Minireview

Contrast effects of autophagy in the treatment of bladder cancer

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Impact statement

Autophagy acts as an intracellular recycling system. Infection and mitochondrial damage, maintaining cellular homeostasis, orchestrating nutrient stress, hypoxia, and oxidative stress are some of the physiological roles associated with autophagy. Autophagy has also context-dependent roles in cancer. Autophagy has a significant impact on tumor initiation and promotion, with both tumor-suppressive and tumor-promoting roles. Unfortunately, conventional systemic chemotherapy for cancer therapy has been reported to have primary limitations such as chemoresistance of targeted cells. The cytoprotective role of autophagy has been postulated as one of the causes of this resistance. Hence, combination therapy using autophagy inhibitors has recently started to emerge as a noteworthy strategy in the treatment of cancer. Therefore, targeting the autophagy pathways may be a potential therapeutic strategy for addressing cancer progression or therapy resistance in the near future. This review will provide a novel insight to understanding the paradoxical roles of autophagy in tumor suppression and tumor promotion.

Abstract

Bladder cancer is a disease that negatively affects patients' quality of life, but treatment options have remained unchanged for a long time. Although promising results have been achieved with current bladder cancer treatments, cancer recurrence, progression, and therapy resistance are the most severe problems preventing the efficiency of bladder cancer treatments. Autophagy refers to an evolutionarily conserved catabolic process in which proteins, damaged organelles, and cytoplasmic components are degraded by lysosomal enzymes. Autophagy regulates the therapeutic response to the chemotherapy drugs, thus determining the effect of therapy on cancer cells. Autophagy is a stress-induced cell survival mechanism and its excessive stimulation can cause resistance of tumor cells to therapeutic agents. Depending on the conditions, an increase in autophagy may cause treatment resistance or autophagic cell death, and it is related to important anti-cancer mechanisms, such as apoptosis. Therefore, understanding the roles of autophagy under different conditions is important for designing effective anti-cancer agents. The dual role of autophagy in cancer has attracted considerable attention in respect of bladder cancer treatment. In this review, we summarize the basic characteristics of autophagy, including its mechanisms, regulation, and functions, and we present examples from current studies concerning the dual role of autophagy in bladder cancer progression and therapy.

Keywords: Autophagy, autophagy inhibitors, bladder cancer, dual role of autophagy, novel therapeutic strategies, signaling of autophagy

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Introduction

Worldwide, bladder cancer is the 11th most commonly diagnosed cancer.[1] Approximately 549,000 new cases and 200,000 deaths occur worldwide according to Global Cancer Statistics 2018 data.[2] Bladder cancer is divided into two classes according to the presence or absence of invasion of tumor cells into the muscle layer at diagnosis. Non-muscle-invasive subtypes account for the majority of bladder cancer cases (approximately 75%), and are associated with good prognosis and high survival rates. These tumors are mostly papillary in type and form finger-like protrusions growing from the lining of the inner surface of the bladder to the lumen. Transurethral resection is the primary treatment option for non-muscle-invasive bladder cancer, but the tumor recurrence rate is high after resection; hence, this treatment is followed by other treatments, using chemotherapeutic agents, or Bacillus Calmette–Guerin (BCG) vaccine administered

intravesically to the bladder, to prevent disease recurrence and progression. The remaining bladder cancer cases (approximately 25%) consist of muscle-invasive bladder cancer that is associated with poor prognosis. The five-year survival rate for these invasive tumors is around 50%. In addition to developing from non-muscle-invasive tumors, muscle-invasive tumors can also develop from non-papillary urothelial dysplasia, known as carcinoma *in situ*.[3]

Autophagy is a highly conserved catabolic multistage process involving the degradation of cytoplasmic macromolecules by lysosomal enzymes. During this process, misfolded proteins and injured organelles sequester from cytoplasm to form covered double-membrane vesicles called *autophagosomes* and are transferred to lysosomes for digestion. Digested cellular constituents are then released into the cytoplasm for reuse. Since it plays a role in the recycling of cellular macromolecules, deterioration of autophagy relates pathophysiological processes in various disorders, such as neurodegeneration, aging, and cancer.[4] Autophagy comprises successive steps, including induction, nucleation, elongation, maturation, fusion, and degradation, all of which are controlled by multiple signaling pathways. Autophagy-related proteins (ATGs) play an important role in the regulation of these steps. Three different subtypes have been shown to transfer the autophagic cargo to the lysosomes: macroautophagy, microautophagy, and chaperone-mediated autophagy (Figure 1). Autophagy is the term generally used for macroautophagy, in which cytoplasmic molecules are enclosed with double membranes and transferred to lysosomes for degradation to occur. In microautophagy, cytoplasmic content is directly invaginated by the lysosomal membrane and digested. In chaperone-mediated autophagy (CMA), target proteins for digestion have KFERQ- or KFERQ-like sequence motifs that are recognized by the chaperone heat shock cognate protein of 70 kDa (HSC70). HSC70 binds target proteins with these motifs and directs them to the lysosomes through interaction with the lysosome-associated membrane glycoprotein type 2 A (LAMP2A)-specific cargo-receptor proteins.[5] Normally autophagy is responsible for two physiological processes in cells: it enables the cells to continue their normal physiological functioning by removing abnormal structures, thus preventing tumor development by checking the quality of the organelles and ensuring the clearance of defective organelles. It also provides alternative biological material and energy for the destruction of existing non-essential structures to maintain cell viability under stress conditions such as starvation, hypoxia. Autophagy is a dynamic process that prevents the transformation of normal cells into tumors by ensuring the degradation of oncogenic molecules.[4] Depending on the conditions, autophagy may have an inhibitory role on tumor development or proliferative role after the formation of tumor. Although autophagy generally plays a role in cell survival as a stress response, there is evidence that the overstimulation of autophagy is an alternative pathway to a form of cell death called type II programmed cell death.[6]

Genetically engineered mouse (GEM) models are now being significantly used in bladder cancer studies *in vivo*. However, new studies which disclose the molecular changes that are common in bladder cancer provide new ways to develop disease-related genes and/or models. Over the past few years, we have undertaken several studies on bladder cancer cell lines.[7,8] Our findings provided a preclinical framework for identifying new molecular targets for the treatment of bladder cancer cells by targeting the cell death and epigenetic pathways. We have also recently developed a bladder cancer mouse model and investigated apoptosis and autophagy—the two major cell death pathways.[9] This review aims to examine recent studies concerning the dual role of autophagy and its possible relevance to bladder cancer treatment.

Autophagy regulating signal pathways

Autophagy initiation is mediated by four signal-sensing kinases: mammalian target of rapamycin complex 1 (mTORC1), Unc-51-like autophagy-activating kinase 1 (ULK1), AMP-activated protein kinase (AMPK), and protein kinase B (AKT or PKB).[10] mTOR is a serine/threonine kinase that regulates cell growth and division by sensing changes in various external and internal factors. It consists of two complexes, called mTORC1 and mTORC2. mTORC1 has an autophagy inhibitory effect when amino acids and growth factors are available (Figure 2(a)). mTORC1 suppresses autophagy by phosphorylating both ULK1 and ULK2.[11] mTOR also negatively regulates autophagy by targeting regulator proteins in other autophagy steps.[12] AMPK is an important protein kinase that mediates and maintains cellular energy. AMPK detects decreases in energy and then activates autophagy (Figure 2(a)). If growth factors are present in the environment, AKT suppresses autophagy by activating its downstream target, mTORC1. AKT can also stimulate autophagy by direct phosphorylation of PI3K complex proteins.[10] AMPK suppresses mTOR activity by direct phosphorylation of the raptor protein or indirect phosphorylation of the tuberous sclerosis complex 2 (TSC2) protein over the Ras homolog enriched in brain (RHEB) pathway (Figure 2(a)). AMPK can also directly activate autophagy by ULK1 phosphorylation.[13] Autophagy initiation is regulated by the ULK1 initiation complex, which consists of four subunits: ULK1, FIP200, ATG13, and ATG101.[14] In the presence of abundant nutrients, mTORC1 suppresses autophagy by phosphorylating the ULK1 complex. mTORC1 disrupts AMPK-ULK1 interaction via the phosphorylation of ULK1 (on Ser637 and Ser757) and ATG13 (Ser258). In the absence of nutrients, mTORC1 is inactivated and its inhibitory phosphorylation effect is disrupted by phosphatases. Phosphatases eliminate the mTORC1 inhibitory effect by dephosphorylating ULK1, which gets separated from the mTORC1 complex. Released ULK1 is then activated by Thr180 autophosphorylation and subsequently phosphorylates other members of the ULK1 complex (Atg13, FIP200, and Atg101).[12]

Rheb is a GTP-binding protein belonging to the Ras family. Rheb activates mTORC1 in a GTP-bound state. TSC1 and -2 interact with Rheb to inhibit its activation, causing its transition to an inactive GDP-bound state (Figure 2(b)). The existence of growth factors inhibits

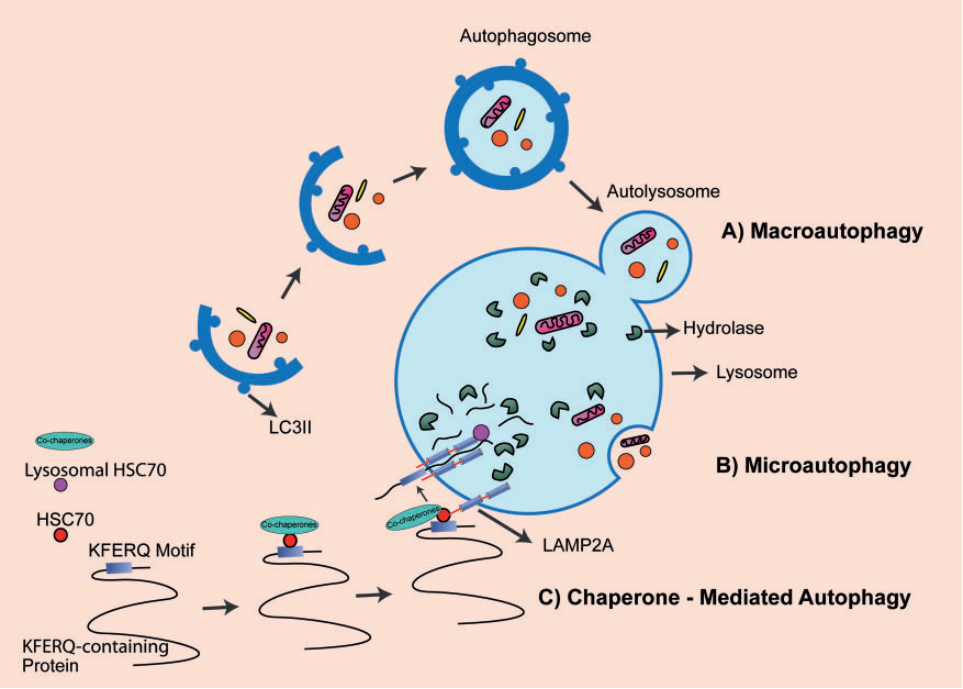

Figure 1. Three morphologically and mechanistically distinct primary types of autophagy. (a) Macroautophagy, (b) microautophagy, and (c) chaperone-mediated autophagy. (A color version of this figure is available in the online journal.)

TSC1/2 functioning through regulation by the PI3K/Akt or the Ras/Raf/ERK pathways. After TSC1/2 inactivation, GTP-bound Rheb leaves the complex and activates mTORC1. mTORC1 can be activated by high amino acids levels, without the TSC1/2 pathway.[15] In the presence of excess amino acids, it is directed to the lysosome by the Ragulator-Rag complex, which resides on the lysosome membrane (Figure 2(b)). The Ragulator-Rag complex works with the lysosome-linked Rheb to regulate autophagy activation.[11]

Apart from these signaling pathways, autophagy is regulated by transcriptional and epigenetic mechanisms. Transcription factor EB (TFEB) regulates autophagy induction, but another transcription factor, ZKSCAN3, has an opposite effect, according to TFEB functioning, and downregulates autophagy-related gene expressions (Figure 2(c)). Some histone modifications have been shown to regulate autophagic flux H3K9 and H3R17 dimethylations caused by autophagy suppression; however, H3K27 trimethylation and H4K16 deacetylation are associated with autophagy activation.[11]

The link between autophagy and apoptosis

Although autophagy and apoptosis involve different mechanisms and morphological characteristics, they are closely linked. Their interactions with each other have been identified in the maintenance of normal cellular homeostasis and the development of cancer.[16] The relationship between autophagy and apoptosis has been described in three ways: (1) Apoptosis and autophagy occur simultaneously and execute cell death independently of each other; (2) one occurs before the other and mediates the activation of the other; (3) autophagy has an opposite effect on apoptosis and this form of autophagy acts cytoprotectively and reduces cell death.[17] These relationships are regulated by various proteins via interactions, among others, between beclin-1 and Bcl-2/Bcl-xL, and between Atg12 and Mcl-1.[4] Autophagy can block apoptosis through various pathways, such as autophagic degradation or initiation of caspase-8, prevention of truncated Bid (tBid) transition to the mitochondria by beclin-1, and inhibition of apoptosis-inducing factors (AIFs) by the suppression of reactive oxygen species (ROS) formation. Simultaneously, autophagy acts as an apoptosis promoter via autophagy-related genes that activate apoptosis. The formation of Atg5 fragments by calpain cleavage initiates mitochondrial apoptosis by releasing cytochrome c from the mitochondrial membrane, inducing mitochondrial apoptosis through Atg12 binding to the anti-apoptotic Bcl-2 protein.[14] p53, DAPK, JNK, BAD, NIX, NOXA, and PUMA are all inducers of apoptosis and autophagy pathway regulators. In addition to their pro-apoptotic function, BH3-only proteins (Bad, Bid, Nix, Noxa, and Puma) disrupt the linkage between Bcl-2 and beclin-1; disentangled beclin-1 then activates autophagy by contacting vps34 protein. The apoptosis inhibitory effects of Bcl-2 also depend on activation by beclin-1. According to one approach, autophagy and apoptosis are activated simultaneously,[18] but according to another approach, autophagy occurs as a first response to stress stimuli and regulates other cell death pathway activations.[19] Autophagy constitutes a threshold for activating apoptosis. If this threshold value is exceeded, cell death is activated via the transition of autophagy to apoptosis, but if this threshold is not exceeded, autophagy blocks the activation of apoptosis and prevents cell death.[20] Yu *et al.*[21]

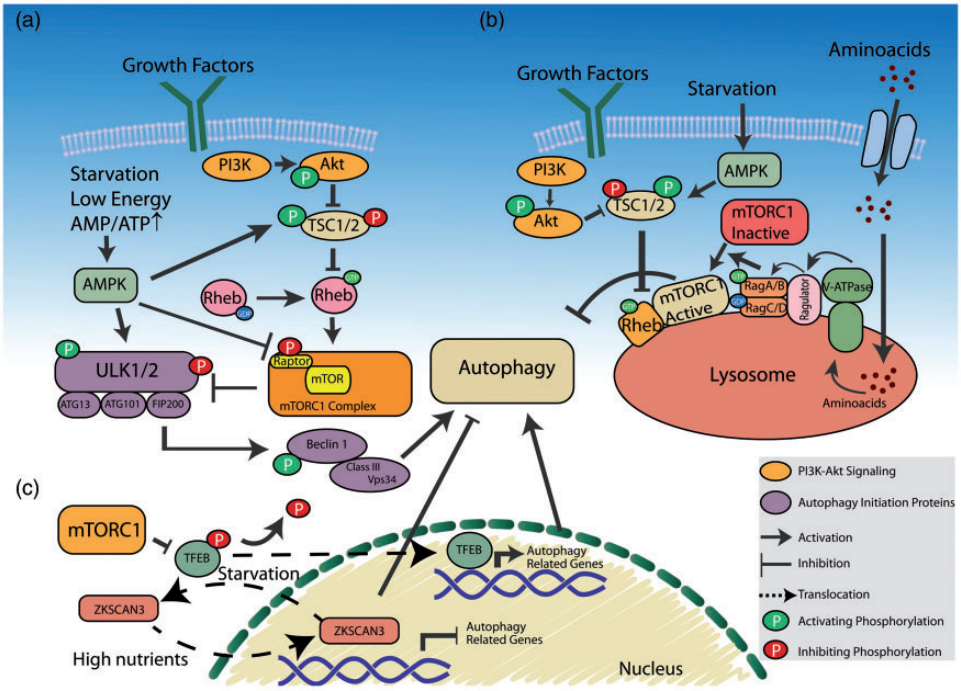

Figure 2. Schematic depiction of major regulatory signaling pathways of autophagy. (a) Possible role of AMPK–mTOR–ULK1/2 signaling cascade. Mammalian target of rapamycin (mTOR) activation is controlled by the tuberous sclerosis complex 1/2 (TSC1/2) and ras homolog enriched in brain (Rheb). Under conditions of low-energy (high AMP/ATP ratio), AMP-activated protein kinase (AMPK) phosphorylates and activates TSC1/2, thereby inhibiting mTORC1. Under glucose starvation, AMPK promotes autophagy by directly activating Unc-51-like autophagy-activating kinase 1/2 (ULK1/2) through phosphorylation. The two protein complexes AMPK and mTORC1 are known to oppositely regulate the autophagy inducing complex ULK1/2-Atg13-Atg101-FIP200. ULK1 directly phosphorylates beclin-1 and enhances pro-autophagic lipid kinase VPS34 to promote autophagy. (b) Amino acid signaling to mechanistic target of mTORC1. mTORC1 interacts with the Ragulator-Rag complex on the surface of the lysosome in response to amino acid levels in the cell. Under amino acid sufficiency, the v-ATPase-Ragulator complex is activated through a lysosomal mechanism, which in turn promotes the GTP-charging of RagA/B via a GEF function of the ragulator. Activated Rags then recruit mTORC1 to the lysosomal surface, where mTORC1 can be activated by Rheb which integrates amino acid signals with other upstream signals converging on the Rheb GTPase. (c) ZKSCAN3, a zinc finger family DNA-binding protein, and transcription factor EB (TFEB) regulate autophagy in opposite directions. The transcriptional repressor ZKSCAN3 downregulates autophagy-related gene expressions. The subcellular localization and activity of TFEB are regulated by mechanistic target of mTORC1-mediated phosphorylation. Under normal nutrient-rich conditions, TFEB is phosphorylated by mTORC1 on the lysosomal surface and is retained in the cytoplasm. During nutrient deprivation (starvation) or lysosomal stress (physical exercise), mTORC1 is inactivated and no longer able to phosphorylate TFEB. Dephosphorylated TFEB can freely translocate to the nucleus, where it activates the transcription of a network of genes that regulates the lysosomal/autophagic pathway. (A color version of this figure is available in the online journal.)

suggested that autophagy is the main executor of cell death and is the transition step for activation of cell death pathways. Therefore, the degree of autophagy determines whether cells survive or die.[16] Simultaneously, autophagic cell death, defined as type II cell death, can occur with excessive stimulation of autophagy. Autophagy can induce cell death in cases where apoptosis is deficient or inadequate.[20]

The relationship between autophagy and tumors

Autophagy plays a dual role in cancer progression[4] and exhibits context-dependent features in tumor development. The tumor type, stage, genetic background, and internal and external stimuli are the basic factors that determine the autophagy characteristics.[5] Gewirtz[22] described four functional features of autophagy in response to therapy: cytoprotective (relating to therapy resistance and apoptosis prevention), cytotoxic (causing cell death and, when blocked, reducing therapeutic efficiency), cytostatic (relating to senescence-dependent extended growth arrest), and non-protective (causing ineffectiveness of treatment if it is suppressed). These features are context-dependent. Only one of these four responses to therapy can be targeted and it is not known which type of autophagy will occur in response to treatment.[22] In the early stages of cancer, autophagy acts as a tumor suppressor and ensures genome integrity by eliminating damaged structures and preventing tumor formation, but in established tumors, autophagy sustains the survival of cancer cells and supports tumor development.[23] Autophagy is an adaptive mechanism under adverse conditions; hence, tumor cells activate autophagy in response to stress conditions, allowing tumor cells to survive in these adverse conditions. Autophagic degradation of non-essential cellular macromolecules provides energy to cancer cells to maintain their viability.[23] Autophagy is responsible for tumor recurrence and progression following treatment, by allowing cancer cells to remain dormant. Autophagy is used by cancer cells as a defense mechanism against cancer treatment. It ensures that cancer cells remain in a malignant state and provides the molecules necessary for their proliferation.[4]Beclin-1 is the main autophagy gene associated with cancer and spontaneous tumor developments were reported in beclin-1-deficient BECN1+/– mouse

models.[24,25]Beclin-1 deletions were observed in various human cancers.[4,26] In these cancers, reduced expression levels of beclin-1 were associated with the tumor suppressing property of autophagy. In our study, we showed that cucurbitacin B (CuB) and cisplatin treatments reduced tumor development in an MB49 mouse syngeneic bladder cancer model by inducing autophagy with increased expression levels of microtubule-associated protein 1 A/1B-light chain 3II (LC3II) and beclin-1.[9] In contrast, an increased beclin-1 expression was found in some cancers.[4,27] In these cancers, it was thought that beclin-1 played a role in tumor formation.[4] Liu *et al*.[28] found that beclin-1 expression was associated with the clinicopathological features of bladder tumors. The researchers used a sample of 147 bladder cancer patients and showed that reductions in beclin-1 expression were associated with increased bladder cancer histological grades and clinical stages.[28] Baspinar *et al*.[29] evaluated the relationship between beclin-1 and bcl-2 expression and clinical parameters in 84 bladder tumors and 10 non-tumor control tissues. They found negative correlations between beclin-1 protein expression and bladder cancer, high histological grades, and high pT stages. By contrast, they showed that increases in bcl-2 expression correlated with tumor stages. They also emphasized that the beclin-1 and bcl-2 expressions were inversely associated with each other in bladder tumors. Chen *et al*.[30] investigated the expression levels of p53, protocadherin-17, and beclin-1 proteins immunohistochemically in 75 bladder cancer patients. They found that these proteins, separately or together, were associated with the clinicopathological features of bladder cancer. They showed that beclin-1 expression, in particular, was associated with the overall survival of bladder cancer patients.

Apart from beclin-1, other autophagy-related genes are known to be associated with autophagy. Deng *et al*.[23] reported tumors' relationships with autophagy-related genes, such as ATG5 and ATG7, in cancer models. Zhu *et al*.[31] demonstrated that ATG7 overexpression played a role in the development of bladder cancer in *in vitro* and *in vivo* bladder cancer models. miRNAs targeting ATG7 were identified in previous studies,[32,33] and therefore detection of miRNAs targeting ATG7 is important in the treatment of bladder cancer. Zhang *et al*.[34] found that ATG7 is the target of miR-154 by using bioinformatics databases. They also described this interaction experimentally using double luciferase reporter analysis. In a subcutaneous mouse tumor model formed by T24 cells stably expressing miR−154, tumor development was more limited in the miR−154 overexpressed group than in the control group.[34] They showed that miR-154 prevented cell proliferation, migration, and invasion by inhibiting ATG7 in bladder cancer cells. Zhu *et al*.[35] showed that ATG7-mediated autophagy played an important role in the invasion of bladder cancer cells. Heterogeneous nuclear ribonucleoprotein D (HNRNPD) is a protein involved in the mRNA degradation of many oncogenes and downstream targets of ATG7, and the autophagic degradation of this protein reduced its interaction with ARHGDIB, which played a role in the cell invasion and migration of bladder cancer cells.[35] Zhu *et al*.[36] indicated that PD-L1 was one of the downstream targets of ATG7. PD-L1 is a co-inhibitory transmembrane protein that allows cancer cells to escape from the immune system. In this study, they showed that the expression levels of PD-L1 were regulated by ATG7-mediated autophagy. Zhu *et al*.[37] showed that ATG7 overexpression facilitated PD-L1 mRNA stability on the FOXO3a/miR-145 axis and increased cancer stem cell characteristics and the invasion of bladder cancer cells. Zhu *et al*.[31] demonstrated that ATG7 causes bladder tumor development through the ETS2/miRNA-196b/FOXO1/p27 pathway. Another study reported that ATG7 promoted sphere formation, invasion, and lung metastasis in human bladder cancer cells by regulating the downstream target CD44 protein.[37]

Buffen *et al*.[38] revealed that autophagy is an important mechanism for regulating trained immunity. A BCG vaccine is widely used in the early stages of bladder cancer to prevent recurrence and progression. BCG vaccines activate trained immunity through the epigenetic regulation (H3K4 methylation) of monocytes. They reported that autophagy suppression reduced the level of H3K4 methylation, which was responsible for the activation of trained immunity in monocytes. They also demonstrated that ATG2B rs3759601 polymorphism was associated with bladder cancer recurrence following BCG treatment in both *in vitro* and *in vivo* models.

Bax-interacting factor-1 (Bif-1) promotes autophagy by regulating beclin-1 activation. Kung *et al*.[39] suggested that Bif-1 acted as a tumor suppressor in cancer development and found decreased expression of Bif-1 in some cancers, including bladder cancer. Autophagy activation by oncogenes also has a tumor-supportive effect. In some cancers, high mutation rates have been observed in some oncogenic genes (rat sarcoma viral oncogene homolog (RAS) and v-Raf murine sarcoma viral oncogene homolog B (BRAF]) associated with increased autophagy levels. In these tumors, autophagy inhibition resulted in cell growth inhibition and reduced tumor development.[5] In 412 bladder cancer patients, Wang *et al*.[40] investigated the relationship between autophagy-related gene expressions and clinicopathological characteristics. They identified three genes (JUN, MYC, and ITGA3) that were clinically related to bladder cancer and had prognostic value. Another protein interacting with autophagy is estrogen-related receptor alpha (ESSRA), a transcription factor involved in autophagy regulation. Kim *et al*.[41] investigated expression levels of LC3B and ESRRA proteins immunohistochemically in muscle-invasive bladder tumors. They found that co-expression of these proteins related to poor overall survival and poor disease-free survival in bladder cancer patients.

Survival effects of autophagy on bladder cancer cells

Various studies have demonstrated the oncogenic role of autophagy in bladder cancer. Overexpression of high mobility group box protein 1 (HMGB1) related to radiation resistance in bladder cancer cells, but the radiosensitization effect of HMGB1 depended on the activation of autophagy. Shrivastava *et al*.[42] found that, in HMGB1-silenced cell lines

and mouse tumor xenograft models, the response to radiotherapy improved due to decreased autophagy. HMGB1 is a nuclear DNA chaperone protein that functions in DNA damage response. It is also related to carcinogenic properties, such as excessive cell proliferation and apoptosis inhibition, invasion, and metastasis. Overexpression levels of HMGB1 were found in bladder cancer cell lines and tumor tissues isolated from primary bladder patients.[43] According to Yin *et al.*[43] expression levels of HMGB1 were correlated with bladder tumor grades and stages. HMGB1-induced autophagy decreased the cytotoxic effect of gemcitabine and HMGB1 induced autophagy through activation of the JNK and ERK pathways. They also put forward that HMGB1-induced autophagy was related to gemcitabine resistance in bladder cancer cells; HMGB1 inhibition was enhanced by the apoptosis induced by gemcitabine in bladder cancer cells.

Hypoxia-inducible factor 1 (HIF-1) regulates the adaptive cell response against hypoxic conditions through gene expression control. These genes also play a role in cancer development; HIF-1α overexpression was observed in bladder cancer.[44] Bcl2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) is a pro-apoptotic protein that belongs to the pro-apoptotic Bcl-2 family. BNIP3 is activated by HIF-1α under hypoxic conditions. Activated BNIP3 dissociates beclin-1 from the Bcl-2/beclin-1 complex and leads to autophagy initiation through released beclin-1. Yang *et al.*[44] showed that suppressing HIF−1α inhibited autophagy as well as BNIP3 and Beclin1, and enhanced gemcitabine-induced apoptosis in bladder cancer cells under hypoxic conditions. In other words, hypoxia-induced cytoprotective autophagy counteracted gemcitabine-induced apoptosis through an increase in HIF−1α expression. Therefore, the researchers suggested that targeting both HIF−1α-associated pathways and autophagy in bladder cancer may be a successful strategy to enhance the sensitivity of bladder cancer chemotherapy.

Regulated in development and DNA damage response-1 (REDD) is a stress protein that allows tumor cells to eliminate apoptosis under stress conditions, such as oxygen and nutrient deficiency and energy stress. This protein promotes cancer cell survival through mTOR inhibition. Zeng *et al.*[45] showed that this protein expression increased in bladder cancer tumor tissues and they found a positive correlation between poor survival rates and high expressions of REDD1 in bladder cancer patients. They reported that REDD1 expression stimulated autophagy through the mTOR-EEF2K pathway and, by inhibiting this gene, the paclitaxel chemosensitivity of T24 and EJ bladder cancer cells increased, depending on autophagy inhibition.

NBR1 is a ubiquitous scaffold protein that acts as an autophagy receptor bridging the LC3 and the autophagosome membrane. The cargo molecules are then degraded by lysosomal enzymes. Kim *et al.*[46] stated that the deterioration in NRB1 functioning restrained autophagosome formation and disrupted the level of p62 protein and autophagosome turnover. They reported that rapamycin activated protective autophagy by increasing the amount of NRB1 in bladder cancer cell lines. NBR1-silenced bladder cancer cells exhibited increased sensitivity to rapamycin due to deficient autophagy, and the suppression of the NRB1 gene increased rapamycin-induced apoptotic cell death. They suggested that the alteration in NBR1 expression determined the response of bladder cancer cells to rapamycin.

Vanzo *et al.*[47] demonstrated that under replication stress, autophagy was activated and promoted DNA synthesis in bladder cancer cells. Their results highlighted that autophagy may support cancer progression, at least in part, by facilitating tumor cell survival and fitness under replication stress, a feature shared by most malignancies. p62 serves as a selective autophagy adaptor and plays a key role in regulating the formation of protein aggregates. This autophagy protein also participated in the Kelch-like ECH-associated protein 1 (KEAP1)-nuclear factor (NF)-E2-related factor 2 (NRF2) anti-oxidative response pathway. NRF2 is degraded by KEAP1 through proteasomal activity under basal oxidative stress conditions. In the presence of excessive oxidative stress, NRF2 activates and regulates oxidative stress response genes. Excessive NRF2 activation protects cancer cells from the lethal effects of ROS accumulation; hence, the NRF2 pathway is one of the pathways involved in cancer formation. p62 is associated with many cancers. Li *et al.*[48] indicated that p62 was overexpressed in bladder cancer cell lines and bladder tumor tissues and prevented NRF2 degradation by binding with KEAP1. They also showed that a syngeneic mouse bladder tumor study, which used p62-silenced 5637 cells, reported downregulated NRF2 and upregulated KEAP1 levels. It was observed that tumor development decreased in mice given p62-silenced bladder cancer cells compared to the control group; therefore, p62 contributed to bladder tumor progression by activating the NRF2 pathway.[48]

Autophagy affects the response of cancer cells to chemotherapy depending on the cell type, inducer intensity, and treatment period.[49] Autophagy restricts the cell-killing effect of anticancer drugs and plays a role in recurrence and metastasis, following treatment, by allowing cancer cells to become dormant under chemotherapy and thereby maintaining their tumoral potential.[21] Ojha *et al.*[50] showed that the percentage of stem cells in bladder cancer primary cell cultures (obtained from patients with low grade, high grade, and muscle-invasive urothelial carcinoma tumor tissues) increased in response to treatment with gemcitabine (GC) and mitomycin (MM). In these stem cells, increased autophagy flux was observed following drug administration. The expression of stem cell-related (Oct-4 and Nanog) and drug resistance-related genes (ABCG2 and MDR) decreased through the inhibition of autophagy. In addition, autophagy inhibition increased the sensitivity of cancer stem cells to the apoptosis induced by GC and MM.[50] Epirubicin is a type of anthracycline-class drug used intravesically in bladder cancer patients after transurethral resection to prevent tumor recurrence. Icaritin is a type of Chinese herbal icaritin that exhibits anti-cancer effects. Pan *et al.*[51] showed that icaritin enhanced the anti-cancer effect of epirubicin via the inhibition of epirubicin-induced autophagy. Simultaneously, they reported that the epirubicin-dependent toxic effect decreased when used with icaritin in BT5637 and T24 bladder cancer cell

models. Zhang *et al.*[52] put forward that 5-Aza-2′-deoxycytidine (5−Aza−CdR) increased the effect of mitomycin-C (MMC) on bladder cancer cells via autophagy inhibition; therefore, co-treatment with 5−Aza−CdR and MMC resulted in increased apoptosis and cell death.

Chloroquine (CQ) and hydroxychloroquine (HCQ) are widely used in the treatment of malaria; they are also agents of late-phase autophagy inhibition and have frequently been used in autophagy studies. Due to their autophagy inhibition features, these drugs have been used in clinical trials to sensitize tumor cells against chemotherapy. CQ and HCQ are lysosomotropic drugs that inhibit autophagy by targeting lysosomal enzymes. When they penetrate lysosomes, they protonate and increase the lysosomal pH; the increased pH inactivates lysosomal enzymes and blocks autophagosome–lysosome fusion. Lin *et al.*[53] investigated the anti-carcinogenic effects of CQ and HCQ on bladder cancer and showed that CQ and HCQ treatment reduced bladder tumor growth and that the tumor inhibition effect of CQ and HCQ was related to basal autophagy suppression and apoptosis activation in bladder cancer cells (RT4, 5637, and T24). Another study showed that CQ treatment enhanced radiosensitivity by inhibiting autophagy *in vitro* (in EJ and T24 cell lines) and *in vivo* (in T24 xenograft bladder cancer models).[54] In this study, co-treatment with CQ and irradiation retarded tumor development and enhanced radiation-induced apoptosis in a T24 xenograft model.

9-ING-41 is a glycogen synthase kinase-3 beta (GSK-3β) inhibitor used in clinical studies. The protective autophagy-inducing properties of 9-ING-41 were demonstrated in bladder cancer cells by Kuroki *et al.*[55] They pointed out that combined treatments with 9-ING-41 and CQ enhanced the toxic effect of 9-ING-41 on bladder cancer cells. They further reported that in chemo-resistant T24 and HT1376 bladder cancer cell lines, 9-ING-41 increased the anti-tumor effect of cisplatin and GC.

Obatoclax (GX15-070) is a BH3 mimetic that inhibits the link between Mcl-1 and Bax or Bak. When this interaction is blocked by obatoclax, Bak is released and apoptosis is activated. Obatoclax also disrupts Mcl-1/beclin-1 interaction, and the beclin-1 cleavage induced by caspase-3 activity leads to autophagy inhibition. High Mcl-1 expression was reported by Jiménez-Guerrero *et al.*[56] in muscle-invasive bladder cancer patients and was shown to be related to bladder cancer recurrence. Paclitaxel is a microtubule toxin that is used in bladder cancer treatment as second-line therapy agent. The researchers also revealed that obatoclax enhanced paclitaxel-mediated apoptosis through autophagy flux blockage in paclitaxel-resistant HT1197 bladder cancer cell lines.

The highest mutation rate is in the epithelial growth factor receptor (EGFR) gene in bladder cancer cells. EGFR inhibitors are largely used in the treatment of bladder cancer. EGFR suppresses autophagy through beclin-1 phosphorylation; however, tyrosine kinase inhibitors, which target this receptor, cause drug resistance by activating the autophagic pathway that supports cancer cell survival. Kang *et al.*[57] stated that cell viability and clonal proliferation were significantly reduced in T24 and J82 bladder cancer cell lines when EGFR inhibitors (lapatinib and gefitinib) and autophagy inhibitors (Bafilomycin A1 (BFA1), chloroquine (CQ), and 3-methyladenine (3-MA)) were used together. They emphasized that autophagy inhibitors increased the apoptotic effect of EGFR receptors.

Cisplatin treatment has been shown to activate protective autophagy and limit apoptosis. Cisplatin-induced autophagy is believed to be one of the mechanisms responsible for drug resistance.[21] Ojha *et al.*[58] demonstrated that autophagy activation related to bladder cancer grades in tumor tissues (obtained from 15 low-grade and 15 high-grade bladder cancer patients) and primary cultured cells grown from those bladder tumor tissues. They indicated that cell protective autophagy was induced in a cisplatin-treated T24 bladder cancer cell line and primary bladder cancer cells, providing evidence of an increased cytotoxic effect of cisplatin when administered with an autophagy inhibitor. Ojha *et al.*[59] identified a mechanism by which autophagy maintained cisplatin resistance through regulation of the immune response in cisplatin-resistant cells, and observed elevated autophagic flux in cisplatin-resistant primary cells (obtained from high-grade and low-grade human bladder tumor tissues) and an T24 bladder cancer-resistant (RT24) cell line treated with GC and MM. According to this study, GC and MM treatments increased autophagy through the Janus kinase/signal transducers and activators of transcription (JAK2/STAT3) pathway, and JAK2-mediated autophagy simultaneously regulated the survival of resistant cells; therefore, the JAK2–STAT3 pathway was associated with chemotherapy resistance and tumor recurrence. Eventually, they highlighted the suppression of autophagy as an actor in overcoming chemotherapy resistance in RT24 cells.[59] Liao *et al.*[60] found that cisplatin treatment led to increased HMGB1 expression and cytoprotective autophagy mediated by HMGB1 in bladder cancer cell lines (EJ, 5637, T24, and BIU-87), and HMGB1 knockdown and knockout bladder cancer cell lines exhibited sensitivity to cisplatin. In the same study, decreased autophagy levels were associated with the enhanced sensitivity of HGBM1-silenced cell lines to cisplatin. Lin *et al.*[61] reported that cisplatin-induced autophagy was regulated by beclin-1 activation and supported cancer cell survival by promoting cisplatin resistance. They further emphasized that pharmacological and genetic inhibition of autophagy overcame cisplatin resistance and increased apoptosis activation in human bladder cancer cells (5637 and T24).

Some of the above-mentioned studies on the inhibition of treatment-induced cytoprotective autophagy in bladder cancer models and involving the combination of the most used chemotherapeutic drugs alongside autophagy inhibitors are summarized in Table 1.

Cell death effects of autophagy on bladder cancer cells

While autophagy is an important factor in chemotherapy resistance, the excessive stimulation of autophagy may cause autophagic cell death. In cancer treatments, the apoptotic pathway is the primary target in the elimination of tumor cells, but this pathway is generally disrupted in

Table 1. Selected studies on the inhibition of treatment-induced cytoprotective autophagy in bladder cancer models.

Agents	Autophagy inhibitors	Autophagy inhibition mechanism/s after combined treatments	Cancer model/s (cell lines and/or animals)	References
Gemcitabine	Wortmannin, CQ, HMGB1 siRNA	HMGB1-mediated JNK/ERK pathway	T24 and BIU-87 cell lines; BC tissues	Yin *et al.*[43]
Gemcitabine	3-MA, CQ, HIF-1α, siRNA	HIF-1α/Bcl-2/BNIP3/Beclin-1 pathway	T24 cell line; BC tissues	Yang *et al.*[44]
Gemcitabine and Mitomycin	Wortmannin, CQ, beclin-1 siRNA	IFN-γ/JAK2/STAT3 pathway	CR-T24 cell line, CR- primary cells established from BC patients; BC tissues	Ojha *et al.*[59]
Rapamycin	Baf-A1, CQ, lentiviral-based shRNA	AMPK/mTOR pathway	T24, 5637, HT1376 and 253J cell lines	Kim *et al.*[46]
Cisplatin	Baf-A1, CQ, lentiviral-based shRNA; beclin-1 siRNA	Beclin-1-dependent autophagy inhibition	5637 and T24 cell lines	Lin *et al.*[61]
Paclitaxel	3-MA, CQ diphosphate salt, miR-22 mimics	REDD1-mediated mTOR/EEF2K pathway	RT4, T24, BIU87 and EJ cell lines; BC tissues; subcutaneous xenograft nude mouse	Zeng *et al.*[45]
Paclitaxel	Obatoclax	Beclin-1-dependent autophagy inhibition	5637 and HT1197 cell lines; BC tissues	Jiménez-Guerrero *et al.*[56]
Epirubicin	Icaritin	MMP suppression	BT5637 and T24 cell lines	Pan *et al.*[51]
Cisplatin under nutritional limiting (starvation) condition	3-MA, Wortmannin, CQ	AMPK/mTOR pathway	T24 cell line; primary cells established from BC patients; BC tissues	Ojha *et al.*[58]
Lapatinib and Gefitinib (EGFR inhibitors)	Baf-A1, CQ, 3-MA, ATG12 siRNA	Decreased expression of LC3II level	T24 and J82 cell lines	Kang *et al.*[57]

3-MA: 3-methyladenine; AMPK: AMP-activated protein kinase; ATG12: autophagy related 12; Baf-A1: bafilomycin A1; BC: bladder cancer; BNIP3: adenovirus E1B 19 kDa protein-interacting protein 3; CQ: chloroquine; CR: cisplatin-resistant; EEF2K: eukaryotic elongation factor 2 kinase; EGFR: epithelial growth factor receptor; ERK: extracellular regulated protein kinase; HIF-1α: hypoxia-inducible factor 1α; HMGB1: high-mobility group box 1; IFN-γ: interferon gamma; JAK2/STAT3: janus kinase/signal transducers and activators of transcription; JNK: c-Jun N-terminal kinase; LC3II: microtubule-associated protein 1 A/1B-light chain 3II; MMP: mitochondrial membrane potential; mTOR: mammalian target of rapamycin; REDD1: regulated in development and DNA damage response-1; shRNA: short hairpin RNA; siRNA: small interfering RNA.

tumor cells, resulting in drug resistance and aggressive tumors. Autophagy provides an alternative target against apoptosis resistance.[16]

Various studies have shown that autophagy is responsible for cell death in bladder cancer. Zeng *et al.*[62] suggested that overexpression of miR-222 inhibited the cisplatin-induced autophagy that caused cell death via the protein phosphatase 2 A subunit B (PPP2R2A)/Akt/mTOR pathway in bladder cancer cells. Matrix metalloproteinase-2 (MMP-2) is an important protease that promotes bladder cancer cell invasion through the degradation of the extracellular matrix. Peng *et al.*[63] revealed that MMP2, degraded by pleckstrin homology domain leucine-rich repeat protein phosphatase 2 (PHLPP2), induced autophagy in bladder cancer cells. G9a is an epigenetic enzyme that regulates gene expression through the methylation of histone proteins. This enzyme transfers methyl groups to the ninth lysine of histone 3 (H3) proteins. Li *et al.*[64] reported that G9a was a negative regulator of autophagy, which bound to the promoter regions of autophagy-related genes and dimethylates H3K9 (a repressive histone mark). Dimethylation of H3K9 resulted in transcriptional repression of autophagy-related gene expressions. They also showed that G9a inhibition resulted in inhibition of cell proliferation by inducing autophagic cell death via the AMPK/mTOR pathway in T24 and UMUC3 bladder cancer cells. Jin *et al.*[65] found that miR-516A downregulated PHLPP2 protein by directly binding to 3′-UTR of its mRNA, and the downregulated PHLPP2 further decreased beclin-1 expression by promoting BECN1 protein degradation, which inhibited autophagy and thereby enhanced the growth of bladder cancer in *in vitro* and *in vivo* models. miR-21 high expression levels and their oncogenic potential have previously been demonstrated in bladder cancer studies. Zhang *et al.*[66] found that miR-21 exhibited a tumor promotion effect through autophagy inhibition. They observed autophagy inhibition and increased tumor properties in T24 bladder cancer cells transfected with miR-21 mimics.

O-linked GlcNAcylation (O-GlcNAc) is a post-translational modification that transfers N-acetyl-glucosamine to the serine/threonine amino acid residues of nuclear and cytoplasmic proteins. This modification is also related to the initiation and development of cancers, including bladder cancer. Glycoside hydrolase O-GlcNAcase (OGA) regulates the removal of acetylated amino sugars from N-acetylglucosamine-attached proteins; therefore, the O-GlcNAcylation of AMPK results in autophagy inhibition, which causes tumor progression in bladder cancer. According to Jin *et al.*[67] a negative correlation existed between AMPK activation and the O-GlcNAcylation of AMPK: the phosphorylation of AMPK decreased with its O-GlcNAcylation.

Cisplatin is one of the most effective systemic chemotherapeutic agents in bladder cancer. However, some patients do not respond to cisplatin-based chemotherapy. The role of autophagy in cisplatin resistance is controversial. Some studies have documented that cisplatin-induced protective autophagy is responsible for drug resistance, but other studies of cisplatin-induced apoptosis have reported autophagy inhibition.[6] Some studies have provided evidence that autophagy inhibition reduced cisplatin resistance, while others have shown increased sensitivity of cancer cells to cisplatin.[49] Li *et al.*[68] demonstrated that a type of fungal immunomodulatory protein (FIP)-Ganoderma tsugae (gts) induced autophagic cell death and apoptosis in both cisplatin-sensitive and -resistant bladder cancer cell lines through autophagosome accumulation. FIP-gts-induced autophagosome accumulation was enhanced by CQ administration. The researchers also reported that cisplatin-resistant N/P bladder cancer cells exposed to both FIP-gts and BAF-A1 reduced beclin-1 expression along with autophagic cell death which has occurred due to excessive autophagosome accumulation. Similar to previous studies, Hsin *et al.*[69] reported that CQ and FIP-gts co-treatment caused excessive autophagosome accumulation stress, leading to caspase-independent cell death in cisplatin-resistant bladder cancer cell lines.

A clinical investigation of a dual phosphatidylinositol 3-kinase (PI3K) /mTOR inhibitor (NVP-BEZ235) revealed that NVP-BEZ235 led to cell death through the autophagic flux activation, without inducing apoptosis, in cisplatin-sensitive NTUB1 and cisplatin-resistant N/P bladder cancer cell lines.[70] Another dual inhibitor is MPT0L145. It is a dual phosphatidylinositol 3-Kinase Catalytic Subunit Type 3 (PIK3C3)/fibroblast growth factor receptors (FGFR) inhibitor that induces bladder cancer cell death in an autophagy-dependent manner. Chen *et al.*[71] reported that MPT0L145 enhanced the sensitivity of cisplatin-resistant N/P bladder cancer to cisplatin and decreased cell viability. They found that MPT0L145 induced autophagy via suppression of FGFR activation, while hindering autophagic flux by inhibiting PIK3C3 activation. They also suggested that accumulation of non-functional autophagic vacuoles in the cancer cytoplasm due to excessive autophagy stimulation resulted in cell death.

Some natural and synthetic compounds, such as troglitazone (TZ),[72] isorhapontigenin (ISO),[73] reversine,[74] cheliensisin A derivative (ChlA-F),[75] and 10-hydroxycamptothecin (HCPT)[76] can induce autophagic cell death in apoptosis-resistant or apoptosis-insufficient cells. HCPT is a natural Chinese plant extract that activates autophagy through the AMPK/mTOR/ULK1 pathway in bladder cancer cells. Autophagy induced by HCPT has a pro-apoptotic effect that reduces cell viability.[76] Analysis of the genetic structure of cancer cells is important for determining the autophagic capacity of cells to respond to stimulants. Drug resistance and cancer recurrence due to defective apoptosis can be overcome by stimulating autophagy. Therefore, the use of autophagy-inducing agents in these cells can be considered as an alternative treatment option. Tetrandrine is an alkaloid used in traditional Chinese medicine and Kou *et al.*[77] showed that the AMPK/mTOR pathway was the main regulator of tetrandrine-induced autophagy, which promoted the apoptotic effect of tetrandrine in bladder cancer cell lines (T24 and 5637). Triacanthine is an alkaloid derived from *Gleditsia* spp. extract and known for its anti-cancer properties. Shin *et al.*[78] showed that this compound induced apoptotic cell death through the caspase-dependent extracellular apoptotic pathway. They also found that triacanthine-induced

Table 2. Selected studies on the induction of cell death through autophagy activation in bladder cancer models.

Agents	Action mechanism/s	Cancer model/s (cell lines and/or animals)	References
miR-21 inhibitor	Increased expression level of Beclin-1, LC3II, Caspase-3	T24 cell line	Zhang *et al.*[66]
miR-516Ai	Inhibition of miR516A-PHLPP2-CUL4A-mediated Beclin-1 protein degradation	UMUC3, J82, RT112 and TCCSUP cell linesUMUC3 Xenograft model in nude mice	Jin *et al.*[67]
FIP-gts and Baf-A1	Increased large amounts of autophagosomes and induced apoptosis	NTUB1 and N/P (cisplatin-resistant sub-line) cell lines	Li *et al.*[68]
FIP-gts	AAA stress; caspase-independent cell death	NTUB1 and N/P (cisplatin-resistant sub-line) cell lines	Hsin *et al.*[69]
NVP-BEZ235	Increased AVO; autophagic flux activation	NTUB1 and N/P (cisplatin-resistant sub-line) cell lines	Li *et al.*[70]
MPT0L145	Accumulation of non-functional autophagic vacuoles due to excessive autophagy stimulation	RT-112, RT-4 and N/P (cisplatin-resistant sub-line) cell lines	Chen *et al.*[71]
HCPT	Activation of AMPK-mTOR-ULK1 pathway	T24 and 5637 cell lines	Wang *et al.*[76]
Triacanthine	Increased levels of Beclin-1 and LC3-II; activated autophagy modulate extracellular apoptotic pathway	EJ cell line; EJ-xenografted nude mice	Shin *et al.*[78]
JQ1	Activation of LKB1/AMPK pathway	T24, 5637 and UMUC-3 cell lines; T24 xenograft tumor model	Li *et al.*[79]
CONPs	ROS-dependent ERK activation	T24, J82, 5637 and UMUC3 cell lines; UMUC3 orthotopic xenograft and T24 subcutaneous nude mouse model	Xiong *et al.*[80]
NaB	Induced AMPK/mTOR pathway-activated autophagy, ROS-mediated apoptosis via the miR-139-5p/Bmi-1 axis	T24 and 5637 cell lines; T24 xenograft nude mice model	Wang *et al.*[81]
Vitamin K2	AMPK-dependent autophagic cell death, PI3K/AKT/HIF-1α-mediated glycolysis promotion	T24, EJ and J82 cell lines; EJ xenograft nude mice tumor model	Duan *et al.*[82]

AAA stress: abundant autophagosome accumulation stress; AKT: protein kinase B; AMPK: adenosine monophosphate-activated protein kinase; ATG: autophagy related genes; AVO: acidic vesicular organelle; Baf-A1: bafilomycin A1; CONPs: cuprous oxide nanoparticles; CUL4A: cullin 4 A; ERK: extracellular signal-regulated kinase; FIP-gts: fungal immunomodulatory proteins-ganoderma tsugae; FIPs: fungal immunomodulatory proteins; HCPT: 10-hydroxycamptothecin; HIF-1α: hypoxia-inducible factor 1α; JQ1: BET bromodomain inhibitor; LC3II: microtubule-associated protein 1 A/1B-light chain 3II; LKB1: liver kinase B1; miR: MicroRNA; miR-516Ai: miR516A sponge inhibitor; MPT0L145: dual inhibitor of phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3) and the fibroblast growth factor receptors (FGFR); mTOR: mammalian target of rapamycin; NaB: sodium butyrate; NVP-BEZ235: dual phosphatidylinositol 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) inhibitor; PHLPP2: PH domain and leucine rich repeat protein phosphatase; PI3K: phosphoinositide 3-kinase; ROS: reactive oxygen species; ULK1: Unc-51 like autophagy activating kinase 1.

autophagy regulated the extracellular apoptotic pathway that led to cell death. Li *et al.*[79] investigated the anti-proliferative effect of bromodomain and extraterminal (BET) inhibitor JQ1 in bladder cancer cell lines and T24 xenografts model. They showed that JQ1 enhanced the binding of the serine–threonine liver kinase B1 (LKB1), is one of the proteins that controls the activation of AMPK, to the AMPKα and activated it. They also reported that AMPKα activation, regulated by JQ1, induced autophagy, which in turn promoted the cell death of bladder cancer cells. Xiong *et al.*[80] showed that cuprous oxide nanoparticles (CONPs) activated ERK-mediated autophagy, which triggered ROS-induced apoptosis in orthotopic xenografts and subcutaneous nude mouse bladder cancer models. Wang *et al.*[81] showed that sodium butyrate (NaB) induced autophagy through AMPKα activation in bladder cancer cells. They also reported that the miR-139-5p/B lymphoma Mo-MLV insertion region 1 homolog (Bmi-1) pathway regulated NaB-induced autophagy in bladder cancer cells. Duan *et al.*[82] showed that vitamin K2 led to metabolic stress through PI3K/AKT/HIF-1α-dependent glycolysis stimulation in bladder cancer cells. Increased metabolic stress was then shown to lead to autophagic cell death through AMPK activation in bladder cancer cell lines and bladder cancer xenografts. Desloratadine (an antagonist of the histamine H1 receptor)[83] and heptaphylline (a carbazole alkaloid)[84] are known agents that have displayed anti-cancer ability against bladder cancer. Both agents were shown to stimulate autophagy in bladder cancer cells, but the effect of this autophagy activation on cell death was not fully explained.

Some of the above-mentioned studies on the induction of cell death through autophagy activation in bladder cancer models are summarized in Table 2. In addition, dual role of autophagy in tumorigenesis is explained by apoptosis-autophagy-tumor progression and recurrence triad, as displayed in Figure 3. On one hand, autophagy is activated as a protective mechanism to mediate the

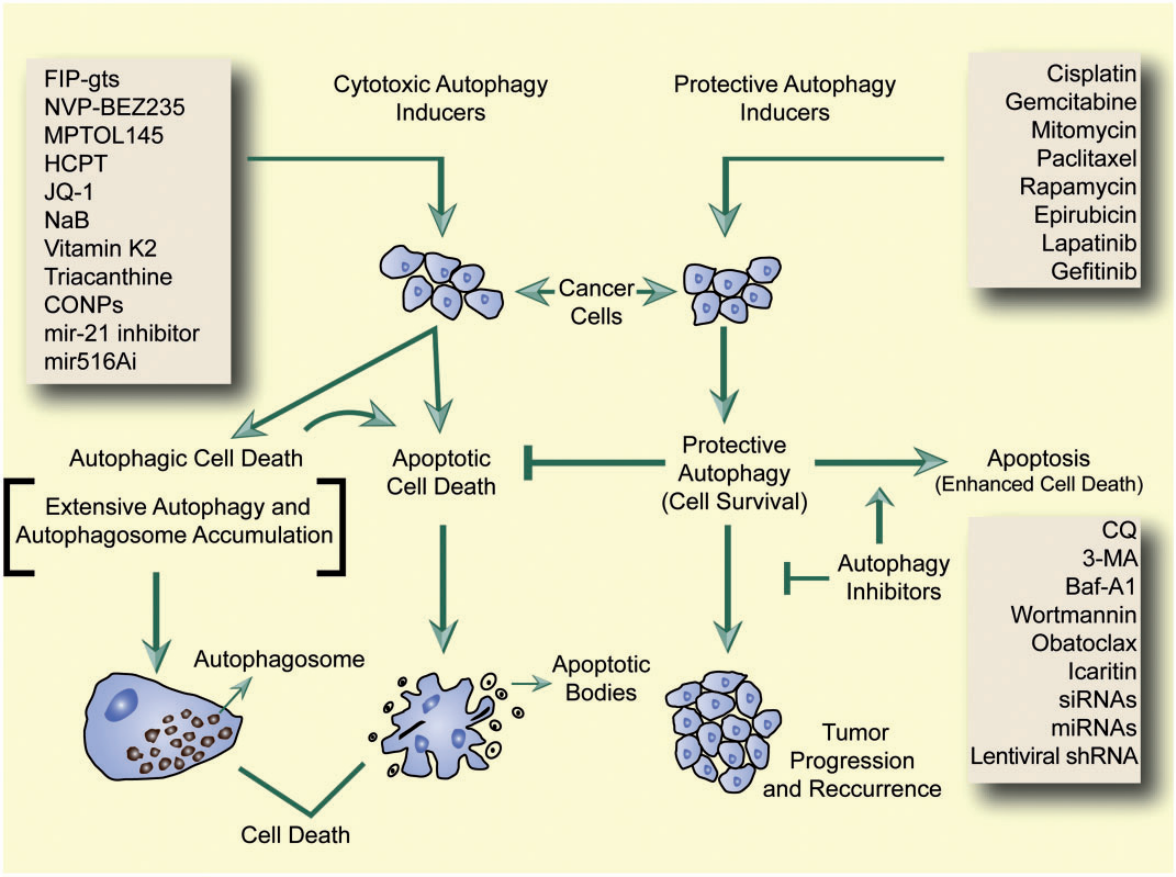

Figure 3. Apoptosis-autophagy-tumor progression and recurrence triad and dual role of autophagy for therapeutic purposes in cancer. The activation of autophagy either leads to cancer cell chemoresistance or potentiates autophagic cell death which depends on the tumor types and treatment characteristics. On one hand, autophagy is activated as a protective mechanism to mediate the acquired resistance phenotype of some cancer cells during chemotherapy. On the other hand, autophagy may also function as a death executioner to induce autophagic cell death. (A color version of this figure is available in the online journal.)

acquired resistance phenotype of some cancer cells during chemotherapy. On the other hand, autophagy may also function as a death executioner to induce autophagic cell death, a form of physiological cell death that is contradictory to apoptosis.

Conclusion

Targeting autophagy regulatory treatments has gained importance and, due to its role in cancer, autophagy modulation has become a preferred approach in recent years for the inhibition of tumor development and the enhancement of treatment. The bi-directional features of autophagy should be taken into account when targeting autophagic therapy. It is necessary to identify the factors that cause the condition-dependent behavior of autophagy, and different genes and signaling pathways should be investigated to better understand the effects of autophagy on bladder cancer. Molecular markers need to be identified to understand how autophagy works at the molecular level and uncovers the autophagic response to bladder cancer treatment. In addition, considering the heterogeneous features of bladder cancer, the assessment of autophagy's role in tumor progression is clinically important; therefore, clinical markers need to be identified to track changes in autophagy in cancer patients. So far, in clinical trials, the anti-tumor effect of autophagy on tumor inhibition has only been investigated using autophagy inhibitors combined with standard chemotherapeutic or radiotopic agents. Autophagic cell death has been well documented in preclinical studies and, considering its effect in stimulating cell death, agents that induce autophagy should be evaluated in clinical trials. Despite promising clinical trials, only one study is currently investigating the role of autophagy in bladder cancer (https://clinicaltrials.gov/ct2/show/NCT03254888). In this retrospectively designed clinical study, autophagy (Atg7, LC3A), endoplasmic reticulum-related stress (ATF6), oxidative stress (MDA), and apoptosis (caspase 3) markers are being investigated in 100 bladder cancer patients (50 low-grade and 50 high-grade) and 50 control individuals. The study completion date is estimated to be 31 December 2022. Understanding the complex behavior of autophagy under different conditions, and identifying autophagy-related pathways in bladder cancer are important for the development of new treatment strategies.

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