Review Article

Autophagy regulation in bladder cancer as the novel therapeutic strategy

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Abstract: Being the most common tumor of the urinary tract, bladder cancer (BC) has a prolonged nature that like many chronic disease requiring careful and invasive long-term surveillance. Among cancers, BC has the highest costs per patient making it an economic burden in medical treatment. The 2016 Nobel Prize in Physiology or Medicine was awarded to the researcher that revealed the mechanism of autophagy. Autophagy is an evolutionally conserved catabolic process that cells degrade cytoplasmic organelles or constituents through lysosome to generate energy and maintain homeostasis for cellular survival and proliferation. Autophagy is now recognized to play important role in both normal tissue and tumor development. In cancer cells, autophagy is usually constitutively activated due to the deregulation of PI3K/Akt/mTOR pathway that enables them to adapt efficiently to an unfavorable hypo-nutrient conditions in the tumor microenvironment. Therefore, targeting autophagy to reduce tumor growth or attenuate treatment resistance has led to greater interest in the field of cancer research, including the role of autophagy in BC. In this review, we summarize the studies from others and our own group regarding the role of autophagy in BC progression and treatment resistant by systematically reviewing treatment modalities including intravesical installation and chemotherapy for non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC).

Keywords: Autophagy; drug resistance, microRNAs; urinary bladder neoplasms

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Introduction

Bladder cancer (BC) is the ninth most common cancer in Taiwanese male according to the Taiwan Cancer Registry Annual Report in 2014 (1). In the United State, BC is the sixth most commonly diagnosed cancer with estimated 79,030 new cases and 16,870 death in 2017 (2). The incidence and mortality rate of BC is just next to prostate cancer among the genitourinary malignancies. Almost 70–80% of patients with bladder tumors present with low-grade, superficial or non-muscle invasive bladder cancer (NMIBC) (3), others are muscle invasive bladder cancer (MIBC). Routine surveillance, repeated transurethral resection of bladder tumor (TUR-BT), and the use of intravesical agents are the standard procedures performed for initial diagnosis, staging and treatment for managing of NMIBC. Despite these efforts, there are still subset of patients who progress to MIBC that drive the mortality of these disease. While new treatment regiments are developed rapidly to manage other cancers, the treatment options for BC remains limited. It is notable that the 50% overall survival at 5 years for MIBC has not improved for almost

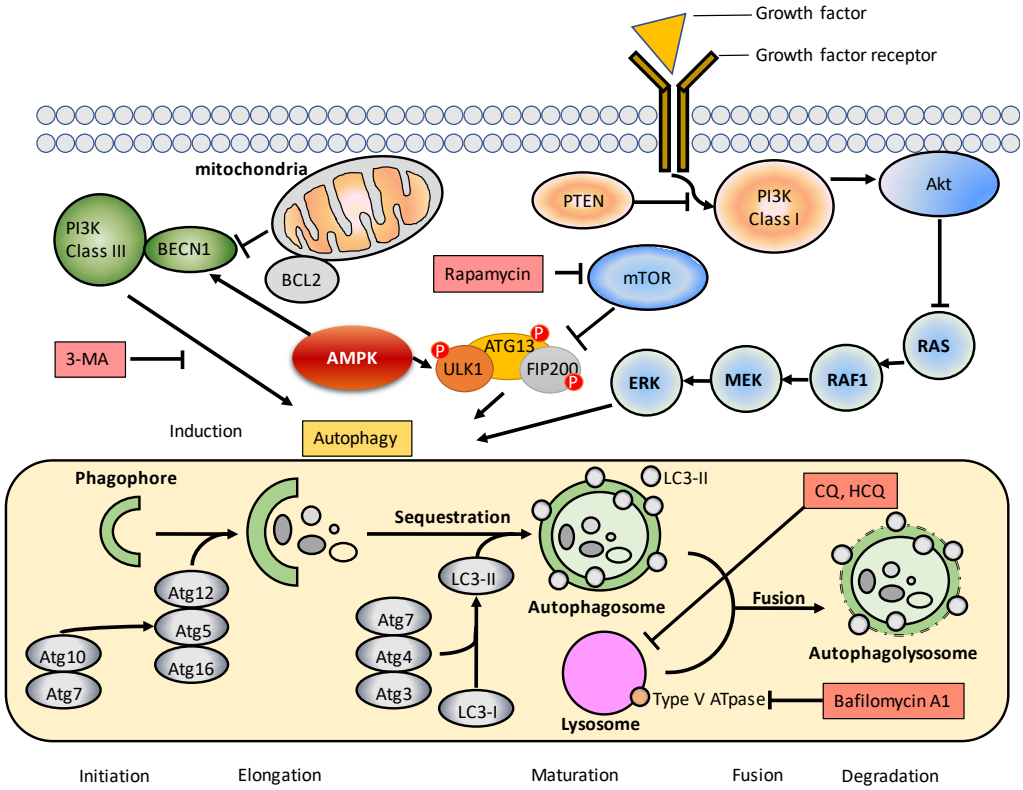

Figure 1 The main steps in the autophagy process. The autophagy process involves six main steps: induction, initiation, elongation and completion, maturation and fusion, and degradation. Several signaling pathways, including AMPK activation, mTOR inhibition, and ERK activation, had been identified to involve in autophagy induction. The ratio between anti-apoptotic protein BCL2 and BH3 containing beclin-1 is considered as a key switch regulating apoptosis and autophagy. There are two types of autophagy inhibitors: The early stage autophagy inhibitor, 3-methyladenine (3-MA) inhibits class III PI3K; and the late stage autophagy inhibitors, including bafilomycin A1 which inhibits the fusion between autophagosomes to lysosomes through blocking vacuolar-type H(+)-ATPase, and chloroquine (CQ) or hydroxychloroquine (HCQ) that disrupt lysosomal function and prevent the degradation of autophagosomes.

20 years. During the past decade, the one major change in management of BC was the introduction of neo adjuvant platinum-based chemotherapy to those patients suffering from non-metastatic MIBC. However, the overall mortality of BC has not changed. Therefore, development of novel therapeutic strategies or improvement of response rate to current therapies are critical to treat BC.

Autophagy and cancer

In 2016, the Nobel Prize in Physiology or Medicine was award to Prof. Yishinori Ohsumi for his discoveries of mechanisms of autophagy (4). Macroautophagy (autophagy, which means "self-eating") is a highly conserved catabolic process that degrades cellular organelles and protein to maintain the cellular biosynthesis during nutrient deprivation or metabolic stress (5). The process of autophagy includes six steps: induction, initiation, elongation, maturation, fusion and degradation (*Figure 1*). These steps involved the formation of double-membranes that extent and engulf cytoplasmic constituents including organelles and protein to from vesicles (also known as autophagosomes) that subsequently fuse with lysosomes (as autophagolysosomes or autolysosomes), where the contents undergo degradation by the enzymes within the lysosome and recycling (5). Sustained, constitutive or basal autophagic activity is also important for all cells to remove damaged organelles as well as long-lived or misfolded proteins. Although autophagy was first observed as a protective mechanism in cells under starvation or metabolic pressures such as nutrition deprivation, hypoxia, ER stress, and chemotherapeutic agents (6), it has been demonstrated

to play important roles during development and in numerous diseases, including infections, neurodegenerative and cardiovascular disease (7). Autophagy defects are known to associated with metabolic stress, genomic damage, and tumorigenesis in preclinical models, indicating its role in tumor suppression (8). Studies also showed that in 40–70% of human breast, prostate and ovarian cancers contain monoallelic loss of beclin-1, an essential autophagy gene, indicating that autophagy may be important in preventing these tumors. On the other hand, autophagic activity is related to the ability for the clearance of damaged organelles and misfolded protein, it also confers stress tolerance of tumor cells that usually under high metabolic rate associated with rapid cell proliferation to survive under adverse conditions. For example, autophagy is induced within hypoxic tumor regions of tumor cells (9). Stress induced autophagy in tumor cells could therefore resulted in tumor dormancy, drug resistant, and subsequently leading to tumor regrowth and procession. Accumulating studies had demonstrated that cancer cells induced autophagy to counteract with anticancer treatments by helping cells to evade apoptotic pathway (10). Therefore, many studies demonstrated that coordinated administration of autophagy inhibitors with chemotherapeutic agents suppressed tumor growth and resulted in a greater extent of cell death compared to chemotherapy alone (11). In the cellular or animal models, inhibition of pro-survival autophagy using pharmacological or genetic inhibitors was demonstrated to enhance apoptotic cell death and increased anti-cancer efficacy (12-16). These results reveal that targeting the prosurvival (or protective) autophagy in tumor cells may represent a novel strategy to develop successful cancer treatment. However, autophagy may also play dual roles (or referred as double-edged sword) in certain cellular contexts. It has been demonstrated that autophagic cell death was occurred in tumor cells, particularly in apoptosis-defective cells, that exhibit excessive or sustained autophagy (17). Thus, it is critical to understand the role of autophagy in cancer treatment as well as the elucidating the mechanisms involved in autophagy induction that influences tumorigenesis and treatment response. Analyzing the signaling pathway involved in autophagy induction may provide and identify new therapeutic targets for developing novel treatments that improve the outcomes.

The molecular mechanism of autophagy regulation involves mammalian target of rapamycin (mTOR), 5'-AMP-activated protein kinase (AMPK), and the extracellular-signal-regulated kinase (ERK) (18). mTOR kinase that governing the expression of cellular protein is the major regulator to inhibit autophagy in the presence of growth factors or nutrition-rich conditions, while AMPK control autophagy in response to low energy or nutrient deprivation (19). Autophagy achieved by multiple processes and many autophagy-related genes that either participates in *de novo* membrane formation, autophagosomes formation, and fusion of lysosomes to autophagosomes for degradation or reuse of engulfed macromolecules (20). In our previous studies, we demonstrated that inhibition of autophagy is critical to improve the efficacy of RAD001, a mTOR inhibitor, in treating human BC cells (16). We also found that human bladder tumor exhibits high basal level of autophagy (15). It is possible that the high level of autophagy limits the effects of cisplatin treatment in BC cells, therefore, combination of chemotherapeutic regiment are required for treating BC. Recent studies indicated that cisplatin treatment induces protective autophagy in esophageal, lung and ovarian cancer cells (21-23). Hence, understanding the role of autophagy in BC progression and drug-resistant is important for designing novel strategies to improve current therapies.

The role of autophagy in BC progression

As mentioned above, most of the patients (~80%) present NMIBC at their first diagnosis. NMIBC has a high recurrence rate, and despite all the medical interventions, approximately 10-30 % patients progress to MIBC (24). However, the exact mechanism of how NMIBC progression to MIBC is not fully understand. Several recent studies have shown the role of autophagy in BC progression. In 2013, Sivridis and his colleagues investigated the autophagy activity in BC tissues from 210 TUR specimens by detection of the expression pattern of microtubule-associated protein LC3A by immunohistochemical staining for its relevance with muscle invasion (25). They reported the detection of a grade-dependent increased number of “Stone like” structures (SLS) within cytoplasmic vacuoles under light microscopy. In this study, LC3A reactivity and the number of SLS were higher in high grade MIBC then in high grade NMIBC, suggesting that autophagy activity may also contribute to the progression of NMIBC to MIBC.

In 2013, Baspinar and his colleagues found a significant inverse correlation between the expression of beclin-1, an important mediator protein during autophagy initiation, and pT stages of BC using 84 tumor samples and 10 non-tumoral bladder tissues (26). They also identified that

the expression level of Bcl-2, an antiapoptotic protein, correlated with histological grade. They concluded that Bcl-2 overexpression and down-regulation of beclin-1 play an important role in the progression and aggressiveness of BC. Like the proapoptotic proteins, beclin-1 contains a BH3 domain which is necessary and sufficient for binding to antiapoptotic Bcl-2 homologs (27). Therefore, interaction between beclin-1 and Bcl-2 is suggested to be a key switch mediating the crosstalk between the autophagy and apoptosis. According to the data from Baspinar *et al.*, BC progression is correlated to the antiapoptotic properties by enhancing Bcl-2 expression but suppressing beclin-1. Another report by Liu *et al.* support this finding that beclin-1 is down-regulated in both mRNA and protein level during the progression of BC (28). However, whether down-regulation of beclin-1 indicating the inhibition of autophagy during the progression of BC is still inconclusive. More recent work by Wang *et al.* demonstrated the knockout and knockdown of retinoblastoma (Rb), a well-known tumor suppressor, resulted in autophagy and apoptosis inhibition via suppressing p53 and caspase-3 signaling, enhancing BC development *in vitro* and *in vivo* (29), supporting the finding that suppression of autophagy and apoptosis may be critical in BC progression.

In 2014, a group in India first published a paper demonstrating an increased grade-dependent autophagy in BC (30). They collected 15 high-grade and 15 low-grade tumor tissue samples, according to WHO criteria, from patients undergoing TUR-BT of NMIBC. Using transmission electron microscopy, they found a significant increase of autophagic vesicles in the high-grade specimens than in the low-grade specimens when compared with the benign tissues which obtained from patients undergoing TUR of prostate for benign prostatic hyperplasia. The expression levels of LC3-II protein, usually used as a marker for autophagic induction, and ATG7 and Beclin-1, key proteins involved in autophagosomes biogenesis, were increased in a grade-dependent manner in high- and low-grade BC tissues. To determine how cells from different grades of BC would respond to a common stress such as starvation, the primary tumor cells were grown under starvation for 0–48 h. The results showed that both high-grade and low-grade BC cells were more susceptible to starvation induced autophagy compared to normal urothelial cells. Furthermore, they identified that activation of AMPK signaling and inhibition of mTOR were involved in the induced autophagy under starvation in these BC cells. In addition, inhibition of starvation-induced autophagy using autophagy inhibitors, such as wortmannin, 3-methyladenine (3-MA), and chloroquine (CQ), increased cancer cell death also in a grade-dependent manner by triggering intrinsic apoptotic pathway.

In 2016, we demonstrated that BC exhibits high basal level of autophagy by showing the increased LC3B expression levels in BC tissues from NMIBC patients compared with their paired adjacent normal tissues (15). Using well established BC cell lines, the basal autophagy activity that detected by the processing of LC3-II and LC3-positive puncta within the cytoplasm, was elevated in a grade-dependent manner in BC cells when compared to immortalized normal urothelial cells, or other reference cell lines including breast, prostate, and kidney cancer cells. Inhibition of basal autophagy using bafilomycin A1 (Baf A1, a specific inhibitor of vacuolar-type H(+)-ATPase which blocks the fusion of autophagosomes to lysosomes) or small hairpin RNA (shRNA) targeting ATG-7 decreased cancer cell viability through apoptosis induction. Our data demonstrated that BC tissues exhibits high basal level of autophagy that leads to two major conclusions: First, targeting basal autophagy may represent a novel therapeutic approach to treat BC. Second, the high basal autophagy is likely contributing to the drug resistant in BC against current chemotherapeutic agents. Since the growth of BC cells is depend on the highly activated basal autophagy, we therefore tried to treat the cancer cells using autophagy inhibitors alone and see if inhibition of basal autophagy decreased the cell viability efficiently. To our surprise, single treatment of CQ or HCQ significantly decreased cell viability via enhanced apoptosis in BC cells (31). The use of autophagy inhibitors exhibits severe cytotoxicity compared to standard chemotherapeutic agents such as Everolimus (RAD001) or cisplatin (16). To further explore the mechanism related to these autophagy inhibitors against BC cell, we investigated the apoptosis induction pathways in CQ-treated BC cells. The results demonstrated that CQ not only disrupted the autophagic flux by inhibiting the fusion of autophagosomes to lysosomes, but it targeted lysosomal functions to amplify apoptotic signals and ultimately leading to cancer cell death (*Figure 2*) (31). The metabolic activities of tumor cells function primarily to support the unusually high rates of cell growth and proliferation. Therefore, it is possible that high basal level of autophagy and lysosomal function are critical to support the growth and survival of BC cells. Targeting autophagy or lysosomal function may present a novel therapeutic approach for treating BC.

We also developed another potential therapeutic

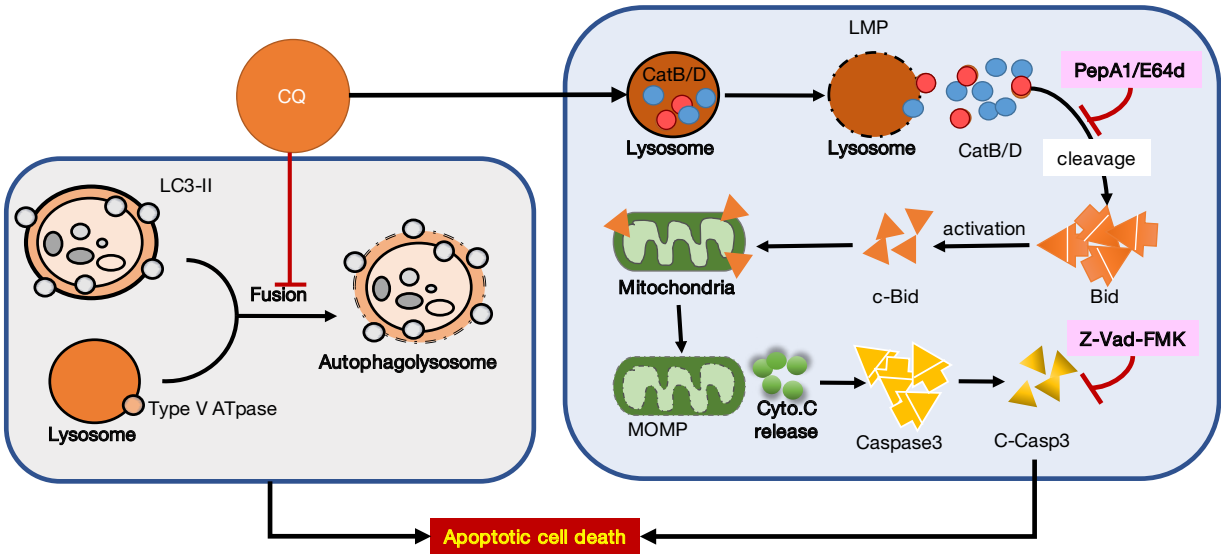

Figure 2 Chloroquine (CQ) single treatment leads to apoptotic cell death in human bladder cancer cells by not only disrupting autophagy but inducing lysosomal membrane permeabilization (LMP). In CQ-treated bladder cancer cells, the inhibition of autophagy was detected by the increased level of LC3-II protein processing and the accumulation of LC3-positive puncta; both are evidences of autophagic flux disruption. Furthermore, our data suggested that CQ as a lysosomotropic reagent is able to induce LMP which leading to mitochondria outer membrane permeabilization (MOMP) and ultimately causing caspase-dependent apoptosis in human bladder cancer cells. In addition, since human bladder cancer cells exhibits high basal level of autophagy with increased numbers of acidified vesicles, the CQ induces cytotoxicity was found to be more profound in cancer cells compared to normal urothelial cells.

approach by taking advantages of high basal autophagy in the BC cells. Acridine orange (AO) vital staining is a conventional approach to detect acidic vesicles upon induction of autophagy. AO shows green fluorescence when bound to DNA and red when bound to acidic regions which includes lysosomes and autophagolysosomes. Increased number of AO-positive red puncta could serve as an autophagy indicator when studying autophagy induction *in vitro*. However, we constantly failed to detect autophagy induction using AO staining in BC cells, even when the detection of LC3-II progression suggests that autophagy was induced. By a closing look at the fluorescent pattern in AO-treated BC cells, we found a phenomenon which is called photooxidative damage that targeting lysosomes to enhance cytotoxicity effect of AO against BC cells. We discovered that AO treatment significantly reduced cell viability of BC cells under blue light exposure while had small impact on immortalized urothelial cells; because cancer cells contain increased number of acidified organelles including autophagolysosomes and lysosomes (32). This type of treatment was designated as AO photodynamic treatment (AO-PDT), and has the potential in further clinical application since current endoscopic light technology that urologist commonly utilized in TUR-BT could be equipped with blue light source. Strategies targeting autophagy to improve BC treatment in our research are illustrated in *Figure 3*. Our recent studies regarding the role of autophagy in urological cancers, including prostate and BC, are listed in *Table 1*.

As described above, the role of autophagy during BC progression is still controversial. However, based on these studies, autophagy becomes an important therapeutic target for it clearly plays a role in BC progression. Inhibition of autophagy activated during BC progression is likely to improve the responses to current therapies.

Coordinate autophagy inhibition with current approved therapies in BC treatment

Intravesical instillation

Standard treatment for NMIBC consists of complete TURBT of all visible lesions. Intravesical therapies followed by TURBT seems to reduce the recurrence rate to 25–50% in 2 years of follow-up, and is the current armamentarium

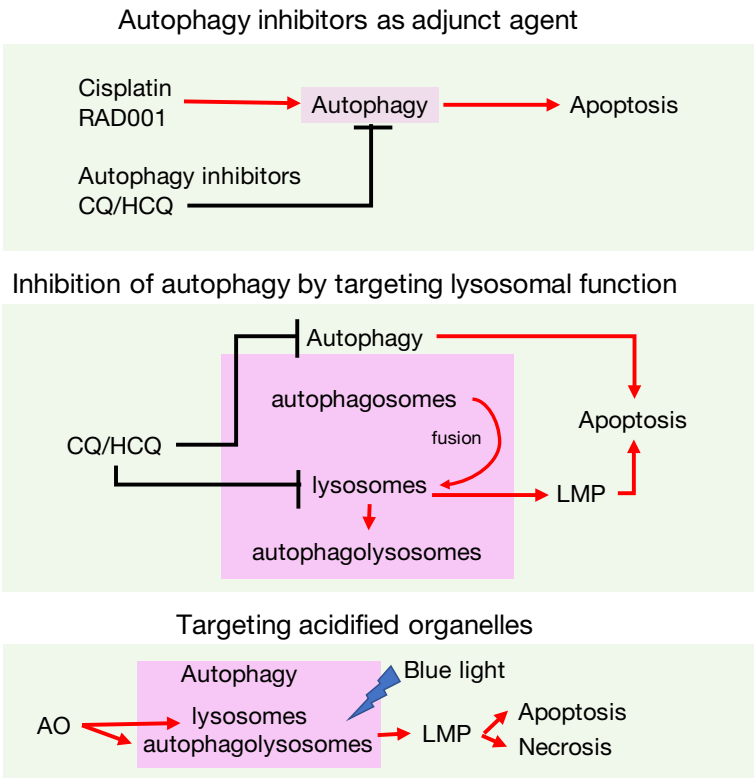

Figure 3 Novel strategies targeting autophagy to treat bladder cancer. Autophagy inhibitors as adjunct agent: bladder cancer cells induce autophagy as a protective mechanism upon cisplatin treatment. Coordinate inhibition of autophagy has been demonstrated to enhance apoptotic cell death. Although the comparison of effectiveness of cisplatin plus autophagy inhibitor as adjunct agent with the current treatment regimens [gemcitabine plus cisplatin (GC) or methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC)] need further investigation, it may provide a novel approach to reduce side effects. Inhibition of autophagy by targeting lysosomal function: our data demonstrated that CQ targeting lysosomes not only disrupts autophagy but also enhances apoptosis. Because bladder cancer exhibits high basal level of autophagy with increased numbers of acidified vesicles, agents such as CQ or HCQ that target lysosomal function may selectively induce cytotoxicity to cancer cells but not the normal urothelial cells. Targeting acidified organelles: acridine orange (AO) is a vital staining dye that commonly used in the detection of autophagy induction. Our data suggested that blue-light irradiation caused photodamages to AO-stained bladder cancer cells via disruption of acidified organelles. This photodynamic treatment leads to rapid necrosis and apoptosis. Agents with similar characteristics as AO may have potential to develop as novel therapeutic drug to treat bladder cancer. With the development of narrow band imaging technology, it is possible to introduce fluorescent light source when performing the transurethral resection of bladder tumors (TURBT). Installation of AO during TURBT may be an option to reduce recurrence rate of bladder cancer.

for the management of NMIBC. Current intravesical instillation are classified as intravesical chemotherapy and immunotherapy, using agents such as mitomycin C (MM) and the bacillus Calmette-Guerin (BCG), respectively.

MM inhibits DNA synthesis is a chemotherapeutic agent that decreases the recurrence rate of NMIBC from 54% to 38% with no impact on the risk of progression (35). It has been demonstrated that other intravesical chemotherapeutic agents including doxorubicin, epirubicin and thiotepa are utilized for intravesical therapy and found no superiority of one drug over the others (36). Cancer stem cells (CSC) has been suggested to help tumor progression, and in some cases evade chemotherapeutics (37). Ojha's group reported the isolation of side population (SP) cells which share characteristics of CSCs from BC cell lines (38). These SP cells showed substantial resistance to MM treatment, and exhibited higher autophagic flux. Inhibition of autophagy by CQ or siRNA against beclin-1

Table 1 Recent studies from our group regarding the role of autophagy in prostate and bladder cancers

Cancer type	Role of autophagy	Title	References
Prostate	Autophagic cell death	Zoledronic acid induces autophagic cell death in human prostate cancer cells	(33)
Prostate	Protective autophagy	Benzyl isothiocyanate induces protective autophagy in human prostate cancer cells via inhibition of mTOR signaling	(34)
Bladder	Basal autophagy	Inhibition of High Basal Level of Autophagy Induces Apoptosis in Human Bladder Cancer Cells	(15)
Bladder	Protective autophagy	Chloroquine and hydroxychloroquine inhibit bladder cancer cell growth by targeting basal autophagy and enhancing apoptosis	(31)
Bladder	Basal autophagy	Acridine orange exhibits photodamage in human bladder cancer cells under blue light exposure	(32)

potentiated the chemotherapeutic effects of MM in these SP cells. In a followed-up study, they showed that MM treatment increased the percentage of CSCs in primary cultured urothelial carcinoma cells (39). These CSCs exhibits high level of autophagy. Inhibition of autophagy by CQ decreased the expression of drug resistance genes (*MDR1* and *ABCG2*) and enhanced MM induced apoptosis. Therefore, synergistic cytotoxicity effect of MM with autophagy inhibitor may help to improve the outcome in NMIBC patients.

BCG, a live attenuated strain of *Mycobacterium bovis*, is one of the germ that causes tuberculosis (TB) (40). It has been used to treat NMIBC for more than three decades and is currently the only agent approved by the US Food and Drug Administration for the therapy of NMIBC. Despite the long term clinical experiences with BCG, the exact mechanism of its therapeutic effect against BC is still largely unknown. It is suggested that BCG triggers an immune response that immune cells such as macrophages and lymphocytes move into the tissues, as a part of the inflammatory reaction, when the bacteria attach and absorb to the tumor cells. Study showed that BCG is markedly superior to MM in high-risk than in low risk patients (41), making it a standard of care in high-risk NMIBC to prevent recurrence and progression (42). The induction of autophagy has been shown to be a defense mechanism inhibiting BCG survival in macrophages (43), thus modulating the immune response to BCG treatment. Recently, Buffen *et al.* reported that inhibition of autophagy blocked trained immunity induced by BCG (44). They also found that polymorphisms (SNPs) in the autophagy gene *ATG2B* and *ATG5* negatively influence trained immunity in monocytes. These studies demonstrate the role of autophagy in successful BCG treatment, and point out future directions for improving BCG therapy.

Radical cystectomy is the standard of care following BCG failure (45). As an alternative therapy, valrubicin is the only agent approved in the US for BCG refractory (46). Other intravesical agents, while not recommended for primary treatment, have been studied for the treatment of NMIBC. The guidelines do allow for the consideration of alternative treatment of NMIBC in the setting of BCG failure. It has been demonstrated that salvage intravesical therapy using interferon in combination with BCG is effective (47). Zhang *et al.* reported that adenoviral-mediated interferon alpha treatment induces autophagy in BC, and inhibition of induced autophagy using 3-MA increases cytotoxicity (48). In other studies, promising results were reported using intravesical gemcitabine alone or in combination with MM in patients with BCG failure (49). The study by Ojha *et al.* using CQ as an autophagy inhibitor demonstrated similar effects as MM on a gemcitabine treated cell lines that reduces the expression of drug resistance genes (39). Addition of CQ also sensitized the CSCs to gemcitabine induced apoptosis, providing evidence that autophagy inhibition could be synergistic to the combination of gemcitabine/MM for BCG failure patients. A recent study by Amantini *et al.* demonstrated that capsaicin (CPS) triggers autophagy which drives epithelial mesenchymal transition (EMT) and chemoresistance in BC cells (50). CPS is the active alkaloid found primarily in the chili peppers and used as an intravesical drug for overactive bladder. They showed that the CPS-resistant EMP-positive BC cells displayed an increased drug-resistance to MM, gemcitabine, and doxorubicine which commonly used in BC therapy. Another study was conducted by Pan *et al.* (51) to investigate the effect and mechanisms of icaritin, a hydrolytic form of icariin which is one of the traditional

Chinese herbals, against human BC cells. Although the exact mechanism is still unclear, they found that icaritin not only suppressed the basal autophagy, but inhibited epirubicin induced autophagy. Therefore, icaritin act synergistically with epirubicin to suppress the proliferation of BC cells. Induction of autophagy had been reported using another intravesical chemotherapy agent, pirarubicin, for BC (52). In this study, Li *et al.* showed that pirarubicin-induced autophagy was mediated via mTOR signaling pathway; and inhibition of induced autophagy using siRNA against ATG3, 3-MA, or hydroxychloroquine (HCQ) significantly induced apoptosis. These studies further supported the idea that autophagy activities in BC is related to its progression and drug resistance.

Chemotherapy

Approximately 30% of newly diagnosed BC patients present with MIBC, and as much as 30% of patients with NMIBC eventually progress to muscle-invasion. Surgery, specifically radical cystectomy is the standard procedure in treating MIBC (53). A major change in treatment of MIBC is the development of platinum-based chemotherapies which are a standard of care (54,55). The two regimens of neoadjuvant chemotherapy are either methotrexate, vinblastine, doxorubicin and cisplatin (MVAC) or gemcitabine and cisplatin (GC). As mentioned above, autophagy is considered as a survival mechanism in cancer cells to evade apoptosis against chemotherapeutic agents. Therefore, a significant amount of studies focus on the potential relationship of autophagy induced by these drugs. CQ, the antimalarial drug and a well-known autophagy inhibitor, enhanced cisplatin mediated cytotoxicity in BC cells without affecting normal urothelial cells (30). Administration of 3-MA, a class III phosphoinositide 3-kinase (PI3K) inhibitor, known to inhibit early stage of autophagy processing enhanced the cytotoxicity of (–)-gossypol, a pan Bcl-2 inhibitor, in cisplatin-resistant BC cell lines (56). Everolimus (RAD001), a specific inhibitor of mTORC1 complex, is used for the treatment of metastatic renal cell carcinoma, but is not effective in the treatment of BC (57). Inhibition of mTOR is known to induce autophagy in cells under starvation (58). Therefore, it is possible that chemotherapeutic agents targeting mTOR induce autophagy which promotes tumor survival, and thus these agents potentially limit their own efficacy. As a prove of principle study, we demonstrated that inhibition of RAD001-induced autophagy using inhibitors, including 3-MA, Baf A1, CQ, or HCQ, significantly decreased the cell viability by enhancing apoptosis of BC cell lines (16). In consist with our findings (59), Fan *et al.* demonstrated that cisplatin treatment induced significant autophagy in BC T24 cell line (60). They reported that cisplatin induces autophagy through inhibition of mTOR pathway, and targeting *ATG8* (LC3) significantly reduced the cell viability in cisplatin-treated cells. Recently, Ojha *et al.* took this one step further (61). They established cisplatin resistant patient derived primary culture cells and treated these cells with gemcitabine and MM. The results showed that resistant cells have higher basal autophagic flux. Gemcitabine and MM further induced autophagy in cisplatin-resistant cells; and combination of autophagy inhibitors (CQ or knockdown of beclin-1) synergistically inhibited BC cell growth. They also reported that INF-γ mediated JAK2 pathway is responsible for the autophagy-related drug resistant in BC.

MicroRNAs (miRNAs) are small noncoding RNAs (~20–24 nucleotides) that regulates their targeted gene expression through translational blockage or mRNA degradation (62). To date, there are 2,588 matured human miRNAs registered in the miRNA database (miRbase, release 21; http://www.mirbase.org). Increasing studies have shown that miRNAs play important roles that regulate tumor formation and progression (63). The important role of dysregulated miRNAs in tumorogenesis has emerged as a new field in cancer research. In BC, profiling of miRNA expression patterns by large scale microarray approaches started at 2007 (64). After the first publication, 7 studies were published in different countries (65-71). And only a few number of validated miRNA target genes were found. Using a microarray approach, we also showed that 11 and 19 miRNAs were up-regulated and down-regulated, respectively, in human BC tissues (72). Among these dysregulated miRNAs, the miR-99 family which are down-regulated was found to target the mTOR signaling pathway in other cancers (73-76). In addition, miR-30a which also found to be down-regulated in BC, was demonstrated to mediated autophagy inhibition that sensitizes renal cell carcinoma cells to sorafenib (77). Although further studies are needed, these miRNAs may involve in the regulation of autophagy in BC. On the other hand, miR-222 that up-regulated in BC was found to be associated with a poor prognosis in BC (78). Zeng *et al.* demonstrated that overexpression of miR-222 activated the Akt/mTOR pathway and inhibited cisplatin-induced autophagy by directly targeting protein phosphatase 2A subunit B (PPP2R2A) (79). Therefore, miR-222 may present a viable

future target for attenuating cisplatin-resistance. More recently, the up-regulation of miR-24-3p was reported in BC (80). In this study, miR-24-3p was found to induce autophagy by suppressing the expression of DEDD, a member of the death effector domain-containing protein family. However, the role of miR-24-3p induced autophagy and its association with drug-resistance remained further investigation.

These studies involving the administration of autophagy inhibitors, including pharmaceutical drugs, such as CQ and HCQ, or molecular inhibitors, such as siRNAs/miRNAs targeting autophagic proteins, demonstrate the further research targeting autophagy process can not only improve the response to current therapies but provide new strategies and targets for future development of novel therapies against BC.

Conclusions

As a major protective mechanism, autophagy is now recognized to play a key role in the stress response against various conditions such as nutrient starvation, hypoxia, infection, and tumor development. There are several on-going clinical trials using autophagy-modulating compounds alone or combined with conventional anti-cancer drugs to treat various types of cancer, including prostate, renal, lung, breast, pancreatic and kidney, but not BC (81). Furthermore, there is no breakthrough to the management of BC, despite its high morbidity in the past decades. The involvement of autophagy in the progression and in treatment resistance in BC is gaining more attraction. Targeting autophagy may help to increase the current therapies and develop novel therapeutic strategies against BC.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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