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# Dual Roles of Autophagy and Their Potential Drugs for Improving Cancer Therapeutics

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## Abstract

Autophagy is a major catabolic process that maintains cell metabolism by degrading damaged organelles and other dysfunctional proteins via the lysosome. Abnormal regulation of this process has been known to be involved in the progression of pathophysiological diseases, such as cancer and neurodegenerative disorders. Although the mechanisms for the regulation of autophagic pathways are relatively well known, the precise regulation of this pathway in the treatment of cancer remains largely unknown. It is still complicated whether the regulation of autophagy is beneficial in improving cancer. Many studies have demonstrated that autophagy plays a dual role in cancer by suppressing the growth of tumors or the progression of cancer development, which seems to be dependent on unknown characteristics of various cancer types. This review summarizes the key targets involved in autophagy and malignant transformation. In addition, the opposing tumor-suppressive and oncogenic roles of autophagy in cancer, as well as potential clinical therapeutics utilizing either regulators of autophagy or combinatorial therapeutics with anti-cancer drugs have been discussed.

**Key Words:** Autophagy, Cancer, Target, Anti-tumor drug, Combinational therapy

## INTRODUCTION

Cancer is characterized by excessive cell growth and malignancy due to the accumulation of genetic defects or abnormal metabolic processes (Sell *et al*., 2016; Dang and Kim, 2018). Upregulated catabolism in cancer cells promotes tumor growth and metastasis (Danhier *et al*., 2017). Moreover, the rapid proliferation of cancer cells is dependent on the recycling of cellular components (Danhier *et al*., 2017; Momcilovic and Shackelford, 2018). Recent studies have demonstrated that the metabolic processes of tumor cells are correlated with autophagy (Su *et al*., 2015; Mowers *et al*., 2017). Autophagy is a mechanism by which abnormal proteins or damaged cellular organelles undergo lysosomal degradation, which provides energy and macromolecular precursors (Yorimitsu and Klionsky, 2005; Mizushima, 2007). Autophagy is involved in the initiation and development of cancer by preventing toxic accumulation of carcinogenic factors (Guo and White, 2016).

Cancer cells meet their high metabolic energy demand by utilizing degraded biomolecules derived from autophagy (Galati *et al*., 2019). Compared to normal tissues, cancerous tissues are highly dependent on autophagy. Thus, targeting autophagy may be a potential therapeutic strategy against cancer (White *et al*., 2015). The role of autophagy in malignant transformation is complex and varies depending on the cancer cell type (Galluzzi *et al*., 2015). Further elucidation of the interaction between autophagy regulation and the key molecules involved in each cancer type is crucial for the identification of an effective therapeutic.

Autophagy-related genes function as either tumor suppressors or oncogenes. Whereas suppression of autophagy inhibits the growth of cancer cells, the induction of autophagy has also been reported to decrease tumor growth (Kimmelman and White, 2017). Many studies suggest that autophagy may function as a tumor suppressor in the early stages of cancer progression, but exert pro-tumor effects in later stages of cancer (Kimmelman and White, 2017; Kocaturk *et al*., 2019).

This review summarizes recent findings on the molecular regulation of autophagy in cancer. In addition, the therapeutic potential of various autophagy inducers, and inhibitors alone and in combination with known anti-cancer drugs is discussed.

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## MOLECULAR MECHANISM OF AUTOPHAGY

Autophagy is classified into the following three types: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). Macroautophagy (called autophagy) is a highly evolutionarily conserved pathway for the degradation of dysfunctional proteins (Yorimitsu and Klionsky, 2005; Kuma *et al*., 2017; Kaushik and Cuervo, 2018). This process is accompanied by evolutionarily well-conserved autophagy-related (Atg) proteins and the formation of a vesicle structure known as an autophagosome. The contents of autophagosomes are degraded by lysosomal hydrolase upon fusion with the lysosome (Yorimitsu and Klionsky, 2005; Kuma *et al*., 2017; Kaushik and Cuervo, 2018). Microautophagy involves direct uptake of cytosolic components by lysosomes (Li and Hochstrasser, 2020). CMA is a complex proteolytic system involving lysosomal membrane-associated protein 2 A and heat-shock protein 70 (Kaushik and Cuervo, 2018).

Nutrient signaling modulates the formation of autophagosomes via the mammalian target of rapamycin (mTOR) and adenine monophosphate-activated protein kinase (AMPK) (Tamargo-Gomez and Marino, 2018). Under physiological conditions, mTORC1 inhibits autophagy by phosphorylating unc-51-like kinase (ULK1), which initiates autophagosome biogenesis. In contrast, mTORC1 is inhibited during starvation (Yorimitsu and Klionsky, 2005; Russell *et al*., 2014). The ULK1 complex, which is activated by autophosphorylation, phosphorylates FIP200, ATG13, ATG101, and other ATG proteins (Park *et al*., 2016). Nutrient deprivation or starvation causes the depletion of ATP and leads to increased ADP and AMP levels, which activates AMPK (Tamargo-Gomez and Marino, 2018; Li and Chen, 2019). Further, AMPK contributes to the restoration of cellular energy levels through autophagy by inhibiting mTORC1 and phosphorylating ULK1 (Li and Chen, 2019).

The ULK1 complex triggers autophagosome nucleation by activating a multi-domain complex comprising Beclin 1, ATG14, and VPS34 (Russell *et al*., 2013). Autophagosomes are modulated by the ATG5-ATG12-ATG16L1 complex, which facilitates the conjugation of LC3-I to phosphatidylethanolamine to form LC3-II (Russell *et al*., 2014). Meanwhile, misfolded proteins or damaged organelles are marked for degradation through ubiquitination. Polyubiquitinated target proteins destined for degradation are recognized by sequestosome-1 (SQSTM1/p62) (Moscat and Diaz-Meco, 2009; Kuma *et al*., 2017; Zaffagnini *et al*., 2018). LC3-II recruits the ubiquitinated p62-cargo complex, which is engulfed by autophagosomes (Zaffagnini *et al*., 2018). ATG4B mediates the recycling of the LC3-II protein to LC3-I (Zaffagnini *et al*., 2018). The fully mature autophagosomes fuse with acidic lysosomes, which results in autolysosome formation. Lysosomal hydrolases degrade the target molecules or organelles, which are then used as metabolite substrates or precursors for macromolecule biosynthesis (Fig. 1) (Mizushima, 2007; Russell *et al*., 2014).

## CORRELATION BETWEEN AUTOPHAGY AND TUMOR FORMATION

Autophagy contributes to the inhibition of tumorigenesis at

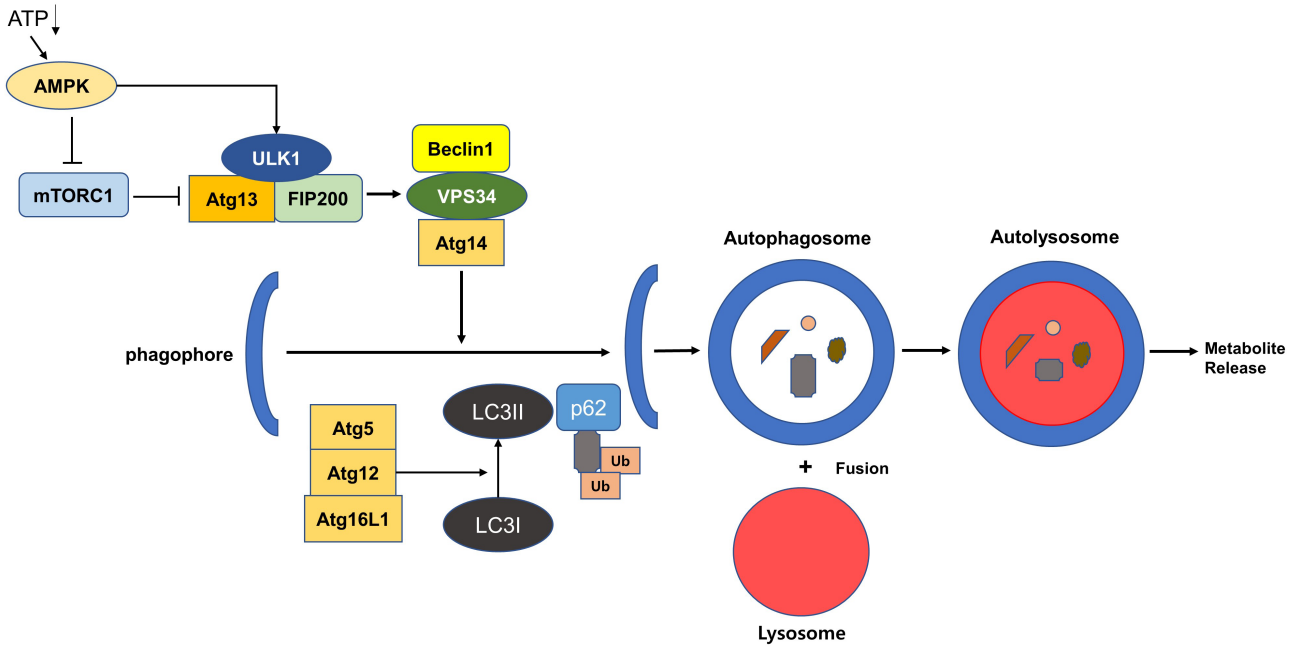


**Fig. 1.** Overview of Autophagy Process. The depletion of ATP activates AMPK under fasting conditions. The AMPK activates autophagy by suppressing the activity of mTORC1 and by phosphorylating the ULK1. The ULK1 complex initiates the formation of autophagosome by stimulating Beclin 1/ ATG14/VPS34 complex. The Atg5–Atg12-Atg16L1 complex, which regulates the autophagosomes, converts LC3-I into LC3-II. Abnormal organelles or proteins after their ubiquitination (Ub) is recognized by p62. LC3-II interacts with the ubiquitinated p62-cargo complex and is engulfed into the autophagosomes. The autophagosomes fuse with lysosome and leads to the formation of autolysosome. The target molecules or organelles are finally degraded by lysosomal proteases and are utilized as metabolite substrates.

multiple stages (Galluzzi *et al*., 2015; Amaravadi *et al*., 2016). ATGs in the autophagy pathway are reported to suppress tumor formation. Deletion of ATG4C has been shown to promote tumorigenesis in mice (Marino *et al*., 2007). Similarly, ATG5 deficiency induces benign tumor formation in mouse livers (Takamura *et al*., 2011).

ULK1 (human ATG1) stimulates autophagic flux, which results in the inhibition of cell proliferation during starvation (Jung *et al*., 2011). In addition, ULK2 is markedly downregulated in glioma. These findings indicate that autophagy inhibition through ULK1/2 downregulation promotes tumor progression (Shukla *et al*., 2014).

Beclin-1, which is involved in autophagy, functions as a tumor suppressor, and its expression is inhibited in various human cancers, such as prostate and colon cancers (Chen and Karantza-Wadsworth, 2009). Monoallelic Beclin-1 gene deletions result in malignancies in humans (Qu *et al*., 2003). Further, Beclin-1 overexpression markedly suppresses proliferation and induces apoptosis in human laryngeal squamous carcinoma cells (Wan *et al*., 2018). Beclin-1-mediated autophagy suppresses cell death and promotes survival under stress. The activity of Beclin-1 is repressed in the presence of the anti-apoptotic proteins BCL-2 and BCL-XL. Moreover, BCL-2, which is upregulated in several cancers (Huang, 2000), negatively regulates Beclin-1-mediated autophagy (Ramakrishnan *et al*., 2007). The interaction between BCL-2 and Beclin-1 promotes autophagy or apoptosis in various cancer cells (Marquez and Xu, 2012). Caspase or calpain, which plays an important role in apoptosis, inhibits Beclin-1-mediated autophagy (Kang *et al*., 2011). p53 levels also regulate Beclin-1 in the autophagic flux. Specifically, overexpression of p53 results in the degradation of Beclin-1 and leads to the suppression of autophagy in embryonal carcinoma (Tripathi *et al*., 2014). Therefore, proteins interacting with Beclin-1 are essential for determining whether cells undergo autophagy or apoptosis.

p62 (or sequestosome-1, SQSTM1) is a signaling scaffold that is upregulated in human cancers (Liu *et al*., 2012; Umemura *et al*., 2016). Autophagy suppression results in p62 accumulation, which promotes oncogenesis by increasing DNA damage and endoplasmic reticulum stress (Moscat and Diaz-Meco, 2009). Benign tumors in ATG5 or ATG7 knockout mice have been shown to exhibit high levels of p62. Loss of p62 in these mice inhibits the growth of tumors (Takamura *et al*., 2011; Liu *et al*., 2012). Diethylnitrosamine, a potent liver carcinogen, stimulates the tumorigenic activity of p62 and aggravates hepatocellular carcinoma (Umemura *et al*., 2016). Therefore, p62 inhibition during autophagy is a potential therapeutic strategy against cancer. These data suggest that autophagy-related gene regulation plays a key role in determining cancer progression.

## AUTOPHAGY INDUCERS IN CANCER THERAPY

Activation of autophagy may be a direct strategy to promote tumor cell death (Table 1). Some tumor cells exhibit resistance to apoptosis. Thus, autophagy may provide an alternative cell death mechanism for cancer cells with defective apoptosis (Tsujimoto and Shimizu, 2005).

As autophagy is negatively regulated by mTOR, some autophagy-inducing drugs target the mTOR pathway. Rapamycin and its derivative, everolimus, inhibit mTORC1, which promotes the dissociation of the ULK1 complex (Vignot *et al*., 2005; Cicchini *et al*., 2015). Rapamycin has been reported to suppress the proliferation of MCF-7 breast cancer (Chang *et al*., 2007), B16 melanoma (Busca *et al*., 1996), and PANC-1 pancreatic carcinoma cell lines (Grewe *et al*., 1999). Similarly, everolimus inhibits the growth of various malignancies (Hare and Harvey, 2017; Bhat *et al*., 2018; Kocaturk *et al*., 2019). For example, it suppresses human vascular endothelial cell growth by delaying the cell cycle in lymphoblastic B cells (Neri *et al*., 2014). Everolimus, although not as effective as rapamycin, exerts similar anti-tumor effects *in vivo*. Further, temsirolimus (CCI-779), a rapamycin analog, activates autophagy by inhibiting mTOR in adenoid cystic carcinoma (Liu *et al*., 2014).

**Table 1.** Autophagy inducers for improving cancer

| Compound | Target or mode of action | Cancer types | References |
|---|---|---|---|
| Rapamycin | mTOR | MCF-7 Breast Cancer, B16 melanoma, Panic-1 pancreatic carcinoma | Busca *et al.*, 1996; Grewe *et al.*, 1999; Chang *et al.*, 2007 |
| Everolimus | mTOR | Lymphoblastic B cells | Neri *et al.*, 2014 |
| Temsirolimus (CCI-779) | mTOR | Adenoid cystic carcinoma | Liu *et al.*, 2014 |
| Metformin | AMPK | Prostate cancer cells, myeloma | Sesen *et al.*, 2015; Wang *et al.*, 2018; Mishra and Dingli, 2019 |
| Perifosine | Akt | Multiple myeloma, neuroblastoma, colorectal cancer, neuroblastoma cells | Li *et al.*, 2010, 2011; Richardson *et al.*, 2012 |
| Ibrutinib | Bruton's Tyrosine Kinase (BTK) | Skin cancer cell lines, HS-4 and A431 | Sun *et al.*, 2018 |
| Suberoylanilidehydroxamic acid (SAHA) | Histone deacetylase (HDAC), mTOR | Cutaneous T cell lymphoma, glioblastoma stem cells | Gammoh *et al.*, 2012; Chiao *et al.*, 2013 |
| Magnolin | LIF/Stat-3/Mcl-1 | Colorectal cancer cells | Yu *et al.*, 2018 |
| Resveratrol | SIRT1 | Prostate cancer cells, MCF-7 cells | El-Mowafy and Alkhalaf, 2003; Li *et al.*, 2014 |
| Spermidine | AMPK | Colon cancer cells | Morselli *et al.*, 2011 |

Metformin (250 mg/kg body weight), an anti-diabetic drug, stimulates autophagy through AMPK activation and markedly inhibits prostate cancer cell proliferation in HimYC mice (Ben Sahra *et al*., 2010). Additionally, metformin is reported to inhibit the growth of glioblastoma cells (Sesen *et al*., 2015). Metformin arrests the cell cycle at the G0/G1 phase and suppresses myeloma cell proliferation by regulating AMPK and mTORC (Wang *et al*., 2018; Mishra and Dingli, 2019). Furthermore, metformin has been shown to enhance the dual effects of chemotherapy and radiation therapy (Saha *et al*., 2015; Sesen *et al*., 2015).

Perifosine induces autophagy through the suppression of Akt, which interferes with the phosphatidylinositol-3-kinase (PI3K) signaling pathway. The therapeutic effect of perifosine on multiple myeloma, neuroblastoma, and colorectal cancer has been evaluated in phase III clinical trials (Li *et al*., 2010; Richardson *et al*., 2012). Akt inhibition suppresses TrKb/neurotropic factor-induced resistance to chemotherapy, which increases the neuroblastoma cell sensitivity to chemotherapy and radiation therapy. Furthermore, perifosine promotes apoptosis in neuroblastoma cells (Li *et al*., 2010) and suppresses the proliferation of colorectal cancer cells (Li *et al*., 2011). Ibrutinib, a Bruton's tyrosine kinase inhibitor, stimulates autophagy by upregulating ATG7 and promoting LC3-II formation. Additionally, ibrutinib has been shown to exhibit growth-inhibiting activity against the skin cancer cell lines HS-4 and A431 (Sun *et al*., 2018). Histone deacetylase (HDAC) inhibitors are also reported to affect the autophagic flux (Bhat *et al*., 2018). Suberoylanilide hydroxamic acid (SAHA), an HDAC inhibitor, promotes autophagy through mTOR inhibition and enhances apoptosis in cutaneous T cell lymphoma (Gammoh *et al*., 2012). Another study reported that SAHA induces autophagy, which delays tumor growth in glioblastoma stem cells (Chiao *et al*., 2013).

In addition, some natural compounds treat cancer via activating the autophagic flux. Magnolin, a bioactive natural compound, activates autophagy and arrests the cell cycle in human colorectal cancer cells by inhibiting the LIF/Stat-3/Mcl-1 pathway (Yu *et al*., 2018). Resveratrol stimulates autophagy by activating sirtuin 1, which promotes autophagy through ATG5 and ATG7 deacetylation (Lee *et al*., 2008; Morselli *et al*., 2011). Further, resveratrol suppresses prostate cancer cell proliferation (Li *et al*., 2014) and promotes apoptosis in MCF-7 cells (El-Mowafy and Alkhalaf, 2003). Spermidine, a natural polyamine, promotes autophagy through an mTOR-independent pathway. Additionally, spermidine phosphorylates protein tyrosine kinase 2β and cyclin-dependent kinase inhibitor 1B via AMPK (Morselli *et al*., 2011) and regulates the acetylation status of ATG5 and LC3 in human colon cancer cells (Morselli *et al*., 2011). Therefore, elucidating the regulatory mechanisms underlying autophagy and cell death in cancer cells will contribute to the development of novel therapeutic strategies.

## AUTOPHAGY INHIBITORS IN CANCER THERAPY

Several autophagy inhibitors effectively inhibit cancer cell proliferation (Table 2). Some inhibitors target kinases in the phagophore or inhibit the fusion of autophagosomes and lysosomes during the autophagic flux (Yang *et al*., 2013; Yuan *et al*., 2015; Mauthe *et al*., 2018).

3-Methyladenine (3-MA) and 2-(4-morpholinyl)-8-phenylchromone (LY294002) inhibit autophagy by suppressing the activity of PI3K, which is involved in the production of phosphatidylinositol (3,4,5)-triphosphate and the nucleation and extension of the phagophore (Yang *et al*., 2013). Class I PI3Ks negatively regulate autophagy, whereas class III PI3Ks directly promote autophagy (Bilanges *et al*., 2019). LY294002 and 3-MA inactivate class III PI3Ks, which has been shown to induce caspase-induced death in HeLa cells (Hou *et al*., 2012).

Bafilomycin A1 promotes the binding of Beclin-1 to BCL-2, which inhibits autophagy (Yuan *et al*., 2015) and suppresses the invasion and migration of gastric cancer cells, as well as promoting the apoptosis of these cells (Li *et al*., 2016). Bafilomycin A1 is also a lysosomal H+-ATPase inhibitor and impairs autophagy by inhibiting the fusion of autophagosomes with lysosomes (Bhat *et al*., 2018).

Chloroquine (CQ) and hydroxychloroquine (HCQ) are clinically approved by the U.S. Food and Drug Administration as anti-malarial agents. CQ acts as an autophagy inhibitor by suppressing the fusion process of autophagosomes and lysosomes (Mauthe *et al*., 2018). CQ also promotes the production of cellular reactive oxygen species and enhances the cytotoxicity of temozolomide by suppressing mitophagy in glioma cells (Hori *et al*., 2015). In addition, CQ, in combination with different anti-cancer drugs, exhibits an anti-cancer effect in breast (Cave *et al*., 2018) and colon cancer (Liu *et al*., 2019).

HCQ enhances anti-tumor immunity and suppresses autophagy by promoting p62 accumulation and upregulating the lysosomal protease cathepsin D in metastatic colorectal cancer (Patel *et al*., 2016). Additionally, HCQ suppresses autophagy to potentiate the anti-estrogen responsiveness of breast cancer (Cook *et al*., 2014). Quinacrine, a synthetic anti-malarial drug, has been reported to suppress autophagic flux (Golden *et al*., 2015). Quinacrine also increases the expression of p21/p27 independent of p53 owing to the downregulation of the p62-Skp2 axis in ovarian cancer (Jung *et al*., 2018).

BCL-2 proteins, which are representative regulators of apoptosis signaling pathways, regulate autophagy (Xu and Qin, 2019). ABT-737, a BCL-2 inhibitor, has been reported to be an autophagy inhibitor. Moreover, colorectal cancer cell sensitivity to ixazomib, used to treat multiple myeloma, is enhanced upon ABT-737 treatment through MCL-1 downregulation and autophagy inhibition (Yang *et al*., 2016). Obatoclax, which inhibits Pan-BCL-2, suppresses autophagy in the bladder (Jimenez-Guerrero *et al*., 2018) and colorectal cancer at a late stage (Koehler *et al*., 2015).

Autophagy is associated with proteasome pathways. Bortezomib, a proteasome inhibitor, is an approved anti-cancer drug for multiple myeloma. Bortezomib treatment initiates autophagosome formation, induces autophagic flux, and upregulates ATG5 in human prostate cancer cells (Zhu *et al*., 2010). Bortezomib also regulates both apoptosis and autophagic pathways via mitogen-activated protein kinase activation in osteocarcinoma (Lou *et al*., 2013). In breast cancer cell lines, bortezomib suppresses autophagy by inhibiting the cathepsin activity and promoting caspase-dependent apoptosis (Periyasamy-Thandavan *et al*., 2010). Bortezomib also promotes apoptosis in ovarian cancer cells by suppressing autophagic flux (Kao *et al*., 2014). The treatment combination of bortezomib and 3-MA has been shown to promote apoptosis in the human glioblastoma cell lines, U87 and U251 (Zhang *et al*., 2014).

**Table 2.** Autophagy inhibitors for treating cancer

| Compound | Target or mode of action | Cancer types | References |
|---|---|---|---|
| 3-Methyladenine (3-MA), LY294002 | Class III PI3K | HeLa cells | Hou *et al.*, 2012 |
| Bafilomycin A1 | Beclin-1, lysosomal $H^+$-ATPase inhibitor | Gastric cancer cells | Li *et al.*, 2016 |
| Chloroquine (CQ) | Fusion process of autophagosome and lysosome | Glioma cells, breast cancer, colon cancer | Hori *et al.*, 2015; Cave *et al.*, 2018; Liu *et al.*, 2019 |
| Hydroxychloroquine (HCQ) | Lysosomal capthepsin D | Colorectal cancer, breast cancer | Cook *et al.*, 2014; Patel *et al.*, 2016 |
| Quinacrine | p21/p27 | Ovarian cancer | Jung *et al.*, 2018 |
| ABT-737 | Bcl-2 | Multiple myeoloma, colorectal cancer cells | Sun *et al.*, 2018 |
| Obatoclax | Bcl-2 | Bladder cancer, colorectal cancer | Koehler *et al.*, 2015; Jimenez-Guerrero *et al.*, 2018 |
| Bortezomib | Proteasome | Multiple myeloma, prostate cancer cells, osteocarcinoma, breast cancer cells, ovarian cancer cells, glioblastoma | Periyasamy-Thandavan *et al.*, 2010; Zhu *et al.*, 2010; Lou *et al.*, 2013; Kao *et al.*, 2014; Zhang *et al.*, 2014 |
| Elaiophylin | Inhibition of autophagy flux | Ovarian cancer cells, multiple myeloma | Zhao *et al.*, 2015; Wang *et al.*, 2017 |
| 4-Acetylantroquinonol B | Inhibition of autophagy flux | Epithelial cancer cells | Liu *et al.*, 2017 |
| Thymoquinone | Permeabilization of the lysosome membrane | Glioblastoma cells | Racoma *et al.*, 2013 |

**Table 3.** Combinatorial treatment by targeting autophagy and anti-tumor drug in clinical trials

| Compounds | Cancer types | References |
|---|---|---|
| CQ+temozolomide | Glioblastoma multiforme, glioblastomas, glioma cells | Hori *et al.*, 2015; Zanotto-Filho *et al.*, 2015 |
| HCQ+temozolomide | Solid tumors and melanoma | Rangwala *et al.*, 2014a |
| HCQ+temsirolimus (CCI-779) | Melanoma | Rangwala *et al.*, 2014b |
| HCQ+bortezomib | Myeloma | Vogl *et al.*, 2014 |
| HCQ and vorinostat | Solid tumors, metastatic colorectal cancer | Mahalingam *et al.*, 2014; Patel *et al.*, 2016 |
| Pantoprazole+doxorubicin | Solid tumors | Brana *et al.*, 2014 |

Clarithromycin, a macrolide antibiotic, is used to treat upper and lower respiratory tract and *Helicobacter pylori* infections (Crowe, 2019). Clarithromycin suppresses autophagy by inhibiting the interaction between the hERG1 potassium channel and PI3K in colorectal cancer (Altman and Platanias, 2012; Petroni *et al.*, 2020). Elaiophylin, a macrolide antibiotic with immunosuppressive properties, inhibits the autophagic flux and has anti-tumor activity in ovarian cancer (Zhao *et al.*, 2015) and multiple myeloma with mutant TP53 (Wang *et al.*, 2017).

4-Acetylantroquinonol B, a novel compound derived from antroquinonol, enhances the sensitivity of cisplatin in epithelial cancer cells by inhibiting the autophagic flux (Liu *et al.*, 2017). Thymoquinone stimulates the permeabilization of the lysosome membrane and inhibits autophagy, which induces cathepsin-mediated death in glioblastoma cells (Racoma *et al.*, 2013). Further studies are required to elucidate the effect of autophagy inhibitors on cancer cells, to develop potential therapeutic agents against cancer (Table 2).

## COMBINATION TREATMENT OF AN AUTOPHAGY REGULATOR AND AN ANTI-CANCER DRUG

Studies have suggested that the efficacy of anti-cancer drugs is enhanced upon co-administration of autophagic flux regulators (Table 3).

CQ enhances the efficacy of temozolomide, which is an alkylating agent used to treat brain cancers, such as glioblastoma multiforme and glioblastomas (Zanotto-Filho *et al.*, 2015) by suppressing mitophagy in glioma cells (Hori *et al.*, 2015). Additionally, CQ promotes apoptotic cell death induced by vorinostat, an HDAC inhibitor, in colon cancer (Carew *et al.*, 2010).

HCQ, in combination with temozolomide, is effective in patients suffering from solid tumors and melanoma, in a phase 1 study (Rangwala *et al*., 2014a). Another phase 1 dose-escalation study has demonstrated that the anti-tumor effect of HCQ and temsirolimus (CCI-779; an mTOR inhibitor) treatment combination is stronger than that of HCQ alone in patients with melanoma (Rangwala *et al*., 2014b). These studies suggest that autophagy inhibitors markedly enhance the therapeutic effects of anti-cancer drugs. The suppression of autophagy may promote apoptosis, which can enhance the sensitivity of cancer cells to radiation therapy. Vogl *et al*. (2014) demonstrated that the treatment combination of HCQ and bortezomib increases the efficacy of proteasome inhibition in patients with myeloma. Additionally, the efficacy of proteasome inhibitors is strongly dependent on the ability of malignant cells to degrade misfolded proteins (Vogl *et al*., 2014). Furthermore, Mahalingam *et al*. (2014) demonstrated the pharmacokinetic and pharmacodynamic properties, as well as the safety of the HCQ and vorinostat treatment combination in patients with advanced solid tumors. This combination treatment suppresses autophagy and markedly enhances anti-tumor immunity in metastatic colorectal cancer (Patel *et al*., 2016).

Similarly, a phase I clinical study revealed that pantoprazole, a proton pump inhibitor, enhances the anti-cancer efficacy of doxorubicin by inhibiting autophagy in patients with solid tumors (Brana *et al*., 2014). These results suggest that the anti-cancer efficacy of an autophagy inhibitor and anti-cancer drug treatment combination is higher than that of anti-tumor drugs alone (Table 3).

## CONCLUSION

Malignant transformation involves dramatic changes in cellular metabolism owing to the high energy demand. During carcinogenesis, a continuous supply of macromolecule precursors is required to support abnormal cell proliferation. Thus, autophagy regulation has recently emerged as a potential therapeutic strategy against cancer.

Autophagy exhibits either cytoprotective or cytotoxic activity as dual roles during carcinogenesis, depending on the heterogeneity of each tumor. Studies have suggested that autophagy suppresses tumor growth in the early stages, but accelerates tumor progression in the later stages (Kimmelman and White, 2017; Kocaturk *et al*., 2019).

Autophagy and cancer pathways have been annotated. However, the interactions between these pathways have not been elucidated. The physiological role of autophagy must be analyzed in each tumor type. Various drugs, either alone or in combination, inhibit cancer progression by regulating autophagy (Table 1-3). However, further studies are required to determine the timing, intervals, and dosage of potent autophagy regulator administration in each cancer type. Additionally, clinical studies are required to further examine the anti-cancer efficacy of treatment combinations of autophagy inhibitors and anti-cancer drugs.

## CONFLICT OF INTEREST

The author declares that there are no conflicts of interest.

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