been stimulated during infection by numerous carcinogenic pathogens. For instance, viruses including, hepatitis B virus, human papillomavirus (HPV), Epstein–Barr virus have been associated with the pathogenesis of HCC, cervical and gastric carcinoma, respectively. On the other hand, bacterial pathogens including, Streptococcus bovis, Chlamydia pneumonia and Helicobacter pylori have been associated with colorectal, lung and gastric carcinoma, respectively. In addition, Salmonella enterica has been associated with an increased incidence of Crohn's disease, hence sustaining colorectal carcinogenesis, and gallbladder carcinoma.

This selective autophagy induced by and against pathogens known as xenophagy [3]. According to studies, xenophagic response is required for the rapid clearance of intracellular pathogens as well as for the stimulation of pathogen-specific immune responses. Consequently, reduced levels of autophagic markers including Becn1 have been correlated with HPV-16 and HPV-18 infection in a cohort of cervical carcinoma patients. Therefore, autophagy may exert oncosuppressive effects also by virtue of its antiviral and antibacterial activity.

Promotion of Tumor Progression

Despite its role in preventing early tumor development, once tumors are established, tumor cell autophagy-related survival function can lead to tumor dormancy, progression, and therapeutic resistance. Furthermore, advanced human cancers exhibited an amplified autophagic flux, linked with invasive phenotype, high nuclear grade, and poor disease outcome. Proposed mechanisms for tumor-supporting functions of autophagy in established tumors includes resistance to stress induced by hypoxia, starvation and cancer therapy, survival of senescent cancer cells, maintenance of cancer stem cells (CSCs), resistance to epithelial-to-mesenchymal transition (EMT) [4].

Supports Survival of Tumor Cells Under a Variety of Stresses

In established tumor, metabolic stress known to induce autophagy so as to seek an alternative source of energy and metabolites. Furthermore, autophagy may also be induced as an adaptive cellular response to various cancer therapies, leading to chemo-resistance and cancer cell survival.

As shown in Table 2, inhibition of autophagy through treatment with 3-MA, bafilomycin A (a specific inhibitor of vacuolar-type H+-ATPase), chloroquine (CQ) or hydroxyl-chloroquine (HCQ) {lysosome-tropic agents that impair fusion between autophagosomes and lysosomes}, or silencing of some essential autophagy genes, such as ATG3, ATG4B, ATG5, Atg6/BECN1, ATG7, ATG10, and ATG12 has been found to increase cell death to a wide spectrum of therapeutic stresses. Moreover, activation of autophagy has been considered as one mechanism for acquired resistance of tumor cells to chemotherapeutic agents.

Sustains Survival of Senescent Cancer Cells

Tumor dormancy, characterized by asymptomatic nature of tumor for extended periods of time, can be present as one of the earliest stages in tumor development, as well as in the in micro-metastasis stage, and can occur when minimal residual disease remains after surgical removal or treatment of primary tumors. It can result from angiogenesis arrest, in balance between apoptosis and cell proliferation, cell cycle arrest, immune surveillance and cancer therapy. It is a poorly understood phase of cancer progression and only recently have its underlying molecular mechanisms started to be revealed. In a study done by Lu et al. revealed that, when the level of ARHI remains at normal autophagic activity, tumor dormancy is likely to occur, as removed tumors can recover their abilities that are consistent with the autophagic process. In the study, the inhibition of ARHI-induced autophagy can dramatically reduce the regrowth of xenografted tumors upon the reduction of ARHI levels, suggesting that autophagy may contribute to the survival of dormant cells; therefore, ARHI can induce ACD as well as promoting tumor dormancy in the presence of factors that promote survival in the tumor microenvironment.

Furthermore, cancer cells exposed to therapeutic interventions can also undergo senescence. In this regard, senescent cells do not proliferate, but they may support disease relapse by releasing a wide panel of pro-inflammatory and mitogenic cytokines into the microenvironment the so-called senescence-associated secretory phenotype (SASP). However, according to studies, these cells are greatly reliant on autophagy for survival, and inhibitions of autophagy have been shown to synergize with several cancer therapeutics in experimental models of lymphoma that are susceptible to acquire the SASP in response to treatment.

Ensures the Maintenance of the Cancer Stem Cell Compartment

It has been known that, autophagy has played a role in the development of resistance to cancer therapy, self-renewal, differentiation and tumorigenic abilities of CSCs. A study done by Song et al. showed that CD133+ liver CSCs (LCSCs) significantly enrichment with higher autophagy levels after hypoxia and nutrient starvation in HCC Huh-

7 cells. It is therefore, the study reported the autophagy's essential role in LCSC maintenance. Furthermore, conversion of microtubule-associated protein LC3-I to LC3-II as well as improved buildups of ATG7 and Beclin-1 has been observed in pancreatic CSCs treated with Rottlerin (ROT), which has been commonly used protein kinase C-delta (PKC-δ) inhibitor. Additionally, the gene silencing of ATG7 and Beclin-1, or co-treatment with 3-MA, can inhibit ROT-induced ACD.

In addition, expressions of ATG5, ATG12 and LC3B in dormant stem cell-like breast cancer cells by farnesyl transferase inhibitors (FTIs) in breast cancer cells having low metastatic abilities induced a reversible state of dormancy. Moreover, JNK-mediated autophagic pathway was also up-regulated in these breast CSCs with the periods of FTI-induced dormancy. On the other hand, irradiation of CD133+ glioma stem cells (GSCs) has been associated with induction of autophagy in a short time and then autophagy slightly decreases the viability of the cells. According to the study, the gamma-radiation also induced a large degree of autophagy in the CD133+ GSCs, characterized by high level expression of ATG proteins such as ATG5, LC3 and ATG12.

Modulation of Autophagy in Cancer Therapy

The role and regulation of autophagy in cancer is apparently quite complex, but when tumor cells induce protective autophagy, inhibition of autophagy may provide a way of sensitizing tumor cells to therapy by activating apoptosis. Conversely, excessive autophagy induced by drugs can potentially result in tumor cell elimination [12]. On top of this, role of autophagy as a protector of cellular homeostasis and genome integrity may be particularly important regarding cancer prevention.

Induction of Autophagic Cell Death (ACD)

Defects in apoptosis are often associated in many cancer cells; hence targeting alternative cell death pathways is an attractive strategy for improving anti-tumor therapy. Thus, the induction of cell death by autophagy may serve as a novel therapeutic strategy, especially in cancer cells with high thresholds to apoptosis.

Moreover, the consequence of promoting autophagy depends on multiple factors, including extent of induction, duration, and cellular context. Accumulating evidence indicates that promotion of autophagy that leads to ACD contributes to in vivo antitumor effects (Table 2). For instance, a natural BH3-mimetic, small-molecule inhibitor of Bcl2, (-)-gossypol, shows potent antitumor activity against human prostate cancer. Accordingly, the antitumor activity by (-)-gossypol is mediated through induction of both apoptosis and ACD. However, autophagy appears to serve as a death program primarily when the apoptotic machinery is defective, as observed in most tumors.

Inhibition of Protective Autophagy

Many anti-cancer therapies such as DNA damaging agents, radiation therapy and targeted therapies can induce autophagy in cancer cells. According to studies, cytotoxic chemotherapeutics and targeted therapies induce autophagy through a number of signaling pathways including the DNA damage response, mTOR and AMPK signaling, the ER stress response and others. In this regard, autophagy is commonly up-regulated in both tumor and normal cells exposed to cancer therapies, but the greater reliance of tumor cells (versus normal cells) on the cytoprotective effects of autophagy and its potential clinical relevance in modulating drug resistance. Consistence with this notion, several studies have found that inhibition of chemotherapy-induced autophagy can enhance the sensitivity of cancer cells to different chemotherapies, leading to cell death and tumor regression.

For instance, the apoptosis potential of melanoma differentiation-associated gene-7/Interleukin-24 (MDA-7/IL-24), a member of IL-10 gene family that has been shown a nearly ubiquitous antitumor properties in vitro and in vivo through induction of cancer-specific apoptosis, increases by inhibiting protective autophagy with 3-MA (Table 2) in prostate cancer cells. Similarly, inhibition of autophagy by 3-MA or Atg7 knockdown induced apoptosis in colon cancer cells treated with 5-FU. Moreover, inhibition of protective autophagy was shown to sensitize resistant cells to TRAIL-mediated apoptosis in apoptosis-defective leukemic and colon cancer cell lines (Table 2). In this regard, these apoptosis-resistant cells only become apoptotic after inhibition of autophagy.

Furthermore, the potential to inhibit autophagy and sensitize tumor cells to metabolic stress is another promising approach for cancer therapy. Many current cancer therapies including angiogenesis, growth factor, and receptor inhibitors when combined with autophagy inhibition produced synergistic anticancer effects. Moreover, combining organelle-damaging drugs, such as sigma-2 receptor agonists, with an autophagy inhibitor might be an effective means of cancer therapy. It is likely that ER stress inducers, including thapsigargin and tunicamycin, that trigger cell death in cancer cells will increase cell killing when autophagy is inhibited. On the other hand, targeting both proteasome- and autophagy-mediated protein degradations might be an effective antitumor approach for highly metabolically active tumor cells. The proteasome inhibitor bortezomib has the approval of the US FDA and has demonstrated potent efficiency in treating multiple myeloma.

Moreover, metastasis prone state of cancer cells may be principally subject to autophagy inhibition as cells in isolation are likely to be more dependent autophagy, even though this likelihood remains to be established. Incidentally, CQ that has been known to inhibit lysosome by acidification and thereby inhibition of autophagy, in conjunction with alkylating agents, displayed remarkable efficacy in inhibiting tumor growth in mice as well as in clinical studies. Synergy between CQ and the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) in killing imatinib refractory chronic myeloid leukemia cells also supports a protective role for autophagy, reinforcing the therapeutic use of autophagy inhibitors in cancer therapy [6]. Similarly, the synergy between CQ and the PI3K/mTOR inhibitor NVP-BEZ235 induced apoptosis in glioma xenografts. By the same token, cyclophosphamide-induced tumor cell death in a Myc-induced murine lymphoma enhanced by CQ in a similar fashion produced by shRNA knock down of Atg5, and it hindered the time-to-tumor recurred. These studies documented that CQ, or its analog (HCQ), when used as autophagy inhibitors in combination with proapoptotic drugs, increases twofold the median survival of cancer patients.

Currently, multiple clinical trials are assessing the effects of combined treatments with various anti-cancer drugs plus HCQ for patients with various refractory malignancies (Table 3). Meanwhile, one underlying concern is that autophagy inhibitors approved for cancer patients might actually act as promoters of tumor development. However, the tumor-promoting effect of autophagy inhibitors, which depends on necrotic cell lysis that follows the inflammatory response, could prevent this undesirable effect upon co-treatment with immunosuppressive drugs.

Cancer type	Clinical trials	Drug (s)	Phase
Advanced solid tumors	NCT00909831	HCQ, temsirolimus I	I
	NCT01266057	HCQ, sirolimus, vorinostat	I
	NCT01023737	HCQ, vorinostat	I
	NCT01480154	HCQ, MK2206	I
Breast cancer	NCT01292408	HCQ	II
Colorectal cancer	NCT01206530	HCQ, leucovorin, 5-FU, oxaliplatin, bevacizumab	I/II
	NCT01006369	HCQ, capecitabine, oxaliplatin, bevacizumab	II
Ductal carcinoma *in situ*	NCT01023477	CQ	I/II
Epithelial ovarian cancer	NCT01634893	HCQ, sorafenib	I
Extraovarian peritoneal carcinoma	NCT01634893	HCQ, sorafenib	I
Fallopian tube carcinoma	NCT01634893	HCQ, sorafenib	I
Kidney cancer	NCT01480154	HCQ, MK2206	I
Lymphangioleiomyomatosis	NCT01687179	HCQ, sirolimus	I
Melanoma	NCT00962845	HCQ	0
Multiple myeloma	NCT00568880	HCQ, bortezomib	I/II
	NCT01438177	CQ, velcade, cyclophosphamide	II
Non-small cell lung cancer	NCT00933803	HCQ, carboplatin, paclitaxel, bevacizumab	I/II
	NCT01649947	HCQ, carboplatin, paclitaxel, bevacizumab	II
Pancreatic cancer	NCT01506973	HCQ, gemcitabine	I/II
	NCT01128296	HCQ, gemcitabine	I/II
Prostate cancer	NCT01480154	HCQ, MK2206	I
Renal cell carcinoma	NCT01144169	HCQ	I
	NCT01510119	HCQ, RAD001	I/II
Small cell lung cancer	NCT00969306 I	CQ, A-CQ 100	I

Table 3: Ongoing clinical trials evaluating the efficiency of CQ/HCQ anticancer activity.

Future Perspectives

There are still several questions that remains to be answered even though, incredible amount of progress have been achieved about role of autophagy in cancer. For instance, the switch in regulation of autophagy from low levels, i.e. during tumorigenesis to elevated levels during tumor progression, has not been clarified. Moreover, the delineation of the autophagic cargo in tumors with elevated autophagy will allow for a greater understanding of how this process is integrated into the metabolism of the cell. Most likely, there will be differences in the role of autophagy in normal metabolism versus that of its tumorigenic counterpart. Given the progress that has been made in proteomic and metabolomics approaches, this type of analysis is certainly feasible.

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