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 (Galia 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 phosphatidyethanolamine 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

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**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 autophagosomes 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-mediated autophagy (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., 2015). 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,               | Busca et al., 1996; Grewe et al., 1999; Chang et al., 2007                |
| Everolimus     | mTOR                     | Lymphoblastic B cells                             | Neri et al., 2014                                                         |
| Temsirolimus   | 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) | mTOR | Histone deacetylase (HDAC), 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 Alkhalaif, 2003; Li et al., 2014                           |
| Spermidine     | AMPK                     | Colon cancer cells                                | Morselli et al., 2011                                                     |

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).
Metformin (250 mg/kg body weight), an anti-diabetic drug, stimulates autophagy through AMPK activation and markedly inhibits prostate cancer cell proliferation in HinYc 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 phosphatidylinositool-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. Magnololin, a bioactive natural compound, activates autophagy and arrests the cell cycle in human colorectal cancer cells by inhibiting the LIF/Stat3/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)-B-phenyl-chromone (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).

Bafloymycin 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). Bafloymycin 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).

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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; Cavé et al., 2018; Liu et al., 2019). Additionally, CQ promotes apoptotic cell death induced by vorinostat, an HDAC inhibitor, in colon cancer (Carew et al., 2010).

**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 cathepsin 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 myeloma, 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 | Penyasamy-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 |
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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REFERENCES

Altman, J. K. and Plataniias, L. C. (2012) A new purpose for an old drug: inhibiting autophagy with chloroquine. Leuk. Lymphoma 53, 1255-1256.

Amaravadi, R., Kimmelman, A. C. and White, E. (2016) Recent insights into the function of autophagy in cancer. Genes Dev. 30, 1913-1930.

Ben Sahra, I., Tanti, J. F. and Bost, F. (2010) The combination of metformin and 2-deoxyglucose inhibits autophagy and induces AMPK-dependent apoptosis in prostate cancer cells. Autophagy 6, 670-671.

Bhat, P., Kriel, J., Shubha Priya, B., Basappa, Shivananj, N. S. and Loos, B. (2018) Modulating autophagy in cancer therapy: advances and challenges for cancer cell death sensitization. Biochem. Pharmacol. 147, 170-182.

Bilanges, B., Posor, Y. and Vanhaesebroeck, B. (2019) PI3K isoforms in cell signalling and vesicle trafficking. Nat. Rev. Mol. Cell Biol. 20, 515-534.

Brana, I., Ocana, A., Chen, E. X., Razak, A. R., Haines, C., Lee, C., Douglas, S., Wang, L., Siu, L. L., Tannock, I. F. and Bedard, P. L. (2014) A phase I trial of pantoprazole in combination with doxorubicin in patients with advanced solid tumors: evaluation of pharmacokinetics of both drugs and tissue penetration of doxorubicin. Invest. New Drugs 32, 1269-1277.

Busca, R., Bertolotto, C., Ortonne, J. P. and Ballotti, R. (1996) Inhibition of the phosphatidylinositol 3-kinase/PI3K(3)-kinase pathway induces B16 melanoma cell differentiation. J. Biol. Chem. 271, 31824-31830.

Carew, J. S., Medina, E. C., Esquivel, J. A., 2nd, Mahalingham, D., Swords, R., Kelly, K., Zhang, H., Huang, P., Mita, A. C., Mita, M. M., Giles, F. J. and Nawrocki, S. T. 2010 Autophagy inhibition enhances vorinostat-induced apoptosis via ubiquilinated protein accumulation. J. Cell. Mol. Med. 14, 2448-2459.

Cave, D. D., Desiderio, V., Mosca, L., Illeso, C. P., Mele, L., Caraglia, M., Cacciapuoti, G. and Porcelli, M. (2018) S-Adenosylmethionine-mediated apoptosis is potentiated by autophagy induction induced by chloroquine in human breast cancer cells. J. Cell. Physiol. 233, 1370-1383.

Chang, S. B., Miron, P., Miron, A. and Iglehart, J. D. (2007) Rapamycin inhibits proliferation of estrogen-receptor-positive breast cancer cells. J. Surg. Res. 138, 37-44.

Chen, N. and Karantza-Wadsworth, V. (2009) Role and regulation of autophagy in cancer--a matter of timing and context. Clin. Cancer Res. 21, 498-504.

Cook, K. L., Warri, A., Soto-Pantoja, D. R., Clarke, P. A., Cruz, M. I., Zwart, A. and Clarke, R. (2014) Hydroxycarbamide inhibits autophagy to potentiate antisterogen responsiveness in ER+ breast cancer cells. Clin. Cancer Res. 20, 3222-3232.

Crowe, S. E. (2019) Helicobacter pylori infection. N. Engl. J. Med. 380, 1158-1165.

Dang, C. V. and Kim, J. W. (2018) Convergence of cancer metabolism and immunity: an overview. Biomol. Ther. (Seoul) 26, 4-9.

Danhier, P., Sanski, P., Payen, V. L., Grasso, D., Ippolito, L., Sonveaux, P. and Porporato, P. E. (2017) Cancer metabolism in space and time: beyond the Warburg effect. Biochim. Biophys. Acta 1858, 556-572.

El-Mowafy, A. M. and Alkhalaf, M. (2003) Resveratrol activates adenylyl-cyclase in human breast cancer cells: a novel, estrogen
receptor-independent cytostatic mechanism. Carcinogenesis 24, 869-873.
Galati, S., Boni, C., Gerra, M. C., Lazzaretto, M. and Buschini, A. (2019) Autophagy: a player in response to oxidative stress and DNA damage. Oxid. Med. Cell. Longev. 2019, 5692958.
Galluzzi, L., Pietrocristi, F., Bravo-San Pedro, J. M., Galluzzi, L., Pietrocola, F., Bravo-San Pedro, J. M., Amaravadi, R. K., Grewe, M., Gansauge, F., Schmid, R. M., Adler, G. and Seufferlein, T. (2017) Autophagy-monitoring receptor-independent cytostatic mechanism. Carcinogenesis 38, E12.
Grewal, S. C., Kaur, S. and Iyer, A. J. (2017) mtORF function and therapeutic targeting in breast cancer. Am. J. Cancer Res. 7, 383-404.
Hori, Y. S., Kosaka, T., Iyama, M., Kato, S., Sato, T., Suzuki, K., Morishima, K., Mikoshiba, K., Hara, J., Kato, N., Nishida, E. and Ohsumi, Y. (2000) Beclin1, a Bcl-2 interacting protein, is required for autophagosome formation. Cell 102, 1071-1082.
Huang, C. C., Hao, C. Y., Wu, H. S. and Peng, H. C. (2018) AMPK and autophagy. Adv. Exp. Med. Biol. 1026, 6561-6565.
Golden, E. B., Cho, H. Y., Hofman, F. M., Louie, S. G., Schonthal, A. H., Gammoh, N., Lam, D., Puente, C., Gammoh, N., Lam, D., Puente, C., Ganley, I., Marks, P. A. and Jiang, X. (2012) Role of autophagy in histone deacetylase inhibitor-induced apoptotic and nonapoptotic cell death. Proc. Natl. Acad. Sci. U.S.A. 109, 6551-6556.
Huang, Z. (2000) Small molecule inhibitors of Bcl-2 function: modulation of apoptosis and promotion of cancer cell death. Curr. Opin. Drug Discov. Devel. 3, 565-574.
Jimenez-Guerrero, R., Gasca, J., Flores, M. L., Perez-Valderrama, B., Kao, C., Chao, A., Tsai, C. L., Chuang, W. C., Huang, W. P., Chen, G. C., Lin, C. Y., Wang, T. H., Wang, H. S. and Lai, C. H. (2014) A novel anticancer effect of resveratrol: reversal of epigenetic modifiers regulating autophagy/apoptosis toggle switch. J. Biol. Chem. 289, 18573-18583.
Kuma, A., Komatsu, M. and Mizushima, N. (2017) Autophagy-monitoring receptor-independent cytostatic mechanism. Carcinogenesis 24, 869-873.
Galati, S., Boni, C., Gerra, M. C., Lazzaretto, M. and Buschini, A. (2019) Autophagy: a player in response to oxidative stress and DNA damage. Oxid. Med. Cell. Longev. 2019, 5692958.
Kroemer, G. (2011) Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteinase. J. Cell Biol. 192, 615-629.

Moscat, J. and Diaz-Meco, M. T. (2009) p62 at the crossroads of autophagy, apoptosis, and cancer. Cell 137, 1001-1004.

Mowers, E. E., Sharfi, M. N. and Macleod, K. F. (2017) Autophagy in cancer metastasis. Oncoogene 36, 1019-1630.

Neri, L. M., Cani, A., Martelli, A. M., Simon, I., Junghanss, C., Tabelini, G., Ricci, F., Tazzari, P. L., Pagliaro, P., McCubrey, J. A. andCapitani, S. (2014) Targeting the PI3K/Akt/mTOR signaling pathway in B-precursor acute lymphoblastic leukemia and its therapeutic potential. Leukemia 28, 739-748.

Park, J. M., Jung, C. H., Seo, M., Otto, N. M., Grunwald, D., Kim, K. H., Moriarity, B., Kim, Y. M., Starker, C., Nho, R. S., Voytas, D. and Kim, D. H. (2016) The ULK1 complex mediates mTORC1 signaling to the autophagy initiation machinery via binding and phosphorylation of ATG14. Autophagy 12, 547-564.

Patel, S., Hurez, V., Narwrocki, S. T., Goros, M., Michalek, J., Saha, A., Blando, J., Tremmel, L. and DiGiovanni, J. (2015) Effect of topofagy, apoptosis, and cancer metastasis. Cancer Res. 75, 615-629.

Kroemer, G. (2011) Spermidine and resveratrol induce autophagy in metastatic colorectal cancer. Oncotarget 2, 59087-59097.

Periyasamy-Thanandas, S., Jackson, W. H., Samaddar, J. S., Erickson, B., Barrett, J. R., Rainey, L., Gopal, E., Ganapathy, V., Hill, W. D., Bhalla, K. N. and Schoenlein, P. V. (2010) Bortezomib blocks the catabolic process of autophagy via a cathepsin-dependent mechanism, affects endoplasmic reticulum stress and induces caspase-dependent cell death in antiestrogen-sensitive and resistant ER+ breast cancer cells. Autophagy 6, 19-35.

Patel, S., Hurez, V., Narwrocki, S. T., Goros, M., Michalek, J., Saha, A., Blando, J., Tremmel, L. and DiGiovanni, J. (2015) Effect of autophagy inhibition: phase I trial of hydroxychloroquine and temsirolimus in patients with advanced solid tumors and melanoma. J. Cell Biol. 209, 851-869.

Rangwala, R., Leone, R., Chang, Y. C., Fecher, L. A., Schuchter, L. M., Kramer, A., Tan, K. S., Heitjan, D. F., Rodgers, G., Gallagher, M., Piao, S., Troxel, A. B., Evans, T. L., DeMichele, A. M., Nathanson, K. L., O’Dwyer, P. J., Kaiser, J., Pontiggia, L., Davis, L. E. and Amaravadi, R. K. (2014a) Phase I trial of hydroxychloroquine with dose-intense temozolomide in patients with advanced solid tumors and melanoma. Cancer Cell 25, 935-948.

Vogl, D. T., Stadtmauer, E. A., Tan, K. S., Heitjan, D. F., Davis, L. E., Pontiggia, L., Rangwala, R., Piao, S., Chang, Y. C., Scott, E. C., Paul, T. M., Nichols, C. W., Porter, D. L., Kaplan, J., Mallon, G., Shukla, S., Patric, I. R., Patil, V., Shwetha, S. D., Hegde, A. S., Chandramouli, B. A., Arivazhagan, A., Santosh, V. and Somasundaram, K. (2014) Metformin inhibits growth of human glioblastoma cells and enhances therapeutic response. PLoS ONE 10, e0123721.

Shukla, S., Patric, I. R., Patil, V., Shwetha, S. D., Hegde, A. S., Chandramouli, B. A., Arivazhagan, A., Santosh, V. and Somasundaram, K. (2014) Methylation silencing of ULK2, an autophagy gene, is essential for astrocyte transformation and tumor growth. J. Biol. Chem. 289, 22306-22318.

Yu, Z., Yang, Z., Yu, X., Chen, Y. and Yu, Q. (2015) Apoptosis, autophagy, necroptosis, and cancer metastasis. Mol. Cancer 14, 48.

Sun, F. D., Wang, P. C., Shang, J., Zou, S. H. and Du, X. (2018) Ibrutinib presents antitumor activity in skin cancer and induces autophagy. Eur. Rev. Med. Pharmacol. Sci. 22, 561-566.

Takamura, A., Komatsu, M., Hara, T., Sakamoto, A., Kishi, C., Waguri, S., Eishi, Y., Hino, O., Tanaka, K. and Mizushima, N. (2011) Autophagy-deficient mice develop multiple liver tumors. Genes Dev. 25, 795-800.

Tamagno-Gomez, I. and Marino, G. (2018) AMPK: regulation of metabolic dynamics in the context of autophagy. Int. J. Mol. Sci. 19, 3812.

Tripathi, R., Ash, D. and Shaha, C. (2014) Beclin-1-p53 interaction is crucial for cell fate determination in embryonal carcinoma cells. J. Cell. Mol. Med. 18, 2275-2286.

Tsujimoto, Y. and Shimizu, S. (2015) Another way to die: autophagic programmed cell death. Cell Death Diff. 12, 1528-1534.

Umemura, A., He, F., Taniguchi, K., Nakagawa, H., Yamachika, S., Font-Burgada, J., Zhong, Z., Subramaniam, S., Raghunandan, S., Duran, A., Linares, J. F., Reina-Campos, M., Umemura, S., Vasilek, M. A., Seki, E., Yamaguchi, K., Koike, K., Itoh, Y., Diaz-Meco, M. T., Moscat, J. and Karin, M. (2016) p62, upregulated during pre-neoplasia, induces hepatocellular carcinogenesis by maintaining survival of stressed HCC-initiating cells. Cancer Cell 29, 935-948.

Vignot, S., Faivre, S., Aguirre, D. and Raymond, E. (2005) mTOR-targeted therapy of cancer with rapamycin derivatives. Ann. Oncol. 16, 525-537.

Vogl, D. T., Stadtmauer, E. A., Tan, K. S., Heitjan, D. F., Davis, L. E., Pontiggia, L., Rangwala, R., Piao, S., Chang, Y. C., Scott, E. C., Paul, T. M., Nichols, C. W., Porter, D. L., Kaplan, J., Mallon, G., Bradner, J. E. and Amaravadi, R. K. (2014) Combined autophagy and proteasome inhibition: a phase 1 trial of hydroxychloroquine and bortezomib in patients with relapsed/refractory myeloma. Autophagy 10, 1380-1390.

Wan, B., Zang, Y. and Wang, L. (2018) Overexpression of Beclin1 inhibits proliferation and promotes apoptosis of human laryngeal squamous carcinoma cell HeP-2. Onco Targets Ther. 11, 3827-3833.

Wang, G., Zhou, P., Chen, X., Zhao, L., Tan, J., Yang, Y. and Zhou, J. (2017) The novel autophagy inhibitor eliapolyphenol exerts antitumor activity against multiple myeloma with mutant TP53 in part through endoplasmic reticulum stress-induced apoptosis. Cancer Biol. Ther. 18, 584-595.

Wang, Y., Xu, W., Yan, Z., Zhao, W., Mi, J., Li, J. and Yan, H. (2018) Metformin induces autophagy and G0/G1 phase cell cycle arrest in myeloma by targeting the AMPK/mTORC1 and mTORC2 pathways. J. Exp. Clin. Cancer Res. 37, 63.

White, E., Mehner, J. M. and Chan, C. S. (2015) Autophagy, metabolism, and cancer. Clin. Cancer Res. 21, 5037-5046.

Xu, H. D. and Qin, Z. H. (2019) Beclin 1, bcl-2 and autophagy. Adv. Exp. Med. Biol. 1206, 109-126.

Yang, L., Wang, J., Xiao, S., Barkhouse, D., Zhu, J., Li, G., Lu, B. and Zhang, Z. (2016) BH3 mimetic ABT-737 sensitizes colorectal cancer cells to ixazomib through MCL-1 downregulation and autophagy inhibition. Am. J. Cancer Res. 6, 1345-1357.

Yang, Y. P., Hu, L. F., Zheng, H. F., Mao, C. J., Hu, W. D., Xiong, K. P., Wang, F. and Liu, C. F. (2013) Application and interpretation of
current autophagy inhibitors and activators. Acta Pharmacol. Sin. 34, 625-635.
Yorimitsu, T. and Klionsky, D. J. (2005) Autophagy: molecular machinery for self-eating. Cell Death Differ. 12, 1542-1552.
Yu, H., Yin, S., Zhou, S., Shao, Y., Sun, J., Pang, X., Han, L., Zhang, Y., Gao, X., Jin, C., Qiu, Y. and Wang, T. (2018) Magnolin promotes autophagy and cell cycle arrest via blocking LIF/Stat3/Mcl-1 axis in human colorectal cancers. Cell Death Dis. 9, 702.
Yuan, N., Song, L., Zhang, S., Lin, W., Cao, Y., Xu, F., Fang, Y., Wang, Z., Zhang, H., Li, X., Wang, Z., Cai, J., Wang, J., Zhang, Y., Mao, X., Zhao, W., Hu, S., Chen, S. and Wang, J. (2015) Bafilomycin A1 targets both autophagy and apoptosis pathways in pediatric B-cell acute lymphoblastic leukemia. Haematologica 100, 345-356.
Zaffagnini, G., Savova, A., Danieli, A., Romanov, J., Tremel, S., Ebner, M., Peterbauer, T., Sztacho, M., Trapannone, R., Tarafder, A. K., Sachse, C. and Martens, S. (2018) p62 filaments capture and present ubiquitinated cargos for autophagy. EMBO J. 37, e98308.
Zanotto-Filho, A., Braganhol, E., Klaafke, K., Figueiro, F., Terra, S. R., Paludo, F. J., Morrone, M., Bristol, I. J., Battastini, A. M., Forcelini, C. M., Bishop, A. J. R., Gelain, D. P. and Moreira, J. C. F. (2015) Autophagy inhibition improves the efficacy of curcumin/temozolomide combination therapy in glioblastomas. Cancer Lett. 358, 220-231.
Zhang, X., Li, W., Wang, C., Leng, X., Lian, S., Feng, J., Li, J. and Wang, H. (2014) Inhibition of autophagy enhances apoptosis induced by proteasome inhibitor bortezomib in human glioblastoma U87 and U251 cells. Mol. Cell. Biochem. 385, 265-276.
Zhao, X., Fang, Y., Yang, Y., Qin, Y., Wu, P., Wang, T., Lai, H., Meng, L., Wang, D., Zheng, Z., Lu, X., Zhang, H., Gao, Q., Zhou, J. and Ma, D. (2015) Elaiophylin, a novel autophagy inhibitor, exerts anti-tumor activity as a single agent in ovarian cancer cells. Autophagy 11, 1849-1863.
Zhu, K., Dunner, K., Jr. and McConkey, D. J. (2010) Proteasome inhibitors activate autophagy as a cytoprotective response in human prostate cancer cells. Oncogene 29, 451-462.